Degradation of DDT by a Soil Amoeba

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INTRODUCTION

It is well known that some chlorinated plaguicides remain on the soil for a long time.Among them DDT occupies a remarkable place,and in some cases a mean life over 25 years has been established (WHEATLEY 1965).Such a persistency is caused by its resistance both to chemical and biological degradation.

However,it has been proved that some microorganisms in the soil may convert DDT into its commonest metabolites (DDD and DDE).These degradations mainly occur in anaerobic conditions.

WEDEMEYER (1967) showed DDT metabolization up to dichlorobenzophenone (DBP) by Aerobacter aerogenes and, further works proved that some others microorganisms may also cause such degradations.Anyway,these further degradations seem to be restricted to a few microorganisms.

FOCHT and ALEXANDER (1971) and PFAENDER and ALEXANDER (1972) assaying with Hydrogenomones,for the first time showed the rupture of DDT ring by microorganisms.

Some DDT metabolites found in higher animals are metabolic products due to intestinal microflora and not to animal enzymatic systems,as it was thought at the beginning.This led to increase the microbial metabolic studies.

In the present work DDT degradation by Acanthamoeba castellanii cultures is studied,as a contribution to the elucidation of DDT metabolism in microorganisms,and particularly,because this amoeba is a microbiological cons-

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tituent in soil ecosystems where this plaguicide results persistent.

MATERIALS AND METHODS

Acanthamoeba castellanii,Neff strain (ATCC 30.010) was obtained from the American Type Culture Collection U.S.A.- A liquid medium consisting of proteose-peptone, glucose,inorganic salts,aminoacids and vitamins (KORN-1963),was used for the maintenance of the strain and for the experimental cultures.

In the assays with whole cells,Acanthamoeba was inoculated into the sterile culture medium containing 5 p.p.m. of pure DDT.The cultures were kept in 50 ml flasks in aerobiosis,without shaking and at 24°C for the periods in each case required.

Because of the possibility that nucleophilic sites on the cell wall might cause dechlorination reactions, control assays were performed with autoclaved cells. Thus,the cultures were incubated until the desired cell concentration was achieved and then autoclaved for 15 min. at 120 °C.After the addition of the plaguicide they were again incubated under the same conditions as the experimental cultures.The biological inactivity of these cells was assured after observing them coloured with trypan blue.

Employing the culture medium without cells,controls were also performed and incubated with DDT under the conditions above described.

After the incubations,extractions of plaguicides were made on 10 ml aliquots.Each aliquot was extracted shaking for 10 min. with 1 ml acetone and 5 ml petroleum ether;the extractions were repeated twice with 2.5 ml portions of petroleum ether.The extracts collected were dried with anhydrous SO_4Na and,without previous clean-up,a-

nalyzed.The analyses by gas-liquid chromatography were carried out in a Carlo Erba GT chromatograpf fitted with an electronic capture detector,under the following operating conditions: 1% SE-30 on Gas-Chromosorb P column at 170 °C;detector of Ni-63 at 250 °C;the gas carrier flow (N_2) was 50 ml per min.Chromatographic peaks were identified through the use of standards of pp'DDD, pp'DDE (Nanogens) and pp'Kelthane (Rohm & Haas) run under the same conditions.The area of peaks were determined by triangulation.A column packed with 3% QF-1 + 1% DC-200 on Gas-Chromosorb P,and TLC on plates coated with aluminium oxide,developed with n-heptane,were used as confirmatory methods.

It was intented to detect the possible presence of breakdown products of DDT with carboxyl groups by methylation previous to gas chromatography.Samples were treated with MeOH-ClH 3N for 1 hour at 65 °C;plaguicides were extracted and analyzed by gas chromatography.

RESULTS AND DISCUSSION

In some instances,after incubations,the cells were separated by centrifugation,washed twice with physiological solution,and the plaguicides extracted from them. The analyses showed the presence of DDT degradation products.Since this washing procedure does not ensure a complete removal of the plaguicides adhered to the cell membrane,in further experiments it was prefered to extract the plaguicides from the total culture.The results given in the tables were obtained by this latter procedure.

Table 1 shows the results average of two determinations,obtained when performing the incubations with 5 p.p.m. DDT for 12 days.

TABLE 1

Incubation of Acanthamoeba castellanii cultures with 5 p.p.m. of DDT.
% of DDT and its degradation products.

INCUBATION	DDT	DDE	DDD	DBP
Culture	80.10	8.09	9.20	2.60
Control with autoclaved cells	94.55	2.07	3.38	traces
Control without cells	95.63	1.97	2.40	traces

DDT degradation products analyzed by gas-liquid chromatography had the chromatographic characteristics of DDE,DDD and DBP. Identification of DBP was done injecting pp'Kelthane,which originates pp'DBP as a product of its thermic degradation (MORGAN 1967).The corroboration of those products was carried out by gas chromatography in columns of different polarity and by thin layer chromatography.There also appeared in some chromatograms smaller peaks with retention times corresponding to DDOH and DDM,but its identity could not be corroborated.

Controls show the same degradation products as cultures,but in a much lower proportion.The autoclaved cells reveal a nonbiological degradation of the same order as that produced in culture media without cells incubated under equal conditions.This suggests that no degradation is produced on cell surface;neither would have influence in degradation the cell waste products, nor the raising of pH that takes place when cultures are grown.It is for this reason,and a greater easiness to operate,that in further experiments only controls without cells were used.

In this nonbiological degradation the possibility of photodecomposition (FLECK 1949) must be discarded, since the assays were carried out in a dark culture chamber.

Table 2 gives the results average of two determinations, obtained when incubating amoeba with DDT for different periods. At the moment incubations were detained, count cells were made in each culture; in the first and second week it was proved that cultures were at their exponential growth period.

TABLE 2

Incubation of Acanthamoeba castellanii with 5 p.p.m. of DDT for different periods. % of DDT and its degradation products.

WEEK	INCUBATION	DDT	DDE	DDD	DBP
1st	Culture	80.85	5.85	4.87	8.66
	Control	97.87	1.45	0.67	traces
2nd	Culture	79.50	4.77	9.63	6.10
	Control	94.70	2.54	1.77	0.98
3rd	Culture	70.88	8.23	9.34	11.46
	Control	94.83	2.22	traces	2.94

It is observed that as cultures are growing a decrease in DDT concentration is produced, until it reaches 70% of the initial value by the third week. Correspondingly, an increase in degradation products is present.

The relatevely low value of DDE during the second week may be due to a tendency to form other metabolic products such as DDD and DBP.

The data obtained by assay controls indicate that in these incubation conditions a nonbiological degradation of DDT is also produced. Nevertheless, the values obtained, though appreciable, are significantly different from those found with the cultures.

In some cases the detection of degradation products traces with chromatographic characteristics similar to DDOH and DDM, though not confirmed, might suggest a degradative metabolic pathway including dechlorinations, hydration, oxidation and decarboxylation (FISHBEIN 1974) until reaching DBP.

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