piece did not decline further. The rapid appearance of a peak of ^{14}C at some distance from the apical end, coupled with the decrease of radioactivity in the first 2 mm, is a polar movement of IAA and indicates that within 15 minutes after removal of the donor the bulk of ^{14}C-IAA is being transported. During the first hour, ^{14}C moved down the aerobic section (Fig. 1, parts D and E) and began to appear in the basal receiving block. As the peak of ^{14}C moved down the aerobic coleoptile, it became flatter and more diffuse (for example, Fig. 1, parts A, D, and E). Nevertheless the position of maximum radioactivity can be estimated with reasonable accuracy for at least 1 hour, and an average velocity of the bulk of the auxin molecules can be obtained. The location of the peak shifted from the vicinity of 4 mm (Fig. 1, part A) to approximately 10 mm (Fig. 1, part D) in the first ½ interval and to about 19 mm (Fig. 1, part E) in the next; therefore, it moved at an average rate of about 15 mm/hr.

Routinely, the velocity of auxin transport in various tissues has been determined by the method introduced by van der Weij (*2*). Although the present method lacks precision, it does have the advantage over van der Weij's procedure that the time required for uptake from the donor and exit to the receiver is not lumped with the time of movement through the section. The present average rate of 15 mm/hr agrees with the velocity of IAA movements through coleoptiles of corn as determined by the classical method (*6*), and this supports the suggestion of McCready (*7*) that loading and unloading are not rate-limiting in the method of van der Weij.

Although it is usually assumed that all the IAA moves with the same velocity, as if, for example, it were being carried in a stream (*2, 8*), the rapidly changing shape of the peak indicates that individual auxin molecules move at different rates. This could be the result of (i) auxin molecules moving largely independently of each other, (ii) several independent streams moving at different velocities, or (iii) a decreasing capacity of transport toward the base of the section, so that auxin molecules depart from the stream and either diffuse in the tissue or equilibrate with the immobile phase. In view of the experimental results, it seems appropriate to reevaluate the confining concept of a single constant velocity of auxin transport.

The movement of the pulse of radioactivity down the section was sharply inhibited by anaerobic conditions. In coleoptiles that were rapidly equilibrated with an anaerobic atmosphere as soon as the ^{14}C-IAA had been transported as far as shown in Fig. 1, part A, the peak shifted less than 2 mm during the following hour (Fig. 1, part C). In this particular experiment, no significant change in the distribution of ^{14}C was detected during the first half hour in nitrogen (Fig. 1, part B), but with cuts at only 2-mm intervals a movement of as much as 1 mm could have been just below the level of detection. Although the inhibition appeared nearly immediately in this particular experiment, the time of onset of inhibition varied somewhat in different experiments. This variation may reflect differences in the rate at which the sections equilibrated with the anaerobic atmosphere. The inhibition established under anaerobic conditions appears to be completely reversible. If after 1 hour under nitrogen, coleoptiles were rapidly reequilibrated with air by evacuation and release to air, the amount of movement in them was not significantly different from control aerobic sections that had never been inhibited (compare parts E and F, Fig. 1).

Obviously anaerobic conditions, at least for 1 hour, had no permanent adverse effect on the transport. Furthermore, since the amount of movement was the same after an hour's inhibition as in control sections, transport must have either (i) resumed within 10 minutes of return to air, or (ii) if it resumed somewhat later it must have been at a greater than normal rate. The second alternative is suggested by the report that the growth rate of sections is stimulated following an anaerobic period (*9*).

Naqvi *et al.* reported that anaerobic conditions inhibit the overall transport in corn coleoptiles by virtue of inhibiting uptake alone (*3*). On the other hand, the present results show that the effect of anaerobic conditions is not limited to uptake; the rate of movement down the section can be reversibly inhibited by an anaerobic atmosphere. The explanation for the discrepancy between these results and those of Naqvi *et al.* is that the present procedures must render the tissues more nearly anaerobic.

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Dechlorination of DDT in Frozen Blood

Abstract. *Dechlorination of a commercial mixture of* o,p′-*DDT and* p,p′-*DDT to* p,p′-*DDD and* p,p′-*DDE occurs in avian blood stored at −20°C, indicating nonenzymatic degradation. The subsequent disappearance of these two metabolites suggests conversion to other metabolites which cannot be detected by gas chromatography with an electron-capture detector.*

During the routine analysis of DDT (*1*) in avian blood samples stored at −20°C, one sample was accidentally repeated at an interval of 3 weeks after the initial analysis. We were surprised to observe that the quantities (in parts per million) of DDT and the metabolites DDD and DDE were different from those of the first analysis, the amount of the metabolites being higher and that of DDT being much lower. Because this presented a possible source of error in the accurate analysis of insecticide residues, we investigated this apparent degradation of DDT in frozen (−20°C) whole blood.

DDT can be dechlorinated under anaerobic conditions by yeast (*2*), by the bacteria *Proteus vulgaris* (*3*), *Serratia marcescens* and *Escherichia coli* (*4, 5*), and *Aerobacter aerogenes* (*5, 6*), DDD being the major product with a small amount of DDE being formed. Castro (*7*) and Miskus *et al.* (*8*) observed a conversion of DDT to DDD by dilute solutions of Fe (II) porphyrin complexes, and Wedemeyer (*6*) observed a similar conversion by reduced

Fe (II) cytochrome oxidase from *Aerobacter aerogenes*. Fleck and Haller (*9*) reported the dehydrochlorination of DDT by traces of iron, chromium, and aluminum salts.

The ability of whole blood to degrade DDT was tested in the following manner. A sample of heparinized chicken blood (25 ml), sufficient for an analysis lasting 12 weeks, was placed in a clean bottle, and a small volume of an acetone solution of commercial DDT (77.2 percent *p,p'*-isomer and 22.8 percent *o,p'*-isomer) (*10*) was added to produce a final concentration of 1.0 ppm total DDT per milliliter of blood. For comparison, heparinized chicken plasma devoid of hemolysis was treated in the same manner. The bottles were sealed tightly and frozen at −20°C. Each week, the samples were quickly thawed, 2.0 ml was removed for analysis, and the samples were again sealed and frozen.

The samples of blood and serum were homogenized in a Waring Blendor with a mixture of 45 ml of acetonitrile, 15 ml of acetone, and 5 g of anhydrous sodium sulfate. The homogenate was filtered through a fritted glass funnel under suction, and the residue was washed with two 10-ml portions of acetonitrile. The combined filtrate was adjusted with enough distilled water to produce a ratio of acetonitrile to water of 2.5 : 1 and was passed through a column of polyethylene-coated alumina (*10*). This column was subsequently washed twice with 35 ml of a mixture of acetonitrile and water (2.5 : 1) and finally with 25 ml of acetonitrile (*11*). The combined eluates were extracted twice with 45 ml of *n*-heptane; the upper (heptane) layers were pooled, filtered under suction through anhydrous sodium sulfate, and concentrated by flash evaporation to near dryness; the concentrate was then taken up in a suitable volume of acetone.

An Aerograph gas chromatograph model 600-D (*10*) with an electron-capture detector was used to detect the DDT and the degradation products. For routine determinations, a pyrex glass column (1.2 m by 0.32 cm) containing 4 percent SE-30 and 6 percent QF-1 on acid-washed Chromasorb W (60/80 mesh) was used (*12*). The column and detector temperatures were 180°C; the injector temperature was 200°C. The flow rate of purified nitrogen was 80 to 85 ml per minute. The results were calculated and presented in parts per million.

Controlled experiments on plasma and whole blood of adult roosters were performed to quantitate the recovery of insecticide by the clean-up procedure. Known amounts of DDT and metabolites were added to the samples before homogenizing. The extraction procedure was carried out, and the final extracts were quantitatively analyzed by gas-liquid chromatography. The percentage of average recovery from 14 experiments with plasma was: DDE, 100.3; DDD, 82.3; *o,p'*-DDT, 94.1; *p,p'*-DDT, 94.3 (*10*). The percentage of average recovery from six experiments with whole blood was: DDE, 99.2; DDD, 76.9; *o,p'*-DDT, 92.3; *p,p'*-DDT, 89.0.

Figure 1 shows the disappearance of the 1.54 ppm *p,p'*-DDT and 0.45 ppm *o,p'*-DDT initially present in each 2.0 ml of whole blood over a period of 12 weeks and the simultaneous appearance of the metabolites DDE and DDD, the former being present in the highest concentration. After the 7th or 8th week, there was a slow decline in the concentration of the metabolites. At the same time, the plasma sample that contained the commerical DDT mixture showed no evidence of degradation, DDE and DDD being absent from the extracts.

The total insecticide plus metabolites recovered did not add up to 100 percent of the concentration at week zero. Figure 2 shows the results for the whole blood calculated as percentage of the insecticide added and plotted against time in weeks. In addition, the line designated "unknown" shows the percentage of degradative products which were not detected by our instrumentation.

In view of the fact that the data were adjusted for the percentage of recovery of each compound, this observation suggests possible formation of metabolites that could not be analyzed by an electron-capture detector because they had lost the ability to adsorb electrons. This may well be the case because, after the 8th week, the concentrations of DDE and DDD declined

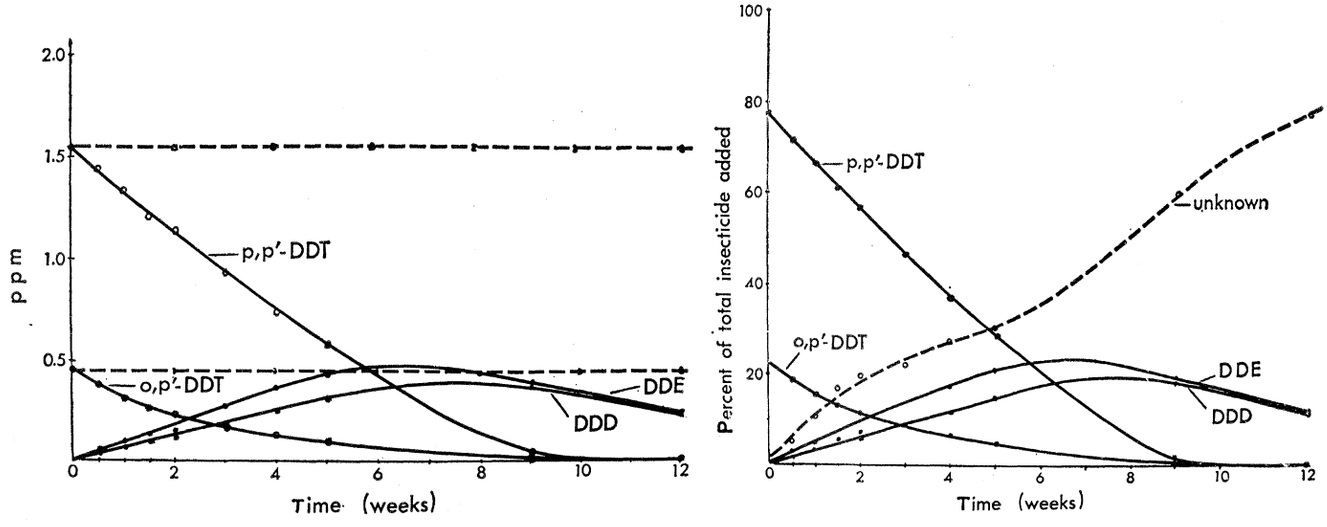

Fig. 1 (left). Degradation of *o,p'*-DDT and *p,p'*-DDT after the addition of 2.0 ppm of a commercial mixture to avian blood (——) and plasma (----), and subsequent storage at −20°C over a prolonged period of time. Fig. 2 (right). Progressive degradation of *o,p'*-DDT and *p,p'*-DDT expressed as a percentage of the total DDT added to avian blood, showing the formation of compound (or compounds) not measurable by an electron-capture detector.

slowly, indicating that degradation of DDT was proceeding beyond these two metabolites. Others have suggested a number of other degradative products, most of which do not have chlorine atoms on the ethane or ethene portion of the molecule and which are considerably more polar (*13*, *14*). The loss of chlorine, if accompanied by an increased polarity, may account for a loss in electron sensitivity. The analysis by gas chromatography of a sample of pure DDA synthesized in our laboratory failed to detect a peak even at high concentrations, indicating that this carboxylic acid is electron-insensitive.

The possible role of reduced porphyrins in the degradation of DDT (*6*, *8*) is of interest because, with repeated freezing and thawing, the erythrocytes would be hemolyzed, exposing the insecticide to a high concentration of free hemoglobin. Experiments in which we added purified hemoglobin to chicken serum showed that degradation proceeded at approximately the same rate, the results being quite similar to those shown in Fig. 1. We have also mixed DDT with serum samples showing a small amount of hemolysis and have observed an initial decline in the concentrations of *o,p'*-DDT and *p,p'*-DDT for approximately 2 weeks, after which the plotted line was parallel to the base line for the remainder of the experiment.

Considering the above results and those reported for degradation by microorganisms (*2–6*), we question the concept of enzymatic dechlorination of DDT. Possibly, tissues and microorganisms which contain large quantities of reduced coenzymes, porphyrins, and other metalloproteins could carry out these degradative steps by a simple chemical redox reaction.

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References and Notes

1. 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; *o,p'*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane.
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Plasma Membranes of Rat Liver: Isolation of Lipoprotein Macromolecules

Abstract. *Three high-density lipoprotein classes and one protein were separated from rat-liver plasma membranes that had been treated with mild sonic oscillation. The lipoproteins were separated and identified by techniques in which ultracentrifugation was used. Enzyme markers and electron-microscopic examination revealed membrane preparations essentially free of contaminating cellular particulates.*

Electron micrographs of the unit membrane structure of various cells led several investigators to postulate that these membranes were constructed of layers of lipids and proteins (*1*). Chemical analyses of membranes from atypical cells, such as erythrocytes (*2*) and Schwann cells (*3*), suggested that they consist of lipids and proteins, which exist as bimolecular leaflets. A new structure for membranes in general, consisting of base pieces with lipoproteins attached, was proposed by Green and Perdue (*4*).

Lipoprotein macromolecules of plasma membranes from rat liver can be isolated and separated by the classical procedures for flotation of lipoproteins by increasing density gradients. Their presence can be verified in the optical ultracentrifuge (*5*), as well as by the chemical analytical procedures that give their lipid (*6*, *7*) and protein components (*8*).

Plasma membranes were isolated from rat liver by a modification of the procedure of Neville (*9*), in which an additional sucrose density (*d*) gradient [$d = 1.18$ g/ml (*7*)] that efficiently separates contaminating mitochondria from the plasma membranes was used.

The bulk of each sample consisted of membranes of various lengths and configurations (Fig. 1). A considerable portion of this membranous material consisted of pairs of elongated membranes that possessed, at intervals, small, dense regions resembling the desmosomes of liver parenchymal cells. The fractions also contained an occasional swollen mitochondrion with fragmented cristae, and a small amount of amorphous material and small vesicles of unknown origin. Microsomes and free ribosomes were virtually absent.

Several enzyme markers were determined on fresh membrane preparations. To show enrichment of the membrane preparations, we determined the activity of the adenosine triphosphatase dependent upon Na^+, K^+, and Mg^{2+} (*10*). We measured the activities of three mitochondrial enzymes [cytochrome *c* oxidase (*11*), monoamine oxidase (*12*), and malate dehydrogenase (*13*)] to determine the possible presence of mitochondria and the different mitochondrial membranes. We measured the activity of glucose-6-phosphatase (*14*) to determine the presence of rough endoplasmic reticulum.

Isolated membranes from ten preparations (200 g of fresh rat liver) were washed with 0.001*M* sodium bicarbonate buffer (*p*H 7.5). Membranes were resuspended in 0.02*M* phosphate buffer (*p*H 7.5, ionic strength 0.05) and treated with sonic oscillation for 90 seconds (M.S.E. ultrasonic disintegrator, 60 watt, 20 kc/sec). The cloudy suspension of membranes became a clear opalescent solution from which only small amounts of debris could be removed by centrifugation at 10,000*g* (12,500 rev/min) in the Spinco No. 40 rotor for 30 minutes. The resulting supernatant fluid will be referred to as the "sonicated" supernatant (SS).

The lipoproteins in the SS were separated into three classes based upon their flotation in KBr solutions of different densities. Suitable samples were reserved for extraction of total lipid with a mixture of one part methanol and two parts chloroform. The densities of all solutions were measured at 25°C with a micropycnometer. Preparative separations were done in the Spinco Model L ultracentrifuge, No. 50 rotor at 50,000 rev/min for 19 hours, 45 minutes. The top 2.5 to 3.0 ml of floating material was removed and analyzed