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THE METABOLISM *IN VIVO* OF 1,1,1-TRICHLORO-2,2-*BIS*(*p*-CHLOROPHENYL)ETHANE (DDT), 1,1-DICHLORO-2,2-*BIS*(*p*-CHLOROPHENYL)ETHANE (DDD) AND 1,1-DICHLORO-2,2-*BIS*(*p*-CHLOROPHENYL)ETHYLENE (DDE) IN THE CHICK BY EMBRYONIC INJECTION AND DIETARY INGESTION

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Abstract—1,1,1-Trichloro-2,2-*bis*(*p*-chlorophenyl)ethane, (*p,p'*-DDT); 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane, (*p,p'*-DDD); and 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)-ethylene, (DDE) were separately injected in peanut oil into fertile eggs prior to incubation to study their metabolism and effect on the embryo and chicks. No teratogenic effect was detected either in chicks or in dead embryos. Typical DDT poisoning neurological symptoms were observed. In other experiments, 100 ppm of *p,p'*-DDT, *p,p'*-DDD and DDE in the diet were fed to chicks hatched from untreated eggs. Tissues of chicks in both the injection and feeding experiments were analyzed for metabolites by electron capture gas-liquid and thin-layer chromatography (TLC). Infrared spectroscopy was used in connection with TLC for confirmation of the identity of some polar metabolites. No significant differences in the pattern of metabolites between the two treatments were observed. The following metabolites were detected from *p,p'*-DDT: 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane, (*o,p'*-DDT); DDE; *p,p'*-DDD; 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1-dichloroethane, (*o,p'*-DDD); 1-chloro-2,2-*bis*-(*p*-chlorophenyl)ethylene, (DDMU); 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethane, (DDMS); unsym-bis(*p*-chlorophenyl)ethylene, (DDNU); 2,2-*bis*(*p*-chlorophenyl)-ethanol, (DDOH); *bis*(*p*-chlorophenyl)acetic acid, (DDA); *bis*(*p*-chlorophenyl)-methane, (DDM) and 4,4'-dichlorobenzophenone, (DBP). The following metabolites were detected from *p,p'*-DDD: *o,p*-DDD; DDMU; DDMS; DDNU; DDOH; DDA; DDM and DBP. DBP was detected as a metabolite of DDE.

THE WIDESPREAD use of DDT‡ as an agricultural pesticide has prompted the need for evaluation of the hazards of such material to man and wildlife. Significant amounts of

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‡ Abbreviations used in this paper are: 1,1,1-trichloro-2,2-*bis*(*p*-chlorophenyl)ethane, (*p,p'*-DDT); 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane, (*p,p'*-DDD); 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene, (DDE); 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1,1-trichloroethane,(*o,p'*-DDT); 2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-1,1-dichloroethane, (*o,p'*-DDD); 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethylene, (DDMU); 1-chloro-2,2-*bis*(*p*-chlorophenyl)ethane, (DDMS); unsym-*bis*(*p*-chlorophenyl)ethylene, (DDNU); 2,2-*bis*(*p*-chlorophenyl)-ethanol, (DDOH); *bis*(*p*-chlorophenyl)acetic acid, (DDA); *bis*(*p*-chlorophenyl)-methane, (DDM); and 4,4'dichlorobenzophenone, (DBP); *bis*(*p*-chlorophenyl) methanol (DBH); 1,1-*bis*(*p*-chlorophenyl) 2,2,2-trichloroethanol (k elthane).

DDT are retained as residues in fish and in various agricultural products and are ultimately consumed by man and birds. DDT and its nonpolar breakdown products tend to be stored more extensively in adipose tissue than in other tissues.[1] The rather high levels of DDT in the adrenal[2] and in the ovary[3] may reflect the high fat content of these organs. In a recent report from this laboratory, it has been shown that DDT, DDD and DDMU are present in marine birds.[4] The chick embryo was selected for more detailed study on the effect of DDT on birds by injection into the yolk sac of fertile eggs prior to incubation and subsequent observation of the embryonic development of the chick. The chick embryo is a unique system, since drugs, even when introduced early in development, do not produce their effects until a susceptible embryonic system appears. Furthermore, this method permitted the observation of possible teratogenic effects, since the injected chemicals were in direct contact with the embryo throughout the development.

The toxicity of various chemicals on the chick embryo has been widely studied.[5-8] The acute and subacute toxicity of certain pesticides, primarily the chlorinated hydrocarbons, have been investigated in several species of game birds.[9-14] DDT at dietary levels up to 100 ppm has been reported to have no effect on Japanese Quail.[15]

Despite the widespread use of DDT, knowledge of its metabolism in birds is incomplete and is based almost entirely on experiments carried out on mammals *in vivo*. The presence of DDD has been reported[16] in many environments where only DDT had been used. The conversion of DDT to DDD in various biological systems (yeast[17] and bacteria[18]) has been reported. DDD was found in liver, but not in kidneys of rats fed DDT, while no DDD was detected from feeding DDE.[19] DDD was found in fish and crayfish taken from a pond treated with DDT.[20] DDD, DDE and *o,p*-DDT were found in anchovy (*Engraulis mordax*) obtained from San Francisco Bay.[21] Rat liver microsomal enzymes *in vitro* catalyze the conversion of DDT to DDD, which also requires oxygen and NADPH.[22] DDT was converted to DDD in the presence of ferrous deuteroporphyrin in an anhydrous and anaerobic solution.[23] It was demonstrated that porphyrin under proper conditions can convert DDT to DDD.[24] A metabolic pathway for DDT, which was based on feeding the pesticide and its postulated metabolites to rats, has been suggested.[25] Minor amounts of DDMU and a material having a chromatographic and partition coefficient characteristic of DDA were found in blood, urine and feces of rabbits that inhaled ^{14}C-DDD.[26] DDD was found in the liver of rats fed with DDT; also *p,p'*-DDT and *p,p'*-DDD were found in livers of rats fed with *o,p*-DDT.[27] *p,p'*-DDA was isolated as a crystalline product from both urine and feces of rats given *p,p'*-DDT.[28] A conjugated form of DDA containing equimolar amounts of aspartic acid and serine was also isolated, but the neutral metabolities such as DDM, DBP, DBH and DDE were not positively identified. The conversion *in vitro* of DDT to DDD and DDE by incubation both of homogenates and slices of pigeon liver was reported.[29] A considerable conversion of DDT to DDD was found under anaerobic conditions, while a very small amount of DDE, similar in magnitude to that found under aerobic conditions, was also reported. Neither DDA nor any other nonpolar metabolite was found. It has been suggested that DDD production may be a nonenzymatic reaction, since it occurred under nitrogen at 75°, similar to that of DDT with reduced prophyrins recently described. In this report is described the metabolism *in vivo* of DDT, DDD and DDE in chicks hatched from

eggs which had been injected with these chemicals separately and in chicks which had been fed a diet containing DDT, DDD and DDE.

MATERIALS AND METHODS

p,p'-*DDT and related compounds.* The following compounds were used as described before:[30] *p,p'*-DDT; *o,p'*-DDT, DDE; *p,p'*-DDD; *o,p'*-DDD; DDMU; DDNU; DDM; DBP; DDOH, Kelthane; DBH; and DDA. The purity of all compounds was checked by gas-liquid chromatography with an electron capture detector.

Injection of eggs. Leghorn fertile eggs were obtained from the Department of Poultry Husbandry, University of California, Berkeley. Eggs to be injected were first candled in order to discard those that were defective and to outline with a pencil the exact location of the air cell. The hatch of control eggs averaged 95 per cent. All eggs weighed from 52 to 60 g. All pure chemicals were dissolved in peanut oil in the desired concentrations and 0·1 ml was injected per egg. The large end of the egg was wiped with a sterile gauze pad moistened with 70% ethanol and a hole was drilled in the shell in the center of the surface over the air cell. A 1-in. long disposable No. 22 gauge needle was inserted vertically through the air cell into the yolk. The needle was wiped with a sterile gauze pad between each injection. As soon as the egg had been injected, the hole in the shell was sealed with nail polish. In order to determine the rate of diffusion, uptake and tissue distribution of DDT in injected embryos, 30 mμc of uniformly ring-labeled ^{14}C-DDT, 0·1 mc/m-mole (New England Nuclear Corp.) was injected with 0·1 mg carrier DDT. Radioactivity was determined in aliquots by liquid scintillation counting.

Incubation and hatching. The injected eggs were placed in the incubator, large end up. The incubator (model 50, Humidaire Incubator Co., New Madison, Ohio) automatically rotated the eggs every 2 hr and was maintained at an optimum temperature of 38° and a relative humidity of 60 per cent. The eggs were candled on the fifth day of incubation and every day thereafter. Clear eggs and dead embryos were removed for examination. The eggs were allowed to hatch in the same incubator.

Dietary ingestion. Twenty-five chicks, 1 day old and hatched from untreated eggs, were fed *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE. Chick starter mash containing 100 ppm of each hydrocarbon separately was supplied *ad libitum* to the chicks. The chemicals were distributed in the mash by the evaporation of an acetone solution containing appropriate concentrations. After 5 days, the chicks were sacrificed and the tissues analyzed as below.

Extraction and cleaning up. Immediately after dissection, the tissues were cut into small pieces and placed in vacuum bottles and were frozen with a dry-ice acetone slurry, then lyophilized. After 24–48 hr, the tissues were thoroughly ground with a mortar and pestle to produce a dry free-flowing powder. These dry samples were extracted with 10% ethyl ether in petroleum ether (v/v) in a Soxhlet extractor for 24 hr, and with chloroform–methanol (2:1, v/v) for another 24 hr. These treatments were sufficient to extract all DDT and its breakdown products. The solvents were evaporated under vacuum and the residues were dissolved in petroleum ether for clean-up. The sulfuric acid–celite column described by Stanley and LeFavoure[31] was found adequate. A preliminary experiment indicated that this procedure was suitable for all of the 14 DDT-type compounds suspected and for efficiently removed interfering impurities. Extracts were concentrated to 5–10 ml under vacuum.

Analytical methods. The concentrated extracts were chromatographed by direct injection into a gas chromatograph or applied directly to thin-layer chromatography. Electron capture gas chromatography was employed utilizing three columns: 5% (w/w) QF-1, 5% (w/w) SE-30 plus 5% (w/w) QF-1, and 10% (w/w) DC-200; the nitrogen flow rates were 70, 100 and 120 ml/min respectively. The column temperature was 195° and the detector temperature was 200° for the three columns. The polar column, 5% QF-1 (Fig. 1), could not resolve the following pairs: DDM–DDNU,

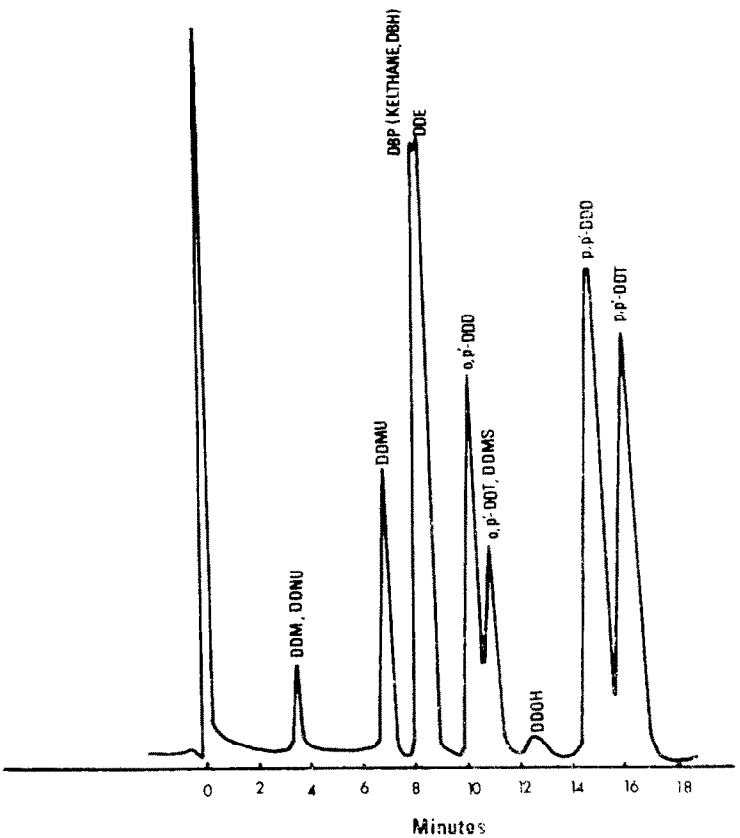

FIG. 1. Electron capture gas chromatography on 5% QF-1 column; standards.

DDE–DBP and *o,p'*-DDT–DDMS. The nonpolar column, 10% DC-200 (Fig. 2), could not resolve DDE and *o,p'*-DDD. The mixed column, 5% SE-30 plus 5% QF-1 (Fig. 3) could not resolve DDE and DDMS. For analysis of polar metabolites, two-dimensional TLC on silica gel G was used. The solvent systems were 10% ethyl ether in *n*-hexane and 50% chloroform:methanol (2:1) in *n*-hexane. This technique has been described in detail.[30] For more confirmation of the identity of Kelthane, DDA and DDOH, infrared spectroscopy by using potassium bromide pellets was used. Identification by i.r. spectra was accomplished by two-dimensional chromatography followed by elution and spectroscopic examination. Samples were applied to two plates. Both were developed and one was sprayed with

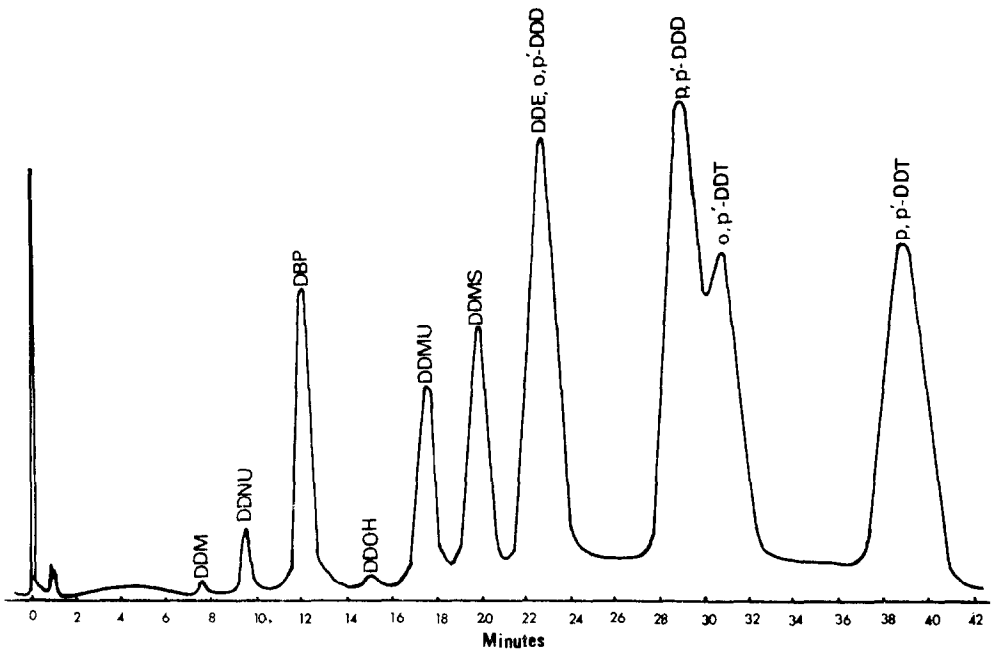

FIG. 2. Electron capture gas chromatography on 10% DC-200 column; standards.

2% *o*-toluidine in acetone and exposed to u.v. light to reveal the location of the metabolite. The corresponding unsprayed plate was marked and the metabolite was isolated by removing the silica gel with a razor blade. The metabolite was eulted from the silica gel with acetone which was evaporated onto a few milligrams of anhydrous DBr. One-mm thick disks of KBr were prepared by evacuated compression and examined for the characteristic spectra.

RESULTS

Diffusion and distribution. The solubility of the injected chemical is quite important, since its solubility in the egg would partially determine its availability to the chick embryo. However, insoluble chemicals can be injected as suspensions or emulsions, which would minimize the solubility problem. An attempt to inject DDT in an emulsion was unsatisfactory because the emulsion itself was toxic. The emulsion had been made of 4 ml peanut oil, 240 mg Tween 20, 50 mg Span 20, 320 mg Lexinol FC and 16 glycerine, and emulsified by sonication for a few minutes (Branson Sinifier). The hatchability was 10 per cent in the eggs which had been injected with 0·1 ml of the emulsion, while it was 94 per cent in the eggs which had been injected with 0·1 peanut oil.

Three oils were tried as carriers for DDT: peanut oil, olive oil and corn oil. All three were colored with the dye, Sudan IV, before injection into the eggs. After several days, diffusion was observed best in peanut oil. Corn oil was not inferior to peanut oil, but it was avoided because it had been reported[39] to produce a significant reduction of esterase activity of the liver. In order to determine the distribution of DDT between yolk and embryo, on various days ^{14}-C-DDT was injected as described above. Table 1 summarizes these results.

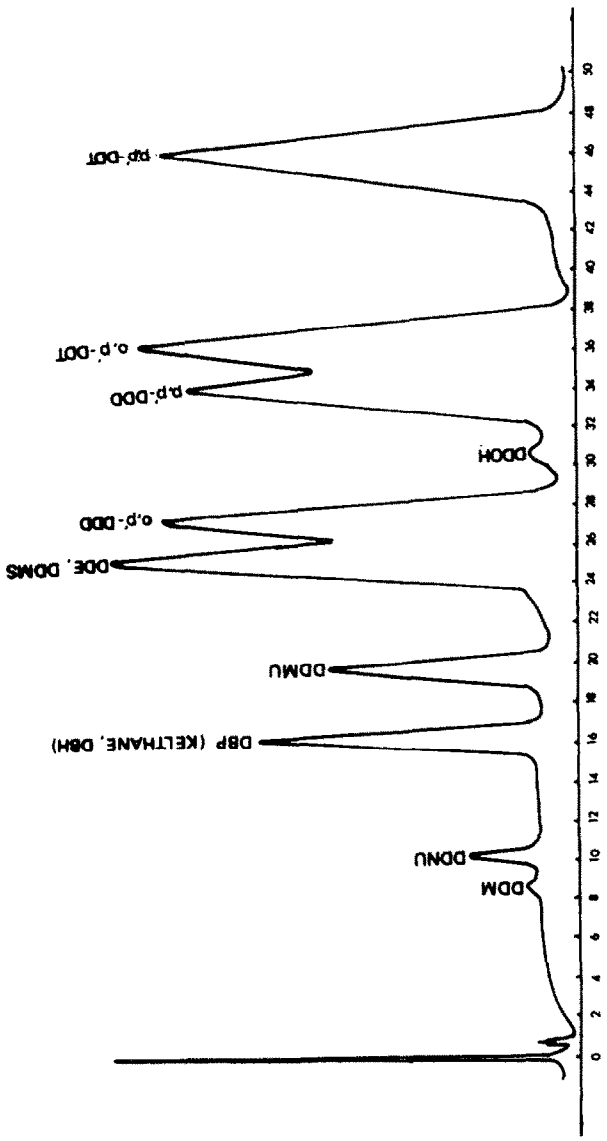

FIG. 3. Electron capture gas chromatography on 5% SE-30 plus 5% QF-1 column; standards.

TABLE 1. TISSUE DISTRIBUTION AND RECOVERY OF ^{14}C-DDT INJECTED IN PEANUT OIL INTO CHICK EMBRYOS

Tissue	Recovered radioactivity			Per cent recovery
	10% Ethyl ether in petroleum ether (dpm)	$CHCl_3$:MeOH (2:1) (dpm)	Total (dpm)	
11-Day embryo	2501*	2679	5180	7·9
Yolk	27,479	20,711	58,190	89·3
13-Day embryo	8400	6633	15,033	23·1
Yolk	24,903	23,700	48,603	74·7
15-Day embryo	15,031	14,922	29,953	46·0
Yolk	17,971	16,097	34,068	52·5
17-Day embryo	23,134	22,043	45,177	69·5
Yolk	9930	8930	18,860	29·0
19-Day embryo	25,197	24,113	49,310	75·8
Yolk	7071	7309	14,380	22·1
1-Day chick†				
Liver	3912	2119	6031	9·2
Brain	1107	907	2014	3·1
Gut	12,150	13,631	25,781	39·6
Carcass	15,978	14,200	30,178	46·4
Feces			1325‡	1·7

* Each value represents the average of 2 determinations.
† Low recovery results from difficulties in feces collection and extraction.
‡ Calculated by difference.

Beginning with the eleventh day, embryos and egg yolks were isolated, lyophilized and extracted as described under Methods. The first solvent, 10% ethyl ether in petroleum ether, extracted only about 50 per cent of the radioactivity. The succeeding extraction with chloroform–methanol (2:1) for 24 hr was sufficient to extract almost quantitatively the rest of the radioactivity. These results are listed in Table 1. After the eleventh day, DDT accumulation by the embryo was rapid. In the chick, a considerable amount was found in the eviscerated carcass and gut. The gut contained almost equal amounts with the carcass.

Hatchability and general observations. When 10 mg *p,p'*-DDT, *p,p'*-DDD and DDE was injected· the hatchability was 80, 80 and 90 per cent respectively, while it was 99, 98 and 100 per cent respectively when 1 mg was injected. These values were corrected for the hatchability of the peanut oil-injected eggs (94 per cent). Since as much as 30 per cent of the yolk remains at the time of htaching and is absorbed during the first 7 days,[8] the chicks were kept under observation for a week in order to detect any delayed effects. The toxicity of the injected chemicals was evaluated from the percentage of hatch as compared to the control (peanut oil-injected eggs) and from the appearance and development of the chicks that did hatch.

Chicks hatched from eggs which had been injected with DDT had the most prominent signs of intoxication, i.e. muscle tremor, incoordination and convulsions. Some of the chicks had few feathers and their eyes remained closed, while others were paralyzed. Some chicks had to be helped out of the shell. In agreement with others,[33] no teratogenic effect was observed either in the hatched chicks or in the dead embryos. Within a few days after hatching, some of the severely paralyzed chicks died, but the survivors suffered no apparent nerve damage.

DDD caused symptons similar to those observed with DDT, but the symptoms were less severe. No additional mortality occurred within 7 days after hatching.

TABLE 2. *p,p'*-DDT AND ITS BREAKDOWN PRODUCTS DETERMINED IN DIFFERENT TISSUES OF CHICKS HATCHED FROM EGGS INJECTED WITH 1 mg DDT IN PEANUT OIL AND FROM CHICKS FED 100 ppm DDT

Tissue	Dry tissue weight (ppm)											
	p,p'-DDT	*o,p'*-DDT	*p,p'*-DDD	*o,p'*-DDD	DDE	DDMU	DDMS	DDNU	DDOH	DDM	DBP	DDA*
Liver												
Injected	150	18	45	10	40	9	6	11	12	10	17	+++
Dietary	120	20	47	12	41	8	6	11	10	7	15	++
Gut												
Injected	65		20		17	3	2	4	18	3	6	—
Dietary	40		17		15	2	1	5	10	2	5	—
Carcass												
Injected	38		11		12	3		3	4		5	—
Dietary	35		12		14	3	1	4	6		4	—
Brain												
Injected	17		5		4			1				—
Dietary	16		4		3	1		2				—
Feces												
Dietary	19		10		7	4	2	6	10	8	12	+++

* Estimated by TLC: ++ = relatively strong test; +++ = very strong; — = none detectable.

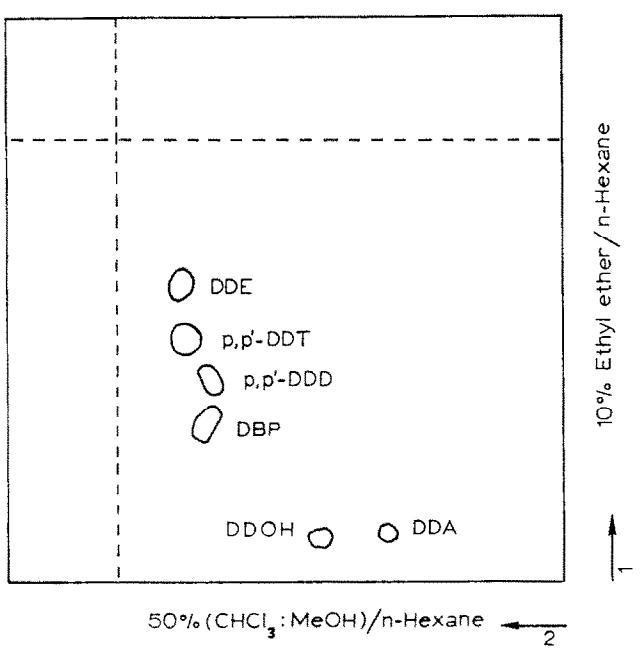

FIG. 4. Two-dimentional separation of the principle DDT metabolites in chick liver. Chromatography was accomplished by migration first in 10% ethyl ether in *n*-hexane followed by migration in 50% chloroform–methanol (2:1) in *n*-hexane. Spots were detected with *o*-toluidine.

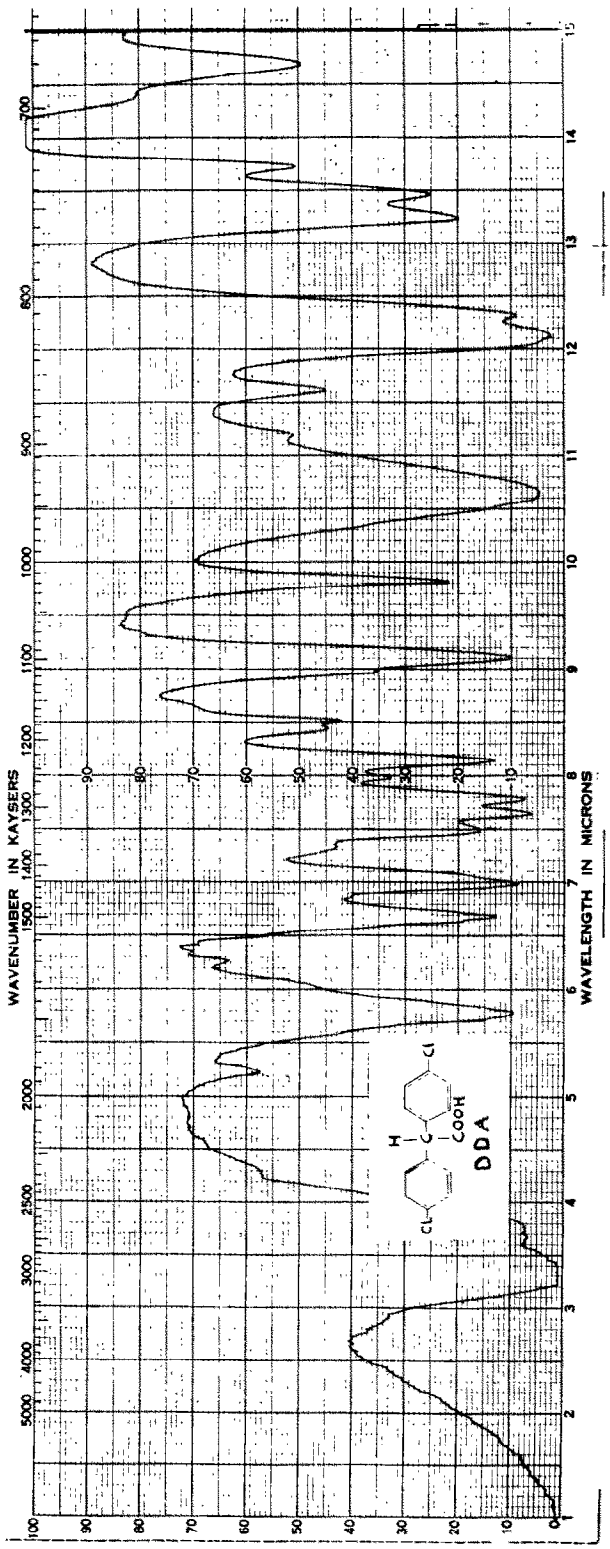

FIG. 5. Infrared spectrum of DDA standard in chick liver.

Chicks hatched from eggs which had been injected with 1 mg DDE per egg appeared normal.

Only five of the chicks fed 100 ppm of DDT showed clinical signs of illness consistent with DDT poisoning and were dead within 3–4 days (20 per cent mortality). No such symptoms were observed with DDD or DDE ingestion at 100 ppm.

Metabolites. DDT and its breakdown products were determined and expressed as ppm of the dry weight of the tissues (Table 2). Each value represents the average of 6 determinations from 6 chicks. The S.E.M.'s ranged from 0·1 to 2·3. In order to avoid any breakdown products from microorganisms or chemical decomposition, tissues were lyophilized immediately after dissection.

DDA could not be detected with any of the electron capture gas chromatography systems used. Kelthane and DBH had the same retention times as DBP on gas chromatography in all systems. Thus, for the detection and identification of DDA, Kelthane and DBH, two-dimensional TLC was used.

Table 2 sets forth the metabolites identified in the tissues of chicks hatched from eggs injected with DDT and of chicks fed DDT respectively. By both routes of administration, the metabolites are essentially the same, but the quantity and relative proportions differ slightly.

In addition to the nonpolar metabolites, DDOH, DBP and DDA were detected. *p,p'*-DDD was found in all tissues at slightly higher concentrations than DDE. DDMU was less than DDNU. DDM was detected only in the liver and gut of both exposure routes and in the feces of dietarily exposed chicks.

Unexpectedly, *p,p'*-DDD was found in the liver of chicks exposed by both routes. DDA was found only in the liver in both treatments, also feces from DDT-fed chicks contained significant amounts of DDA. DDA was identified by TLC (Fig. 4) and infrared spectroscopy. Figs. 5 and 6 represent the i.r. spectra of isolated and synthetic DDA.

Kelthane was not identified in any tissue or feces. DBH could not be identified because of its destruction on gas chromatography to DBP, and because DBH migrated close to DDOH on TLC.

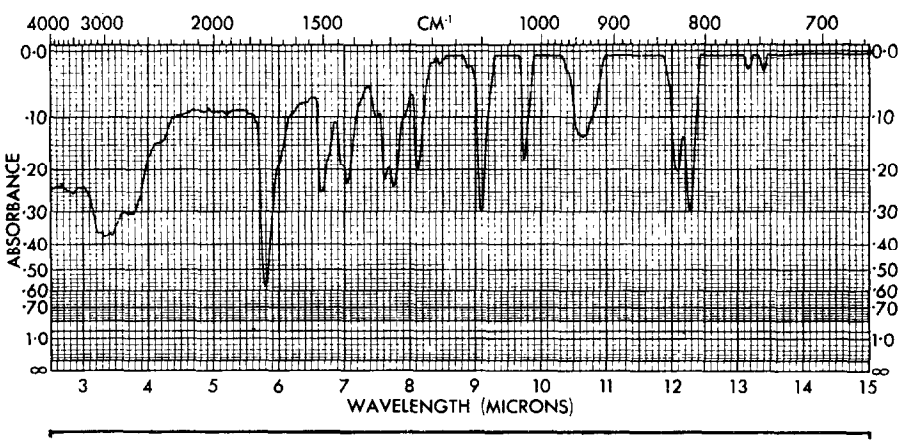

FIG. 6. Infrared spectrum of DDT metabolite in chick liver

Fig. 7 presents typical electron capture gas chromatograms, utilizing the 5% QF-1 column, for the metabolites extracted from the liver of chicks hatched from embryos exposed to *p,p'*-DDT. The nonpolar column, 10% DC-200, and the mixed column, 5% SE-30 plus 5% QF-1, were utilized to resolve DDNU–DDM, DDE–DBP and *o,p'*-DDT– DDMS as described under Methods.

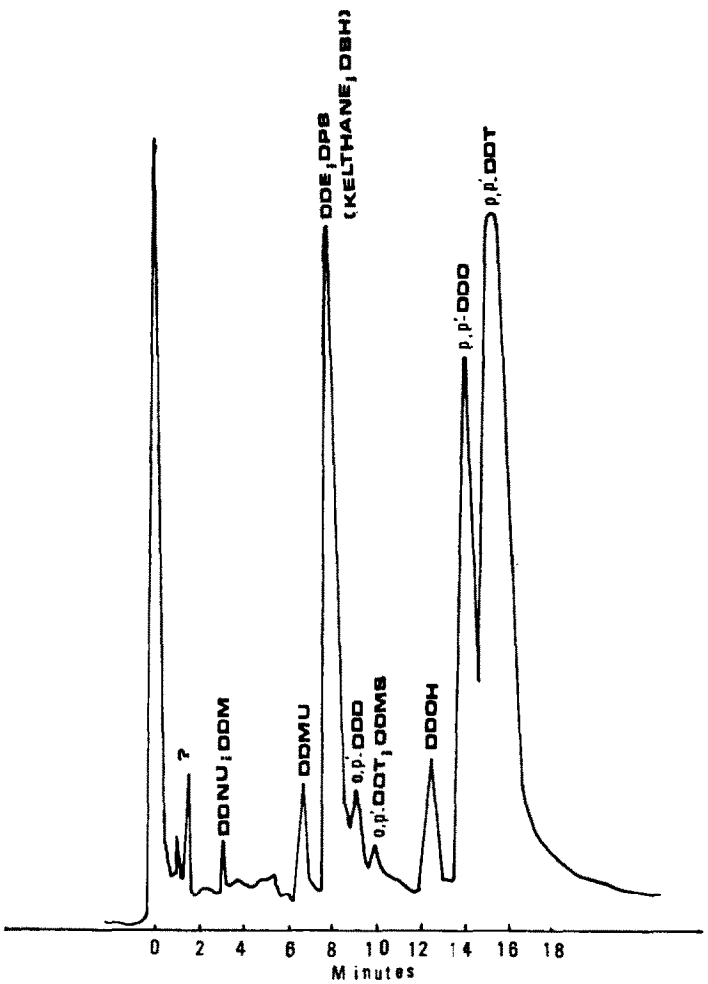

FIG. 7. Electron capture gas chromatography on 5% QF-1 column; *p,p'*-DDT metabolites in chick liver.

The metabolites found in the tissues of chicks exposed by injection and dietary ingestion of *p,p'*-DDD are presented in Table 3. Neither DDT nor DDE was detected. DDMU, DDMS and DDNU were present in much higher concentrations than those observed in the DDT experiments. DDMS was less than DDMU which, in turn, was less than DDNU. DDM was found in all of the tissues. DDOH and DBP were present in all tissues in considerable amounts, as in the DDT exposure. DDA was found in the liver of both injected and dietarily exposed chicks and, as again in the

TABLE 3. *p,p'*-DDD AND ITS BREAKDOWN PRODUCTS DETERMINED IN DIFFERENT TISSUES OF CHICKS HATCHED FROM EGGS INJECTED WITH 1 mg DDD IN PEANUT OIL AND FROM CHICKS FED 100 ppm DDD

Tissue	Dry tissue weight (ppm)								
	p,p'-DDD	*o,p'*-DDD	DDMU	DDMS	DDNU	DDOH	DDM	DBP	DDA*
Liver									
Injected	190	25	24	15	27	13	14	20	+
Dietary	190	21	17	11	21	10	10	15	—
Gut									
Injected	80		10	7	12	8	5	7	—
Dietary	85		6	4	7	4	3	5	—
Carcass									
Injected	55		6	4	8		3	5	—
Dietary	24		4	3	5	3	2	4	—
Brain									
Injected	20		3		5		2		—
Dietary	15		3	1	4		2		—
Feces									
Dietary	24		7	5	10	12	8	17	++

* Estimated by TLC: + — relatively strong test; ++ — very strong; — — non detectable.

DDT experiments, was found in the feces of the dietarily exposed chicks. Infrared spectra were used to demonstrate DDA.

Fig. 8 illustrates typical chromatograms of the metabolites extracted from the liver of chicks exposed to DDD as embryos. The nonpolar column, 10% DC-200, and the mixed column, 5% SE-30 plus 5% QF-1, were utilized to resolve DDM–DDNU, as described under Methods.

Exposure to DDE by either embryonic injection or dietary ingestion resulted in the presence of DBP as the only metabolite (Table 4). DBP was found in all tissues,

TABLE 4. *p,p'*-DDE AND ITS BREAKDOWN PRODUCT DETERMINED IN DIFFERENT TISSUES OF CHICKS HATCHED FROM EGGS INJECTED WITH 1 mg DDE IN PEANUT OIL AND FROM CHICKS FED 100 ppm DDE

Tissue	Dry tissue weight (ppm)	
	DDE	DBP
Liver		
Injected	289	44
Dietary	219	81
Gut		
Injected	113	20
Dietary	87	11
Carcass		
Injected	69	12
Dietary	72	8
Brain		
Injected	30	
Dietary	25	
Feces		
Dietary	70	

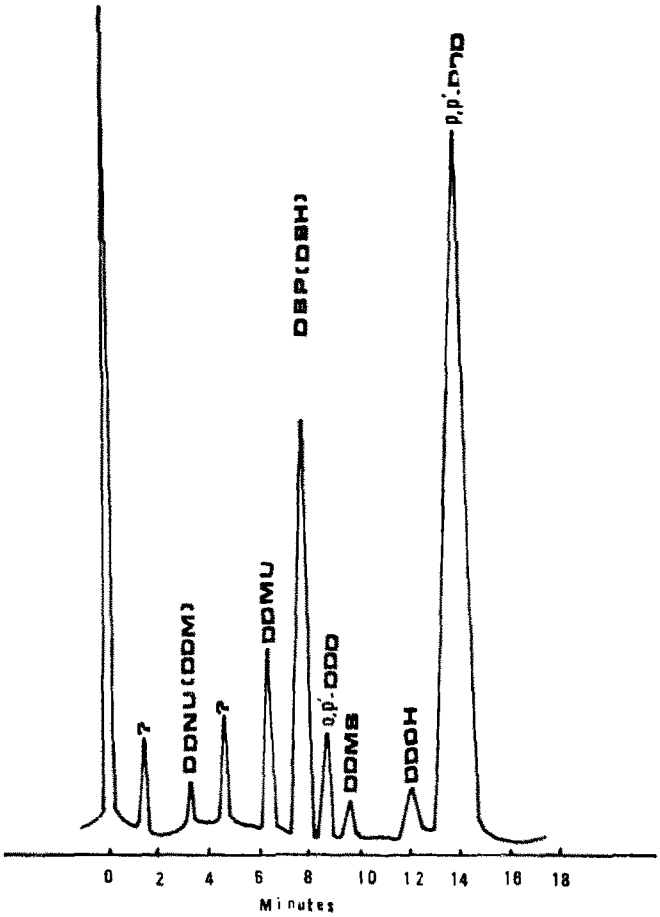

FIG. 8. Electron capture gas chromatography on 5% QF-1 column; *p,p'*-DDD metabolites in chick liver.

except brain, which contained DDE only. DBP was confirmed by gas chromatography on two columns, 5% QF-1 and 10% DC-200 (Fig. 9), by two-dimensional TCL and by i.r. spectroscopy (Fig. 10 and 11).

DISCUSSION

Until this report, the metabolism of DDT in birds had not yet been fully elucidated. This report illustrates the major pathways of metabolism of DDT, DDD and DDE in the chick and chick embryo.

Injection of lipid-soluble compounds into the essential aqueous environment of the embryo raised questions as to the extent of availability of the material to the embryo. From a toxiocological point of view, the present data illustrate that the actual dose accumulated by the embryo prior to 11 days is small (7·9 per cent) and increases rapidly with the maturity of the embryo. The lack of teratological effects for any of the compounds studied may be related to the relatively low proportion of the dose actually present in the embryo during the early stages of development. Our observations

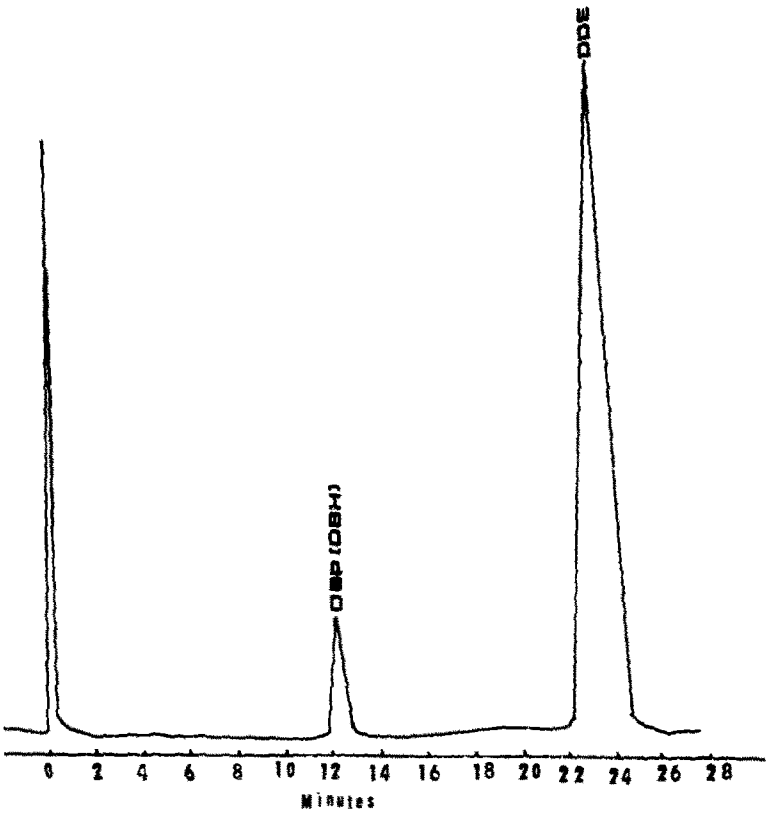

FIG. 9. Electron capture gas chromatography on 10% DC-200 column; DDE metabolites in chick liver.

confirm those previously reported,[33] but do not preclude the possibility that naturally occurring residues, being intimately associated with the embryo, might not produce abnormalities.

The level of DDT found in the brain of exposed animals was in agreement with the neurological symptoms in the chicks and similar to that reported in rats.[34]

Quantitative extraction of DDT and its metabolites from tissues could not be accomplished by the reported procedure,[25] i.e. 10% ethyl ether in petroleum ether (v/v), even though the extraction was extended from the original method of 4·5–6 hr to 24 hr. Extraction with a much more polar solvent was necessary for quantitative recoveries.

It has been recently shown[35] that gas chromatography with a 3-foot long stainless steel column, operated at 228° with 18·5% fractionated HiVac grease on acid-washed 42/60 mesh GC-22 fireback, caused destruction of DDT when it was injected in benzene to some compounds of which DDE, DDD and DDMU were identified. In the present investigations, all pure compounds were injected in an all-glass gas chromatographic apparatus and each compound gave only one peak in the three employed columns. The fact that chromosorb W in a glass column at lower temperature (195°) was used may account for the stability of the standard compounds in the

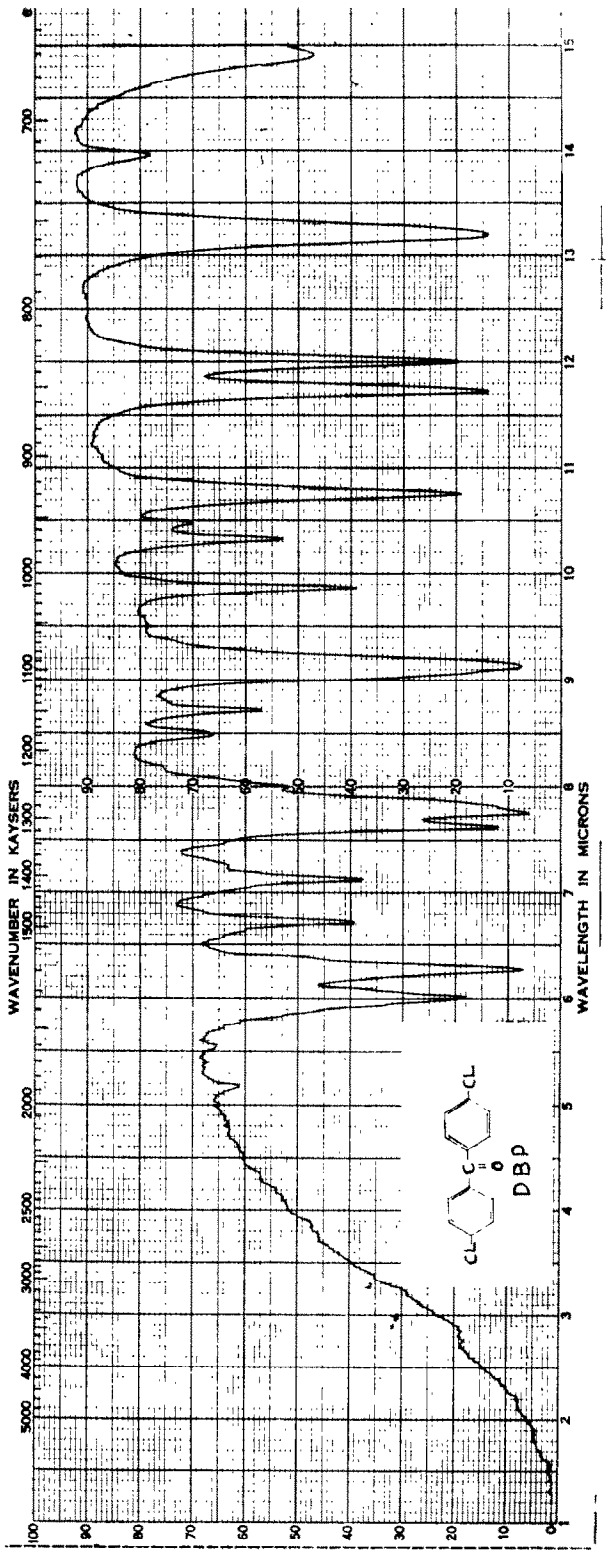

FIG. 10. Infrared spectrum of DBP standard in chick liver.

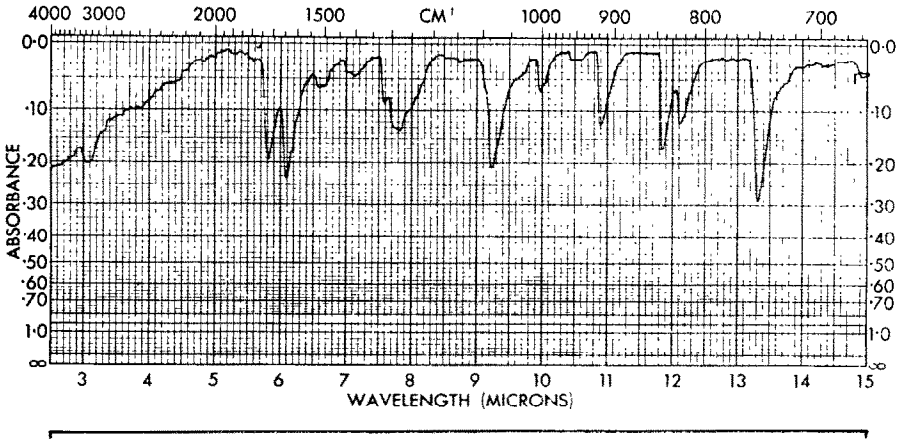

FIG. 11. Infrared spectrum of DDE metabolite in chick liver.

present work. Stainless steel at higher temperatures might have catalyzed the decomposition of DDT.

DDA does not have chlorine in the ethane portion of the molecule. This may account for DDA electron insensitivity and the failure to detect any peak even at high concentrations, as was recently suggested.[36]

Judged by symptomological observations, it was clear that the actual target of DDT in chicks is the central nervous system, as has been found in insects and mammals.[37] By utilizing the electron microscope,[38] the production of a characteristic histologic lesion has been demonstrated in adult rat liver upon chronic exposure to dietary DDT. It has been reported that technical DDD caused adrenal cortical atrophy in dogs.[39] It has been shown that *m,p'*-DDD was the most active of the three isomers.[40] The reduced hatchability of chicken eggs which had been injected or dipped in a solution containing *o,p'*-DDD has been reported.[41, 42] DDA has been shown to inhibit the acylation of choline.[43] Recently, it has been shown that DDA completely disrupted the growth of cultured KB and HeLa cells.[44] Most of these compounds have been identified in this investigation as DDT metabolites in the chick. The effect of the majority of DDT biotransformation products on cell structure and function is not known and more research is needed before the impact of environmental residues from DDT is fully elucidated.

The metabolic conversion of DDT to DDA has been elucidated in the rat[25] as the following sequence: DDT→ DDD→ DDMU→ DDMS→ DDNU→ DDOH→(hypothetical aldehyde)→ DDA. In the chick, all these postulated compounds were detected and identified with the exception of the hypothetical aldehyde. Moreover, DDM, DDOH and DBP were also detected and identified as metabolites of DDT. DBP was reported,[45] along with DDE and DDA, as DDT metabolites formed by human louse homogenate. In addition to these metabolites, *p,p'*-DDT and *o,p'*-DDD were also formed in the chick liver.

In the present study when DDD was applied, all these metabolites were formed except DDE and the back transformation to DDT.

Some identified metabolites of DDT were not formed, as one would expect. For

example, when DDA was discovered, it was postulated on chemical evidence that DDE would be intermediary in its formation.[46] However, chicks which produced both DDE and DDA from DDT were incapable of forming DDA when DDE was applied in both experiments. This observation was in good agreement with the results in the rat.[25]

It has long been thought that DDE did not undergo any change in different organisms and it was excreted as unchanged DDE. By utilizing the paper chromatographic technique, it has been reported that liver and kidney samples from rats fed with DDE yielded nothing but unchanged DDE.[25] This finding contradicted the earlier results[47] of the metabolism of DDE in houseflies. In that study, the authors were able to recover almost the entire topically applied dosage from DDT-susceptible flies, while only 40–57 per cent of DDE could be recovered from DDT-resistant flies. Accordingly, it has been concluded that an unidentified metabolic product "X" was formed; this conclusion was confirmed later.[48] It has been reported[27] that when DDE was fed to rats, DDD, *p,p'*-DDT or *o,p'*-DDT, could not be detected by electron capture gas chromatography with polar isophthalate and nonpolar SF-96 columns. In the present investigation, by utilizing electron capture gas chromatography with the polar 5% QF-1, no compound was detected other than DDE in all tissues of chicks treated with DDE. As it was reported, this column does not separate DDE and DBP. When the nonpolar 10% DC-200 column was used for analysis of the same tissue extracts, DBP was found. The quantity of DBP was not very high, but it was reproducible and its identity was established by the TLC technique in conjunction with infrared spectroscopy. DBP might have been the compound "X" isolated from houseflies treated with DDT.[47, 48]

It has been found that a commercial grade of *p,p'*-DDT was converted to *p,p'*-DDD and *p,p'*-DDE by avian blood stored at −20° suggesting a nonenzymatic degradation.[36] In the present investigation, it was found that even "pure" commercially available DDT-type compounds showed many peaks when chromatographed in electron capture G.L.C. These compounds had to be recrystallized at least three times in ethanol in order to show only one peak for each compound in the three columns utilized. The fact that these authors have used commercial DDT may have accounted for the peaks which were identified and for the catalytic degradation of these compounds.

Isomerization of the aromatic chlorine of the DDT structure appears to be a general phenomenon. Both *o,p'*-DDT and *o,p'*-DDD were found in liver extracts and not elsewhere. Isomerization apparently takes place from both *p,p'*-DDT and *p,p'*-DDD. The occurence of *o,p'*-DDD in the liver of chicks exposed only to *p,p'*-DDT supports the validity of the interconversions. The site of such isomerization is clearly the liver and argues for enzymatic interconversion.

Fig. 12 presents a scheme illustrating a suggested sequence of *p,p'*-DDT degradation in the chick. All compounds in this sequence were detected except DBH, and Kelthane, which might have been rapidly oxidized to DBP. The reactions that have been implied in this sequence of bio-transformation of DDT, DDD and DDE in the chick are as follows: Dehydrochlorination is involved in the transformation of DDT to DDE. Reductive dechlorination is responsible for the formation of DDD from DDT. Dehydrogenation took place in the transformation of DDD to DDMU. Hydrogenation was involved in the formation of DDMS from DDMU, which was consequently dehydrochlorinated to DDNU. Reductive hydroxylation converted DDNU to DDOH. Oxidation was exemplified by conversion of DDOH to DDA. Decarboxy-

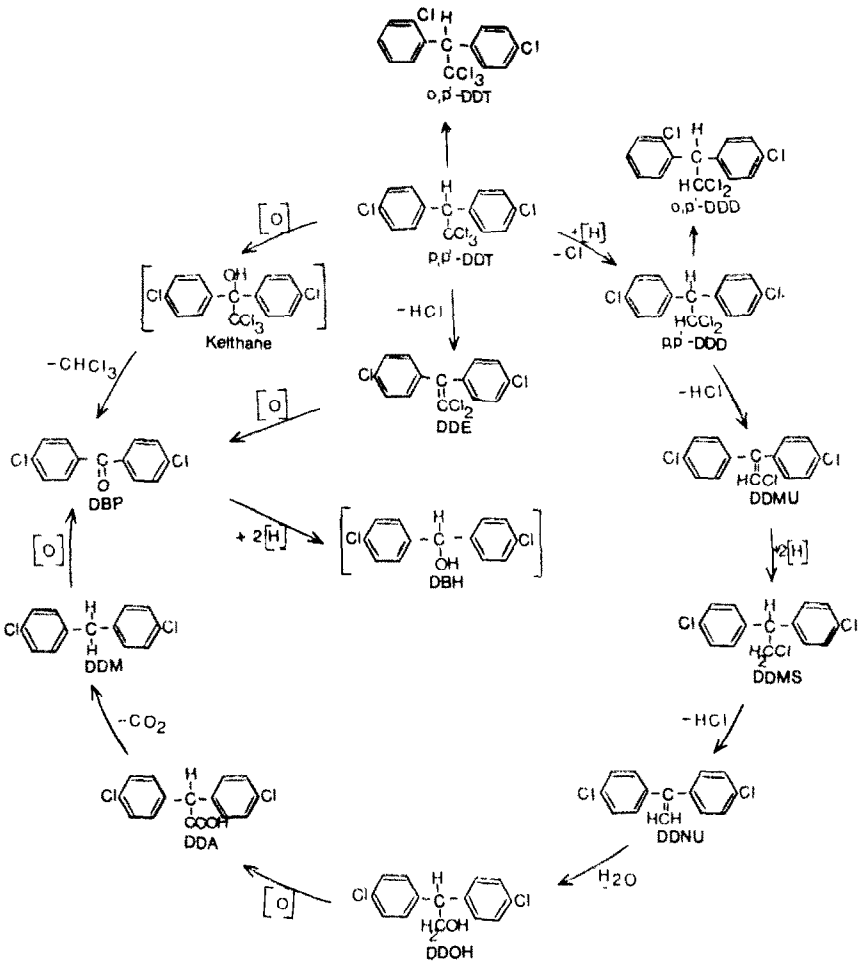

FIG. 12. Suggested DDT metabolic pathways in the chick.

lation was involved in the transformation of DDA to DDM. Oxidation converted DDM into DBP, which might also be formed by the oxidation of Kelthane and DDE. Finally, DBP could be reduced to form DBH. All polar compounds, DDOH, DBH, Kelthane and DDA, can be conjugated to form more soluble products which can easily be excreted.

Isomerization was responsible for the formation of *o,p'*-DDT and of *o,p'*-DDD from *p,p'*-DDT and *p,p'*-DDD respectively. DDE is slowly oxidized to DBP which may undergo reduction reaction to form DBH.

It is interesting to note that DDD was more stable than DDT, but DDE was more stable than either. This fact may account for reports of delayed metabolism of both DDD and DDE.

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