Metabolism of DDT in Human Embryonic Lung Cell Cultures

Cultures of primary human embryonic lung (HEL) cells have been utilized to study DDT degradation. Incubation of ring-^{14}C-DDT for 48 hr with primary HEL cells in monolayer cultures resulted in metabolism of DDT by reductive dechlorination to DDD (38%) and DDA (4%). No other metabolites were found. This pathway is quite similar to that seen in mammals and microorganisms.

In an early study of DDT metabolism in mammals, White and Sweeney (1945) treated rabbits orally with DDT and then analyzed the urine for DDT and metabolites. X-Ray diffraction analysis revealed DDA [bis(*p*-chlorophenyl) acetic acid] as the only metabolite present in the urine; no DDT was found. In another study (Klein *et al.*, 1964), DDT was administered to rats orally in their feed. By analysis of hepatic tissue, it was determined that DDT had been reductively dechlorinated to DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane] and dehydrochlorinated to DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene]. These authors further determined that *o,p'*-DDT, when fed to rats, underwent a biological isomeric transformation to *p,p'*-DDT in the liver, although other work (Cranmer, 1972) indicates that this does not occur. A conflicting report by Barker and Morrison (1964) implied that DDT was probably not metabolized to DDD in living mice. These researchers presented evidence that DDD was formed in mouse tissue that was decomposing after the mice were sacrificed. However, Peterson and Robison (1964) established that DDD was indeed a metabolite of ingested DDT in the rat. These authors further elucidated the specific steps through which DDT passed on its way to elimination in the urine as DDA. In a somewhat related study, sheep were administered DDT orally and their fat was assayed for DDT and metabolites (Hunnego and Harrison, 1971). After 2 weeks of dosing, DDT, DDD, and DDE were present in the fat in relative amounts of DDE > DDD > DDT.

DDD seems to be the major metabolite of DDT metabolism by microorganisms. *Proteus vulgaris*, isolated from the intestine of a mouse, metabolized DDT in an *in vitro* culture (Barker *et al.*, 1965). DDD was the only metabolite recovered from the cultures. DDE was also incubated with the bacteria in culture, but no DDD was produced, thus excluding DDE as an intermediate in the production of DDD from DDT. The authors felt the bacteria could be the major producer of DDD from DDT in the rat. Similar findings were reported by Wedemeyer (1966) in *Aerobacter aerogenes*. The efficacy of the conversion of DDT to DDD was inversely proportional to the availability of oxygen to the system. Using deuterated DDT and mass spectroscopy, Plimmer *et al.* (1967, 1968) showed that the conversion of DDT to DDD proceeds by reductive dechlorination and not by dehydrochlorination and subsequent reduction. Johnson *et al.* (1967) used 27 microorganisms to metabolize DDT to DDD. They also noted that the conversion is enhanced by low oxygen levels. Evidence was presented for the presence of additional metabolites. Fries *et al.* (1969) have investigated DDT metabolism *in vitro* by rumen microorganisms. The major product of this metabolism was DDD, with an accompanying small amount of a polar metabolite. DDT conversion to DDD was concluded to be ubiquitous among microorganisms.

A study of DDT metabolism *in vitro* by HeLa S tissue culture cells was reported by Huang *et al.* (1970). In this system, DDT was degraded to DDD, DDE, DBP (4,4'-dichlorobenzophenone), and DDM (4,4-dichlorodiphenylmethane). These results were interpreted as showing that many reactions were involved in DDT degradation by HeLa S cells, rather than merely a dechlorination reaction to DDD. A criticism of this study is that no control cultures were included, so it is possible that DDT could have been degraded by components of the incubation medium. Also, amounts of recovered radioactivity from original inocula of radioactive DDT varied as much as threefold from one incubation interval to another. Spalding *et al.* (1971) reported that mouse L 5178 lymphoma cells in culture did not metabolize DDT in a 72-hr incubation. These two studies are the only reports of DDT metabolism in cell culture. The present study utilizes a primary cell culture system from a nonmalignant tissue source.

METHODS AND MATERIALS

Cell Cultures. Human embryonic lung cells (HEL cells) were procured from Flow Laboratories, Rockville, Md. These cells were freshly explanted from *in vivo* sources and seeded in culture flasks with a medium composed of 80% Eagle's Minimum Essential Medium (Eagle, 1959), 19% fetal bovine serum, and 1% glutamine.

Chemicals. DDT was uniformly ring-labeled with ^{14}C (specific activity, 4.6 mCi/m*M*), purchased from Nuclear-Chicago Corp., Chicago, Ill.

Unlabeled DDT was synthesized by the method of Adams and Johnson (1949). DDE [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene], a possible metabolite of DDT, was synthesized by the method of Peterson and Robison (1964). Other standard reference compounds were obtained from the indicated sources as follows: DDA [bis(*p*-chlorophenyl)acetic acid], Aldrich Chemical Co., Cedar Knolls, N. J.; DBP (4,4-dichlorobenzophenone), Aldrich Chemical Co.; DDD [2,2-bis(*p*-chlorophenyl)-1,1-dichloroethane], Aldrich Chemical Co.; dicofol [1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol], Rohm and Haas Co., Philadelphia, Pa.; DDM [bis(*p*-chlorophenyl)methane], Eastman Organic Chemicals, Rochester, N. Y.; and *p*-chlorobenzoic acid, Aldrich Chemical Co.

Incubation Conditions. A benzene solution of radiolabeled DDT was carefully added to sterile culture flasks (100,000–300,000 dpm) in such a way that the material was dispersed over the surface of each flask upon which the cells would be growing when added to the flasks. A stream of air previously passed through a sterile filter was used to evaporate the benzene. Cells were trypsinized from a donor flask (North and Menzer, 1972) and added to the flasks with fresh nutrient medium. The flasks were tightly stoppered and placed in an incubator at 37°. Control flasks containing all the above components except cells were similarly prepared and incubated.

Extraction and Chromatography. After 48 hr of incubation and growth, hexane was added to both test and control flasks to stop metabolic activity. The hexane was separated and the remaining aqueous fraction was extracted four times with additional hexane. The hexane extracts were pooled.

Silica Gel-G and Silica Gel-F254 thin-layer chromatography was used to separate DDT and its metabolites. Radiolabeled materials were spotted with known reference compounds on the plates and developed in the following solvent systems: heptane–ethanol–acetone (98:0.1:2) (Huang *et al.*, 1970); cyclohexane–carbon tetrachloride (1:1) (Plimmer *et al.*, 1968); benzene–dioxane–acetic acid

(10:3:1) (Focht and Alexander, 1971); hexane–carbon tetrachloride (7:3) (Hemphill *et al.*, 1967); diethyl ether–hexane (3:1) (Kapoors *et al.*, 1970); acetonitrile–water–ammonium hydroxide (8:1.8:0.2) (Bull and Lindquist, 1964); and hexane–diethyl ether–acetic acid (100:1:1) (Bishara *et al.*, 1971).

After tlc resolution of radioactivity and unlabeled standards, the plates were either observed under ultraviolet light or sprayed with a solution of silver nitrate in 2-phenoxyethanol (Mitchell, 1963), followed by exposure to uv light for 10 min. The location of radioactive metabolites was determined by exposing the plates to Kodak No-Screen medical X-Ray film or scanning on a Berthold Radiochromatogram Scanner. Other radioassay procedures were according to North and Menzer (1972). Final identification was afforded by demonstration of cochromatography of an unlabeled standard metabolite with a radioactive candidate metabolite suspected of being identical in structure.

RESULTS AND DISCUSSION

DDT was incubated for 48 hr with HEL monolayer cells and with noncell, medium-only controls. All flasks were extracted with hexane. In test and control flasks, 96–100% of the original radioactive inoculum was recovered. All radioactive material recovered from control flasks was in the hexane fraction. This material cochromatographed with standard reference DDT on Silica Gel-G tlc, using the listed solvent systems. Only DDT was recovered from control flasks.

From test flasks, 96% of the radioactivity partitioned into hexane, with 4% in the aqueous fraction. Tlc on Silica Gel-G chromatoplates showed radioactive materials in the hexane fraction separating into two areas. The upper spot constituted 60% of the spotted radioactivity, while the lower spot accounted for 40%. Further tlc showed cochromatography between the upper spot and standard reference DDT. Cochromatography between the lower spot and standard reference DDD was seen on the same tlc solvent systems. Thus, originally inoculated DDT appears to have been metabolized by reductive dechlorination to DDD.

Aqueous fraction aliquots were evaporated, and the radioactive residue was resuspended in acetone for spotting on Silica Gel-G chromatoplates. Tlc showed one radioactive spot. Further tlc showed cochromatography of this spot with standard reference DDA. This was the only metabolite found in the aqueous fraction. Thus, HEL cells metabolized DDT to DDD (38%) and DDA (4%) as the only detectable metabolites. These experiments were repeated four times.

HEL cell metabolism of DDT to form DDD and DDA mirrors DDT metabolism by microorganisms. Another similarity is the absence of DDE as a metabolite. Thus, HEL cells seem to reductively dechlorinate DDT to DDD. DDD may be further dechlorinated and oxidized to DDA by HEL cells. None of the intermediate metabolites found by Peterson and Robison (1964) were found in this study.

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