Table 2. Effect of a single dose of tetrahydrohomopteroate on normal monkeys, measured as blood urea nitrogen (BUN) on the 8th and 12th days after injection.

Monkey	Dose (mg/kg)	BUN (mg/100 ml)	
		Day 8	Day 12
3600	80	66	35
3589	80	125	25
3627	40	146	52
3607	40	27	22
3356	20	38	22
3605	20	33	28

Eight days after the injection, monkeys 3600, 3589, and 3627 showed an elevated blood urea nitrogen (Table 2), which decreased to normal 4 days later. Monkey 3607 showed no ill effects, and in monkeys 3605 and 3356 only slight rise was detected. This effect of the drug appears to be completely reversible. After a single dose of the drug (40 mg/kg) was administered by stomach tube, detectable blood levels were not observed.

The evidence presented shows that tetrahydrohomopteroic acid is a new type of antifolate which is effective against parasites which are resistant to pyrimethamine, which is a 2, 4-diaminopyrimidine. Pyrimethamine is considered to exert its antimalarial effect by inhibiting dihydrofolate reductase (*4*). The new agent probably does not exert its major action here since it has been shown that dihydrohomopteroate serves as a substrate for mouse tumor dihydrofolate reductase (*5*). In the presence of reduced nicotinamide-adenine dinucleotide phosphate the reductase catalyzes the formation of tetrahydro- from dihydrohomopteroic acid at one half the rate observed for the reduction of dihydrofolate to tetrahydrofolate.

Sulfonamides are believed to have antimalarial activity because they inhibit the incorporation of *p*-aminobenzoic acid into dihydrofolic acid (*4*). Since tetrahydrohomopteroic acid has a *p*-aminobenzoic acid moiety it might act at the same site as the sulfonamides. The presence of the pteridine moiety in the new drug introduces the additional possibility that enzymes of dihydrofolate biosynthesis which involve pteridine but not *p*-aminobenzoic acid may be inhibited. Two examples of enzymes in this category are (i) the enzyme that catalyzes the pyrophosphorylation of 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine (*6*) and (ii) the enzyme that catalyzes the addition of glutamate to dihydropteroate (*7*).

Two other possibilities should also be considered. (i) Tetrahydrohomopteroic acid might inhibit an enzyme involved in the feedback control of folate biosynthesis; (ii) it might compete with tetrahydrofolate for coenzyme sites involved in the synthesis of essential metabolites (*8*). For example, it inhibits *Escherichia coli* thymidylate synthetase by 35 percent at $3.4 \times 10^{-5} M$ (*5*). The possibility that the drug inhibits folate metabolism at sites other than those attacked by pyrimethamine or sulfonamides suggests that combinations of these drugs should be tested for synergistic effects.

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Pesticide Transformation to Aniline and Azo Compounds in Soil

Abstract. *The herbicide 3′,4′-dichloropropionanilide decomposes in soil to carbon dioxide and 3,4-dichloroaniline, and two molecules of the latter compound are condensed to form 3,3′,4,4′-tetrachloroazobenzene. Soil microorganisms are involved in both transformations.*

The herbicide 3′,4′-dichloropropionanilide (DCPA) was transformed by soil microorganisms; it was suggested that the aliphatic side chain of the molecule was oxidized in part to carbon dioxide and that the aromatic moiety was liberated as a toxic residue that depressed soil respiration (*1*). To establish this possibility as fact and to enable the isolation and identification of metabolites, we treated 500 g of soil (Nixon sandy loam, *p*H 5.3) with 1.0 g of DCPA, moistened it to 60 percent of capacity, and incubated it at 28°C for 17 days. By the extraction and fractionation procedures summarized below, two decomposition products of the herbicide were concentrated, crystallized, and characterized chemically as 3,4-dichloroaniline (DCA) and 3,3′,4,4′-tetrachloroazobenzene (TCAB).

For isolation of DCA, the soil was extracted with 2 liters of acetone. The filtered extract was diluted with three volumes of water, adjusted to *p*H 11.0 with NaOH, saturated with NaCl, and partitioned with hexane. The hexane fraction was extracted with 1.0*N* HCl saturated with NaCl, and the acid extract was adjusted to *p*H 11.0 with NaOH and then partitioned with chloroform. The solvent was evaporated to dryness, and the residue was recrystallized twice from ligroin and established as identical with an authentic sample of DCA by comparison of their movements on thin-layer plates (Eastman Chromagram type K301R developed with benzene; R_F values: DCPA, 0.20, DCA, 0.67, and TCAB, 0.94), by retention times in a gas chromatograph (Aerograph 660, 1.5 meters long and 0.3 cm outside diameter stainless steel column, packed with 5 percent SE-30 on Chromosorb W; carrier: 60 ml N_2 per minute; flame ionization detector; retention at 155°C, DCPA, 5 minutes, and DCA, 1 minute; retention at 200°C, DCPA, 1 minute, and TCAB, 6 minutes), by infrared spectra, and by melting points (71°C).

For the isolation of TCAB, the hexane-soluble material extracted from soil was evaporated to dryness, and the residue was dissolved in benzene and applied to a silica-gel column. Elution with benzene yielded a fast-moving orange fraction which was purified by

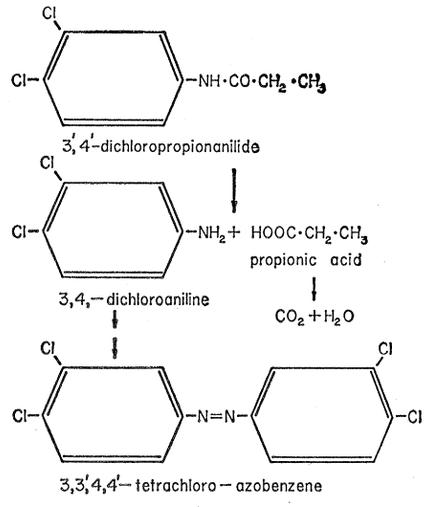

Fig. 1. Scheme of transformation of 3',4'-dichloropropionanilide in soil.

recrystallization as orange needles from absolute ethanol and from hexane. The mass spectrum indicated that the compound had a molecular weight of 322 and an empirical formula of $C_{12}H_6N_2Cl_4$. Authentic TCAB was synthesized by the use of $LiAlH_4$ for the reductive condensation of 3,4-dichloronitrobenzene (*2*). The identity of the synthetic TCAB and of the pesticide transformation product isolated from soil was established by comparing their movements on thin-layer plates, retention times in a gas chromatograph, infrared spectra, and melting points (157°C).

No DCA or TCAB could be isolated, from soil that had not received pesticide or from soil that was sterilized, treated with filter-sterilized herbicide solutions, and incubated at 28°C for 14 days before analysis. Moreover, tests were performed to exclude the possibility that DCA and TCAB were produced chemically during extraction or isolation procedures.

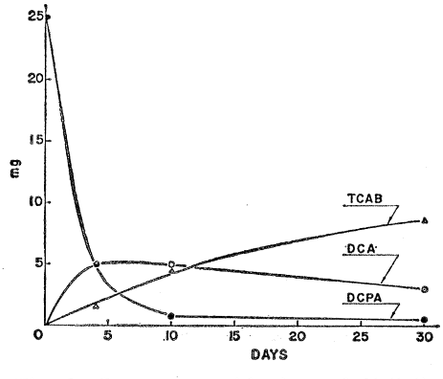

Fig. 2. Conversion with time of 3',4'-dichloropropionanilide (DCPA) to 3,4-dichloroaniline (DCA) and 3,3',4,4'-tetrachloroazobenzene (TCAB) in soil treated with 500 parts per million of the herbicide.

Apparently, in natural soil an acylamidase of microbial origin catalyzes the cleavage of the herbicide molecule DCPA into DCA and propionic acid. The latter compound is utilized as a source of carbon and energy by soil microorganisms and transformed to carbon dioxide, water, and cell substance. The condensation that produces TCAB from DCA may be a direct oxidative condensation of two molecules of DCA, or DCA may be first transformed in part to 3,4-dichloronitrosobenzene, after which a spontaneous condensation of one molecule of the aniline compound with one molecule of the nitroso compound may then occur. In this reaction the nitroso compound would be produced by the biological oxidation of the aniline compound, but coupling would be accomplished chemically rather than biochemically (Fig. 1).

The conversion of DCPA to DCA and ultimately to the azo compound during 30 days in the soil is illustrated in Fig. 2. Quantitative analysis of the pesticide and of its metabolites was performed by gas chromatography. The concentration of DCA reached a maximum within 10 days and then declined, but TCAB continued to accumulate for the duration of the experiment. In 30 days, 46 percent of the aromatic moiety of the added DCPA was recovered as the azo compound, but this value may be low because some TCAB may have been retained by the soil.

The TCAB was formed in soil that received DCPA or DCA only; this supports the conclusion that DCA is an intermediate in the transformation of the herbicide to TCAB. In view of the fact that anilines are products of the degradation of various phenylurea and phenylcarbamate herbicides (*3*), the possibility of condensation reactions and the formation of TCAB or other azo compounds in soil should be explored. The biological activity of TCAB has not been investigated; however, the fact that certain azo compounds are known to be carcinogenic (*4*) necessitates further study of the production and fate of these compounds during pesticide decomposition in soil.

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Azure Mutants: A Type of Host-Dependent Mutant of the Bacteriophage f2

Abstract. *A new type of host-dependent mutant,* azure *mutant, of bacteriophage f2 has been isolated. Growth of these mutants was restricted specifically by* amber *suppressor genes in the host bacteria. Restriction of the formation of infective centers by different bacterial suppressor genes was 98 percent, 90 percent, and 70 percent with Su-3, Su-1, and Su-2 genes, respectively. Restriction, like suppression, was the dominant phenotype. The block in growth of the mutants occurred in an early stage of the infection cycle. Once infection was established, however, an infected cell produced approximately the same number of progeny phage as a cell without the suppressor genes did. It is proposed that the* azure *codon is the same as the* amber *codon (uracil, adenine, guanine) and that restriction results from improper termination of protein chains of the phage RNA polymerase. Similar mutants may exist in other systems.*

Amber mutants have been isolated from various bacteriophages, such as T4 (*1–4*) and RNA phage f2 (*5*). They are defined as mutants that grow in bacteria that have certain suppressor genes (Su+) but not in bacteria lacking these genes (Su−). We report here the isolation and preliminary characterization of a set of mutants of f2, the growth of which, contrary to *amber* mutants, is restricted by *amber* suppressor genes in the bacteria. Edgar (*6*) isolated a similar set of mutants of T4 that grow in *Escherichia coli* B but not in *E. coli* K12 harboring a suppressor gene and named them *azure* mutants. However,