3. A. Siccardi, *Genet. Res.* **8**, 219 (1966); personal communication; D. H. Smith, *Lancet* **1967-I**, 252 (1967), and unpublished observations.
4. T. Watanabe, *Method. Med. Res.* **10**, 202 (1964); L. Leive, *Biochem. Biophys. Res. Commun.* **18**, 13 (1965).
5. R. Novick, *Fed. Proc.* **26**, 29 (1967).
6. T. Watanabe, *J. Bact.* **88**, 716 (1964).
7. I thank Lorraine Sitewicz for technical assistance. Supported by PHS grant GM-14119-01. The author is a Fellow of the Medical Foundation of Boston.

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Anaerobic Biodegradation of DDT to DDD in Soil

Abstract. *DDT labeled with carbon-14 was added to soil, and the mixture was incubated anaerobically for 2 weeks and 4 weeks. DDT and seven possible decomposition products were separated by thin-layer chromatography, and the radioactivity of material from individual spots was determined by liquid scintillation. The DDT was dechlorinated by soil microorganisms to DDD, and only traces of other degradation products were detected. No degradation of DDT was detected in sterile soil.*

Persistence of some chlorinated insecticides in soils is well documented (*1*), and methods for accelerating decay of these materials in soils would be of monumental importance whenever an undesirable accumulation occurs. In this study, results showed that a considerable amount of DDT (*2*) was converted rather rapidly to DDD, and only 57 percent of the initial DDT was recovered in identifiable products after 4 weeks of incubation.

A number of investigators studied the conversion of DDT to DDD in animals by microorganisms. DDT was dechlorinated to DDD by *Serratia marcescens* isolated from stable flies (*3*), *Escherichia coli* and *Aerobacter aerogenes* isolated from the gastrointestinal tract of rats (*4*), and *Proteus vulgaris* from the intestinal flora of a mouse (*5*). The anaerobic condition required for the conversion process was shown by Stenersen (*3*). Wedemeyer (*6*), using sonically disrupted cell suspensions of *Aerobacter aerogenes* and selected metabolic inhibitors, showed that DDT was dechlorinated in the presence of reduced Fe (II) cytochrome oxidase. DDT was converted to DDD by yeast (*7*) and shake-cultures of actinomycetes (*8*); but shake-cultures of selected fungi had no effect on DDT (*8*).

The objectives of this study were: (i) to determine if DDT could be degraded anaerobically by soil microflora and (ii) to determine the metabolic decomposition products. The *p,p'*-DDT uniformly ring-labeled with C^{14} and carrier *p,p'*-DDT were made up in *n*-hexane so that 2 ml of standard solution, which was added to 10 g of soil (*9*), contained 0.1 mg of DDT with a C^{14} activity of 1.1 μc. After evaporation of the organic solvent, the soils were wet to 28 percent water on a dry weight basis (⅓ bar suction) and were incubated anaerobically in an atmosphere of 20 percent CO_2 and 80 percent N_2 at 30°C. Prior to extraction, the incubation chambers were flushed with N_2 and the exhaust gases were passed through two traps connected in series. The first trap (*n*-hexane) collected any loss of pesticide in the vapor phase, and the second trap (NaOH) absorbed the CO_2. Carbonate in the base was precipitated with $BaCl_2$. The resulting $BaCO_3$ was acidified, and the CO_2 evolved was collected quantitatively in 4 ml of 0.6*N* NCS (*10*). Soils were extracted at the completion of the incubation periods with a mixture of acetone, *n*-hexane, and acetic acid (50 : 50 : 1). Extracts were transferred to separatory funnels containing 300 ml of water, and the decomposition products were partitioned into the *n*-hexane layer. The *n*-hexane was dried with Na_2SO_4 and concentrated to 2 ml.

Decomposition products were separated on a two-dimensional aluminum oxide plate for the detection of DDT, DDD, DDE, kelthane, DBP, and DBM (*2*). After development, the aluminum oxide plates (impregnated with $AgNO_3$) were exposed to ultraviolet light to produce a visible dark spot. Initially, the developed thin-layer plates were exposed to x-ray film to be sure that all the activity was associated with products having predetermined R_F values from authentic samples (Table 1). Since the activity coincided with the standard compounds, the visible dark spot produced by AgCl and ultraviolet light was used to identify the products. Material from individual dark spots was transferred into counting vials containing 5 ml of counting solvent (*11*) with a suction apparatus. Both DDA and BA (*2*) were separated on an Adsorbosil-1 plate developed in a mixture of benzene, methanol, and acetic acid (50 : 8 : 2). The R_F values for DDA and BA were 0.55 and 0.48, respectively. Standard authentic samples of these two acids were spotted on each side of the soil extracts, and only the standards were sprayed with bromcresol green, which gave a yellow spot on a blue background. Material from the extracts having the same R_F values as the adjoining acids was removed by suction into counting vials. Radioactivity was determined in a liquid scintillation counter. Confirmation of the major constituents (DDT, DDD, DDE, and DDA) was made by using gas chromatography (*12*) with an electron-affinity detector.

Table 1. Two-dimensional R_F values of DDT derivatives. Layer: aluminum oxide G impregnated with $AgNO_3$ (20 mg per plate) and activated for 30 minutes at 130°C. Solvent No. 1 is *n*-heptane; solvent No. 2, a mixture of *n*-heptane, ethanol, and acetone (98:0.1:2).

Compound	R_F values in solvent	
	No. 1	No. 2
DDA and BA	0	0
Kelthane	0.02	0.11
DBP	.05	.53
DDD	.18	.45
DDT	.37	.58
DBM	.46	.65
DDE	.53	.70

DDT was dechlorinated to DDD by soil microorganisms under anaerobic conditions (Table 2). To ascertain if dechlorination was a result of microbial conversion or of catalytic conversion, a complete set of soil samples was sterilized for 1 hour in an autoclave. After each incubation period, the sterilized soil samples were plated and in all cases the soils remained sterile. DDT was not degraded in soils that were sterilized prior to incubation, which indicates that the conversion was a microbial process. After 2 weeks of incubation, 10 percent of the radioactivity in the extract was in DDD, 88 percent in DDT, and less than 2 percent in the other products. After 4 weeks of incubation, 62 percent of the radioactivity recovered was in DDD, 34 percent in DDT, and 4 percent in the other prod-

Table 2. Decomposition products of DDT isolated from soil after anaerobic incubation for 2 weeks and 4 weeks. Values, in micrograms (calculated from measurements of radioactivity), are means of two replicates.

Product	Recovery (in micrograms) after	
	2 weeks	4 weeks
DDA	0.37	0.51
BA	.24	.59
Kelthane	.15	.61
DBP	.39	.64
DDD	7.1	35
DDT	62	19
DBM	0.09	0.03
DDE	.19	.25
Total	71	57

ucts. The results indicate that during anaerobic decomposition, DDT was converted directly to DDD, and further breakdown did not result in an appreciable buildup of any of the other identified products.

The total amount of radioactivity in the partitioned *n*-hexane layer of the soil extract decreased with increasing length of incubation. After 2 and 4 weeks of incubation, 29 and 43 percent, respectively, was unaccounted for as products soluble in *n*-hexane. Part of the activity unaccounted for in the *n*-hexane was found in the water layer after partitioning the other products into the *n*-hexane. Isolation and identification of water-soluble compounds have not been made. No activity was found in the hexane trap or as $C^{14}O_2$ (NaOH trap). Therefore, DDT was dechlorinated to DDD, and further degradation products included some water-soluble materials.

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References and Notes

1. M. Alexander, *Soil Sci. Soc. Amer. Proc.* **29**, 1 (1965).
2. Abbreviations used are: 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; DBP: 4,4′-dichlorobenzophenone; DDA: bis-(*p*-chlorophenyl)acetic acid; BA: *p*-chlorobenzoic acid; kelthane: 1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol; and DBM: 4,4′-dichlorodiphenylmethane.
3. J. H. V. Stenersen, *Nature* **207**, 660 (1965).
4. J. L. Mendel and M. S. Walton, *Science* **151**, 1527 (1966).
5. P. S. Barker, F. O. Morrison, R. S. Whitaker, *Nature* **205**, 621 (1965).
6. G. Wedemeyer, *Science* **152**, 647 (1966).
7. B. J. Kallman and A. K. Andrews, *ibid.* **141**, 1050 (1963).
8. C. I. Chacko, J. L. Lockwood, M. Zabik, *ibid.* **154**, 893 (1966).
9. Pawnee silt loam with the following properties: *p*H, 5.2; total nitrogen, 0.154 percent; organic matter, 3.03 percent; and surface area, 142 m^2/g.
10. NCS, a strong organic base (quaternary ammonium salt dissolved in toluene) developed by Nuclear of Chicago, was used to absorb CO_2 for counting in liquid scintillation. Trade names are included as a matter of convenience to the reader, and such inclusion does not constitute any preferential endorsement of products named over similar products available on the market.
11. Counting solvent: 4 g of PPO (2,5-diphenyloxazole) and 50 mg of POPOP [*p*-bis(2-5-phenyloxazolyl)benzene] in 1 liter of toluene.
12. Gas chromatography: A 1.8-m pyrex glass column (outside diameter, 6 mm; inside diameter, 4 mm) was packed with equal portions of 10 percent Dow-Corning 200 silicone fluid [(12,500 centistokes) on 80/90 mesh] on Anakrom ABS and 15 percent QF-1 on Gas Chrom Q. Operating conditions of the chromatograph were: column temperature, 210°C; detector, 235°C; carrier gas (He) flow rate, 100 ml/min.
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Anemia phyllitidis: Inducibility of Physiological State Antagonistic to Antheridium Formation

Abstract. *It has been reported that a preparation obtained from prothalli of the fern species* Anemia phyllitidis *controls antheridium formation in this species. If this same preparation is applied at the spore stage it induces a physiological state antagonistic to antheridium formation. The inhibitory state is inducible only at a very early stage of development, but, once induced, it remains manifest at much later stages. Quite possibly, the inhibitory state arises in response to the factor that induces formation of antheridia. The inhibitory state may have an important function in fern development.*

Prothalli of *Pteridium aquilinum* elaborate a hormone that controls antheridium formation in many if not all species of the fern family Polypodiaceae (*1*, *2*). This hormone has been isolated and termed an antheridiogen (*3*). Native antheridiogens have also been demonstrated in the fern species *Blechnum gibbum*, *Lygodium japonicum*, *Anemia phyllitidis* (*4*), and *Anemia hirsuta*.

The antheridiogen of *P. aquilinum* does not affect *A. phyllitidis*, a species belonging to the fern family Schizaeaceae. The prothalli of this species elaborate a different antheridiogen (A_{An}, *4*). The phytohormone gibberellic acid (A_3) also induces formation of antheridia in *A. phyllitidis* (*5*, *6*). However, like A_{An} (*4*), A_3 has no effect on a species used to assay for the antheridiogen of *P. aquilinum* (*2*). Other gibberellins also cause antheridium formation in *A. phyllitidis*, some of them at an exceedingly low concentration (*7*, *8*). The antheridiogen A_{An} does not seem to be identical with any of the several tested gibberellins (*9*).

The sensitivity of fern prothalli to antheridiogen varies with the developmental stage (*1*–*6*). If *Anemia* prothalli are exposed to A_{An} at the 0-day stage (before, or immediately after, spore inoculation), then the interval between the appearance of antheridia at the highest concentration of the antheridiogen and that at the lowest concentration is 14 days. In contrast, the corresponding interval is approximately 1 day if A_{An} is supplied at the 11-day stage (*10*). If we take this interval as a basis for comparison, the sensitivity of 0-day-old prothalli to differences in antheridiogen concentration exceeds that of 11-day-old prothalli by a factor of about 14. The delayed appearance of antheridia in prothalli exposed at the 0-day stage might be interpreted to mean that the juvenile prothalli are refractory to the action of A_{An}. If this were true, prothalli exposed at the 0-day stage would be expected to form antheridia no later than the time at which they are formed by prothalli exposed at the 11-day stage. Instead, prothalli exposed to the lower concentrations of A_{An} at the 0-day stage formed antheridia much later than prothalli exposed at the 11-day stage did, and they also formed a much larger number of vegetative cells before they attained the antheridial phase (*10*) (Table 2). Clearly, an inhibitory state becomes operative in the juvenile prothallus, greatly delaying the appearance of antheridia but permitting the continuance of vegetative cell division. This inhibitory state may not be an obligatory characteristic of development but may be, instead, induced by the preparation containing A_{An}. On this assumption, inducibility of the inhibitory state decays before the prothallus attains the 11-day stage, but, once it is induced in the juvenile prothallus, the inhibitory state remains manifest beyond the stage at which it ceases to be inducible.

For all experiments, prothalli were cultured in 50-ml erlenmeyer flasks on 10 ml of an agar-solidified, inorganic medium. Conditions of culture and the method of spore sterilization have been described (*11*). The antheridiogen A_{An} was applied as the liquid medium harvested from 7-week-old cultures of *A. phyllitidis*. It induced formation of antheridia to a dilution of one part in a thousand in most spore samples; it is termed the active preparation below.

Prothalli were first grown for 11 days, some on nonsupplemented medium, others on medium supplemented with dilutions of the active preparation at full strength ranging from 1:100 to 1:3000. Eleven days after spore inoculation, prothalli grown on the nonsupplemented medium were transferred to the 11-day-old cultures of prothalli grown on supplemented medium. Prothalli on agar slivers about 60 mm^2 and 2 mm thick were transferred from the nonsupplemented cul-