Dechlorination of DDT by Aerobacter aerogenes

Abstract. *Dechlorination of DDT to DDD in higher animals requires the presence of molecular oxygen, but in microorganisms the presence of oxygen hinders dechlorination. In cell-free preparations of* Aerobacter aerogenes, *the use of selected metabolic inhibitors indicated that reduced Fe(II) cytochrome oxidase was responsible for DDT dechlorination. This finding may possibly explain the persistence of DDT residues in soils and sediments.*

DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane] residues in soils strongly resist microbial degradation, although dechlorination of DDT to DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] can occur in higher organisms (*1*). In the rat, liver microsomal enzymes catalyze this reaction, which also requires molecular oxygen and reduced triphosphopyridine nucleotide (*2*). Dechlorination of DDT by microorganisms also is possible; *Proteus vulgaris* and baker's yeast reductively dechlorinate DDT to DDD (*3*). However, the efficacy of conversion by microorganisms is inversely proportional to the availability of oxygen in the system (*4*); and DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene] is also produced. Castro (*5*) and Miskus *et al.* (*6*) found that dilute solutions of iron (II) porphyrin complexes were oxidized by DDT in vitro to the corresponding iron (III) halides (hemins) and DDD. Thus, it seemed likely that the microbial dechlorination of DDT might be explained as a process implicating the cytochromes of the respiratory chain, the absence of oxygen serving to keep the cytochromes in the reduced (Fe II) state.

To test this hypothesis, three facultatively anaerobic organisms not previously studied, *Escherichia coli, Aerobacter aerogenes*, and *Klebsiella pneumoniae*, were tested for ability to dechlorinate DDT (5 parts per million) to DDD. The organisms were grown in trypticase–soy broth or thioglycolate media (BBL). Anaerobic conditions markedly increased the yield of DDD. Although 2- to 3-percent conversion to DDE always occurred, this amount could be completely accounted for by the controls. There was no lag period in DDD production and DDE was not an intermediate, as shown by failure to metabolize it to DDD. Moreover, reduced glutathione, which is a required cofactor for DDT-dehydrochlorinase (*7*), did not promote DDE production even in mineral media. Because *A. aerogenes* effects up to 80-percent conversion to DDD, it was chosen for more detailed study. Because DDT is slowly absorbed by whole cells, cell-free systems were used in the remainder of the work so that incubation times could be shortened.

Cell suspensions of 10 mg/ml (dry wt) were sonically disrupted in 0.07*M* phosphate buffer (*p*H 7) at 15°C. Buffer (5 ml) containing 10 ppm DDT (added in 0.5 ml acetone) was combined with 5 ml of the sonorate in Thunberg tubes which were evacuated, flushed with nitrogen or carbon monoxide, and incubated overnight at 30°C. The controls were either boiled for 5 minutes or had 0.1 percent *p*-chloromercuribenzoate added. Endogenous metabolism was usually so rapid that it was not necessary to add Krebs cycle intermediates to maintain the cytochromes in the reduced state. After incubation, the suspensions were extracted with hexane and the extracts were dried with Na_2SO_4 and assayed for DDD, DDE, and DDT by use of a Wilkens-204 gas chromotograph with an electron-capture detector.

Under these conditions the average conversion to DDD was about 70 percent. Again the small amounts of DDE that always formed could be accounted for by the controls. Addition of 0.001*M* cyanide, which blocks the reoxidation of cytochromes but not their initial reduction by way of the dehydrogenase system (*8*), completely inhibited DDD production, as did an atmosphere of carbon monoxide. Significantly, the inhibition by carbon monoxide was completely reversible by 1614-mphot illumination and partially reversible by 484 mphot, but not by methylene blue, even though reduction of the methylene blue still occurred (that is, the dehydrogenase systems were still operative). Inhibition by CN^- was not reversible by either methylene blue or illumination. Also, 0.001*M* nitrate, ferricyanide, malonate, and antimycin A (2 μg/ml) all inhibited dechlorination of DDT, even though methylene blue could still be reduced. Inhibition by antimycin A was partially reversible by 0.01*M* ascorbate and completely reversible if 100 ppm cytochrome *c* also was added. However, addition of cytochrome *c* alone was ineffective.

The fact that the cytochrome oxidase–iron carbonyl complex is dissociable by light (*9*), together with the other data, leads to the conclusion that reduced cytochrome oxidase is probably the cellular agent in the reductive dechlorination of DDT to DDD. This idea may possibly explain the persistence of DDT residues in aerobic soils and sediments. Because DDT evidently does not keep the cytochromes in the oxidized state if oxygen is present, the suggestion by Castro (*5*) that the oxidation of Fe (II) in the respiratory chain is a mechanism of DDT toxicity is probably untenable.

GARY WEDEMEYER
Fish-Pesticide Research Laboratory, U.S. Fish and Wildlife Service, Denver, Colorado

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Separation of Living and Dead Cells by Dielectrophoresis

Abstract. *High-frequency non-uniform electric fields were used to cause selective dielectrophoresis of yeast cells in an aqueous medium. Living cells separated from admixed dead ones remained viable after the separation process.*

Differences in dielectric behavior in a non-uniform field can be used to produce selective migration of single-cell organisms, here represented by yeast cells.

The process described here is not electrophoresis, but dielectrophoresis. Electrophoresis is the motion of charged particles in an electric field. Dielectrophoresis is the motion resulting from the force set up by the action of a non-uniform electric field on a neutral