Accumulation of [^{14}C]Aldrin by Organochlorine Insecticide Sensitive and Resistant Bacteria

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(Received 13 June 1972)

The accumulation of chlorinated insecticides from the surrounding environment by micro-organisms has been described by several workers. Chacko & Lockwood (1967) observed the accumulation of DDT and dieldrin from liquid culture media by fungi, streptomycetes and bacteria; Ko & Lockwood (1968) extended these investigations to the soil environment, where fungi and streptomycetes were shown to accumulate DDT and dieldrin to above ambient concentrations. Kokke (1970) demonstrated that many bacteria from a mixed soil population accumulated DDT during growth on nutrient agar containing 0.8 μg per ml of [^{14}C]DDT. Blue-green algae exposed to DDT at 1 p.p.m. for 7 days accumulated it at levels 94 to 964 times greater than that in the surrounding medium (Gregory, Reed & Priester, 1969). Yeasts have been shown to absorb γ -hexachlorocyclohexane and dieldrin (Voerman & Tammes, 1969).

Organochlorine insecticides have recently been studied for effects upon the growth, viability and metabolism of a range of Gram-positive, Gram-variable and Gram-negative bacteria (Trudgill, Widdus & Rees, 1971; Widdus, Trudgill & Maliszewski, 1971; Widdus, Trudgill & Turnell, 1971). Without exception growth of the Gram-positive and Gram-variable bacteria was inhibited by at least one of the organochlorine insecticides studied while all the Gram-negative organisms were resistant.

In this communication we report on investigations into the ability of a group of test organisms to accumulate [^{14}C]aldrin from nutrient agar at subinhibitory concentrations.

METHODS AND RESULTS

Organisms. The sources of all the bacteria used were previously described (Trudgill *et al.* 1971). Stock cultures were maintained on nutrient agar slopes.

Growth media. Organisms were grown on nutrient broth and used to inoculate nutrient agar. Additions of [^{14}C]aldrin were made to nutrient agar by adding a solution in acetone, diluted with the appropriate amount of unlabelled carrier, to the hot molten agar. After thorough mixing the plates were poured, allowed to cool, and dried at 37 °C for 36 h.

Materials. Nutrient agar and nutrient broth were supplied by Oxoid Ltd, London. [^{14}C]Aldrin (68 mCi/mmol) was supplied by the Radiochemical Centre, Amersham, Buckinghamshire, and analytical-grade aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-endo-1,4-exo-5,8-dimethanonaphthalene) was a gift from Shell Research Ltd, Sittingbourne, Kent.

Growth of bacteria in the presence of [^{14}C]aldrin. Inocula were grown in 5 ml of nutrient broth contained in 25 ml Erlenmeyer flasks and shaken on a New Brunswick Gyrotory shaker (New Brunswick Co., New Brunswick, New Jersey, U.S.A.) at 200 rev./min and

Fig. 1. Accumulation of $[^{14}\text{C}]$ aldrin by (a) Gram-positive and Gram-variable, (b) Gram-negative bacteria. Photographs of autoradiographs from 3 to 6 mm diameter patches of organisms grown on nutrient agar containing $[^{14}\text{C}]$ aldrin were scanned, using the reflectance mode, in a Chromoscan with a 3×0.4 mm light source aperture and a 60° angle wedge. Dark patches on the photographs, corresponding with blackened areas of the autoradiographs due to accumulation of $[^{14}\text{C}]$ aldrin, caused increases in the percentage deflection of the recorder pen. The bacteria used in this study, together with their sensitivity to organochlorine insecticides, given in parentheses as the no. of insecticides that inhibited growth out of a total of nine tested (Trudgill *et al.* 1971), are as follows.

(a) Gram-positive and Gram-variable: 1, *Bacillus megaterium* (9); 2, *Streptomyces antibioticus* (8); 3, *B. subtilis* (7); 4, *B. cereus* (6 to 7); 5, *Corynebacterium* sp. T1 (6); 6, *Microbacterium flavum* (5); 7, *Micrococcus lysodeikticus* (5); 8, *Staphylococcus aureus* (4); 9, *Arthrobacter simplex* (4); 10, *Sarcina lutea* (2); 11, *Pseudomonas iodinum* (at least 1).

(b) Gram-negative: 1, *Achromobacter butyri*; 2, *Achromobacter* sp. PC4; 3, *Escherichia coli*; 4, *Klebsiella aerogenes*; 5, *Pseudomonas acidovorans*; 6, *P. aureofaciens*; 7, *P. dehalogens*; 8, *P. fluorescens*; 9, *P. fluorescens* (NCIB 9392); 10, *P. multivorans*; 11, *P. putida*. None of the organochlorine insecticides tested (Trudgill *et al.* 1971) caused significant inhibition of growth of any of this group of Gram-negative bacteria.

30°C . After 24 h of growth these cultures were patch plated (about 5 mm diameter) on to nutrient agar plates containing $0.04 \mu\text{Ci}$ of radioactivity and $1 \mu\text{g}$ of aldrin/ml and incubated at 30°C .

Autoradiography of nutrient agar plates. After 48 h of growth in the presence of $[^{14}\text{C}]$ aldrin when the patches had grown, the concentration of aldrin chosen being too low to inhibit the growth of the sensitive Gram-positive organisms (Trudgill *et al.* 1971), the agar was covered with a waterproof plastic foil (5 mg/cm^2) and an X-ray film then placed in close contact with it as described by Kokke (1970) and the plates stored at 2°C to check microbial growth. The film was exposed in this way for 7 days, developed with Kodak DX-80 developer and fixed with Kodak FX-40 X-ray fixer (Kodak Ltd, London).

The autoradiographs were then photographed against a white background using a Kodak Panatomic X film and printed at the original size on Ilfobrom 1B2.1P paper. Prints were then cut so as to fit the carriage of a Chromoscan (Joyce Loebel & Co. Ltd, Gateshead, Durham) and scanned in the reflectance mode (Fig. 1), increase in pen deflection being in response to accumulated radioactivity.

Without exception the organochlorine insecticide-sensitive Gram-positive bacteria produced strongly radioactive bacterial patches. In some instances, where they were individually dilution plated on to nutrient agar containing [^{14}C]aldrin so as to yield about 100 colonies, it appeared that the bacteria had sequestered most of the [^{14}C]aldrin at the surface of the agar in view of the negligible background radiation remaining in comparison with an uninoculated control.

Most of the organochlorine insecticide-resistant Gram-negative bacteria accumulated no [^{14}C]aldrin as judged by visual observation of the radioautographs and confirmed by the Chromoscan trace (Fig. 1). Weak accumulation did however occur in *Pseudomonas aureofaciens* and *P. dehalogens*.

DISCUSSION

Chacko & Lockwood (1967) observed that when cultured on a glucose-peptone-yeast extract liquid medium containing 0.1 to 1 p.p.m. DDT or dieldrin the Gram-negative bacteria *Serratia marcescens* and *Agrobacterium tumefaciens* accumulated larger quantities of the two insecticides than did *Bacillus subtilis*. This can be considered as a logically expected result in view of the generally greater proportion of lipid components in the walls of Gram-negative bacteria (Salton, 1964), and the lipophilic nature of the tested compounds. However, Trudgill *et al.* (1971) have presented evidence, based on studies with benzyl penicillin-induced sphaeroplasts of *Escherichia coli*, that the complex wall of this and probably other Gram-negative organisms acts as an effective barrier to organochlorine insecticide penetration.

One suggestion that could reconcile these contradictory observations is that the lipid components of the Gram-negative bacterial cell wall absorb the organochlorine insecticide, become swollen, and seal the cell against insecticide incursion to target areas, thus rendering it resistant.

On the basis of the results obtained (Fig. 1) we discount this rather unlikely hypothesis as far as our group of organisms is concerned in favour of the view that it is the highly organized structure of the Gram-negative bacterial cell wall that effectively excludes the organochlorine insecticide from these organisms and renders them resistant. In contrast, the more amorphous and in most instances almost lipid-free walls of the Gram-positive bacteria (Salton, 1964) are ineffective at preventing the incursion of [^{14}C]aldrin and probably other organochlorine insecticides of similar structure and polarity.

The situation is, however, probably more complex than this since *Staphylococcus aureus*, *Sarcina lutea* and *Arthrobacter simplex*, which all accumulate clearly detectable amounts of [^{14}C]aldrin (Fig. 1), are resistant to the action of aldrin as a growth inhibitor (Trudgill *et al.* 1971).

We wish to thank Miss A. J. Evans and Miss M. M. Williams for preparation of the photographs.

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