Effects of Chlorinated Insecticides on Metabolic Processes in Bacteria

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Experimental findings accumulated over a number of years have frequently been at variance with regard to the effects of pesticide application on the microbial populations of soils. Such variation may be a result of differing application rates and procedures, heterogeneous and diverse soil environments within which studies have been made (Basson, 1966; Edwards, 1966) and, as yet not understood, protection conferred on susceptible organisms by resistant organisms in a mixed culture (Bounds, Magee & Colmer, 1969). Increased populations and metabolic activities of soil bacteria in the presence of aldrin or lindane have been reported (Verona, 1952; Fletcher & Bollen, 1954; Roberts & Bollen, 1955), whereas aldrin, chlordane, dieldrin, endrin, heptachlor and lindane have been shown to have no effect (Gray, 1954; Wegorek, 1957; Martin, Harding, Connell & Anderson, 1959) or to be toxic (Jones, 1956; Grossman & Steckhan, 1960).

On agar-plate culture under laboratory conditions benzene hexachloride inhibits the growth of Gram-positive organisms only (Gray & Rogers, 1955). *Pseudomonas fluorescens* is sensitive to DDT but not dieldrin or heptachlor, and *Staphylococcus aureus* is inhibited by heptachlor and DDT but not dieldrin (Collins & Langois, 1968). Aldrin, chlordane and lindane are potently antifungal on agar-plate cultures (Grossmann & Steckhan, 1960), and inhibit nitrification by chemolithotropic bacteria in liquid culture over concentration ranges 1–1000 p.p.m. (Garretson & San Clemente, 1968).

Research in this laboratory is directed towards the elucidation of cyclodiene-sensitive sites of susceptible bacteria and the characteristics that confer immunity on the remainder. Initial screening experiments with organisms in pure culture were carried out on nutrient agar plates with the pesticide incorporated in a uniformly distributed finely divided or emulsified form (1000 p.p.m.) or as a thin surface film (1 mg/cm²). Relatively large amounts of insecticides were used because of the difficulty of assessing active concentrations of compounds of such low water-solubility, and with the object of compressing the time-scale of events that may result from the known ability of micro-organisms to accumulate chlorinated insecticides from the surrounding medium over a period of time (Choko & Lockwood, 1967; Gregory, Reed & Priester, 1969). Growth of the following Gram-positive organisms was arrested by one or more chlorinated insecticides: *Bacillus subtilis* strain 4E, *B. cereus* strain 22C, *B. megaterium* (N.C.T.C. 9848), *Microbacterium*

flavum (N.C.I.B. 8707), *Micrococcus lysodeikticus* (N.C.I.B. 9278), two untyped strains of *Nocardia*, *Sarcina lutea* (N.C.I.B. 8553), *Streptomyces antibioticus* (N.C.I.B. 8504), *Streptococcus faecalis* (N.C.I.B. 8661), an untyped strain of *Corynebacterium*, and the Gram-variable organism *Arthrobacter simplex* (N.C.I.B. 8929). *B. megaterium* was the most sensitive organism, growth being inhibited by aldrin, chlordane, dieldrin, endrin, telodrin, lindane and DDT, and *S. lutea* the least sensitive, growth being inhibited only by chlordane. Chlordane was the most potent inhibitor, inhibiting growth of all the Gram-positive organisms tested, DDT the least, inhibiting only one. Growth of the Gram-negative organisms *Klebsiella aerogenes* W5, *Achromobacter butyri* (N.C.I.B. 9404), *Escherichia coli* (N.C.I.B. 8545) and several strains of *Pseudomonas fluorescens*, *P. putida*, *P. acidovorans* and *P. multivorans* was not inhibited by any of the insecticides tested, thus extending the observation of Gray & Rogers (1955) and Fritsch, Moore, Meyer, Shklair & Devine (1969).

Respiration by whole cells of Gram-positive organisms was inhibited by technical chlordane (660 p.p.m.) as follows: *B. subtilis*, *B. megaterium* and *B. cereus*, 60%; *Nocardia* sp., 63%; *M. flavum*, 52%; *S. lutea*, 53%; *Corynebacterium* sp. 34%; *M. lysodeikticus*, 30%. Similar experiments with the range of Gram-negative organisms previously tested for growth sensitivity revealed no significant inhibitory effects. Resistance of Gram-negative organisms to chlorinated insecticides may, like the resistance of *E. coli* to hexachlorophene (Joswick, 1961), anacardic acids (Gellerman, Walsh, Werner & Shlenk, 1969) and tetrachlorosalicylanilide (Hamilton, 1968), be associated with cell-wall structure and composition, since respiration of sucrose-stabilized spheroplasts prepared from *E. coli* by the EDTA-lysozyme method (Repaske, 1958) or by penicillin treatment (Lederberg, 1956) was sensitive to chlordane, inhibition being 43 and 33% respectively. Less drastic treatment with 5 mM-EDTA, which is known to remove a portion of the lipopolysaccharide from the walls (Leive, 1965) and increase cell permeability without loss of viability, does not produce chlordane-sensitive cells.

NADH oxidase activity of membrane fractions from *B. subtilis*, *M. lysodeikticus*, a *Nocardia* sp., *E. coli*, *P. fluorescens* and *A. butyri* was markedly inhibited (90–33%) by aldrin, chlordane, bandane, thiodan and dieldrin, with lindane and DDT showing less severe effects. NADH oxidase of membranes from *B. subtilis* and *P. fluorescens* was sensitive to chlordane at 66 p.p.m. Succinate dehydrogenase and NADH dehydrogenase assays on membrane preparations from *B. subtilis* and *E. coli* have shown only the NADH dehydrogenase of *E. coli* to be inhibited by chlordane. Experiments

with u.v. irradiation (360 nm) to destroy vitamin K₂ (Kashket & Brodie, 1962), with triphenyltetrazolium chloride, 2,6-dichlorophenol-indophenol, phenazine methosulphate and horse heart cytochrome *c* as electron acceptors and tetramethylphenylene-diamine and paraquat (*E. coli* nitrate reductase) as electron donors indicate that chlordane inhibition of NADH oxidation by *B. subtilis* may occur at all steps subsequent to the dehydrogenase. Differences in the relative sensitivities of NADH and succinate oxidation displayed by *E. coli* membranes are a manifestation of the insensitivity of the succinate dehydrogenase. Electron transport from succinate to triphenyltetrazolium or horse heart cytochrome *c* is weakly inhibited as is transport to oxygen, and similar studies with NADH as electron donor show marked inhibition at all stages. These results are an interesting parallel to the reported inhibitory effects of DDT on the tricarboxylic acid cycle in housefly homogenates (Sacklin, Terriere & Remmert, 1955), of aldrin, dieldrin, chlordane and heptachlor on cytochrome oxidase of the American cockroach (Morrison & Brown, 1954) and the inhibition of catfish liver mitochondrial succinoxidase and cytochrome oxidase by endrin (Colvin & Philips, 1968).

The possibility that electron transport is not the key target site responsible for total inhibition of growth of Gram-positive organisms is suggested by the frequently incomplete inhibition of this activity by high concentrations of insecticides, and is reinforced by the observation that *S. faecalis*, which does not possess a cytochrome-mediated electron-transport chain, is insecticide-sensitive. Effects on NADH oxidation by this organism must be ruled out since neither the soluble nor the membrane-bound oxidase- and peroxidase-mediated reactions are sensitive. The consistent but weak inhibition of glycolysis by K⁺-rich or K⁺-depleted cells maintained at pH 6 or pH 7 in aqueous or Na⁺-containing systems suggests that unlike gramicidin and valinomycin (Harold & Baarda, 1967) chlordane does not have any dramatic effect on membrane

permeability to cations. Other possible cyclodiene-sensitive target sites will be discussed.

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