Biochemical Pharmacology, Vol. 19, pp. 637–639. Pergamon Press. 1970. Printed in Great Britain

Degradation of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane by HeLa S cells

(*Received* 25 *June* 1969; *accepted* 15 *August* 1969)

DDT* IS AN effective and most widely used insecticide nowadays. The presence of this chlorinated organic compound has been found almost everywhere, including in soils, plants, foods and even in the human body.[1, 2]

Persistence of DDT in natural surroundings is well known; however, several *Actinomycetes* were reported to degrade DDT to DDD,[3] and recently Korte *et al.*[4] also found a number of microorganisms can degrade all representative chlorinated cyclodiene insecticides slowly except dieldrin. The presence of DDT metabolites, DDD and DDE, was found in rat liver,[5] organs of fishes,[6] and in avian liver,[7] indicating that DDT is metabolized in mammalian systems. It is of interest to know how DDT is catabolized in other biological systems. In this experiment, DDT-^{14}C was incubated with HeLa S cells. DDT and its metabolites were extracted with hexane, concentrated, developed by two-dimensional thin-layer chromatography; then the radioactive compounds were identified by autoradiography.

EXPERIMENTAL

HeLa S cells were obtained from the Carver Research Foundation laboratory. Accurately measured cells (4×10^6) were grown in 10 ml of medium (80% medium No. 199, 20% calf serum) which contained 0·05 μc of DDT-^{14}C (specific activity, 2·73 mc/mM, New England Nuclear Corp., Mass.) for periods of 12, 24, 36 and 48 hr at 37°. At the termination of the desired incubation periods, the culture medium was collected by centrifugation. A 10-ml aliquot of *n*-hexane was used to extract DDT and its metabolites from the aqueous phase three times. The combined extract was concentrated to 1 ml by vacuum evaporator. DDT and its metabolites were separated by two-dimensional thin-layer chromatography using Silica gel G-coated plate (2 mm thickness, 20 × 20 cm) as stationary phase. The first developing solvent used was *n*-hexane and a solvent mixture consisting of *n*-heptane, ethanol, acetone at a ratio of 98:0·1:2 was the second-dimensional developing solvent. The resulting chromatogram was subjected to autoradiography with Kodak, medical X-ray film, NS-54 T for 1 month. The spots appearing on the film were identified by comparing with R_f values of authentic compounds. The portions of silica gel absorbent that correspond to the radioactive spots as judged by the X-ray film were scraped and transferred to a counting vial for radioactivity determination.

Radioactivity was determined in a Packard Tri-Carb liquid scintillation counter. The composition of toluene scintillator fluid was: 4 g BBOT [2,5 bis-(5-*tert*-butylbenzoxazoyl) thiophenol] plus 80 g of napthalene in 400 ml of methyl cellusolve and 600 ml of toluene.

RESULTS AND DISCUSSION

The positions of radioactive spots appearing on the X-ray film are shown in Fig. 1. Five of them were judged as DDE, DBM, DDT, DDD and DBP. Assuming from the low mobility with solvent systems, a spot remaining near the origin could be DDA and BA.

Distribution of radioactivities of DDT and its metabolites at different incubation periods is shown in Table 1. The majority of activities were found in DDT and in DDE. The proportion of radioactivities among DDT, DDE, DBM, DBP, unidentified spot and DDD after 48 hr was 72, 13, 4·9, 4·3, 3·5 and 2·26 respectively. DDT and its metabolites tended to decrease at 12 hr, reached a minimum at 24 hr, then increased again and finally leveled after 36 hr. This trend might reflect growth phases of the cells whereby DDT was absorbed faster together with nutrients in the media by the cell at the initial stage

* Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane; DDE, 1,1-dichloro-2,2 bis(*p*-chlorophenyl) ethylene; DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane; BA, *p*-chlorobenzoic acid; DDA, bis-(*p*-chlorophenyl) acetic acid; DDMU, 1-chloro-2,2-bis(*p*-chlorophenyl) ethylene; DDOH, 2,2-bis-(*p*-chlorophenyl) ethanol; DBP, 4,4-dichlorobenzophenone; DBM, 4,4-dichlorodiphenyl methane.

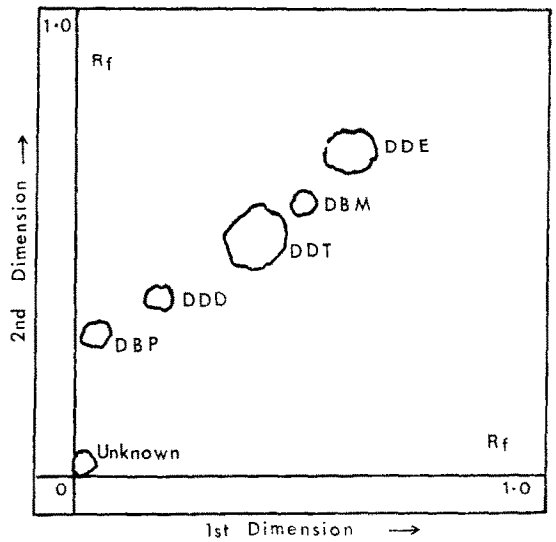

FIG. 1. The degradation pattern of DDT in HeLa S cell system. Solvent No. 1 is *n*-heptane; solvent No. 2, a mixture of *n*-heptane, ethanol and acetone (98:0·1:2 v/v).

TABLE 1. DISTRIBUTION OF DDT AND ITS METABOLITES EXTRACTED FROM HELA S CELL MEDIA AT DIFFERENT INCUBATION PERIODS

Compound	Incubation period (hr)			
	12	24	36	48
Unidentified	82 ± 3*	57 ± 5	80 ± 5	67 ± 2
DBP	76 ± 4	66 ± 1	67 ± 3	82 ± 2
DDD	35 ± 3	19 ± 5	41 ± 1	43 ± 5
DDT	866 ± 8	440 ± 9	1373 ± 2	1364 ± 9
DBM	53 ± 5	10 ± 3	57 ± 2	95 ± 6
DDE	143 ± 3	57 ± 10	212 ± 4	248 ± 2

* Values are expressed as cpm and are mean ± S.E. of six determinations.

of propagation. After reaching a plateau of growth, possibly due to deficiency of some nutrients in the medium, absorption of DDT was decreased and, in the mean time, absorbed DDT and its metabolites were excreted out in the medium from inside the cells.

DDT is a relatively stable compound and its major metabolite is considered to be DDD.[3, 5, 8] It is reported, however, that *Fusarium oxysporum* decomposed DDT to DDD, DDE, DDMU, DDA and DDOH.[9] Guenzi and Beard[10] added DDT-^{14}C to soil and later identified seven radioactive metabolites

In mammals, Vessey *et al.*[8] found DDT, DDD and DDE in cellular fractions of rat liver. Peterson and Robinson[5] reported that rat-liver cells metabolized DDT to DDD and to a lesser extent to DDE. However, they reported that DDE was a terminal metabolite whereas DDD was further metabolized to more polar analogs. Thus once formed, DDE seems to be transferred back to the blood where it is found as the principal metabolite.

In this experiment, five of the radioactive compounds were identified as DDT, DDD, DDE, DBM and DBP. This result shows that many reactions are involved in degradation of DDT in HeLa S cells rather than merely a dechlorination reaction to DDD. The presence of larger amounts of DDE may

suggest that DDE is a terminal metabolite of DDT.[5] It is also possible that the presence of iron porphyrin complexes in the medium might have enhanced dehydrochlorination of DDT,[11] resulting in accumulation of DDE.

Acknowledgement—This work was supported by Grant No. RO 1-ES 00095 from the National Institute of Health.

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