REDUCTIVE DECHLORINATION OF P,P'-DDT BY ESCHERICHIA COLI AND PSEUDOMONAS AERUGINOSA

Thesis for the Degree of Ph. D.
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Allen L. French

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This is to certify that the

thesis entitled

REDUCTIVE DECHLORINATION
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AND PSEUDOMONAS AERUGINOSA

presented by

Allen L. French

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Roger Hoopingarner
Major professor

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ABSTRACT

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The metabolism and uptake of C^{14}-labeled p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) by intact cells of the bacteria Escherichia coli and Pseudomonas aeruginosa were investigated. The phenyl rings of the p,p'-DDT molecules were uniformly labeled with C^{14} atoms. The cultures were incubated for 1, 2, or 3 days in Anderson's minimal synthetic broth medium (Scheenhard, 1961) in the presence or absence of atmospheric oxygen. Autoclaved cultures served as controls.

In addition, washed membrane fractions were obtained from E. coli by lysozyme treatment followed by osomotic shock. The capacity of cellular components to metabolize p,p'-DDT was investigated. The effect of exogenous Krebs cofactors and intermediates on the metabolism of p,p'-DDT by particulate components of the bacterial cell was evaluated.

The p,p'-DDT and its metabolites were identified by thin-layer and gas-liquid chromatography. Metabolites containing C^{14} were determined by autoradiography of thin-layer chromatograms and compared to authentic samples of p,p'-DDT

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ABSTRACT

BIOCONCENTRATION AND BIOTRANSFORMATION OF p,p'-DDT BY MICROBACTERIA OCCI AND FERROOXIDANS AZOTOBACTER

by

Allen I. Laskin

The metabolism and uptake of C_{14}-labeled p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) by intact cells of the bacteria Brevibacteria ocCi and Ferrooxidans azotobacter were investigated. The phenyl rings of the p,p'-DDT molecules were uniformly labeled with C_{14} atoms. The cultures were incubated for 7, 5, or 3 days in Anderson's minimal synthetic broth medium (Bonnemann, 1961) in the presence or absence of atmospheric oxygen. Autoclaved cultures served as controls.

In addition, washed membrane fractions were obtained from B. ocCi by lysozyme treatment followed by osmotic shock. The capacity of cellular components to metabolize p,p'-DDT was investigated. The effect of exogenous Krebs cofactors and intermediates on the metabolism of p,p'-DDT by particulate components of the bacterial cell was evaluated.

The p,p'-DDT and its metabolites were identified by chromatograms and compared to authentic samples of p,p'-DDT determined C_{14} were determined by autoradiography of thin-layer thin-layer and gas-liquid chromatography. Metabolites com-

and its metabolites. Quantification was accomplished by gas-liquid chromatography and liquid scintillation counting. C^{14}-labeled compounds were collected from the column effluent and their radioactivity determined by liquid scintillation counting. Each assay was performed 3 times, and each incubant was replicated 2 times.

Aerobic and anaerobic cultures of E. coli and P. aeruginosa degraded p,p'-DDT to p,p'-DDD (1,1-dichloro-2,2-bis-(p-chlorophenyl)ethane). The capacity to degrade p,p'-DDT increased with the exclusion of atmospheric oxygen from the incubation medium. Over 90% of the p,p'-DDT was degraded to p,p'-DDD by anaerobic cultures of E. coli incubated 3 days. Less than 10% conversion occurred in autoclaved cell cultures incubated anaerobically. The pattern of p,p'-DDT metabolism by P. aeruginosa was similar to that found in the E. coli incubations. However, anaerobic cultures of P. aeruginosa were able to metabolize p,p'-DDT to p,p'-DDD more rapidly. Over 85% of the p,p'-DDT was reductively dechlorinated to p,p'-DDD by anaerobic cultures incubated 2 days.

Uptake of p,p'DDT was not increased by its metabolism. After 3 days of incubation, 71% of the radioactivity was associated with the cells of E. coli cultured anaerobically, and 80.1% was associated with the cells of aerobic cultures. Autoclaved cells were able to take up 47.2% of the radioactivity.

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cytoplasm (100,000 g supernatant).

After 4 hr of anaerobic incubation, neither the particulate
nor the cytoplasmic fractions of E. coli and E. aerogenes
reduced p,p'-DDD to p,p'-DDMU. Under the same conditions,
Proteus vulgaris reduced 10% of the p,p'-DDD to p,p'-DDMU.
Aerobic and anaerobic cultures of E. coli and E. aerogenes
were capable of reducing 1 mg of p,p'-DDD (1,1-dichloro-2,2-bis-
(p-chlorophenyl)ethane). The capacity to degrade p,p'-DDD
increased with the oxidation of atmospheric oxygen from the
incubation medium. Over 20% of the p,p'-DDT was degraded to
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components ("shockate" supernatant fraction) of E. coli cells, produced substantial amounts of p,p'-DDD. When the above fractions were combined, conversion of p,p'-DDT to p,p'-DDD occurred (29.8%). However, addition of "shockate" supernatant to boiled membrane fractions did not stimulate p,p'-DDD production.

When 3 ml of washed membrane fractions (25 mg/ml original wet weight of cells) were combined with a mixture of Krebs cycle cofactors and intermediates consisting of 2.0 umole each of NAD, NADP, FAD, malate, pyruvate and 0.1 umole each of ADP and inorganic phosphate and incubated anaerobically for 4 hr, 2.2% conversion of p,p'-DDT to p,p'-DDD occurred. When NAD, NADP, or malate and pyruvate were omitted from the incubations, the conversion was increased by a factor of 10.

Addition of FAD (2.0 umole) to washed membrane fractions resulted in the conversion of 22.5% of the p,p'-DDT to p,p'-DDD. However, addition of exogenous FAD to aerobically incubated membrane fractions did not stimulate p,p'DDD production.

Based on the results of the membrane studies, the following possibilities are suggested. Reductive dechlorination of p,p'-DDT occurs in the membranous portion of the bacterial cell and is not cytoplasmic in origin. It is stimulated by component(s) in the cytoplasm. Reductive dechlorination of p,p'-DDT does not utilize electrons produced by the oxidation of Krebs cycle intermediates and passed through the cytochrome system. Reductive dechlorination of p,p'-DDT

components ("apoenzyme" supernatant fraction) of E. coli cells, produced substantial amounts of b,b'-DDD. When the above fractions were combined, conversion of b,b'-DDT to b,b'-DDD occurred (29.8%). However, addition of "apoenzyme" supernatant to boiled membrane fractions did not stimulate b,b'-DDD production.

When 3 ml of washed membrane fractions (25 mg/ml original wet weight of cells) were combined with a mixture of Krebs cycle cofactors and intermediates containing of 2.0 umole each of NAD, NADP, FAD, malate, pyruvate and 0.1 umole each of ADP and inorganic phosphate and incubated anaerobically for 4 hr, 2.2% conversion of b,b'-DDT to b,b'-DDD occurred. When NAD, NADP, or malate and pyruvate were omitted from the incubations, the conversion was increased by a factor of 10.

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is dependent upon the enzymatic reduction of FAD and occurs only under anaerobic conditions. Reductive dechlorination of p,p'-DDT requires electrons produced by the oxidation of an energy source. Reductive dechlorination of p,p'-DDT may require the formation of free radicals. The oxidation of endogenous substrates can produce the half-reduced form of FAD (FADH·, a semiquinone) and may be the active moiety involved in the enzymatic reduction of p,p'-DDT.

is dependent upon the enzymatic reduction of NAD and occurs only under anaerobic conditions. Reductive decolorination of b'b'-DQI requires electrons produced by the oxidation of an energy source. Reductive decolorination of b'b'-DQI may involve the formation of free radicals. The oxidation of NAD ("NADH," a semiquinone) and may be the major moiety involved in the enzymatic reduction of b'b'-DQI.

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INTRODUCTION

Kallman and Andrews (1963) were the first to demonstrate that an isolated microorganism could degrade p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) to p,p'-DDD (1,1-dichloro-2,2-bis(p-chlorophenyl)ethane). Following this report, interest grew in the role of microorganisms in degradation of "persistent" pesticides. Since p,p'-DDT is extremely stable and has been extensively employed throughout the environment, several investigators have studied its metabolism.

Investigators have employed organisms that were obtained from soils, animal feces, intestines, and laboratory strains. Their ability to degrade p,p'-DDT was measured after various incubation intervals in a variety of broth cultures, agar-based suspensions and soils in the presence and absence of oxygen. The results of these investigations are presented in the literature review.

Although previous investigations established a wide microbiological spectrum of p,p'-DDT degradative capacity, little is known about the biological mechanism involved in bacterial uptake and degradation of p,p'-DDT. The site of metabolism, cytoplasmic or particulate, is still open to question. The present study was undertaken to answer some

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LITERATURE REVIEW

Kallman and Andrews (1963) were the first to demonstrate that microbes could metabolize DDT to DDD when they cultured commercial yeast anaerobically. Eighty-eight % conversion occurred after 3 days compared to only 3% conversion in the boiled controls. They reported that DDE (1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene) was not metabolized by the yeast.

Microorganisms Isolated From Animals

Several investigators have demonstrated the metabolism of DDT to DDD by microorganisms isolated from animals. Miskus et al. (1965) reported partial conversion of DDT to DDD in bovine rumen fluid. Stenersen (1965) determined the ability of Serratia marcescens and Alcaligenes faecalis, isolated from feces of resistant stableflies, Stomoxys calcitrans (L), and laboratory cultures of Escherichia coli, Bacillus brevis and Aerobacter aerogenes to metabolize C^{14}-labeled DDT. Ninety % of the DDT had been metabolized to DDD after 3 days of anaerobic incubation in meat extract bouillon while no conversion was reported in aerobic cultures. Proteus vulgaris, isolated from the intestinal tract of a mouse, was cultured in heart infusion medium with DDT by Baker and Morrison (1965). They found 65% conversion of DDT to DDD in 6 days. Between 6

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and 20 days a steady decline in recoverable DDD was noted, suggesting that other metabolites were formed. Mendel and Walton (1966) cultured E. coli and A. aerogenes, isolated from rat feces, in trypticase soy broth for 2 days with DDT. E. coli degraded 35.9% of the DDT to DDD, and A. aerogenes degraded 33% to DDD.

Microorganisms Isolated From Soil

Chacko et al. (1966) tested 9 actinomycetes and 8 fungi from soil for their ability to degrade DDT in a nutrient medium. None of the fungi displayed any appreciable capacity to degrade DDT while 6 actinomycetes did produce DDD. A maximum of 25% was degraded by Streptomyces aureofaciens in 6 days.

Matsumura and Bousch (1968), employing an unspecified liquid medium containing C^{14}-labeled DDT, incubated 18 soil isolated variants of the fungus Trichoderma viride anaerobically for 3 days. Of the 18 variants tested, 8 cultures produced both DDD and dicofol (1,1-bis(p-chlorophenyl)2,2,2-trichloroethanol) as their major metabolite, 3 produced DDD and 1 produced DDE and DDD. Six variants displayed no ability to degrade DDT under the conditions tested. The authors indicated the presence of unknown water soluble metabolites.

Johnson (1967) cultured 27 species of pathogenic and saprophytic bacteria associated with plants, anaerobically, in thioglycolate medium containing DDT for 7 or 14 days. Only the strict aerobe Sarcina letea and the anaerobe Clostridium sporogenes failed to convert DDT to DDD. None of

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the organisms tested displayed any capacity to degrade DDT to DDD when cultured aerobically.

Guenzi and Beard (1967) recovered 34% of the C^{14}-labeled DDT which had been added to soil and maintained anaerobically for 4 weeks. The major metabolite was DDD (62%) while only 4% was recovered as other products. Although the authors incubated autoclaved soil containing DDT, no values were presented. Bartha et al. (1967) measured the effect of DDT and DDD on carbon dioxide and nitrite production in the soil. The compounds at 150 and 1500 ppm had no appreciable effect on carbon dioxide production but were found to slightly increase nitrification as measured by nitrite production.

Laboratory Isolates

Wedemeyer (1966) tested E. coli, A. aerogenes and Klebsiella pneumoniae for their ability to anaerobically degrade DDT in trypticase soy broth or thioglycolate medium. Maximum conversion to DDD (80%) was achieved by A. aerogenes cultures after an unspecified incubation period. In subsequent reports Wedemeyer (1967 a and 1967 b), using 2 day A. aerogenes cultures, identified 4 additional metabolites, DDMU (1-chloro-2,2-bis(p-chlorophenyl)ethylene), DDMS (1-chloro-2,2-bis(p-chlorophenyl)ethane), DDNU (unsym-bis(p-chlorophenyl)ethylene), and DDE. When the cells were incubated in mineral media containing methionine as a carbon source, only DDD was recovered after 100 hr incubation.

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Degradative Mechanisms

Plemmer et al. (1968), employing deuterated DDT, convincingly demonstrated that DDE was not an intermediate in the metabolism of DDT to DDD. After incubating A. aerogenes anaerobically for 2 days in trypticase broth containing deuterated DDT, 2-deuterioethane was found to be present in the recovered DDD. Ninety-two % conversion was reported with DDD being the only metabolite.

Wedemeyer (1966) employed sonically disrupted cells of A. aerogenes and selected inhibitors to ellucidate the biological mechanism involved in reductive dechlorination of DDT to DDD. Cell suspensions were sonically disrupted in 0.07 M phosphate buffer (pH 7) and added to buffer to which DDT in an acetone solution had been added resulting in a final concentration of 5 ppm. After incubating overnight, anaerobically, an average of 70% conversion to DDD occurred. No other metabolites were reported, and no conversion was found in the boiled controls. Cyanide, nitrate, ferricyanide, malonate, antimycin A and an atmosphere of carbon monoxide completely inhibited DDD production. The carbon monoxide effect was completely reversed by exogenous cytochrome c plus ascorbate. Based on the nature of the inhibition, the author concluded that reduced cytochrome oxidase was probably the agent of reductive dechlorination. In subsequent work, Wedemeyer (1967 a) increased the incubation time to 2 days and determined the influence of temperature, pH and exogenous energy sources on the metabolism of DDT by cell free preparations of

Degradative Mechanisms

Plimmer et al. (1968), employing dechlorinated DDT, conclusively demonstrated that DDE was not an intermediate in the metabolism of DDT to DDD. After incubating A. aerogenes aerobically for 2 days in trypticase broth containing dechlorinated DDT, 2-dechlorobenzene was found to be present in the recovered DDD. Ninety-two % conversion was reported with DDD being the only metabolite.

Wedemeyer (1966) employed aerobically disrupted cells of A. aerogenes and selected inhibitors to elucidate the biological mechanism involved in reductive dechlorination of DDT to DDD. Cell suspensions were aerobically disrupted in 0.07 M phosphate buffer (pH 7) and added to buffer to which DDT in acetone solution had been added resulting in a final concentration of 5 ppm. After incubating overnight, anaerobically, an average of 70% conversion to DDD occurred. No other metabolites were reported, and no conversion was found in the boiled control. Cyanide, nitrate, ferricyanide, methano, anthracin A and an atmosphere of carbon monoxide effectively inhibited DDD production. The carbon monoxide effect was completely reversed by exposures of broth exposure to light. Based on the nature of the inhibition, the author concluded that reduced cytochrome oxidase was probably the agent of reductive dechlorination. In subsequent work, however (1967 a) increased the incubation time to 2 days and determined the influence of temperature, pH and exogenous energy sources on the metabolism of DDT by cell free preparations of

A. aerogenes. The preparation of the cell free system was essentially the same as reported previously. However, he did reduce the acetone concentration to 0.5%, doubled the mass of the preparation and increased the volume of cell free preparation utilized. Five metabolites of DDT were identified; DDD, DDE, DDMU, DDMS, and DDNU with DDD and DDNU being the major metabolites. The recovery of DDT from aerobic incubations averaged 92%. Ninety-five % remained unchanged in the boiled controls. When cultured anaerobically, the relative distribution of metabolites varied with both temperature and pH but not with different carbon sources. Each metabolite was synthesized and incubated with the cell free preparation. Based on the results of these studies, the author proposed the following pathway: DDT → DDD → DDMU → DDMS → DDNU. DDE was not degraded further, while DDA (2,2-bis(p-chlorophenyl)acetate) was produced from DDNU, and DBP (4,4'-dichlorobenzophenone) from DDA but not from DDT. The conversion of DDD to DDMU was inhibited by cyanide, fluoride, iodoacetate and malonate. DDMS conversion to DDNU was inhibited by malonic acid while DDA to DBP was not inhibited by any of the agents employed.

Nonenzymatic Degradation

A number of workers have shown nonenzymatic conversion of DDT to DDD. Castro (1964) demonstrated that dilute solutions of Fe^{++} can be oxidized at room temperature by alkyl halides, including DDT, to the corresponding Fe^{+++} halide

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Monooxygenic Degradation

A number of workers have shown monooxygenic conversion of DDT to DDD. Castro (1964) demonstrated that dithe solutions of Fe^{++} can be oxidized at room temperature by alkyl halides, including DDT, to the corresponding Fe^{+++} halide

complexes. Miskus et al. (1965) showed partial conversion of DDT to DDD in hemoglobin and hematin solutions. Ott and Gunther (1965) established that DDT can be converted to DDD when injected in a stainless steel gas chromatographic column at 228°C. Farrow et al. (1966) showed conversion of residual DDT to DDD during canning of spinach. Ecobichon and Saschenbrecker (1967) observed conversion of DDT to DDE, DDD and other undetermined metabolites in frozen chicken blood. To obtain samples, the blood was repeatedly thawed over a twelve week period.

Animal Degradation

DDE, DDD and dicofol have been reported as metabolites of DDT in insects, while DDD production has been reported as common in mammals. A DDA derivative has been produced by rats, and DDE production has been reported in man (O'Brien, 1967). When rats were fed DDT, DDD was recovered from the liver (Datta, et al., 1964; Klein, et al., 1964; Peterson and Robinson, 1964; Mendel and Walton, 1966). However, when DDT was administered by interperitoneal injection, no conversion to DDD occurred (Baker and Morrison, 1964; Mendel and Walton, 1966). If the excised livers of DDT injected rats were allowed to putrify then DDD was recovered (Baker and Morrison, 1964; Peterson and Robinson, 1964; Mendel and Walton, 1966). Significantly, bacteria isolated from intestinal tracts and feces of animals have shown the ability to degrade DDT to DDD (Baker, et al., 1965; Stenersen, 1965; Mendel and Walton, 1966;

(Baker, et al., 1963; Stenerson, 1963; Mendel and Walton, 1966; Peterson and Robinson, 1964; Mendel and Walton, 1966). Significantly, bacteria isolated from intestinal tracts and joned to butrik then DDD was recovered (Baker and Morrison, 1966). If the excised livers of DDT injected rats were simultaneously incubated with DDT and NADPH, no conversion to DDD occurred (Baker and Morrison, 1964; Mendel and Walton, was administered by intraperitoneal injection, no conversion Hopinson, 1964; Mendel and Walton, 1966). However, when DDT liver (Datta, et al., 1964; Klein, et al., 1964; Peterson and rats, and DDE production has been reported in man (O'Brien, 1963). When rats were fed DDT, DDD was recovered from the common in mammals. A DDA derivative has been produced by of DDT in insects, while DDD production has been reported as

Animal Degradation

DDE, DDD and dicofol have been reported as metabolites obtain samples, the blood was repeatedly thawed over a twelve other undetermined metabolites in frozen sulfuric blood. To dreher (1963) observed conversion of DDT to DDE, DDD and DDT to DDD during cranking of spinach. Hoopingon and Deacheng- et al. 22800. Barrow et al. (1966) showed conversion of residual when injected in a sterilized sterl gas chromatographic column Gunther (1963) established that DDT can be converted to DDD of DDT to DDD in benzylopin and hexatin solutions. Ott and complexes. Miskus et al. (1963) showed partial conversion

Brunberg and Beck, 1968). Mendel and Walton concluded that the microflora of the intestinal tract were responsible for the conversion of DDT to DDD in the rat. However, Morella (1965) isolated microsomes from rat liver that degraded DDT to DDD, and DDT-metabolizing activity was increased after intraperitoneal injections of DDT. The inductive effect of DDT and its metabolites on rat liver microsomes have also resulted in increased epoxidation of Aldrin (1,2,3,4,10,10,-hexachloro-1,4,4$_a$,5,8,8$_a$-hexahydro-1,4-endo-exo-5,8-dimethanophthalene), (O'Brien, 1967; Gillett, 1968). DDT has been shown to induce NAD kinase in Tritoma infestans (Agosin, 1967) and to induce the synthesis of messenger RNA and overall protein synthesis (Litvak, 1968).

Peterson and Robinson (1964) proposed the following pathway of DDT metabolism in rats: DDT → DDD → DDMU → DDMS → DDNU → DDA. The pathway was deduced from the metabolism of orally administered doses of DDT and DDT metabolites. It should be noted, however, that not all metabolites were recovered when DDT was the initial substrate.

Biochemical Inhibitions

DDT and many non-insecticidal derivatives have been reported to inhibit the cytochrome oxidase activity in muscle homogenates of the American roach, Periplaneta americana (Morrison and Brown, 1954), in meal worm homogenates, Pyralis farinalis L. (Ludwig, et al., 1955), by sub-cell particles from the housefly, Musca domestica L. (Sacklin, et al., 1955)

from the housefly, Musca domestica L. (Brattin, et al., 1958) Periplaneta fuliginosa L. (Ludwig, et al., 1955), by rat liver particles (Morrison and Brown, 1954), in many worm homogenates, Pyrella homogenates of the American roach, Periplaneta americana (Perry, 1955), and by mammalian liver homogenates (Brown, 1951). DDT and many non-insecticidal derivatives have been reported to inhibit the cytochrome oxidase activity in muscle

Biochemical Inhibitions

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and in muscle homogenates of mealworm and housefly (Barsa and Ludwig, 1959) and lactate dehydrogenase (Sova, 1966). DDT also inhibits the oxidation of Kreb cycle intermediates and oxidative phosphorylation catalized by sub-cell particles obtained from houseflies (Sacklin, et al., 1955) and glycolytic pyruvate production in cell free preparations of thoracic leg muscle obtained from Triatoma infestans (Agosin, 1961). DDT has been reported to inhibit oxidative phosphorylation of rat liver metochondria (O'Brien, 1967) and housefly mitochondria (Gregg, et al., 1964). However, in most cases concentrations greater than 0.001 M were required.

Metabolic Capacity of Isolated Membranes

That the Krebs cycle is the pathway of terminal respiration in bacteria was first established by cell-free extracts (Kornberg, 1959). Weibull (1953) was the first to successfully employ lysozyme to dissolve the cell wall of Bacillus megaterium to produce protoplasts. Yoshida et al. (1960) demonstrated that the sub-cellular membrane system procuced by lysozyme treatment followed by osmotic shock could produce large membrane fragments of E. coli capable of incorporation of C^{14}-labeled amino acids into protein. The authors also established the necessity of magnesium ions for membrane activity. Utilizing a similar method of preparation, Mizuno et al. (1961) demonstrated the capacity of isolated bacterial membranes to oxidize Krebs cycle intermediates, carbohydrates and casamino acids. The authors further noted

and in muscle homogenates of meadowlark and monarch (Beres and Ludwig, 1959) and jackrabbit dehydrogenase (Sova, 1960). DDT also inhibits the oxidation of Krebs cycle intermediates and oxidative phosphorylation catalyzed by sub-cell particles obtained from monarchs (Beckman, et al., 1955) and glycolytic pyruvate production in cell free preparations of pectoral muscle obtained from Swainsons thrushes (Agosin, 1961). DDT has been reported to inhibit oxidative phosphorylation of rat liver mitochondria (O'Brien, 1957) and monarch mitochondria (Greep, et al., 1954). However, in most cases concentrations greater than 0.001 M were required.

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that the oxidations were stimulated by addition of the "shock-ate" supernatant. This was confirmed by Gray *et al.* (1966) who was able to identify the cytochromes b, a, a_2, c and o associated with *E. coli* membrane fractions, and Cox *et al.* (1968), who employed membranes isolated from a ubiquinone-deficient mutant to study the oxidation of malate. Nagata *et al.* (1966) and Yoshida *et al.* (1966) reported the ability of isolated *E. coli* membranes to incorporate P^{32} into nucleic acids, C^{14}-labeled amino acids into protein and to catalize oxidative phosphorylation.

that the oxidations were stimulated by addition of the "phosphorylase" experiment. This was confirmed by Gray et al. (1936) who was able to identify the cytochromes b, a, and c and to associated with E. coli membrane fractions, and Cox et al. (1936), who employed membrane isolated from a mutant deficient in the oxidation of malate. Pardee et al. (1946) and Vosnida et al. (1946) reported the ability of isolated E. coli membranes to incorporate P^{32} into nucleic acids, O_2-labeled amino acids into protein and to oxidize oxidative phosphorylation.

MATERIALS AND METHODS

Intact Cell Studies

For uptake and metabolism studies with whole cells, sterile Anderson's minimal synthetic broth medium was inoculated with Escherichia coli or Pseudomonas aeruginosa and incubated for 19 hr at 37°C with shaking. At the end of the growth periods, measurements of cell masses were made by observing their optical densities at 650 mμ with a Bausch and Lomb Spectronic 20. Their dry weights were read from a calibration curve relating optical density at 650 mμ to dry weight in mg/ml. The cells were harvested by centrifugation at 12,000 g for 5 minutes. The cells were washed by resuspending in 0.85% saline and recentrifuged.

The experimental inocula consisted of 425 mg (dry weight) of washed cells. The cells were resuspended in 500 ml aliquots of sterile minimal medium to which had been added 0.1 ml of acetone containing 2.22×10^5 dpm of C^{14} ring labeled p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane). The cultures were shaken at 37°C for 1, 2, or 3 days under aerobic or nitrogen atmospheres. Controls consisted of autoclaved cells to which C^{14}-labeled DDT was aseptically added.

Materials and Methods

Inoculum Preparation

For uptake and metabolism studies with whole cells, sterile Anderson's mineral synthetic broth medium was inoculated with Escherichia coli or Pseudomonas aeruginosa and incubated for 18 hr at 37°C with shaking. At the end of the growth period, measurements of cell masses were made by observing their optical densities at 650 mμ with a Bausch and Lomb Spectronic 20. Their dry weights were read from a calibration curve relating optical density at 650 mμ to dry weight in mg/ml. The cells were harvested by centrifugation at 12,000 g for 5 minutes. The cells were washed by resuspending in 0.85% saline and recentrifuged.

The experimental inocula consisted of 425 mg (dry weight) of washed cells. The cells were resuspended in 500 ml aliquots of sterile mineral medium to which had been added 0.1 ml of acetone containing 5.22 x 10^5 dpm of C_{14} ring labeled p,p'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane). The cultures were shaken at 37°C for 1.5, or 3 days under aerobic or nitrogen atmospheres. Control consisted of autoclaved cells to which C_{14}-labeled DDT was aseptically added.

Extraction & "Clean-up"

After incubation the cells were separated from the medium by centrifugation at 12,000 g for 5 minutes. The supernatants were extracted 3 times with 100 ml volumes of hexane and concentrated to 25 ml aliquots. Interfering materials were removed from the concentrates by column chromatography utilizing 10 g aliquots of Florisil and Celite (5:1) deactivated with water (15%). The effluents were concentrated to 0.5 ml and assayed for C^{14} content with a Mark I liquid scintillation computer (Nuclear-Chicago Corporation). Supernatants were assayed for C^{14} content before and after extraction. The cells were extracted 3 times with acetone. The extracts were taken to dryness and "cleaned-up" as above. The effluents were concentrated to 0.5 ml and assayed for C^{14} content.

Analytical Methods

DDT and its metabolites were identified by thin-layer and gas-liquid chromatography. Thirty µg of p,p'-DDT, o,p'-DDT (1,1,1-trichloro-2,o-chlorophenyl-2-p-chlorophenylethane), DDD, and DDE were spotted on silica gel H thin-layer chromatographic plates (Brinkman Instrument Co.) with 15,000 dpm of each experimental concentrate and developed twice through 15 cm. Autoradiograms were produced by exposing Kodak medical X-ray film to the plates for 4 days. After development of the X-ray films, the chromatograms were sprayed lightly with a 0.1% alcoholic Rhodamine B solution and

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Analytical Methods

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treated with sodium carbonate to resolve the authentic standards (Johnson and Goodman, 1967).

Quantification was accomplished by gas-liquid co-chromatography utilizing a 6 ft. X ¼ in. glass column containing 80-100 mesh Gas Chrome-Q (The Anspec Co.) coated with 11% DC QF-1 (Applied Science Laboratories, Inc.) and OV-17 (Applied Science Laboratories, Inc.) in a ratio of 1.3:1.0. Base line separations of the standards were achieved by a column temperature of 190°C, detector temperature of 200°C and nitrogen flow rate of 20 cc/min. A Packard Model 850 gas fraction collector employing cartridges filled with Pyrex glass wool was used to collect C^{14}-labeled components from the effluent stream of the column. Following injection of the sample, collections were made at 5 minute intervals for a total of 75 minutes. The glass wool was removed from the cartridges and the entraped radioactivity determined by liquid scintillation counting. The retention times of the C^{14}-labeled components were compared to the retention times of the 4 authentic standards mentioned above.

Cell Free Studies

For cell free studies, active E. coli membranes were prepared by the method of Nagata et al. (1966). Washed intact cells (0.25 mg/ml dry weight) were incubated at 30°C for 30 minutes with gentle shaking in a medium consisting of 3 parts 0.9 M sucrose in pH 8.0, 0.05 M Tris (2-amino-2-(hydroxymethyl)-1,3-propanediol), 1 part 0.0071 M EDTA

treated with sodium carbonate to resolve the authentic standards (Johnson and Goodman, 1957).

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(ethylenediaminetetraacetic acid), and 1 part of lysozyme (Sigma Corporation) solution at 0.6 mg/ml. The incubation mixture was centrifuged at 15,000 g for 5 minutes and the "protoplasts" harvested. Membranes were obtained from protoplasts as illustrated in Figure 1.

Incubations were carried out in 10 ml Warburg flasks containing 0.1 g glass beads (15 μ) and 350,000 dpm of DDT-C^{14} was added in acetone solution to the surface of the beads and the acetone evaporated prior to the addition of 3 ml aliquots of membrane suspension. The desired Krebs cycle intermediates and cofactors were added to the side arms of the flasks and the contents emptied into the incubation mixtures after 5 minute periods of temperature and atmospheric equilibration. Controls consisted of boiled membrane suspensions. Nitrogen atmospheres were maintained throughout the incubation period. Extraction of C^{14} metabolites, their identification and quantification were carried out as described previously.

The C^{14}-labeled p,p'-DDT was obtained from Nuclear-Chicago Corporation. The p,p'-DDT (unlabeled) was obtained from City Chemical Corp., the o,p'-DDT isomer from Geigy Chemical Corp., and the DDD from City Chemical Corp. The p,p'-DDE was prepared by alkaline dehydrochlorination of p,p'-DDT and Alumina chromatography (Sternburg and Kerns, 1952). The Krebs cycle intermediates, malonate and pyruvate, as well as the cofactors NAD (nicotinamide adenine dinucleotide), NADP (nicotinamide adenine dinucleotide phosphate), FAD

(ethylenediaminetetraacetic acid), and 1 part of lysozyme (Sigma Corporation) solution at 0.6 mg/ml. The incubation mixture was centrifuged at 15,000 g for 5 minutes and the "protoplasts" harvested. Membranes were obtained from protoplasts as illustrated in Figure 1.

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The C_{14}-labeled p,p'-DDT was obtained from Wofser-Chemco Corporation. The p,p'-DDT (unlabeled) was obtained from Olin Chemical Corp., the o,p'-DDT isomer from Geigy Chemical Corp., and the DDD from Olin Chemical Corp. The p,p'-DDE was prepared by alkaline dehydrochlorination of p,p'-DDT and Florisil chromatography (Stenhagg and Kerne, 1952). The Krebs cycle intermediates, malonate and pyruvate, as well as the cofactors NAD (nicotinamide adenine dinucleotide), NADP (nicotinamide adenine dinucleotide phosphate), FAD

(flavin adenine dinucleotide), and ADP (adenosine diphosphate), were obtained from the Sigma Corporation.

Values presented in the results are means of two replicate experiments.

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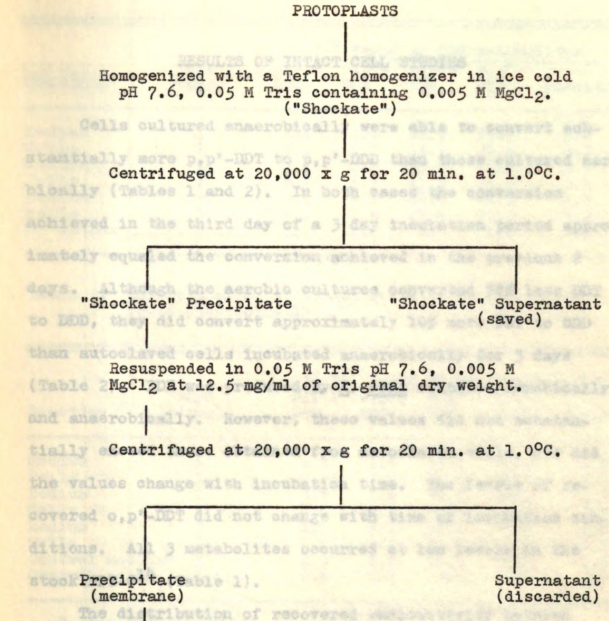

Figure 1. Preparation of membranes from protoplasts. (Nagata, et al., 1966)

Figure 1. Preparation of membrane from protoplasts.
(Mamata, et al., 1965)

RESULTS OF INTACT CELL STUDIES

Cells cultured anaerobically were able to convert substantially more p,p'-DDT to p,p'-DDD than those cultured aerobically (Tables 1 and 2). In both cases the conversion achieved in the third day of a 3 day incubation period approximately equaled the conversion achieved in the previous 2 days. Although the aerobic cultures converted 70% less DDT to DDD, they did convert approximately 10% more DDT to DDD than autoclaved cells incubated anaerobically for 3 days (Table 2). DDE was produced by E. coli cultured aerobically and anaerobically. However, these values did not substantially exceed those obtained from autoclaved cells, nor did the values change with incubation time. The levels of recovered o,p'-DDT did not change with time or incubation conditions. All 3 metabolites occurred at low levels in the stock DDT-C^{14} (Table 1).

The distribution of recovered radioactivity between cells and medium is presented in Table 3. Although bacteria both in aerobic and anaerobic cultures were able to concentrate the radioactivity progressively with time, slightly more radioactivity was concentrated by cells cultured aerobically. After 3 days, 96% of the radioactivity found in the medium was DDD in anaerobic cultures, while 70% of the radioactivity was DDT in the medium of aerobic cultures.

RESULTS OF INTACT CELL STUDIES

Cells cultured anaerobically were able to convert substantially more p,p'-DDT to p,p'-DDD than those cultured aerobically (Tables 1 and 2). In both cases the conversion achieved in the third day of a 3 day incubation period approximately equaled the conversion achieved in the previous 2 days. Although the aerobic cultures converted 20% less DDT to DDD, they did convert approximately 10% more DDT to DDD than autoclaved cells incubated anaerobically for 3 days (Table 2). DDE was produced by H. coli cultured aerobically and anaerobically. However, these values did not substantially exceed those obtained from autoclaved cells, nor did the values change with incubation time. The levels of recovered o,p'-DDT did not change with time or incubation conditions. All 3 metabolites occurred at low levels in the stock DDT-Q_{10} (Table 1).

The distribution of recovered radioactivity between cells and medium is presented in Table 3. Although bacteria in aerobic and anaerobic cultures were able to consume/degrade the radioactivity progressively with time, slightly more radioactivity was consumed by cells cultured aerobically. After 3 days, 96% of the radioactivity found in the medium was DDD in anaerobic cultures, while 20% of the radioactivity was DDT in the medium of aerobic cultures.

Table 1. DDT metabolism by intact cells of E. coli incubated aerobically.

Fraction	Time (days)	% C^{14} found as DDT metabolites: p,p'-DDE	o,p'-DDT	p,p'-DDD	p,p'-DDT	Unknown
Medium	1	5.1	1.6	12.9	75.4	5.0
Cells	1	7.2	0.0	15.2	74.6	3.0
Medium	2	3.9	2.7	13.5	76.6	3.3
Cells	2	8.1	1.7	17.4	70.4	2.4
Medium	3	2.0	1.1	24.0	70.2	2.7
Cells	3	2.1	1.5	22.4	70.3	3.9
C^{14}-Stock	0	3.5	1.7	0.8	91.8	2.2

Table 2. DDT metabolism by intact cells of E. coli incubated anaerobically.

Fraction	Time (days)	% C^{14} found as DDT metabolites: p,p'-DDE	o,p'-DDT	p,p'-DDD	p,p'-DDT	Unknown
Medium	1	2.0	1.8	39.2	53.0	4.0
Cells	1	7.0	1.0	22.3	62.0	7.6
Medium	2	3.4	1.8	54.2	39.1	1.5
Cells	2	5.4	2.0	35.6	54.3	2.7
Medium	3	1.0	1.1	96.1	0.9	0.8
Cells	3	2.2	2.0	92.4	3.0	0.4
Control medium[a]	3	2.9	1.2	10.3	82.7	3.3
Control cells	3	8.6	2.0	9.0	75.1	5.3

[a] Autoclaved cultures were employed as controls.

Table 1. DDT metabolism by intact cells of *E. coli* incubated aerobically.

Fraction	Time (days)	% C_{14} found as DDT metabolites				
		p,p'- DDE	o,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Medium	1	5.1	1.6	12.9	75.4	5.0
Cells	1	1.2	0.6	12.2	79.6	6.4
Medium	2	3.9	2.7	13.5	76.6	3.3
Cells	2	8.1	1.7	17.4	70.4	2.4
Medium	3	2.0	1.1	24.0	70.2	2.7
Cells	3	2.1	1.5	22.4	70.3	3.3
C^{14}-Stock	0	3.5	1.7	0.8	91.8	2.2

Table 2. DDT metabolism by intact cells of *E. coli* incubated anaerobically.

Fraction	Time (days)	% C_{14} found as DDT metabolites				
		p,p'- DDE	o,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Medium	1	2.0	1.8	39.2	53.0	4.0
Cells	1	7.0	1.0	22.3	68.0	2.6
Medium	2	3.4	1.8	34.2	59.1	1.5
Cells	2	5.4	2.0	35.6	54.3	2.7
Medium	3	1.0	1.1	96.1	0.9	0.8
Cells	3	2.2	2.0	92.4	3.0	0.4
Control medium[a]	3	2.9	1.2	10.3	82.7	3.3
Control cells	3	8.6	2.0	9.0	72.1	5.3

[a] Autoclaved cultures were employed as controls.

Table 3. Distribution of radioactivity in cultures of *E. coli*.

Culture	Time(days)	% radioactivity	
		Medium	Cells
Aerobic	1	81.0	19.0
	2	42.3	57.7
	3	19.7	80.3
Anaerobic	1	82.0	18.0
	2	61.0	39.0
	3	29.0	71.0
Anaerobic control	3	52.8	47.2

With autoclaved *E. coli*, 58% of the recovered radioactivity was associated with the medium, while 47.2% was associated with the cells.

The pattern of DDT metabolism by *P. aeruginosa* was similar to that found in the *E. coli* incubations(Tables 4 and 5). However, anaerobic cultures of *P. aeruginosa* were able to produce nearly as much DDD in 2 days as the *E. coli* cultures were able to produce in 3 days. In addition, 2-day-aerobic *P. aeruginosa* cultures were able to produce slightly more DDD from DDT than 1-day-anaerobic *E. coli* cultures.

The percent recovery of C^{14}-labeled metabolites extracted from the medium varied. The highest recovery was attained in 1-day-aerobic cultures (97%) and the lowest from anaerobic cultures incubated 3 days (71%). This non-extractable radioactivity attained a maximum of 4% of the total radioactivity.

An average of 84% of the extracted radioactivity was recovered from injected samples by glass wool trapping.

Table 3. Distribution of radioactivity in cultures of *E. coli*.

Culture	Time(days)	% radioactivity: Medium	% radioactivity: Cells
Aerobic	1	81.0	19.0
	2	43.3	57.2
	3	19.7	80.3
Anaerobic	1	82.0	18.0
	2	61.0	39.0
	3	29.0	71.0
Anaerobic control	3	52.8	47.2

With autolysed *E. coli*, 58% of the recovered radioactivity was associated with the medium, while 42.2% was associated with the cells.

The pattern of DDT metabolism by *E. aerogenes* was similar to that found in the *E. coli* incubations (Tables 4 and 5). However, anaerobic cultures of *E. aerogenes* were able to produce nearly as much DDD in 2 days as the *E. coli* cultures were able to produce in 3 days. In addition, 2-day-aerobic *E. aerogenes* cultures were able to produce slightly more DDD from DDT than 3-day-anaerobic *E. coli* cultures.

The percent recovery of C_{14}-labeled metabolites extracted from the medium varied. The highest recovery was attained in 1-day-aerobic cultures (97%) and the lowest from anaerobic cultures incubated 3 days (71%). This non-extractable radioactivity attained a maximum of 4% of the total radioactivity.

An average of 84% of the extracted radioactivity was recovered from incubated samples by glass wool trapping.

Table 4. DDT metabolism by intact cells of P. aeruginosa incubated aerobically.

Fraction	Time (days)	% C^{14} found as DDT metabolites				
		p,p'- DDE	o,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Medium	2	2.8	0.8	37.0	57.4	2.0
Cells	2	2.2	1.1	40.1	55.1	1.5

Table 5. DDT metabolism by intact cells of P. aeruginosa incubated anaerobically.

Fraction	Time (days)	% C^{14} found as DDT metabolites				
		p,p'- DDE	o,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Medium	2	2.1	0.7	87.4	6.4	3.4
Cells	2	3.3	1.1	86.5	6.9	2.2
Control medium	2	6.2	2.5	4.0	85.3	2.1
Control cells	2	1.5	1.0	5.7	88.3	3.5

Table 4. DDT metabolism by insect cells of P. acerifrons incubated aerobically.

Fraction	Time (days)	% C_{14} found as DDT metabolites				
		DDE	DDT	DDD	DDT	Unknown
		p,p'-	o,p'-	p,p'-	p,p'-	
Medium	2	2.8	0.8	72.0	57.4	2.0
Cells	2	2.2	7.1	40.1	35.1	1.5

Table 5. DDT metabolism by insect cells of P. acerifrons incubated anaerobically.

Fraction	Time (days)	% C_{14} found as DDT metabolites				
		DDE	DDT	DDD	DDT	Unknown
		p,p'-	o,p'-	p,p'-	p,p'-	
Medium	2	2.1	0.7	87.4	6.4	3.4
Cells	2	3.3	1.1	86.2	6.3	2.2
Control medium	2	2.2	2.2	4.0	89.3	2.1
Control cells	2	1.2	1.0	5.7	88.3	3.2

RESULTS OF MEMBRANE STUDIES

Experiments were designed to acertain the site of DDT metabolism in E. coli. Both the cytoplasmic fraction ("shockate" supernatant) alone and the cytoplasmic fraction plus boiled membrane fraction displayed little ability to degrade DDT to DDD (Table 6). On the other hand, cytoplasmic fractions plus unboiled membranes produced substantially more DDD (29.8 vs. 2.4 and 3.8%). Thus the membrane of bacteria is the site of reductive dechlorination of DDT and the cytoplasm contains an essential factor(s).

Table 6. Effect of membrane and cytoplasm of E. coli on conversion of DDT to DDD.

Components[a]	% C^{14} found as DDT metabolites				
	p,p'- DDE	o,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Membrane only	0.4	1.8	4.6	90.5	2.7
Membrane & Cytoplasm[b]	0.3	1.3	29.8	61.9	6.7
Cytoplasm only	0.1	2.0	2.4	92.5	3.0
Boiled membrane & Cytoplasm	0.3	1.5	3.8	90.0	4.4
DDT-Carbon-14 & Buffer	0.4	1.9	0.6	93.9	3.2

[a] The membrane fractions (3 ml aliquots) consisted of Tris buffer containing membranes at 25.0 mg/ml (original dry weight of cells).

[b] The membrane ppt. were resuspended in cytoplasmic fraction at 25.0 mg/ml (original dry weight of cells).

RESULTS OF MEMBRANE STUDIES

Experiments were designed to ascertain the site of DDT metabolism in *E. coli*. Both the cytoplasmic fraction ("enzyme" supernatant) alone and the cytoplasmic fraction plus boiled membrane fraction displayed little ability to degrade DDT to DDD (Table 6). On the other hand, cytoplasmic fractions plus whole unboiled membranes produced substantially more DDD (29.8 vs. 5.4 and 3.8%). Thus the membrane of bacteria is the site of reductive dechlorination of DDT and the cytoplasm contains an essential factor(s).

Table 6. Effect of membrane and cytoplasm of *E. coli* on conversion of DDT to DDD.

Components[a]	% C_{14} found as DDT metabolites				
	DDE	p,p'- DDT	p,p'- DDD	p,p'- DDT	Unknown
Membrane only	0.4	1.8	4.6	90.5	2.7
Membrane & Cytoplasm[b]	0.3	1.3	29.8	61.9	6.7
Cytoplasm only	0.1	2.0	2.4	92.5	3.0
Boiled membrane & Cytoplasm	0.3	1.5	3.8	90.0	4.4
DDT-Carbon-14 & Buffer	0.4	1.9	0.6	93.9	3.2

[a] The membrane fractions (3 ml aliquots) contained of This buffer containing membranes at 25.0 mg/ml (original dry weight of cells).

[b] The membrane ppt. were resuspended in cytoplasmic fraction at 25.0 mg/ml (original dry weight of cells).

In the presence of NAD, NADP, ADP, FAD, inorganic phosphate, malate, and pyruvate, the level of DDD recovered was not substantially different from that of the membranes alone (Table 7). When NAD, NADP, or the Krebs cycle intermediates (malate and pyruvate) were not included in the incubation mixtures, increases in DDD production occurred. Omission of ADP plus inorganic phosphate or FAD from the incubation mixture gave no increase in DDD production. Significantly, substantial DDD production was achieved only in those incubants containing FAD, ADP, and inorganic phosphate.

Since addition of exogenous ADP plus inorganic phosphate or FAD did enhance DDD production by isolated membranes, experiments were conducted to determine the effects of these components singly or in combination (Table 8). Membranes plus exogenous FAD, ADP, and inorganic phosphate or membranes plus FAD only produced over 4 times the DDD then membranes incubated with exogenous FAD plus inorganic phosphate (Table 8). Thus the addition of exogenous FAD to membrane preparations enhances DDD production. Increasing the exogenous FAD from 2 to 8 µmole did not result in a substantial increases in DDD production.

The addition of ADP plus inorganic phosphate, or FAD, or FAD plus ADP and inorganic phosphate to membrane resuspended in cytoplasmic fractions did not increase DDD production beyond that attained by membrane and cytoplasmic combinations only (Tables 6 and 8). These results suggest that the availibility of endogenous enzymes and/or substrates were

limiting the rate of DDD production.

The addition of FAD, ADP, and inorganic phosphate to membrane fractions incubated aerobically did not enhance DDD production. Thus FAD enhancement of DDD production is dependent on anaerobic conditions. This suggests that normally operating oxidative pathways preclude the reductive dechlorination of DDT.

Table 7. Effect of exogenous Krebs cycle intermediates and cofactors on DDT metabolism by membrane preparations of E. coli.

Components[a]	% C^{14} found as DDT metabolites				
	p,p'-DDE	o,p'-DDT	p,p'-DDD	p,p'-DDT	Unknown
Membrane only	0.4	1.8	4.6	90.5	2.7
All Cofactors & Chos.[b]	0.2	1.3	2.2	93.2	3.1
Cofactors & Chos.(0.1X)	0.1	1.6	7.9	86.3	4.1
minus FAD	1.1	1.8	4.9	86.4	5.8
minus ADP & PO_4	0.0	1.2	5.9	89.1	3.8
minus Malate & Pyruvate	0.4	1.4	21.4	73.7	3.1
minus NAD	0.5	1.6	26.8	65.6	5.5
minus NADP	0.8	1.6	21.1	69.3	7.2
minus Chos. (control)[c]	0.1	0.4	1.3	94.7	3.5

[a] Each incubant contained 3 ml of membrane fraction.

[b] Two µmole each of NAD, NADP, FAD, malate, pyruvate, and 0.1 µmole each of ADP and inorganic phosphate.

[c] Components consisted of 3ml boiled membrane fraction plus exogenous cofactors. Malate and pyruvate were not added.

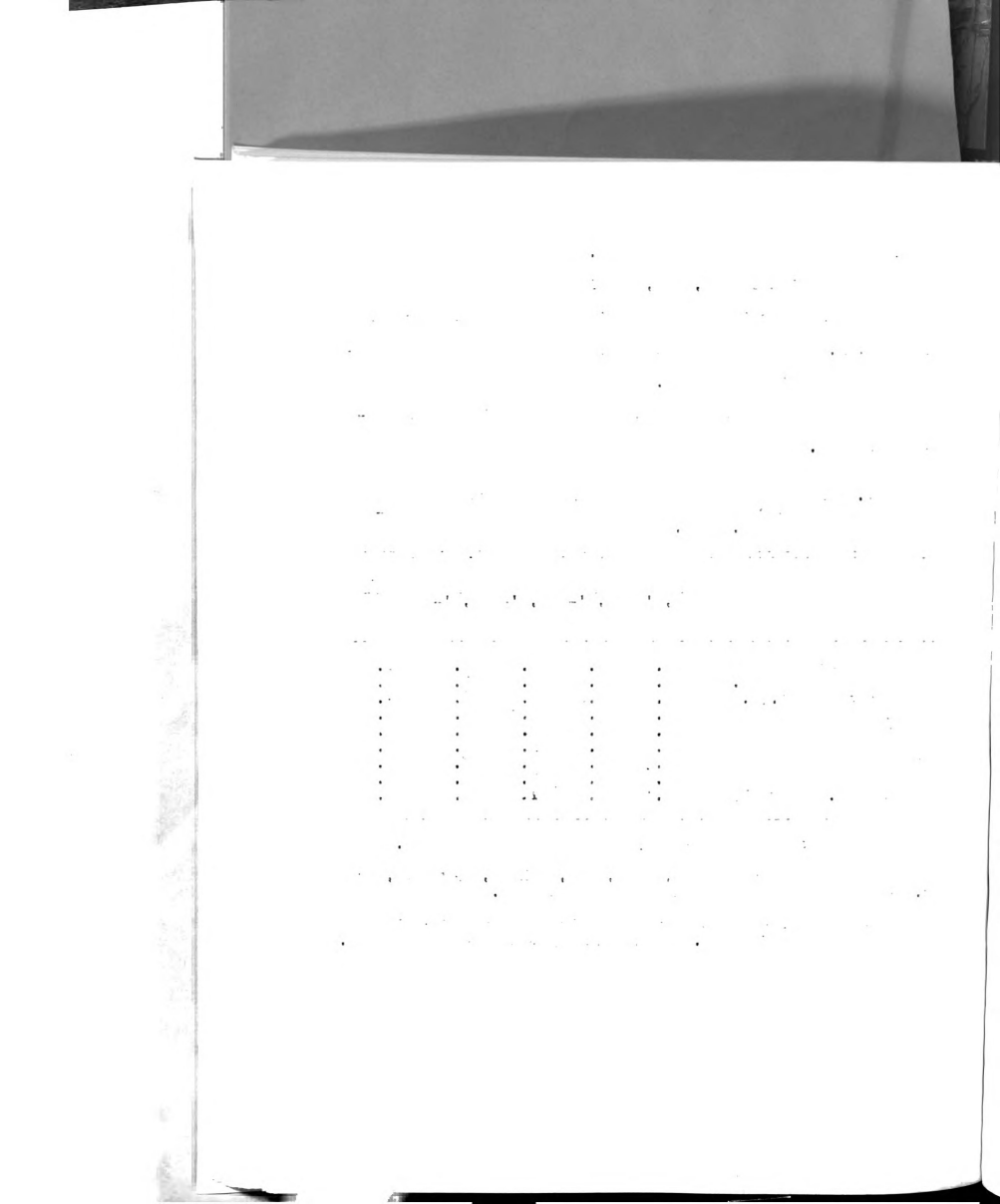

Table 8. Effect of NAD, FAD, ADP, and inorganic phosphate on DDT metabolism by membrane preparations of E. coli.

Components[a]	% C^{14} found as DDT metabolites				
	p,p'-DDE	o,p'-DDT	p,p'-DDD	p,p'-DDT	Unknown
NAD	0.3	1.3	7.7	87.4	3.3
FAD	0.2	1.2	22.5	72.6	3.5
ADP & PO_4	0.5	2.1	5.2	88.3	3.9
FAD, ADP & PO_4	0.5	1.7	20.5	74.2	3.1
4X FAD, ADP & PO_4	0.7	0.8	23.1	71.1	4.3
Cytoplasm & FAD,ADP,PO_4	0.9	1.6	28.9	62.6	6.0
Cytoplasm & FAD	0.1	1.3	26.2	68.8	3.6
Cytoplasm, ADP & PO_4	0.5	1.3	27.9	65.1	5.2
Aerobic Atmosphere, FAD, ADP, & PO_4	0.5	1.6	2.9	90.0	5.0

[a] Each incubant contained 3 ml of membrane fraction.

DISCUSSION

The results of the whole cell studies carried out in this investigation are in general agreement with the observations made by other investigators, that is, the conversion of DDT to DDD is inversely related to the supply of atmospheric oxygen available to the bacteria. However, the present investigation also demonstrated aerobic conversion of DDT to DDD by bacterial cultures. Two investigators have reported that no conversion of DDT to DDD occurs in aerobic bacterial cultures (Stenersen, 1965; Johnson, 1967) while others have reported the contrary (Chacko, et al., 1966; Wedemeyer, 1966). Metabolic differences between species may account for this discrepancy. On the other hand, shaking may not have provided sufficient oxygen to maintain an aerobic state with the bacterial populations employed in this investigation.

The levels of DDE exceeded that of the DDT-C^{14} stock solution. However, the DDE content did not increase with incubation time and did not vary significantly from the levels found in autoclaved cells. Similar results have been obtained by other investigators employing other microorganisms (Kallman and Andrews, 1963; Stenersen, 1965; Wedemeyer, 1966; Plemmer, et al., 1968). Guenzi and

Beard (1967) reported a slight increase in recoverable DDE after incubating nonsterile soil, 4 weeks, anaerobically, with DDT.

Autoclaved cells displayed a limited capacity to convert DDT to DDD under anaerobic conditions. Of those investigators that referred to autoclaved control experiments, none reported conversion of DDT to DDD. Contamination of the control cultures cannot be categorically eliminated as the control cultures were not plated after incubation. The observations by Castro (1964) that dilute solutions of Fe^{++} porphyrins can dechlorinate DDT and Miskus (1965) that dechlorination can be accomplished by hemoglobin and hemitin solutions at room temperature, demonstrate non-biologically catalized degradation can occur with relatively mild conditions. This may account for all the DDE and a fraction of the DDD extracted from aerobic and anaerobic cultures and for the presence of DDE and DDD in autoclaved cultures.

The non-hexane-extractable radioactivity in anaerobic E. coli cultures represented 4% of the radioactivity after 3 days. Stenersen (1965), Guenzi and Beard (1967), and Matsumura and Bousch (1968) also indicated the presence of nonextractable radioactivity associated with water phases. This residual activity may represent water soluble metabolites. The autoradiograms of anaerobic cultures of E. coli, incubated 3 days, and P. aeruginosa, incubated 2 days, possessed 1 slightly exposed spot of extremely low R_f, probably

indicating a high degree of polarity, which did not correspond to any of the standards. This lends support to reports of Wedemeyer (1967 a) and Bousch and Matsumura (1968) that products other than DDD occurred as minor metabolites of bacterial degradation of DDT.

Anaerobic, aerobic and autoclaved cells concentrated DDT and its metabolites (Table 3). Eighty %, 71%, and 47% of the total C^{14} was extracted from the cells of aerobic, anaerobic and autoclaved cultures respectively, after 3 days of incubation. The volume occupied by 0.25 mg (dry weight) of E. coli is approximately 1.0 µl (Roberts, et al., 1963). At the population levels employed in this research, the bacteria occupied a volume of approximately 3.5 µl/ml of culture. Relating this information to the distribution of extractable radioactivity presented in Table 3, a more dramatic representation of the radioactive distribution can be seen. That is, 80% of the extractable radioactivity was associated with less than 0.4% of the incubation volumes in aerobic cultures, and 71% of the extractable radioactivity was associated with 0.4% of the incubation volumes in anaerobic cultures. Washing the bacterial pellets by resuspension in 0.85% sodium chloride solution released an insignificant amount of radioactivity. Since a rather large initial inocula of lag-phase bacteria were employed in these experiments (425 mg/experiment), the increases in population were not sufficient to alter the optical density of the medium. Thus living cells

do concentrate DDT and its metabolites, but this is not a requisite for uptake as autoclaved cells displayed this capacity as well (Table 3). The ability to metabolize DDT does not enhance its uptake under the conditions employed in this investigation. In addition, most of the DDD produced by the bacteria remained associated with the cells. However, the role of the medium cannot be discounted. If media of high lipid content were employed, the partitioning of DDT and its metabolites may show different characteristics.

After cellular lysis, neither the particulate membrane fraction nor the soluble fraction (cytoplasm) could produce significant amounts of DDD (Table 6). If, however, these 2 fractions were combined, conversion of DDT to DDD occurred. Upon boiling the membrane fraction for 5 minutes and combining the boiled membranes with the "shockate" supernatant, one could no longer obtain significant conversion. These observations, plus the fact that the addition of FAD to the membrane fraction enhanced DDD production, suggests that the capacity to metabolize DDT to DDD resides in the membranous portion of the bacterial cell and is not cytoplasmic in origin. Since the cell walls were depolymerized and made soluble by the action of lysozyme (Salton, 1960), it most probably plays no direct role in this aspect of DDT degradation.

The membrane fraction plus NAD, NADP, pyruvate, malate, FAD, ADP, and inorganic phosphate converted little DDT to DDD. However, if NAD, NADP or the 2 Krebs cycle intermediates

do concentrate DDT and its metabolites, but this is not a requisite for uptake as autoclaved cells displayed this capacity as well (Table 3). The ability to metabolize DDT does not enhance its uptake under the conditions employed in this investigation. In addition, most of the DDD produced by the bacteria remained associated with the cells. However, the role of the medium cannot be discounted. If media of high lipid content were employed, the partitioning of DDT and its metabolites may show different characterization.

After cellular lysis, neither the particulate membrane nor the soluble fraction (cytoplasm) could produce significant amounts of DDD (Table 6). It, however, these 2 fractions were combined, conversion of DDT to DDD occurred. Upon boiling the membrane fraction for 5 minutes and combining the boiled membranes with the "anoxic" supernatant, one could no longer obtain significant conversion. These observations, plus the fact that the addition of PAD to the membrane fraction enhanced DDD production, suggests that the capacity to metabolize DDT to DDD resides in the membranous portion of the bacterial cell and is not cytoplasmic in origin. Since the cell walls were depolymerized and made soluble by the action of lysozyme (Salton, 1960), it most probably plays no direct role in this aspect of DDT degradation.

The membrane fraction plus NAD, NADP, pyruvate, malate, PAD, ADP, and inorganic phosphate converted little DDT to DDD. However, if NAD, NADP or the 2 Krebs cycle intermediates

were deleted from the incubation components, significant increases in DDD production occurred (Table 7). Thus the presence of potential Krebs cycle activity inhibits DDD production with the conditions utilized in this study.

Since membrane preparations of E. coli are capable of metabolizing Krebs intermediates (Mizuno, et al., 1961; Gray, et al., 1966; Cox, et al., 1968), and furthermore, contain the cytochromes b, a, a_2 and c (Gray, et al., 1968), one would not expect the results obtained in this investigation. If indeed, reduced cytochrome a_3 (cytochrome oxidase) is the enzyme responsible for the conversion of DDT to DDD (Wedemeyer, 1966) then the deletion of major components of the Krebs cycle should not enhance DDD production. On the contrary, their metabolism should contribute electrons to the cytochrome system and maintain them in a reduced state.

The involvement of FAD in enzymatic electron transfer processes is well documented (White, et al., 1965; Slater, 1966; Wellner, 1967). Addition of exogenous FAD to the membrane fraction significantly enhanced DDD production (Table 8). However, addition of FAD to aerobically incubated membrane fractions did not stimulate DDD production. Thus anaerobic conditions are a requisite to FAD enhancement of DDD production. The results of this investigation suggest that under anaerobic conditions FAD may be a cofactor required for the enzymatic conversion of DDT to DDD. Secondly, the results suggest that FAD may function as a cofactor in an electron transfer process not directly involved in the

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contain the cytochromes b, a, and c (Dyer, et al., 1966),
Dyer, et al., 1966; Cox, et al., 1966), and furthermore,
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function with the conditions utilized in this study.
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increases in DDD production occurred (Table 7). Thus the
were deleted from the incubation components, significant

immediate reduction of DDT to DDD, but in an electron transfer process or processes necessary for the ultimate reduction of DDT. Further investigation would be required to establish the role of FAD in DDT reduction.

Four-fold increments of exogenous FAD added to membrane fractions failed to significantly increase DDD production beyond that obtained by the addition of 2 umole aliquots (Table 8). This suggests that another factor or factors are limiting the rate of DDT reduction. Cytoplasmic stimulation of DDT reduction by membrane preparations was not increased by addition of exogenous FAD. The stimulating factor or factors that were present in the cytomplsmic fraction ("shockate" supernatant) are unknown. The isolation and characterization of this factor or factors required for DDT reduction would contribute valuable information concerning the metabolic processes involved in DDT reduction.

SUMMARY

Aerobic and anaerobic cultures of E. coli and P. aeruginosa degraded DDT to DDD. This conversion was inversely related to the supply of atmospheric oxygen available to the bacteria. Autoclaved cells produced substantially less DDD. The levels of DDE and o,p'-DDT produced after 3 days did not exceed the control levels.

Anaerobic, aerobic and autoclaved cells concentrated DDT and its metabolites. The magnitude of C^{14} uptake was not related to the ability of the cells to metabolize DDT. Thus the ability to concentrate DDT is a passive process.

The membrane fraction or the cytoplasmic fraction (20,000 g "shockate" supernatant) degraded little DDT to DDD. The combined fractions were able to dechlorinate more DDT. Addition of cytoplasmic fractions to boiled membrane fractions did not enhance the reductive dechlorination of DDT.

When NAD, NADP, FAD, ADP, malate, pyruvate, and inorganic phosphate was added to membrane fractions, the levels of recovered DDD did not exceed the levels of DDD produced by membrane fractions only. When NAD, NADP, or malate and pyruvate were omitted from the incubation components, increases in DDD production occurred. Addition of exogenous

FAD to membrane fractions resulted in increased DDD production under anaerobic conditions.

The results of the membrane studies indicate the following:

1. Reductive dechlorination of DDT occurs in the membranous portion of the bacterial cell and is not cytoplasmic in origin.

2. Reductive dechlorination of DDT is stimulated by components in the cytoplasm.

3. Reductive dechlorination of DDT does not utilize electrons produced by the oxidation of Krebs cycle intermediates and passed through the cytochrome system.

4. Reductive dechlorination of DDT is dependent upon the enzymatic reduction of FAD and occurs only under anaerobic conditions.

5. Reductive dechlorination of DDT requires electrons produced by the oxidation of an energy source.

6. Reductive dechlorination of DDT may require the formation of free radicals. The oxidation of endogenous substrates could produce the half-reduced form of FAD (FADH·, a semiquinone) and may be the active moiety involved in the enzymatic reduction of DDT.

LITERATURE CITED

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Agosin, M., Scaramelli, N., and Neghme, A. 1961. Intermediary carbohydrate metabolism of Triatoma infestans. Comp. Biochem. Physiol. 2:143-159.

_____, Ilividky, J., and Litvak, S. 1967. The induction of NADkinase by DDT in Triatoma infestans. Can. J. Biochem. 45:619-626.

Baker, P.S., and Morrison, F.O. 1964. Breakdown of DDT to DDD in mouse tissue. Can. J. Zool. 42:324-325.

_____, and Morrison, F.O. 1965. Conversion of DDT to DDD by Proteus vulgaris, a bacterium isolated from the intestinal flora of a mouse. Nature 205:621-622.

Barsa, M.C., and Ludwig, D. 1959. Effects of DDT on the respiratory enzymes of the mealworm, Tenebrio molitor L. and of the housefly, Musca domestica L. Ann. Entomol. Soc. 52:179-182.

Bartha, R., Lanzilotta, R.P., and Pramer, D. 1967. Stability and effect of some pesticides in soil. J. Appl. Microbiol. 15:67-75.

Brunberg, R.C., and Beck, V. 1968. Interaction of DDT and the gastrointestinal microflora of the rat. J. Ag. and Food Chem. 16:451-453.

Castro, C.A. 1964. The rapid oxidation of Iron (II) porphyrins by alkyl halides. A possible mode of intoxication of organisms by alkyl halides. J. Amer. Chem. Soc. 86:2310-2311.

Chacko, C.I., Lockwood, J.L., and Zabik, M. 1966. Chlorinated hydrocarbon pesticides: degradation by microbes. Science 154:893-895.

Collins, J.A., and Langlois, B.E. 1968. Effect of DDT, Dieldrin and Heptachlor on the growth of selected bacteria. Appl. Microbiol. 16:799-800.

Cox, G.B., Snoswell, A.M., and Gibson, F. 1968. The use of a ubiquinone-deficient mutant in the study of malate oxidation in E. coli. Biochim. Biophy. Acta 153:1-12.

Datta, P.R., Laug, E.P., and Klein, A.K. 1964. Conversion of P,P'-DDT to P,P'-DDD in the liver of the rat. Science 145:1052-1053.

Ecobichon, D.L., and Saschenbrecker, P.W. 1967. Dechlorination of DDT in frozen blood. Science 156:663-664.

Farrow, R.P., Eldins, E.R., and Cook, R.W. 1966. Conversion of DDT to TDE in canned spinach. J. Ag. Food Chem. 14:430-434.

Gillett, J.W. 1968. No effect level of DDT in induction of microsomal epoxidation. J. Ag. Food Chem. 16:295-297.

Gray, C.T., Wimpenny, J.M.T., Hughes, D.E., and Mossman, M. 1966. Regulation of metabolism in facultative bacteria. Biochim. Biophys. Acta 117:22-32.

Gregg, C.T., Johnson, J.R., Heisler, C.R., and Remmert, L.F. 1964. Inhibition of oxidative phosphorylation and related reactions in insect mitochondria. Biochim. Biophys. Acta 82:340-343.

Guenzi, W.O., and Beard, W.E. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science 156:1116-1117.

Hayes Jr., W.J. 1965. Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals. Ann. Review Pharmacology 5:27-52.

Johnson, B.T., and Goodman, R.N. 1967. Conversion of DDT to DDD by pathogenic and saprophytic bacteria associated with plants. Science 157:560-561.

Kallman, B.J., and Andrews, A.K. 1963. Reductive dechlorination of DDT to DDD by yeast. Science 141:1050-1051.

Klein, A.K., Laug, E.P., Datta, P.R., Watts, J.O., and Chen, J.T. 1964. Reductive dechlorination of DDT to DDD and isomeric transformation. J. Assoc. Official Ag. Chem. 47:1129-1145.

Kornberg, H.L. 1959. Aspects of terminal respiration in microorganisms. Ann. Rev. Microbiol. 113:49-55.

Litvak, S., Litvak, L., and Pobete, P. 1968. Evidence for the DDT-induced synthesis of messenger ribonucleic acid in Triatoma infestans. Comp. Biochem. Physiol. 26:45-56.

Ludwig, D., Barsa, M.C., and Cali, T. 1955. The effect of DDT on the activity of cytochrome oxidase. Ann. Entomol. Soc. Amer. 48:165-170.

Datta, P.R., Lenz, R.P., and Klein, A.W. 1964. Conversion of P,P'-DDT to P,P'-DDD in the liver of the rat. Science 145:1052-1053.

Goodlohon, D.L., and Sassenbrookeh, L.C. 1967. Decholorination of DDT in frozen blood. Science 156:665-664.

Barrow, R.F., Kiding, R.W., and Cook, J.W. 1966. Conversion of DDT to DDE in canned spinach. J. Ag. Food Chem. 14:430-434.

Gillett, J.W. 1968. No effect level of DDT in induction of microsomal oxidation. J. Ag. Food Chem. 16:295-297.

Grey, C.T., Wimpenny, J.W.T., Andrew, W.A., and Mossman, A. 1966. Regulation of cephalosin in resistant bacteria. Biochim. Biophys. Acta 117:55-56.

Gregg, C.T., Johnson, W.W., Reiter, C.A., and Hammerl, I.E. 1964. Inhibition of oxidative phosphorylation and related reactions in isaeol mitochondria. Biochim. Biophys. Acta 82:340-343.

Guenzi, W.C., and Beard, W.E. 1967. Anaerobic biodegradation of DDT to DDD in soil. Science 156:1116-1117.

Hayes Jr., W.J. 1963. Review of the metabolism of chlorinated hydrocarbon insecticides especially in mammals. Ann. Review Pharmacology 3:27-32.

Johnson, E.T., and Goodman, R.M. 1967. Conversion of DDT to DDD by peptonemia and saprophytic bacteria associated with plants. Science 157:560-561.

Klein, A.W., Lenz, R.P., Datta, R.R., Watts, J.O., and Chen, J.T. 1964. Reductive dechlorination of DDT to DDD and isomeric transformation. J. Assoc. Official Ag. Chem. 47:1139-1145.

Kumlien, E.J., and Andrews, A.K. 1963. Reductive dechlorination of DDT to DDD by yeast. Science 141:1030-1031.

Klein, A.W., Lenz, R.P., Datta, R.R., Watts, J.O., and Chen, J.T. 1964. Reductive dechlorination of DDT to DDD and isomeric transformation. J. Assoc. Official Ag. Chem. 47:1139-1145.

Kombrey, H.E. 1959. Aspects of terminal respiration in microorganisms. Ann. Rev. Microbiol. 113:49-52.

Litvak, S., Litvak, I., and Pobere, R. 1968. Evidence for the DDT-induced synthesis of messenger ribonucleic acid in Trichome tolerans. Comp. Biochem. Physiol. 26:43-56.

Lwhyr, D., Berge, M.G., and Galt, T. 1955. The effect of DDT on the activity of cytochrome oxidase. Ann. Entomol. Soc. Amer. 48:169-170.

Martin, J.P. 1967. Influence of pesticides on soil microbes and soil properties. ASA Special Publication 8. Soil Science of America Inc. 95-108.

Matsumura, F., and Bousch, G.M. 1968. Degradation of insecticides by a soil fungus, Trichoderma viride. J. Econ. Entomol. 61:610-612.

Mendel, J.L., and Walton, M.S. 1966. Conversion of P,P'-DDT to P,P'-DDD by intestinal flora of the rat. Science 151:1527-1528.

Miskus, P., Blair, D.P., and Casida, J.E. 1965. Conversion of DDT to DDD by bovine rumen fluid, lake water, and reduced porphyrins. J. Ag. Food Chem. 13:481-483.

Mizuno, S., Yoshida, E., Takahashi, H., and Maruo, B. 1961. Experimental proof of a compartment of "energy-rich-P" in a subcellular system from Pseudomonas flourscent. Biochim. Biophys. Acta 49:361-381.

Morello, A. 1965. Induction of DDT-metabolizing enzymes in microsomes of rat liver after adminstration of DDT. Can. J. Biochem. 43:1289-1293.

Morrison, P.E., and Brown, A.W.A. 1954. The effects of insecticides on cytochrome oxidase. J. Econ. Entomol. 47:723-730.

Nagata, Y., Mizuno, S., and Maruo, B. 1966. Preparation and properties of active membrane systems from various species of bacteria. J. Biochem. 59:404-410.

O'Brien, R.D. 1967. Insecticides, Action and Metabolism. Academic Press, New York.

Ott, D.E., and Gunther, F.A. 1965. DDD as a decomposition product of DDT. Residue Rev. 10:70-84.

Perry, A.S., and Sacktor, B. 1955. Detoxification of DDT in relation to cytochrome oxidase activity in resistant and susceptible houseflies. Ann. Entomol. Soc. 48:329-333.

_____, 1960. Biochemical aspects of insect resistance to chlorinated hydrocarbons. Miscellaneous Pub. Entomol. Soc. Amer. 2:119-137.

Peterson, J.E., and Robison, Wm. H. 1964. Metabolic products of P,P'-DDT in the rat. Toxicology Appl. Pharmacol. 6: 321-327.

Plemmer, J.R., Kleamey, P.C., and VanEndt, D.W. 1968. Mechanism of conversion of DDT to DDD by Aerobacter aerogenes. J. Ag. Food Chem. 16:594-597.

Roberts, H.B., Abelson, P.H., Bolton, E.T., Cowie, D.B., and Britten, R.J. 1963. Studies of Biosynthesis in Escherichia coli. Carnegie Institution, Washington.

Sacklin, J.A., Terriere, L.C., and Remmert, L.F. 1955. Effect of DDT on enzymatic oxidation and phosphorylation. Science 122:377-378.

Salton, M.R.J. 1960. The properties of lysozymes. Ann. Rev. Microbiol. 115:82-99.

Schoenhard, D.E. 1961. Basic Concepts and Experiments in Microbiology. Burgess Publishing Co., Minneapolis.

Slater, E.C. 1966. Flavin and Flavoproteins. Elsevier Publishing Co., New York.

Sova, C.R. 1966. Lactate dehydroginase activity: effect in vitro of some pesticidal chemicals. Science 154:1661-1662.

Stenersen, V.H.J. 1965. DDT metabolism in resistant and susceptible stableflies and in bacteria. Nature 207: 660-661.

Sternburg, J., and Kearns, W. 1952. Chromatographic separation of DDT and some of its known and possible metabolites. J. Econ. Entol. 45:505-509.

Wedemeyer, G. 1966. Dechlorination of DDT by Aerobacter aerogenes. Science 152:647.

_____, 1967 a. Dechlorination of 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane by Aerobacter aerogenes. 1. Metabolic products. J. Appl. Microbiol. 15:569-574.

_____, 1967 b. Biodegradation of dichlorodiphenyl trichloroethane: Intermediates in dichlorodiphenylacetic acid metabolism by Aerobacter aerogenes. J. Appl. Microbiol. 15:1494-1495.

Weibull, C. 1953. Isolation of protoplasts from Bacillus megaterium by controlled treatment with lysozyme. J. Bacteriol. 66:688-695.

Wellner, D. 1967. Flavoproteins. Ann. Rev. Biochem. 36: 669-687.

White, A., Handler, P., and Smith, E.L. 1964. Principles of Biochemistry. 3rd ed., McGraw-Hill Book Co., New York.

Flemmer, J.R., Klemsey, P.C., and VanAndt, D.W. 1968. Mechanism of conversion of DDT to DMU by Aerobacter aerogenes. J. Ag. Food Chem. 16:598-597.

Goberde, H.E., Abelson, P.H., Bolton, E.T., Cowie, D.B., and Brittem, R.J. 1963. Studies of Bioaccumulation in Saccharomonia coli. Carnegie Institution, Washington.

Hoxlin, J.A., Terriere, L.C., and Hemmert, L.F. 1955. Effect of DDT on enzymatic oxidation and phosphorylation. Science 122:377-378.

Salton, M.R.J. 1960. The properties of lysozymes. Ann. Rev. Microbiol. 11:82-99.

Schoenhard, D.H. 1961. Basic Concepts and Experiments in Microbiology. Burgess Publishing Co., Minneapolis.

Slater, R.C. 1966. Flavin and Flavoproteins. Elsevier Publishing Co., New York.

Sova, G.E. 1966. Lactate dehydrogenase activity: effect in vitro of some pesticidal chemicals. Science 153:1681-1682.

Stenersen, V.H.J. 1965. DDT metabolism in resistant and susceptible ebablefflies and in bacteria. Nature 207: 660-661.

Sternburg, J., and Kearns, W. 1953. Chromatographic separation of DDT and some of its known and possible metabolites. J. Econ. Entol. 46:305-309.

Wedemeyer, G. 1966. Deuhlorination of DDT by Aerobacter aerogenes. Science 152:647.

_____, 1967 a. Deuhlorination of 1,1,1-trichloro-2,2-bis (p-chlorophenyl)ethane by Aerobacter aerogenes. J. Metabolic products. J. Appl. Microbiol. 15:597-574.

_____, 1967 b. Biodegradation of dichlorodiphenyl trichloroethane: Intermediates in dichlorodiphenylacetic acid metabolism by Aerobacter aerogenes. J. Appl. Microbiol. 15:1494-1497.

Walhull, G. 1953. Isolation of protoplasts from Bacillus megaterium by controlled treatment with lysozyme. J. Bacteriol. 66:686-689.

Wellner, D. 1967. Flavoproteins. Ann. Rev. Biochem. 36: 669-687.

White, A., Handler, P., and Smith, E.L. 1964. Principles of Biochemistry. 3rd ed., McGraw-Hill Book Co., New York.

Yoshida, E., Mitsui, H., Takahashi, H., and Maruo, B. 1960. Amino acid incorporation by a bacterial cell free system. J. Biochem. 48:251-261.

APPENDIX

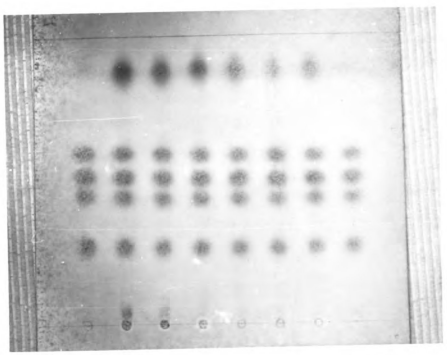


Figure 2. Representative thin-layer chromatogram.

Mobil phase; hexane. Chromogenic agent: 0.1% Rhodamine B. Each point of origin was spotted with 30.0 µg each of p,p'-DDE, o,p'-DDT, p,p'-DDT, and p,p'-DDD. From left to right authentic carbon-14-labeled DDT (1); 3ml of membrane fraction + 2.0 µmoles each of NAD, NADP, FAD, and 0.1 µmoles each of ADP & inorganic PO_4 (2); duplicate experiment using the components of number 2 (3); 3 ml of membrane fraction + 0.2 µmoles each of NAD, NADP, FAD, malate and pyruvate and 0.01µmoles each of ADP & inorganic PO_4 (4); duplicate experiment using the components of number 4 (5); 3 ml of membrane fraction (6); duplicate experiment using the components of number 6 (7); authentic carbon-14-labeled DDT (8). The spots at R_f 0.60, 0.51, 0.44, and 0.26 correspond to DDE, o,p'-DDT, p,p'-DDT, and DDD, respectively. The autoradiogram obtained from this chromatogram is presented in Figure 6B.

Figure 3. Autoradiograms of thin-layer chromatograms of carbon-14-labeled DDT and carbon-14-labeled metabolites produced by *E. coli*.

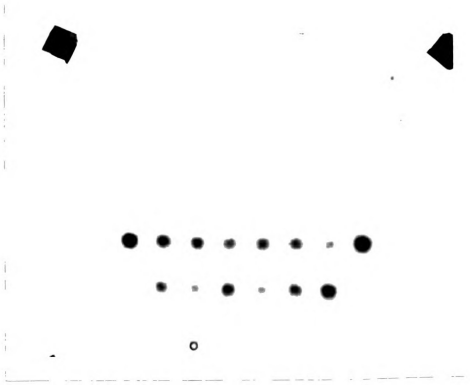


Figure 3A. Anaerobic cultures incubated 1 and 2 days.

From left to right carbon-14-labeled DDT (1); medium of an anaerobic culture incubated 1 day (2); bacteria of an anaerobic culture incubated 1 day (3); medium of an anaerobic culture incubated 1 day (4); bacteria of an anaerobic culture incubated 1 day (5); bacteria of an anaerobic culture incubated 2 days (6); medium of an anaerobic culture incubated 2 days (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

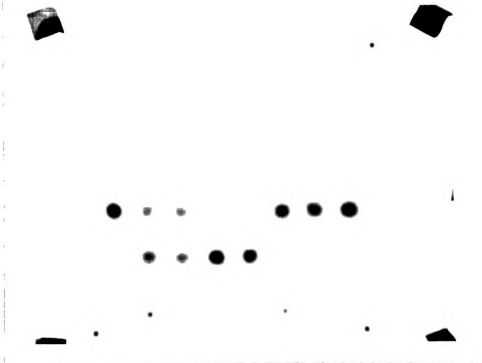


Figure 3B. Anaerobic cultures incubated 2 days, 3 days, and autoclaved cells incubated 3 days.

From left to right authentic carbon-14-labeled DDT (1); medium of an anaerobic culture incubated 2 days (2); bacteria of an anaerobic culture incubated 2 days (3); medium of an anaerobic culture incubated 3 days (4); bacteria of an anaerobic culture incubated 3 days (5); medium of an anaerobic autoclaved culture incubated 3 days (6); bacteria of an anaerobic autoclaved culture incubated 3 days (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

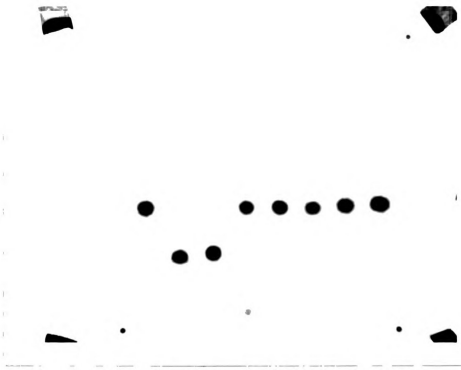


Figure 3C. Anaerobic and aerobic cultures incubated 3 days and autoclaved cells incubated 3 days.

From left to right authentic carbon-14-labeled DDT (1); medium of an anaerobic culture incubated 3 days (2); bacteria of an anaerobic culture incubated 3 days (3); medium of an anaerobic autoclaved culture incubated 3 days (4); bacteria of an anaerobic autoclaved culture incubated 3 days (5); medium of an aerobic culture incubated 3 days (6); bacteria of an aerobic culture incubated 3 days (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

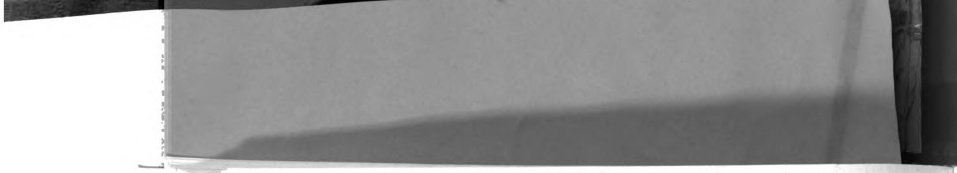


Figure 3D. Aerobic cultures incubated 1 and 2 days.

From left to right authentic carbon-14-labeled DDT (1); bacteria of an aerobic culture incubated 2 days (2); medium of an aerobic culture incubated 2 days (3); medium of an aerobic culture incubated 1 day (4); bacteria of an aerobic culture incubated 1 day (5); medium of an aerobic culture incubated 1 day (6); bacteria of an aerobic culture incubated 1 day (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

Figure 3E. Aerobic culture incubated 2 and 3 days.

From left to right authentic carbon-14-labeled DDT (1); medium of an aerobic culture incubated 2 days (2); bacteria of an aerobic culture incubated 2 days (3); medium of an aerobic culture incubated 3 days (4); bacteria of an aerobic culture incubated 3 days (5); authentic carbon-14-labeled DDT (6). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

Figure 4. Autoradiograms of thin-layer chromatograms of carbon-14-labeled DDT and carbon-14-labeled metabolites produced by P. aeruginosa.

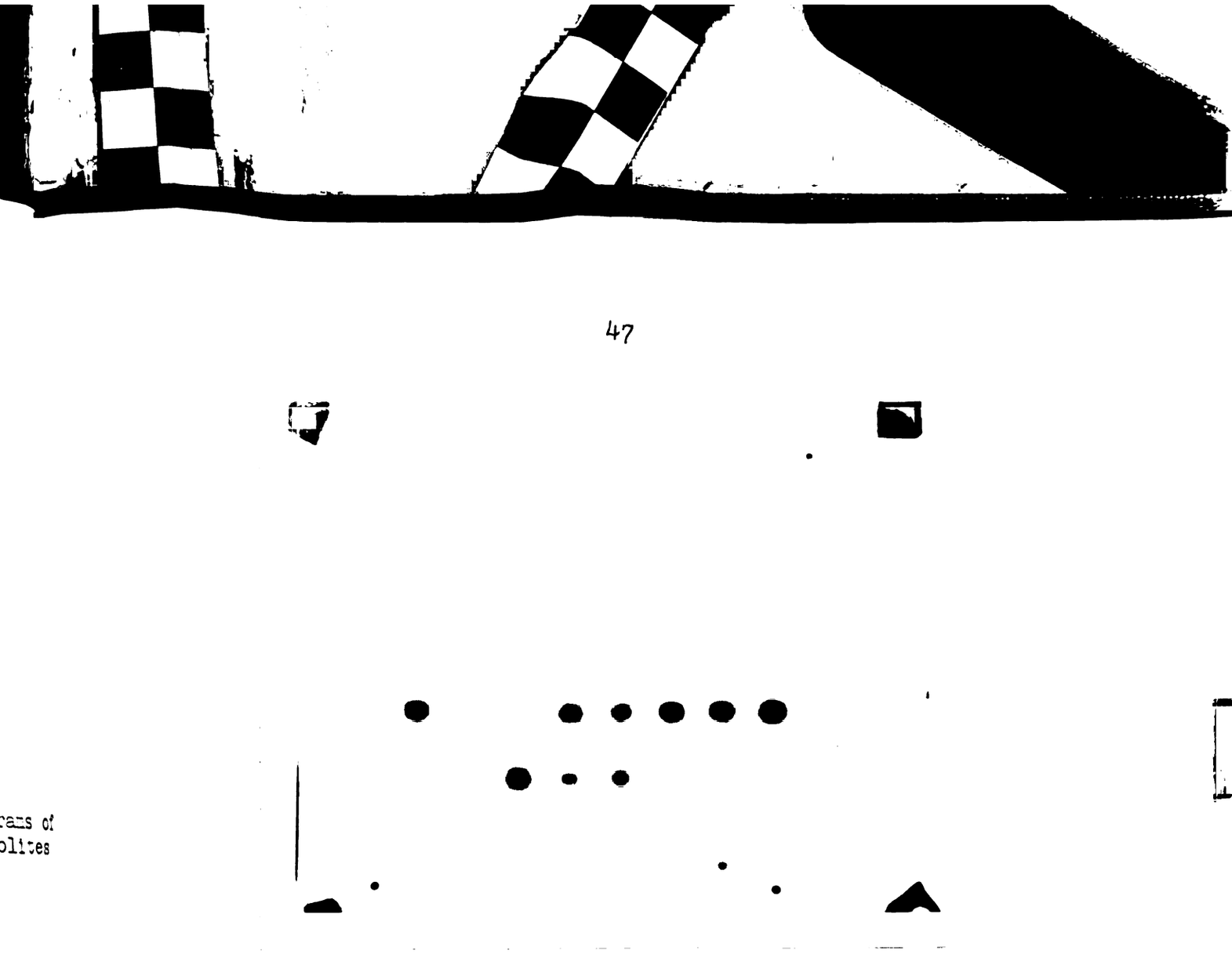


Figure 4A. Anaerobic and aerobic cultures incubated 2 days and autoclaved cells incubated 2 days.

From left to right authentic carbon-14-labeled DDT (1); medium of an anaerobic culture incubated 2 days (2); bacteria of an anaerobic culture incubated 2 days (3); medium of an aerobic culture incubated 2 days (4); bacteria of an aerobic culture incubated 2 days (5); medium of an anaerobic autoclaved culture incubated 2 days (6); bacteria of an anaerobic autoclaved culture incubated 2 days (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

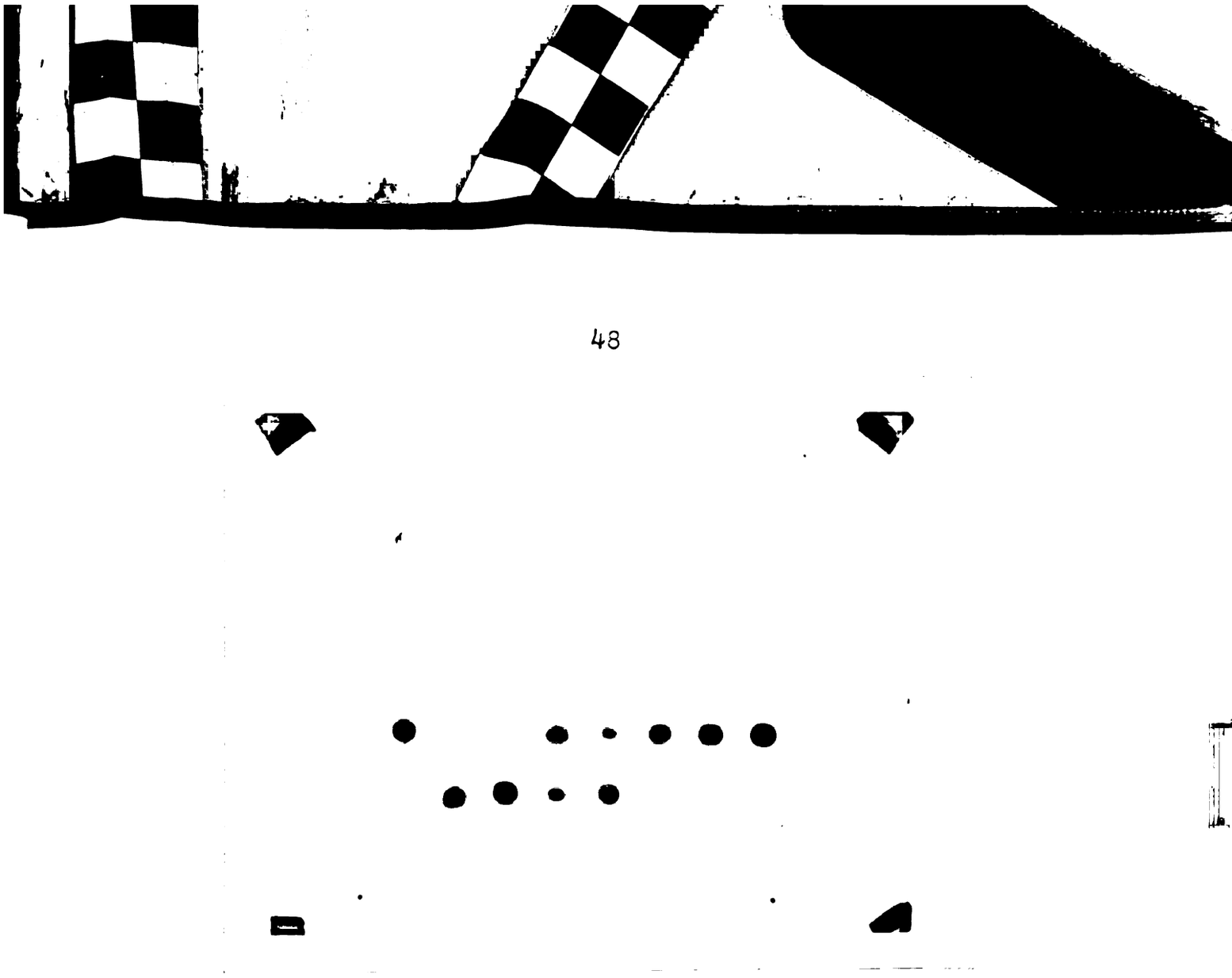


Figure 4B. Anaerobic and aerobic cultures incubated 2 days and autoclaved cells incubated 2 days.

From left to right authentic carbon-14-labeled DDT (1); medium of an anaerobic culture incubated 2 days (2); bacteria of an anaerobic culture incubated 2 days (3); medium of an aerobic culture incubated 2 days (4); bacteria of an aerobic culture incubated 2 days (5); medium of an anaerobic autoclaved culture incubated 2 days (6); bacteria of an anaerobic autoclaved culture incubated 2 days (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

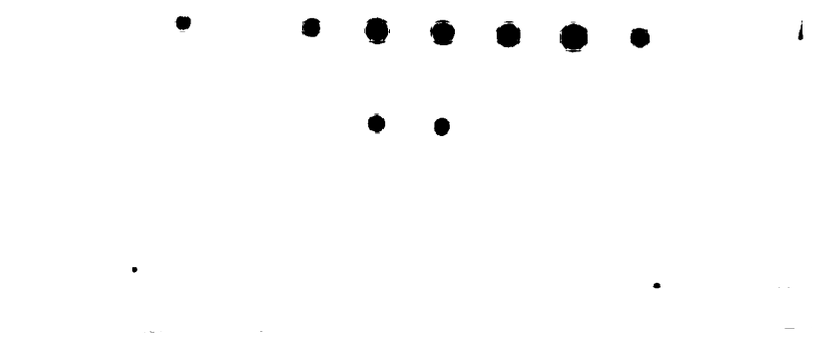


Figure 5. Autoradiogram of a thin-layer chromatogram of carbon-14 labeled DDT and metabolites produced by combining cytoplasmic fractions and membrane preparations of E. coli.

From left to right authentic carbon-14-labeled DDT(1); 3 ml of boiled membrane + cytoplasmic fraction (2); duplicate experiment using the components of 2 (3); 3 ml of membrane + cytoplasmic fraction (4); duplicate experiment using the components of number 4 (5); 3 ml of cytoplasmic fraction only (6); duplicate of 6 (7); authentic carbon-14-labeled DDT (8). The upper and lower spots corresponded to p,p'-DDT and p,p'-DDD, respectively.

Figure 6. Autoradiograms of thin-layer chromatograms of carbon-14-labeled DDT and metabolites produced by the addition of Krebs cycle intermediates or cofactors to membrane preparations of *E. coli*.

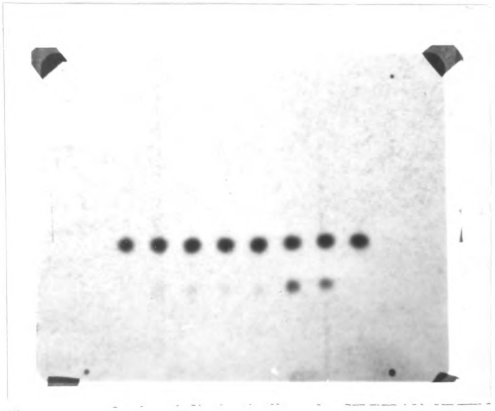


Figure 6A. Addition of intermediates and cofactors and the omission of ADP, inorganic phosphate or NAD.

From left to right authentic carbon-14-labeled DDT (1); 3 ml of membrane + 2.0 µmole each of NAD, NADP, FAD, malate, pyruvate, & 0.1 µmole each of ADP & PO_4 (2); duplicate experiment using the components of 2 (3); 3 ml of membrane + cofactors & intermediates minus ADP & PO_4 (4); duplicate experiment using the components of 4 (5); 3 ml of membrane + cofactors & intermediates minus NAD (6); duplicate experiment using the components of 6 (7); authentic carbon-14 labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

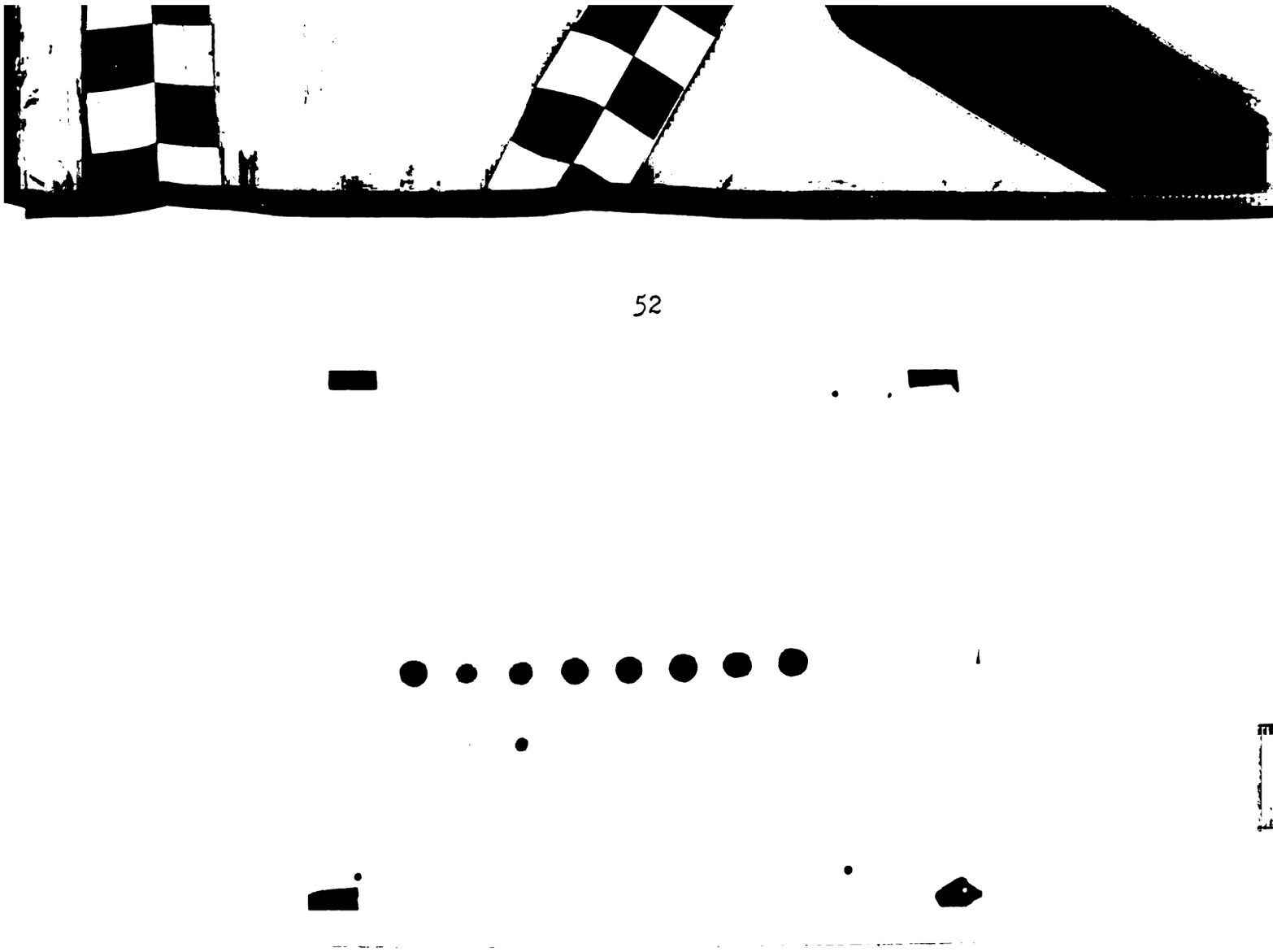


Figure 6B. Addition of intermediates and cofactors, membrane only and omission of intermediates.

From left to right authentic carbon-14-labeled DDT (1); 3 ml of membrane fraction + 2.0 µmole each of cofactors minus intermediates (2); duplicate experiment using the components of 2 (3); 3 ml of membrane + 0.2 µmoles each of intermediates and cofactors & 0.01 µmole each of ADP & PO_4 (4); duplicate experiment using the components of 4 (5); 3 ml of membrane only (6); duplicate experiment using the components of 6 (7); authentic carbon-14-labeled DDT (8). The upper and lower spots corresponded to p,p'-DDT and p,p'-DDD, respectively.

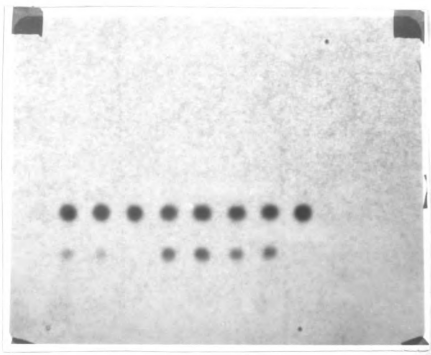
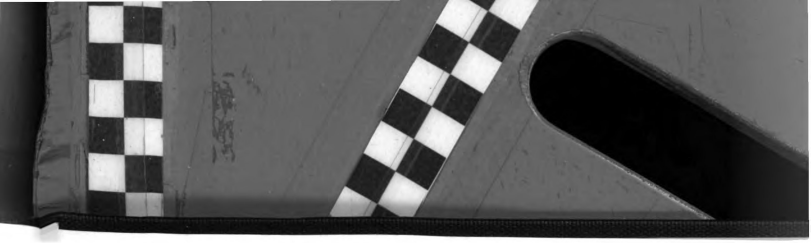


Figure 6C. Omission of FAD, NADP and intermediates.

From left to right 3 ml of membrane + 2.0 µmole of cofactors & intermediates minus FAD (1); duplicate experiment using the components of 1 (2); authentic carbon-14-labeled DDT (3); 3 ml membrane + intermediates, cofactors, ADP & PO_4 minus NADP (4); duplicate experiment using the components of 4 (5); 3 ml of membrane + cofactors, ADP & PO_4 minus intermediates (6); duplicate experiment using the components of 6 (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.

Figure 6D. Addition of FAD, ADP and inorganic phosphate.

From left to right authentic carbon-14-labeled DDT (1); 3 ml of membrane + 2.0 µmole FAD (2); duplicate experiment using the components of 2 (3); 3 ml of membrane + 0.1 µmole each of ADP & PO_4 (4); duplicate experiment using the components of 4 (5); 3 ml of membrane + FAD, ADP & PO_4 (6); duplicate experiment using the components of 6 (7); authentic carbon-14-labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.



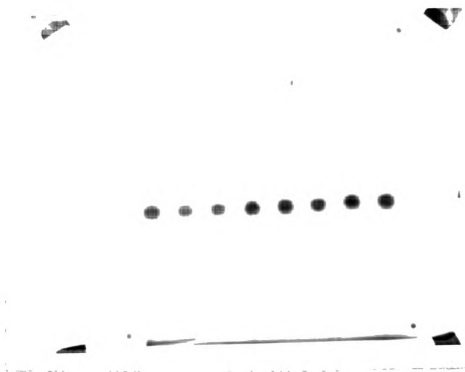
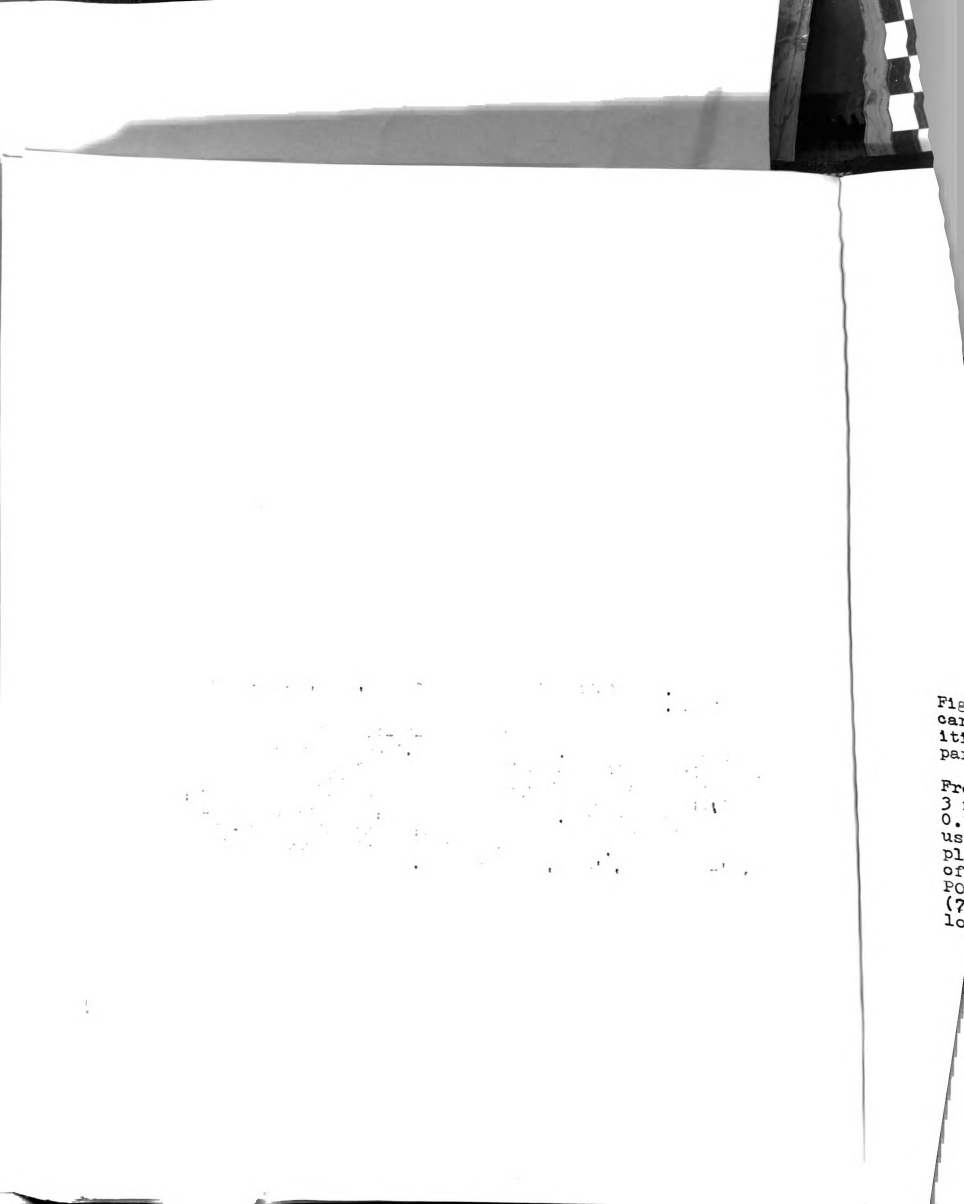


Figure 6E. Addition of FAD or NAD or FAD, ADP, PO_4 & atmospheric O_2.

From left to right authentic carbon-14-labeled DDT (1); 3 ml of membrane + 8.0 µmole FAD & 0.1 µmole each of ADP & PO_4 (2); duplicate experiment using the components of 2 (3); 3 ml of membrane + FAD, ADP & PO_4 with atmospheric O_2 (4); duplicate experiment using the components of 4 (5); 3 ml of membrane + 2.0 µmole of NAD (6); duplicate experiment using the components of 6 (7); authentic carbon-14 labeled DDT (8). The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.



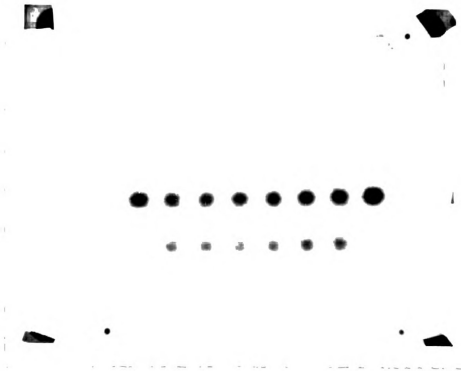


Figure 7. Autoradiogram of a thin-layer chromatogram of carbon-14-labeled DDT and metabolites produced by the addition of FAD, ADP and inorganic phosphate to membrane preparations suspended in cytoplasmic fractions.

From left to right authentic carbon-14-labeled DDT (1); 3 ml of membrane in cytoplasm fraction + 2.0 µmole FAD, 0.1 µmole each of ADP & PO_4 (2); duplicate experiment using the components of 2 (3); 3 ml of membrane in cytoplasm + FAD (4); duplicate experiment using the components of 4 (5); 3 ml of membrane in cytoplasm fraction + ADP & PO_4 (6); duplicate experiment using the components of 6 (7); authentic carbon-14-labeled DDT (8); The upper and lower spots correspond to p,p'-DDT and p,p'-DDD, respectively.