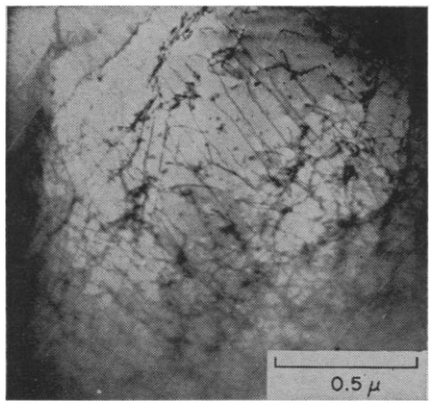

Fig. 1. α-Phase. The foil normal is (201).

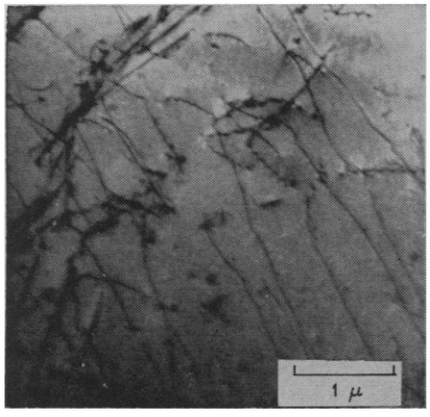

Fig. 2. α-Phase, showing long dislocation lines lying in {110} planes.

quently, the dislocations must be normal slip dislocations and not prismatic dislocations. Further evidence that the dislocations are very strongly pinned is that they did not move during thermal stressing in the electron beam, even though the sample was eventually heated to the melting point. Therefore the dislocations must have originated by multiplication during slip and not merely by, for example, point defect condensation. That plastic deformation must have occurred during the γ- to α-transformation is strongly indicated, and it is probable that such deformation preceded formation of the precipitate.

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2. We thank L. Foran for assistance in preparing the specimens and Fred Park for critical comments. Work supported by NASA.
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Conversion of p,p′-DDT to p,p′-DDD by Intestinal Flora of the Rat

Abstract. p,p′-*DDD* [*1,1-dichloro-2,2-bis*(p-*chlorophenyl*)-*ethane*] *occurs in the feces and livers of rats that are given* p,p-*DDT* [*1,1,1-trichloro-2,2-bis-*(p-*chlorophenyl*)-*ethane*] *by stomach tube, but not of rats injected intraperitoneally with* p,p′-*DDT. Coliform bacteria, isolated from feces of control animals, can effect reductive dechlorination of* p,p′-*DDT to* p,p′-*DDD. These findings indicate that the normal flora of the gastrointestinal tract, rather than the liver, as others have suggested, is the major source of the* p,p′-*DDD that is found in animals fed* p,p′-*DDT.*

When *p,p′*-DDT [1,1,1-trichloro-2,2-bis(*p-chlorophenyl*)-*ethane*] is administered to rabbits (*1*) or rats (*2*), either by stomach tube or in the diet, both *p,p′*-DDD [1,1-dichloro-2,2-bis (*p*-chlorophenyl)-ethane] and *p,p′*-DDT are found in the liver. Although it has been suggested that *p,p′*-DDT is converted to *p,p′*-DDD in the liver, there is no concrete evidence for the liver being the site of conversion. We have obtained evidence that the reductive dechlorination of *p,p′*-DDT to *p,p′*-DDD takes place in the gastrointestinal tract of rats and results from bacterial action.

Twenty adult male and female Osborne-Mendel rats, raised to maturity on a semisynthetic diet low in chlorinated pesticide residues, weighed from 182 to 354 g. Control (eight animals) and test rats (two groups, each of six) were matched according to sex and weight. *p,p′*-DDT (dissolved in corn oil, 2 mg/ml) was administered to the test animals at the rate of 4 mg/kg, either by stomach tube or by intraperitoneal injection. Feces were collected for 48 hours; the animals were then killed and the livers removed. Feces and livers were extracted by grinding with sand and ethyl ether in a mortar with pestle; the final volume of the ether extracts was 50 ml.

Cultures of *Escherichia coli* and *Aerobacter aerogenes*, isolated from control-rat feces, were maintained on 3-percent trypticase soy agar slants (*3*). The cultures were used to inoculate flasks containing 250 ml of sterile 3-percent trypticase soy broth to which 200 μg of *p,p′*-DDT in 0.1 ml of acetone was added. After 24-hour incubation at 37°C, the cultures were extracted twice with equal volumes of ethyl ether by shaking for 2 minutes in a separatory funnel; the combined extracts were reduced to 50 ml on a steam bath.

Portions of the ether extracts of livers, feces, and cultures were cleaned up by evaporating them to dryness on a steam bath, dissolving the residues in petroleum ether, extracting with acetonitrile and reextracting with petroleum ether, and chromatographing the extracts on Florisil (*4*). The purified extracts were then analyzed by gas-liquid chromatography by the method of Klein, Watts, and Damico (*4, 5*).

Representative gas chromatograms of extracts of feces are shown in Fig. 1. The major chlorinated-pesticide residue found in the feces of rats receiving *p,p′*-DDT by stomach tube was *p,p′*-

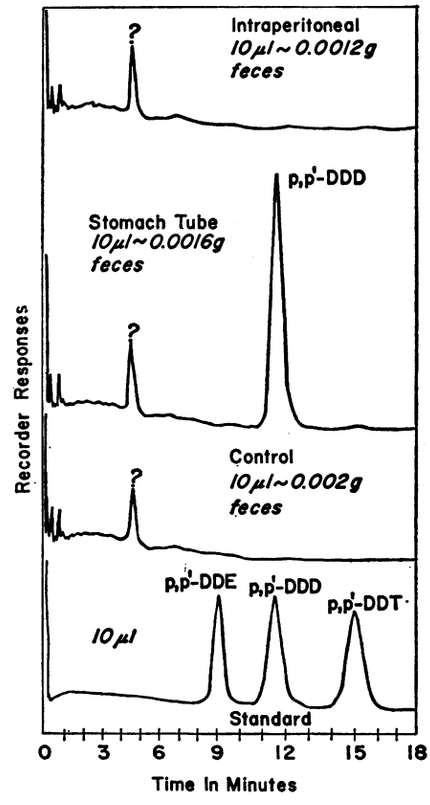

Fig. 1. Representative gas chromatograms of extracts of feces from a control rat and from rats given *p,p′*-DDT by stomach tube or by intraperitoneal injection. The standard solution contains 0.20 μg of *p,p′*-DDT, 0.20 μg of *p,p′*-DDD, and 0.10 μg of *p,p′*-DDE per milliliter of isooctane.

Table 1. Conversion of *p,p'*-DDT to *p,p'*-DDD by coliform bacteria in broth culture; data from gas chromatography. Residues, after 24-hour incubation, from initial contents of 200 μg of *p,p'*-DDT.

Organism	Residue (μg)	
	p,p'-DDD	*p,p'*-DDT
E. coli	35.9	69.5
A. aerogenes	33.0	26.7
None	0	191.6

DDD, with at most a trace of *p,p'*-DDT. Essentially no chlorinated pesticides were found in the feces of the rats injected intraperitoneally; in fact the chromatograms did not differ significantly from those of extracts of the control feces.

Typical gas chromatograms of liver extracts are seen in Fig. 2. Analyses of livers from the animals to which *p,p'*-DDT was administered by stomach tube showed the presence of both *p,p'*-DDD and *p,p'*-DDT, with the ratio DDD : DDT varying from 1 : 3 to 1. On the other hand, livers from rats that received the *p,p'*-DDT intraperitoneally contained principally *p,p'*-DDT, the ratio DDD : DDT ranging from 1:24 to 1:60.

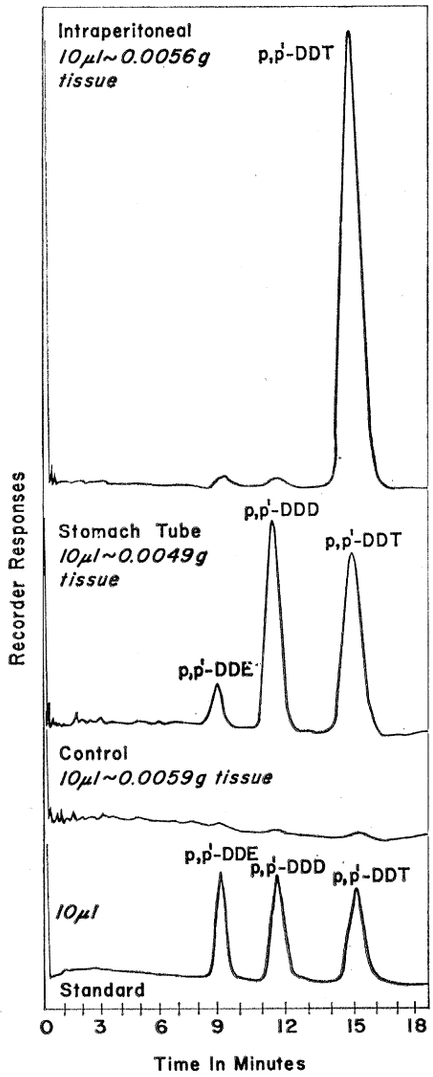

Fig. 2. Representative gas chromatograms of extracts of livers from the same animals and with the same standard as for Fig. 1.

The results of the analyses of feces and liver suggest that, if appreciable *p,p'*-DDD is to be found in the livers of animals administered *p,p'*-DDT, the *p,p'*-DDT must pass through the gastrointestinal tract. Because it has been demonstrated that one microorganism, yeast, can reductively dechlorinate *p,p'*-DDT to *p,p'*-DDD (*6*), and because the growth of Gram-negative rods in general is stimulated by *p,p'*-DDT (*7*), it seemed possible that coliforms—Gram-negative bacteria of the gastrointestinal tract—could be responsible for the conversion of *p,p'*-DDT to *p,p'*-DDD.

Representative studies show that the coliforms *A. aerogenes* and *E. coli* can effect the conversion (Table 1). In the bacterial cultures, *p,p'*-DDD constituted a significant fraction of the chlorinated pesticides recovered, with the DDD : DDT ratio ranging from about 1 to 1:2. Of the *p,p'*-DDT added to the cultures no more than 30 to 55 percent could be accounted for either as unchanged *p,p'*-DDT or as metabolite. However, incubation of uninoculated culture medium with *p,p'*-DDT resulted in neither appreciable loss of pesticide nor formation of *p,p'*-DDD. The loss of pesticide in the growing cultures may well represent the formation of other metabolites of *p,p'*-DDT that were not detected by the analytical methods employed.

Our results indicate that the normal flora of the gastrointestinal tract must be considered as the major agent for formation of *p,p'*-DDD in intact rats fed *p,p'*-DDT; they also indicate that the conversion takes place during the life of the animal, which fact does not support the suggestion of Barker *et al.* (*8*) that the DDD is a postmortem artifact caused by tissue decomposition and by invasion by adventitious microorganisms.

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8 November 1965

Autoradiography with Tritiated Methotrexate and the Cellular Distribution of Folate Reductase

Abstract. *Bound methotrexate has been revealed by an autoradiographic procedure, presumed to introduce a method for cytochemical study of folate reductase. Preferential localization is seen in kidney proximal tubules, intestinal epithelium, and nuclei of parenchymal liver cells in mice. The extremely firm binding and prolonged retention of this drug should render it suitable as an inert label for the autoradiographic study of cell migrations and lifetimes.*

The enzyme folate reductase (tetrahydrofolate dehydrogenase, *1*) catalyzes the conversion of folate and dihydrofolate to tetrahydrofolate. Several forms of this cofactor are required for many reactions that involve the transfer of one-carbon groups, and regeneration of tetrahydrofolate is essential for the synthesis of thymidylate (*2*). No cytochemical reaction has so far been devised for the demonstration of this important enzyme, nor, indeed, of any enzyme of this type. However, the intracellular sites of binding of suitable enzyme inhibitors can be revealed (*3*) by the use of an isotopic label (usually ^3H) in the inhibitor and the methods of tissue autoradiography. Using an irreversible inhibitor and strategies designed to confine it to known types of enzyme, Barnard and Ostrowski and their colleagues have shown that this approach can yield qualitative and quantitative information on the cellular distribution of cholinesterase and nonspecific esterases in various tissues (*3*). In the