AN ABSTRACT OF THE THESIS OF

ALEXANDER CHIN-LOON SOO (Name of Student) for the M. S. (Degree)

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S. C. Fang

A single oral dose of DDT, ranging from 40 to 200 mg/kg, had a drastic effect on the in vivo acetate metabolism in rats. The normal elimination pattern of the expired $^{14}CO_2$ from ^{14}C-labelled acetate was distorted, and the rate of output of the $^{14}CO_2$ was decreased. The ratio of two labelled urinary metabolites, urea and one unknown compound, was also affected by DDT. Both of these were dependent upon the dosage level and the time of post-administration. DDT affected differently the C-1 and C-2 carbons of acetate molecule on the $^{14}CO_2$ elimination patterns and the incorporation into urinary metabolites, suggesting that these two carbons went through different pathways of metabolism.

No such effect was observed when labelled acetate was given intraperitoneally, suggesting that the absorption mechanism of the gastro-intestinal tract may be involved.

DDT slowed down the in vitro active transport of acetate-1-^{14}C across the rat intestine. Within two hours, as much as 57 percent inhibition was observed in the transport of acetate-1-^{14}C from the mucosal to the serosal side of DDT-treated intestine at 25°C.

DDT increased the incorporation of acetate-1-^{14}C into total liver lipids, this increase being due to the increased incorporation of ^{14}C into the neutral lipids and the decreased incorporation into phospholipids. No significant difference was found in the liver neutral lipids of the DDT-treated and control rats, but an alternation in the distribution of ^{14}C-labelled phospholipid was observed in the DDT-treated rats. This change in the abundance of ^{14}C-labelled phospholipids in rat liver was concluded to be due to the inhibition of transmethylation and decarboxylation by DDT.

The Effects of 1, 1, 1-Trichloro-2, 2-bis(p-chlorophenyl)ethane on the *In Vivo* Metabolism of Acetate in Rats

by

Alexander Chin-Loon Soo

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APPROVED:

[redacted]

Associate Professor of Chemistry
in charge of major

[redacted]

Chairman of Department of Biochemistry and Biophysics

[redacted]

Dean of Graduate School

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Typed by Muriel Davis for Alexander Chin-Loon Soo

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INTRODUCTION

Ever since the discovery of the insecticidal properties by Paul Müller in 1939, 1, 1, 1-trichloro-2, 2-bis(p-chlorophenyl)ethane (DDT) has been widely used in agriculture for insect control in crops. Because significant amounts of it are retained as residues in various forms in dairy products and are ultimately consumed by humans, it is therefore important to investigate how DDT will affect the metabolism in humans and animals, and which parts of the body will be affected.

The toxicity of DDT was first studied by R. Domenjoz who observed that the first perceptible effect was abnormal susceptibility to fear, with violent reaction to normal subthreshold stimuli. Spontaneous movement was limited and food intake stopped. All the signs were strengthened by external stimuli. These symptoms usually appeared 4-5 hours after administration of the compound and death followed in 8-12 hours (18). These findings have been very generally confirmed by many other investigators (33, 60) as well as in this laboratory.

DDT has its greatest observed toxicity when given by the intravenous route. This is undoubtedly because more of the material is made available to critical tissues in a shorter period of time. The

lethal dose (L. D.) values for various animals have been studied by many laboratories, and for white adult rats, the single-dose LD_{50} has been determined to be 150 mg/kg, 80 mg/kg and 47 mg/kg for oral, intraperitoneal and intravenous injections, respectively (11, 40, 47).

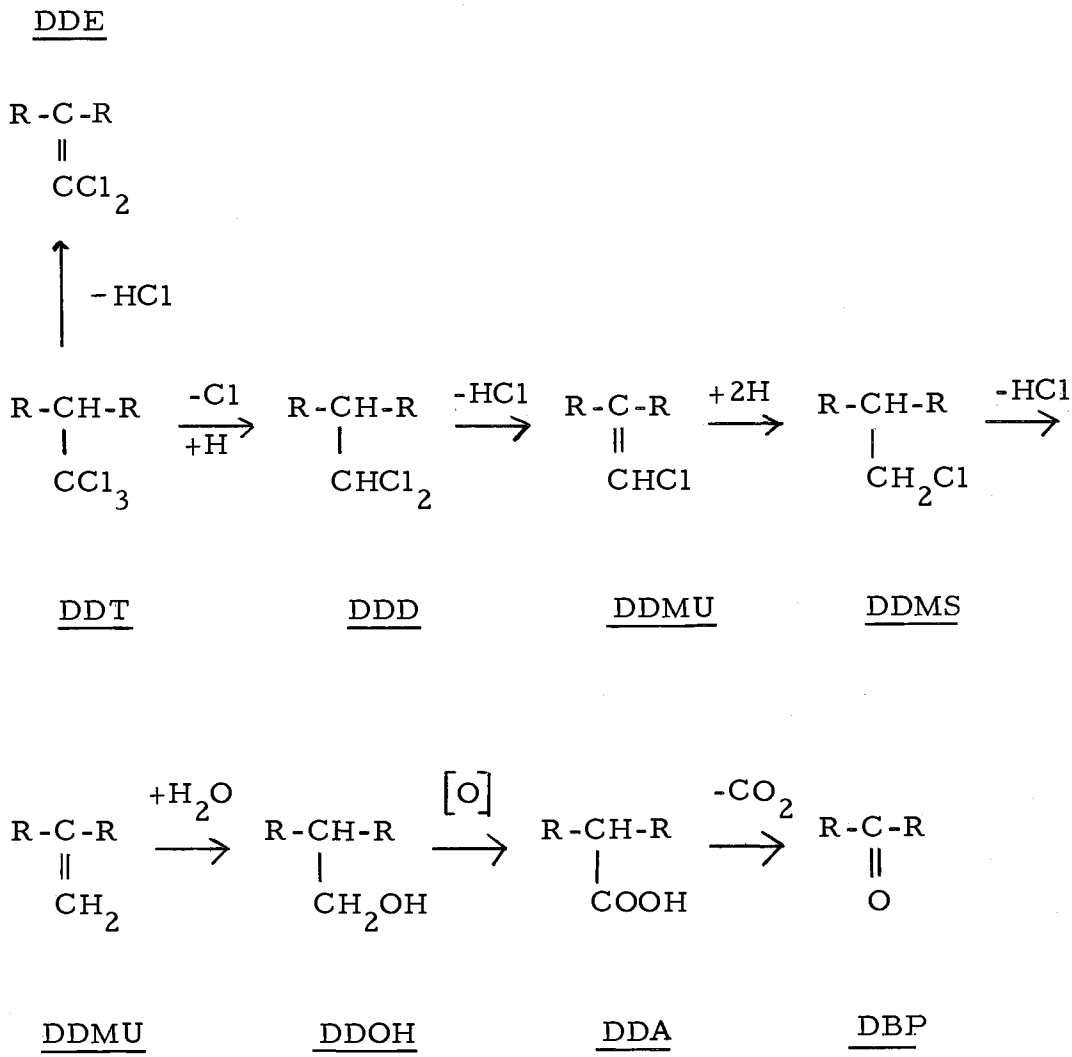
DDT is a contact insecticide. It can be absorbed from the alimentary canal after ingestion with the food so that it may on occasion act as a stomach poison. Contact with the integument may result from the application of liquid sprays or dust or from the insect walking on dry residual films. The intimacy and effectiveness of such contact will be influenced by the anatomy and by the behavior of the insect. This constitutes the problem in the mode of action of DDT.

The metabolic fate of DDT has been well-documented. The primary degradation product of DDT in insects is DDE (43), a relatively non-toxic compound (53). Agosin found that in addition to DDE, DDT is also metabolized to a Kelthane-like material, DBH, in T. infestans. He suggested that Kelthane is the precursor of DBH (2).

DDT $\longrightarrow$ 1, 1-bis(p-chlorophenyl)-2-2-2-trichloroethanol (kelthane)

$\longrightarrow$ 4, 4'-dichlorobenzohydrol (DBH)

Evidence has been gathered indicating that the major metabolite of DDT in animals was DDA (24, 41) and the pathway of conversion has been worked out (37).



"R" represents a p-chlorophenyl group.

DDA is further metabolized into 4, 4'-dichlorobenzophenone by decarboxylation in Aerobacter aerogens (58). The passage of unabsorbed DDT and its metabolite in the feces of many species of animals following oral administration constituted the major route of excretion (35, 48). As much as over 90% of the administered DDT had been reported to be found in the feces, whereas little or no DDT was

found in urine (28, 57).

Just how DDT acts on living cells is still a mystery and is currently under extensive investigation by many workers. The first such detailed studies on the mode of action of DDT were carried out by Judah (26), who found no change in the excretion of sulfur or glycuronic acid which he thought could be attributed to DDT poisoning. No change in the excretion of amino acid was revealed by chromatography. Rats poisoned by intraperitoneal or intravenous injection of DDT showed no ketones, reducing substances, or protein in the urine. However, he found the total urinary nitrogen increased following intravenous injection of the poison at 25 mg/kg. Neal et al (33), on the other hand, did not observe an increase in nitrogen excretion in dogs receiving DDT orally or by insufflation at the rate of 100 mg/kg/day. In his conclusion, Judah stated that his investigation had failed to explain the mode of action of DDT.

Recent studies with the aid of better instrumentation and tracer technique have revealed much information on the mode of action of DDT. Light microscopy demonstrated that the cells of DDT-treated rat liver produce a characteristic histologic lesion. This consists of hepatocellular enlargement, central cytoplasmic hyalinization, peripheral migration of ergastoplasm and cytoplasmic inclusion body formation in hepatocytes. When electron microscopy was used to study these lesions, it was demonstrated that the hyalinization was

due to a marked proliferation of the agranular endoplasmic reticulum. This striking cytoplasmic change was apparently compatible with protein synthesis, glycogen storage and infrequent toxic signs (36).

The signs of DDT poisoning, such as muscular tremor, incoordination and convulsion suggested that the primary effect of DDT was on the central nervous system (8, 22). However, no inhibitory action of DDT on brain or serum cholinesterase has been detected (56). In the last few years, by using the tracer technique, evidence has been accumulated that interaction or binding occurred between DDT and components of the American cockroach nervous system (19, 31). It was thus suggested that the mode of action of DDT owes its activity to the formation of a charge-transfer complex with a component of the nerve axon, with consequent disturbance of the function (34).

That there was an effect on the sympathetic nervous system was indicated by the occurrence after poisoning of increased blood glucose (51) and ventricular fibrillation of the sensitized myocardium (41). Recently, Stavinoha found that rats which had been poisoned with DDT excreted an increased amount of norephinephrine and epinephrine in urine and he suggested that the levels of norepinephrine and epinephrine in urine and in tissue from rats poisoned with DDT could be used to estimate the nature and extent of involvement of the sympathetic nervous system (50).

The literature concerning the effect of DDT on mammalian metabolism and correlating this effect to its mode of action is scant. Tinsley (54) found that in rats that were on DDT ration, the activity of liver glucose-6-phosphate dehydrogenase was greatly decreased while the activity of liver-6-phosphogluconate dehydrogenase was not affected. He suggested that the ratio of the activities of these two enzymes could be employed as an index of DDT stress. Recently, Chung et al.(13) reported the studies of some of the molecular events following exposure of Hela S cell culture to DDT. The authors found that DDT at 0 to 0.5 p.p.m. markedly decreased both RNA and DNA synthesis and at higher levels, protein synthesis was decreased also.

That DDT has an effect on intermediary carbohydrate metabolism had been reported by Agosin et al. who found DDT inhibited anaerobic glycolysis (3) while it stimulated the reactions of the pentose phosphate pathway in Triatoma infestans (4). De Villar and Mosnaim (16) observed that DDT increased the incorporation of glucose into total protein and this increase was paralleled by the amount of protein in the fourth instar larvae. They also found that DDT increased free proline.

The research reported in this thesis covers the in vivo study of the effects of a single oral dose of DDT on the metabolism of labeled acetate in adult female rats, its actions on expired $^{14}CO_2$ elimination pattern, on urinary metabolites, on active transports

and on lipid synthesis. Attempts to correlate these effects to the mode of action of this insecticide were made.

MATERIALS

White adult female rats of an inbred Oregon State Wistar strain, aged four to seven months old, weighing approximately 210 grams to 290 grams, were used.

The pesticide, 1, 1, 1-trichloro-2, 2-bis(p-chloro-phenyl)ethane (p, p'-DDT) was obtained from Aldrich Chemical Company. It has a melting point range of 109 to 110°C with recrystallization twice from ethanol. The pesticide solution was prepared by dissolving 800 mg DDT in 10 ml of Wesson oil in a water bath of 65°C. The amount of Wesson oil administered with DDT was shown to have no significant effect on acetate metabolism. Sodium acetate labelled at carboxyl carbon (-1-^{14}C) or methyl carbon (-2-^{14}C) were purchased from either New England Nuclear Corporation or from International Chemical and Nuclear Corporation with the specific activity of 1 mc. per 41 mg. or 1 mc. per 5.5 mg. respectively. The purity was greater than 98% by paper chromatography.

For oral administration, the sodium acetate solutions were prepared by dissolving it in distilled water. For intraperitoneal injection, the acetate-1-^{14}C was dissolved in 0.9% saline solution and autoclaved for 20 minutes at 110°C and 15 pounds of pressure. The labelled acetate solution was mixed with nonlabelled acetate so that each time, 15 micromole of acetate per ml was administered

into the animal. The activity of the labelled acetate was adjusted according to need.

For the in vitro active transport experiments, the acetate-1-^{14}C was dissolved in single Kreb-Ringer phosphate solution (55), the activity was adjusted to approximately 90,000 DPM or 20 millimicromole per 0.2 ml.

The solvent system used for liquid scintillation counting was made up of 5 ml. each of the following solutions: A. Toluene phosphor solution: 4 grams of 2,5-diphenyloxazol (PPO), and 50 mg. of 1,4-bis-2-(5-phenyloxazol) benzene (POPOP) per liter of toluene. B. Methyl-cellosolve-naphthalene solution: 50 grams of naphthalene dissolved in a liter of methylcellosolve. The counting efficiency of this medium was 44%. Benzoic-7-^{14}C acid was used for internal standardization.

METHODS

Administration of Chemicals

A. Oral Dose. The animal was lightly anesthetized with ether fumes and held vertically in the air by squeezing tightly at the back of the neck. Under such conditions, the animal's mouth was partially opened, and the chemical solution was introduced into the stomach by means of a syringe and a thin Teflon stomach tubing, approximately 0.022 inches in diameter and 3.5 inches long.

B. Intraperitoneal Injection. The acetate-1-^{14}C solution, the syringe and the needle were sterilized for 20 minutes at 15 pounds pressure of steam prior to the injection. The animal was lightly anesthetized with ether fumes and laid on a tray with abdomen up. The acetate-1-^{14}C solution was then injected into the peritoneal cavity. Care was taken not to inject the solution into the intestine or any of the internal organs.

Detection of Radioactivity in the Expired $^{14}CO_2$

Immediately after the labelled acetate solution was administered, the animal was placed into the Delmar metabolism cage. Lubriseal was applied to all the joints to ensure that the cage was air-tight. Room air was pushed into the cage by an oil pump at a flow rate of 426 c.c. per minute. The air which came out from the

metabolism cage was allowed to pass through a column of drierite to remove the moisture. This drieritte-filtered air then entered the electrometer of Cary model 38 with a 500 c. c. ionization chamber and the radioactivity was recorded by the Moseley model 681 strip chart recorder. The chart speed was set at one inch per hour. In most cases the experiments were terminated at the end of the 24th hour period at which time the recorder showed no more radioactive $^{14}CO_2$ was expired from the animal. In a few exceptions, the experiments were carried on as long as to the 48th hour. Only water was given to the animals during the first 12 hours of the experimental period in order to obtain a clean urine. Both water and laboratory Purina chow were available to the animals during the rest of the experimental time.

Collection of Urine and Feces

Urine was separated from the feces by means of the urine separator trap of the Delmar metabolism cage. A few drops of toluene was added to the urine for the purpose of preservation during the experimental period. However, in most cases, the feces was contaminated by urine. The urine sample was collected every 12 hours and the trap was rinsed with 50% ethanol each time, while the fecal sample was collected every 24 hours. An 0.1 aliquot of urine sample was pipetted into the scintillation vial and the radioactivity

was counted in a Packard Tricarb Liquid Scintillation Spectrometer model 314EX-2. The remaining urine samples were freeze-dried and stored in the freezer for later uses.

Fecal samples were extracted repeatedly with 50% ethanol. The solid materials were centrifuged out in an International Centrifuge model PR-1, and air dried. An aliquot of 100 mg of the finely-powdered fecal residue was counted in a Geiger-Mueller counter for radioactivity. The counts were corrected for self-absorption and background. An aliquot of 0.2 ml of the supernatant was counted in the liquid scintillation counter. The radioactivity in feces was obtained by the summation of these two counts.

Paper Chromatography

Paper chromatography was used to study the nature of radioactivity in urine. The urine samples collected at 12 hours and 24 hours after the administration of isotopic acetate were chromatographed one dimensionally on Whatman #1 chromatography paper strips of 1 x 18-1/2 inches. The urine was spotted directly onto the paper in the form of a line and developed at room temperature in the solvent system of BAW (n-butanol: acetic acid: water, 12:3:5, V/V/V). The paper strips were allowed to equilibrate in the solvent chamber for half an hour before adding the BAW solvent which was freshly prepared each time. After being developed, the paper

strips were air-dried in a hood for one hour and the radioactive spots were detected by the Radiochromatogram Scanner Packard model 7201. The relative activity under each peak was determined.

Column Chromatography

Urinary metabolites were separated and isolated out by column chromatography. Whatman standard grade cellulose powder, made slurry in 95% ethanol, was packed to a height of 30 cm in a 1.2 cm x 50 cm column. Applying slight air pressure during packing resulted in a uniform distribution of adsorbent, which was supported by a small glass wool plug. The column was washed with 200 ml of 95% ethanol. The freeze-dried urine samples were dissolved in a minimum amount of water, approximately 2 ml, and placed carefully onto the column. Urea was eluted out with 50 ml of 95% ethanol and the unknown metabolite was eluted out with 40 ml of distilled water. A 5 ml fraction was collected by the Risco automatic fraction collector, and the flowrate was regulated by a Beckman Solution Metering pump at one ml per minute. The activity in each fraction was determined by counting an 0.2 ml aliquot in the liquid scintillation spectrometer.

Identification of Urinary Metabolite

The fractions containing the unknown urinary metabolite were

pooled, freeze-dried and recrystallized once from distilled water.

Molecular Weight Determination

25 mg of Sephadex G-10-120 which has an exclusion limit of 700 average molecular weight, was soaked overnight in 150 ml of acetate buffer (0.1 M NaCl, 0.01 M NaAc). The Sephadex column was packed by gravity to a height of 20 c.c. in a 50 ml standard burette; it was washed with 100 ml of acetate buffer before use. The void volume (V_o) was determined by Blue Dextran 2000. A standard calibration curve was obtained by running two other radioactive substances of known molecular weight (for example glucose-U-^{14}C and urea-^{14}C) through the Sephadex column. Ten mg of crystallized metabolite was then dissolved in 1 ml of water and its eluted volume (V_e) was noted. The approximate molecular weight was determined from the calibrated curve.

Nitrogen Determination

The method used for total nitrogen determination was the method of Johnsons (25). One ml of 2N sulphuric acid containing 0.2 gram per liter of $CuSeO_3$ was added to the sample in the test tube containing approximately 10 to 40 micrograms of nitrogen, and digested overnight in a sand bath at 110°C. A drop of nitrogen-free 30% hydrogen peroxide was added if the digested solution was not

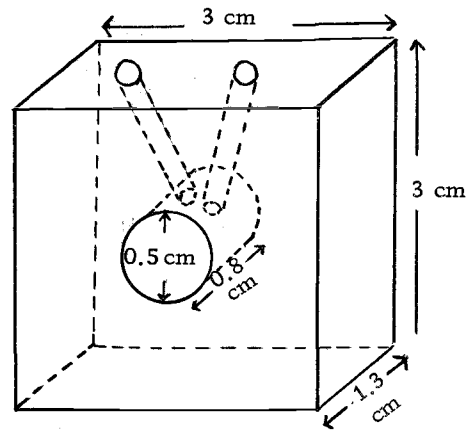
clear. After digestion, 2 ml of distilled water, 2 ml of Nessler's reagent and 3 ml of 2N sodium hydroxide was added to the tube in that order. The tube was then allowed to stand for 15 minutes at room temperature and the color was read at 490 millimicron in a Bausch & Lomb Spectronic 20. Standard nitrogen in microgram amounts was used to construct the calibration curve and the accuracy was checked with urea. The amount of nitrogen in metabolite was determined in a similar manner and the value was obtained from the standard calibrated curve.

Infra-red Spectra Analysis

One mg of metabolite, isolated from cellulose column and re-crystallized once from water, was ground with 500 mg of potassium bromide into a fine homogenous powder. An aliquot of the powder was pressed into a pellet of 0.031 mm thick and 2.5 cm long, using a hydraulic press. The pellet was scanned in Baird-Atomic Infra-red Recording Spectrophotometer, model KM-1.

The Active Transport of Acetate-1-^{14}C across the Rat Intestine

The cell blocks, specially designed for the active transport experiments, were made of plastic and have the dimensions of 3 cm x 3cm x 1.3 cm. The actual volume of the cell has a height of 0.8 cm and a diameter of 0.5 cm. It holds 0.25 ml of water when full.



On the upper part of the cell block, there are two air holes which are at approximately a 15 degree angle with each other. These holes enable one to inject and withdraw the solutions from either side of the cell without taking the two cells apart, and meanwhile permits the air to circulate through the solution during the entire experimental period. The two holes are made at a 15^{o} angle with each other because this allows one to withdraw the solution in the cell completely.

The upper portion of jejunum of the adult female rats was used. The intestine, about one cm long, was cut open along the mesentery with a scissors, rinsed well in the distilled water and immediately was placed carefully in between two specially designed cells. The mucosal side of the intestine was filled three-fourths full with acetate-1-^{14}C in Kreb-Ringer solution, and the serosal side was filled three-fourths full with Kreb-Ringer solution. The whole system was made water-tight and was shaken gently in a temperature-regulated water bath.

The amount of acetate-1-^{14}C transported through the intestine was determined by measuring the activity of the solution in the serosal side of the intestine. The solution was pipetted out by a long stem medicine dropper through one hole and was refilled at once by a one ml automatic syringe through the other hole. The activity of the solution was counted directly in the Tricarb liquid scintillation spectrometer.

Extraction, Isolation and Purification of Liver Lipids

The livers taken from the rats were quickly freeze-dried. The dried samples were ground to a fine powder. An aliquot of 20 mg was dissolved in 2 ml of p-(di-isobutyl-cresoxyethoxyethyl) hydroxide (hyamine 10 x) at 60°C. The radioactivity of the hyamine 10 x solution was determined by liquid scintillation counting (52). An aliquot of one gram of liver powder was weighed out into a 50 ml conical centrifuge tube, the lipids were extracted by shaking the tissue with a mixture of 2:1, chloroform:methanol, V/V, in a shaker for at least 24 hours in a cold room. After centrifugation, the supernatant was decanted and the liver residue was extracted again with the chloroform:methanol mixture. The supernatants from several successive extractions were combined. The lipids obtained from this method usually contain some non-lipids such as amino acids and carbohydrates. These non-lipids were removed by the

method of Bligh and Dyer (10). Briefly, the chloroform:methanol supernatant was shaken with water in the proportion of approximately 2:1:0.8, chloroform:methanol:water, V/V/V. The chloroform phase was separated from the aqueous phase by centrifugation. The lipids in the chloroform layer extracted by the above mentioned procedure contained no significant amounts of non-lipid materials, and the loss of lipids in the methanol:water layer was only about one percent of the total lipid which was considered insignificant, and the lipids remaining in the tissue residue were approximately 6% of the total extracted lipids (10). The partially-purified lipids in the chloroform layer were concentrated at 45°C under a jet stream of nitrogen. The total lipids were further separated into neutral lipids and phospholipids by passing them through a silicic acid-celite column 2:1, V/V (46).

Preparation of Silicic Acid-Celite Column

Silicic acid of reagent grade and celite 535 were activated in an oven at 110°C for at least 24 hours. The column was one cm in diameter, 25 cm height and had a reservoir on the top which has a capacity of 200 ml. Silicic acid and celite were made slurry with 4:1 chloroform:methanol and packed to a height of 20 cm. The column was first washed with 100 ml of 4:1 chloroform:methanol mixture followed by 50 ml straight chloroform. Total

lipids were dissolved in chloroform and placed on top of the column. Neutral lipids were eluted out with 40 ml of chloroform and phospholipids, with 80 ml of methanol.

Analysis of Neutral and Phospholipids by Thin Layer Chromatography

The different classes of neutral lipids and phospholipids were separated by the technique of thin-layer chromatography. A number of methods for this thin-layer chromatography of lipids had been reviewed by Mangold (29). The thin-layer chromatogram plates used here were the Eastman Chromagram sheet, type K301R2, labelled for thin-layer chromatography, and the home-made glass thin-layer plates using silica gel G with $CaSO_4$ binder. The thickness was adjusted to 100 microns. The sheets were activated before use by heating in an oven for 15 to 20 minutes at 110°C.

Application of Samples

The lipid samples were applied onto the sheet by a 10 microliter syringe. The samples were applied either as single spots, about 2-1/2 cm above the lower edge of the plate, or as a band. Each spot was air-dried before the next was added.

Solvent Systems

For neutral lipids: Hexane:diethyl ether:glacial acetic acid, 84:15:1, V/V/V (9).

For phospholipids: Chloroform:methanol:30% ammonium hydroxide, 65:25:4, V/V/V (23).

Development of Chromatoplates

The plates were developed at room temperature and in ascending technique. They were placed on edge in a jar containing about 50 ml of solvent. The jar was covered with an airtight lid and solvent travels up the length of the plate. The jar has an internal dimension of 7.5 x 27.5 x 27.5 cm. The inside of the jar was completely lined with filter paper which saturated the atmosphere in the jar with solvent vapor. This assured an even rate of migration of the solvent over the whole width of the plate and shortened the developing time by about one-third.

Visualization and Analysis of Lipids

After the development was completed the plates were taken out, air-dried for 10 to 30 minutes and scanned directly in a Radiochromatogram Scanner of Packard model 7201. The following indicators were used in thin-layer chromatograms.

Iodine vapors: Brown spots appeared on a white or yellow background. Visualized all unsaturated lipids, also some saturated nitrogenous lipids (30).

Antimony trichloride (saturated solution in chloroform): Various characteristic colors on a white background. (Visualized all the steroids and alicyclic vitamins (52)).

Saturated chromic sulfuric acid solution: Black spots on white background after heating at 180°C. Color changes during heating (32). This indicator was used on glass plates only since the organic binder of the Eastman Chromagram sheet made the normal visualization impossible.

Molybdenum blue reagent: Blue spots on a white or light blue-grey background. Visualized a wide range of compounds containing phosphorous (17).

RESULTS AND DISCUSSION

Effect of DDT on the Elimination Pattern of Radioactivity in Expired $^{14}CO_2$

More than ten separate runs of experiments have revealed that oral doses of labelled acetate, -1-^{14}C or -2-^{14}C, were metabolized and broken down into $^{14}CO_2$ in a well-regulated manner. Figure 1A ID and IB, IC, IE showed the actual tracing of the expired $^{14}CO_2$ of control and DDT treated rats, receiving acetate-1-^{14}C and -2-^{14}C respectively. These diagrams showed clearly that the peak maximum of the expired $^{14}CO_2$ from control rat was reached usually in the first half hour after the oral administration of the acetate-1-^{14}C or -2-^{14}C, and it was then followed by a very smooth decrease in radioactivity. The peak maximum of the expired $^{14}CO_2$ was reached as early as 15 minute after the injection of acetate-1-^{14}C has been reported by some workers who used a smaller metabolic chamber (44). The output of radioactivity in the expired $^{14}CO_2$ became very consistent and leveled off after 9 to 10 hours, and at the end of 24 hours, more than 95% of the administered activity was recovered in $^{14}CO_2$. The results obtained here by the Vibrating Reed Electrometer and recorder was in close agreement with other investigators (14, 21, 38, 44) who used the $BaCO_3$ precipitation technique.

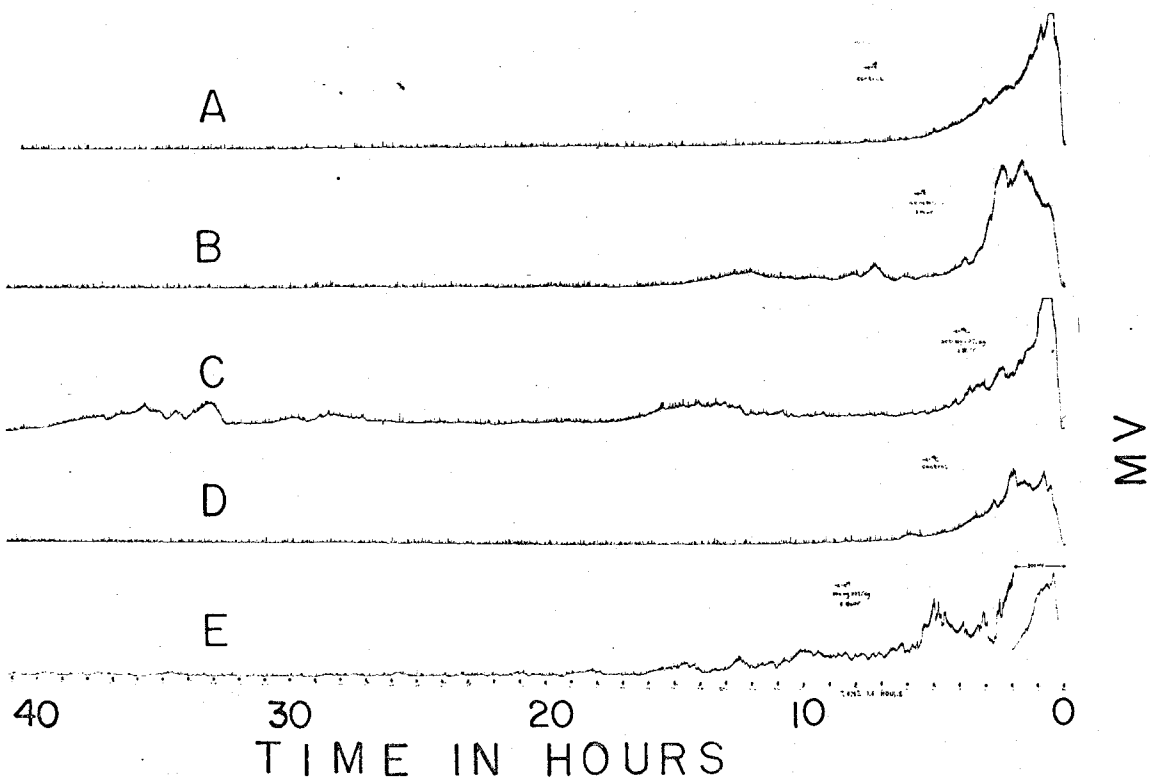


Figure 1. The effect of p, p'-DDT, administered to the rat one hour prior to the oral dose of acetate-1-^{14}C or acetate-2-^{14}C, on the elimination pattern of expired $^{14}CO_2$. The graphs were obtained by a Vibrating Reed Electrometer of Cary model 3810. The ordinate is in mv scale (full deflection equals to 100 mv unless otherwise stated), and abscissa, time in hours. A. Control (4 μCi acetate-1-^{14}C) 0.25 ml of Wesson oil. B. 100 mg DDT per kg body weight, (5 μCi acetate-1-^{14}C). C. 200 mg DDT per kg body weight, (5 μCi acetate-1-^{14}C). D. Control (5 μCi acetate-2-^{14}C) 0.25 ml of Wesson oil. E. 100 mg DDT per kg body weight (20 μCi acetate-2-^{14}C), first two hours was set at 300 mv scale.

The elimination pattern of the expired $^{14}CO_2$ changed drastically when the rat was given orally a single dose of 100 mg DDT/kg in Wesson oil, one hour prior to the acetate-1-^{14}C or -2-^{14}C administration (see Figure 1 B, C and E). The first sign of difference was usually a delay in reaching the peak maximum of the expired $^{14}CO_2$. After the maximum was reached, a very sharp drop in the radioactivity of the expired $^{14}CO_2$ was noticed. This decrease in radioactive $^{14}CO_2$ could be due to the effect of DDT in slowing down the permeability or absorption of acetate through the gastro-intestinal tract or inhibiting the conversion of acetate carbon through the Tricarboxylic Acid cycle. At five to six hours after the acetate-1-^{14}C or -2-^{14}C administration, the expired $^{14}CO_2$ fluctuated very much and became very unsteady until at the end of the 20th hour. Similar effects on <u>in vivo</u> acetate metabolism were obtained by using 2,4-Dichlorophenoxyacetic acid (38). Since 2,4-D is not known to be a nerve poison, the possibility that this fluctuation of radioactive $^{14}CO_2$ elimination pattern was caused by the action of DDT on the nervous system could be ruled out. Also there were no signs of fluctuation of the expired $^{14}CO_2$ in the DDT-treated rats which received the acetate-1-^{14}C intraperitoneally. This again supported the above statement that this effect of DDT on acetate oxidation was not due to the action of DDT on the nervous system.

Figure 2 shows the kinetic plot of the $^{14}CO_2$ expired by normal and DDT-treated rats. Two straight lines representing the elimination rates were obtained from the control rats. The initial rate of elimination, K_I, (1 to 10 hours post-administration) was very rapid and had a biological half-life of 1.38 hours for acetate-1-^{14}C and 1.56 hours for acetate-2-^{14}C. K_I was followed by the second elimination rate, K_{II}, which lasted through the remaining of the experimental time. K_{II} was much slower and had a biological half-life of 21 hours and 53 hours for acetate-1-^{14}C and acetate-2-^{14}C, respectively.

The appearance of two separate rates of $^{14}CO_2$ elimination pattern would indicate two different pathways of acetate metabolism (38). The initial pathway was most likely due to the direct oxidation of acetate molecules in the form of acetyl CoA through the Tricarboxylic Acid cycle. The slower elimination rate of the second pathway was probably derived from the recycling of radioactive acetate carbons which were incorporated into cell constituents and other metabolites such as fatty acids, steroids and amino acids which were subsequently reoxidized and released as $^{14}CO_2$. This may be the reason why the biological half-life of acetate-2-^{14}C is much longer than that of acetate-1-^{14}C in the second pathway, because the methyl carbon of acetate molecule is incorporated into intermediate metabolites in a ratio much higher than acetate-1-^{14}C, through

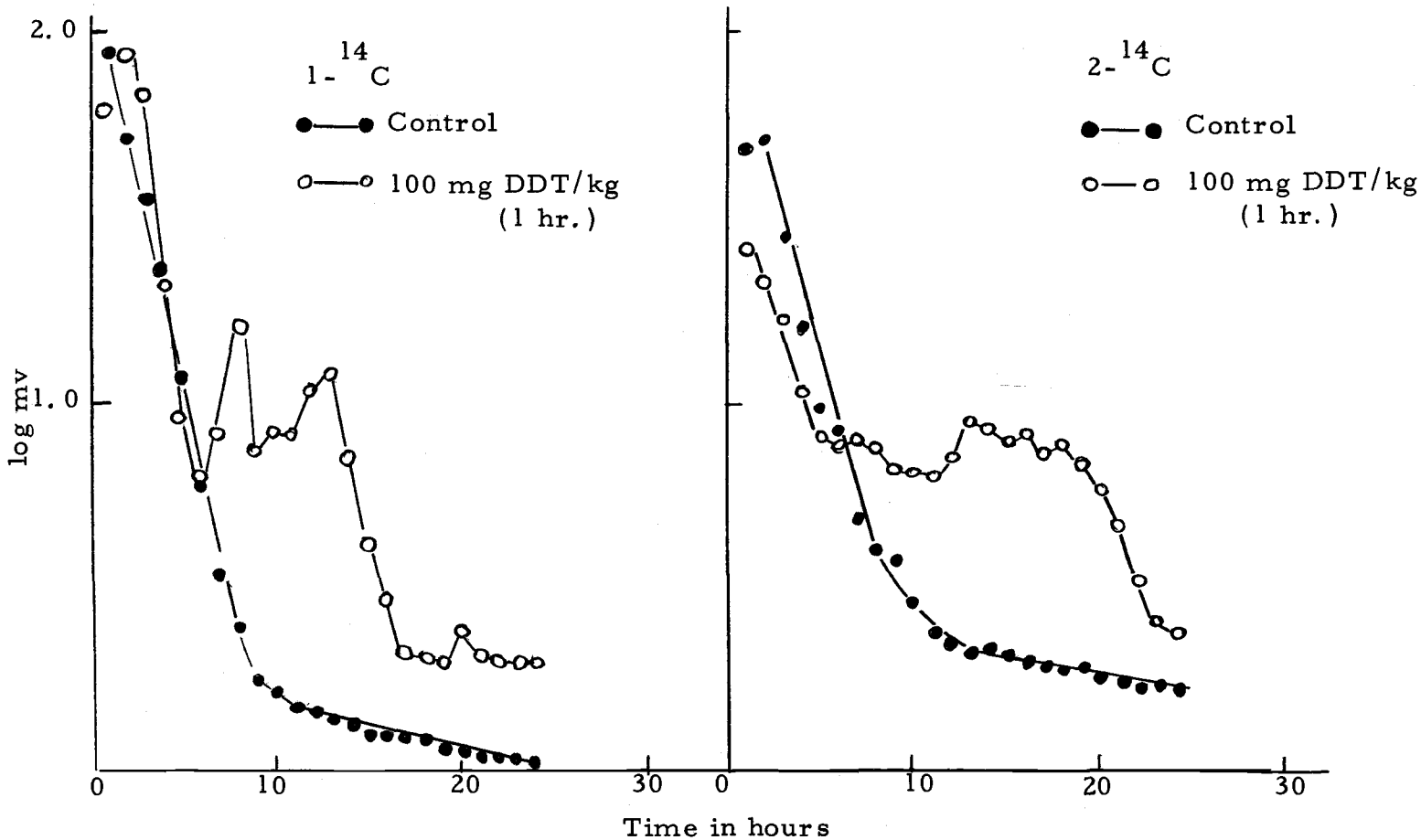


Figure 2. Kinetic plot of the expired $^{14}CO_2$ of adult female control rats and rats receiving 100 mg DDT/kg body weight one hour prior to the administration of ^{14}C-labelled acetate.

methylation and other biological reactions.

These studies of DDT effects on acetate oxidation revealed that acetate-1-^{14}C and acetate-2-^{14}C were not oxidized in an identical pathway. The initial pathway of direct oxidation through the Tricarboxylic Acid cycle seemed to be very much the same for both C-1 and C-2 as would be expected. However, these two carbons of the acetate molecule went through different sequences of biochemical reactions in the second pathway. If they went through an identical pathway, then the effect of DDT on the $^{14}CO_2$ elimination pattern should be the same also, but this was not found. Results indicated that DDT has a longer lasting effect on the metabolism of acetate-2-^{14}C than that of acetate-1-^{14}C (see Figure 1E). This effect of DDT on the $^{14}CO_2$ elimination pattern was both time and dosage dependent (Table 1).

In addition to the disturbance of expired $^{14}CO_2$ elimination pattern, orally administered DDT also showed a reduction of the total recovery of the radioactivity in the expired $^{14}CO_2$ as compared to the control (Figure 3). At the 200 mg DDT per kg level, it took as long as 24 hours to get 68% of the administered acetate-1-^{14}C to be oxidized, while in the case of the control, both acetate-1-^{14}C and acetate-2-^{14}C, more than 95% of the administered activity was oxidized and recovered as $^{14}CO_2$ in 24 hours. Of these, about 75% to 80% came out within the first five hours. This inhibitory effect of

Table 1. Effect of Orally Administered DDT on the Rate Constants of Catabolic Oxidation of Acetate in Rats

Time Between DDT & Acetate[a] Administration	Number of Rats	DDT dosage mg/kg	Rate Constant		Time Range in Hours			Percent Oxidation		
			K_I	K_{II}	K_I	Unsteady Period	K_{II}	K_I	Unsteady Period	K_{II}
				A. Acetate-1-^{14}C						
1 hour	2	100	1.08	----	0-4	4-20	----	68	32	--
24 hours	2	100	1.00	0.023	0-6	4-12	12-24	66	24	10
48 hours	2	100	0.92	0.033	0-5	5-10	10-24	68	25	7
72 hours	2	100	0.52	0.030	0-10	----	10-24	89	--	11
Control	24	0	0.52 ±0.03	0.033 ±0.001	0-10	----	10-24	90	--	10
1 hour	2	20	0.50	0.04	0-10	----	10-24	91	--	9
1 hour	2	40	0.64	0.029	0-6	6-10	10-24	83	7	10
1 hour	2	80	0.69	----	0-4	4-24	----	40	60	--
1 hour	2	100	1.08	----	0-4	4-20	----	68	32	--
1 hour	2	200	0.58	----	0-5	5-36	----	38	62	--
				B. Acetate-2-^{14}C						
1 hour	2	100	0.42	----	0-6	6-24	----	46	54	--
24 hours	2	100	0.48	0.020	0-5	5-17	17-24	72	19	9
48 hours	2	100	0.49	0.020	0-6	6-15	15-24	70	15	15
72 hours	2	100	0.44	0.015	0-5	5-16	16-24	54	32	14
Control	4	0	0.40 ±0.03	0.013 ±0.001	0-9	----	9-24	86	--	14

[a] Each rat was dosed with 5 μCi of ^{14}C-labelled acetate in 1 ml of distilled water and diluted with non-labelled acetate to a concentration of 14.5 μmole per ml.

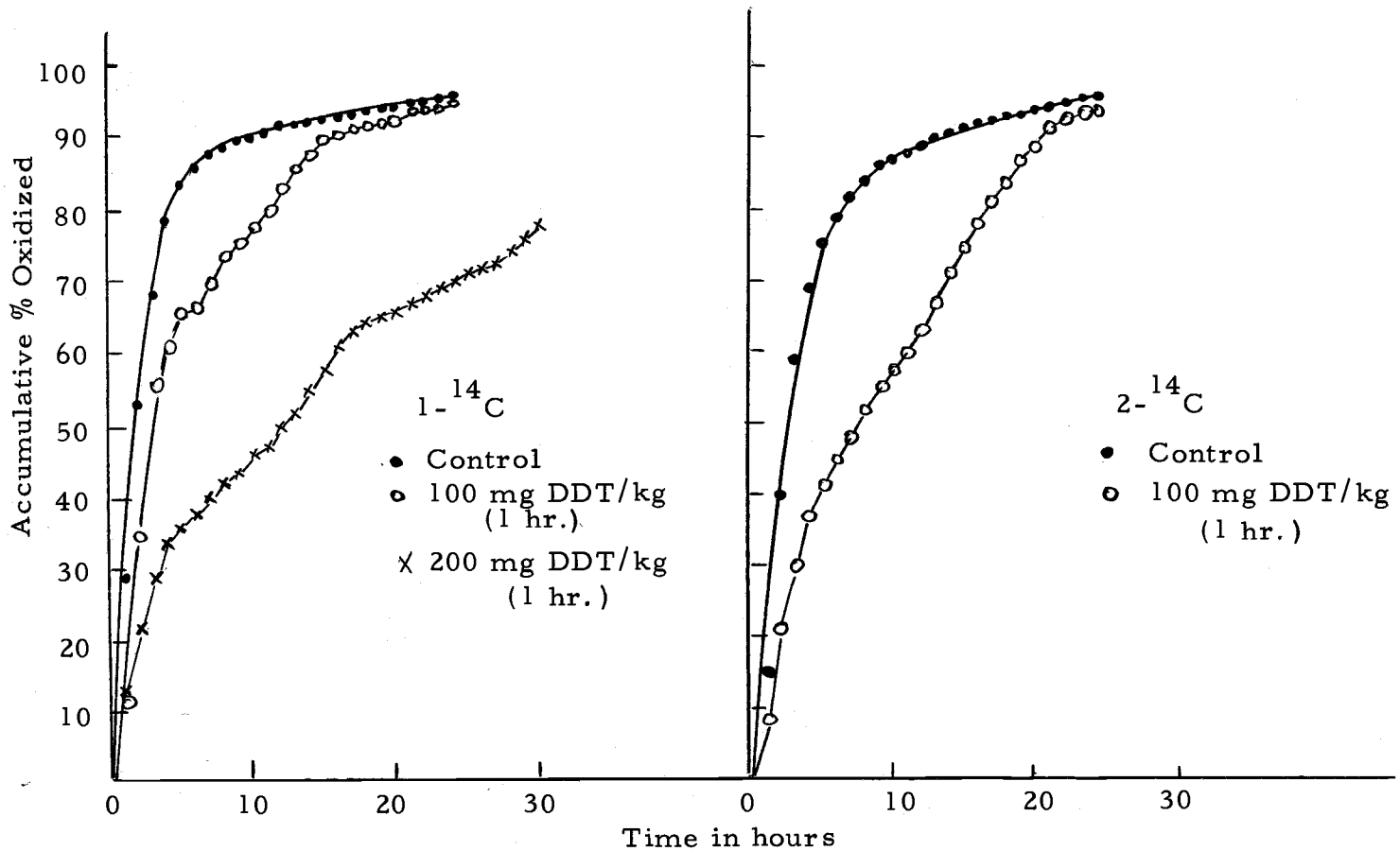


Figure 3. The effect of DDT on the total recovery of expired $^{14}CO_2$ from control and treated rats expressed as percent of dose.

DDT may have resulted from a reduction of acetate absorption in the gastro-intestinal tract or of acetate turnover via the Tricarboxylic Acid cycle.

Radioactivity in the Urine and Feces

A small percentage of the administered radioactivity was recovered in the urine and feces. Normal adult female rats incorporated approximately 3% of the orally administered acetate-1-^{14}C and 2.5% of acetate-2-^{14}C into urine in 24 hours. This incorporation was reduced in the DDT-treated rats. The reduction of acetate-1-^{14}C and acetate-2-^{14}C incorporation into urine in relation to post-medication time and DDT dosage level (for acetate-1-^{14}C only) are given in Table 2. No significant difference in the fecal activities of the control and DDT-treated rats was observed. This was usually 1 to 1.5% of the total administered activity. It is very probable that this radioactivity was partially due to the contamination of feces by the urine since the separation of feces from urine was poor.

The Nature of the Radioactivity in Urine

Paper chromatography technique was used to determine the nature of the radioactivity in urine. Two major radioactive spots were observed in BAW one dimensional chromatograms (see Method). Spot II, Rf = 0.5, which has over 90% of the urinary activity of the

Table 2. Effect of DDT on the Quantity of ^{14}C in Rat Urine after Orally Administered 20 μCi of Acetate-^{14}C.

Time Between DDT & Acetate Administration	No. of Rats	DDT dosage mg/kg	Percent of Incorporation			
			-1-^{14}C		-2-^{14}C	
			0-12 hr	12-24 hr	0-12 hr	12-24 hr
Control	9	0	2.8±0.1	0.3±0.02	2.2±0.1[a]	0.3±0.01
1 hour	2	80	1.2	0.5	0.7	0.7
24 hours	2	80	1.6	0.8	1.6	0.5
48 hours	2	80	1.8	0.4	1.3	0.5
72 hours	2	80	2.1	0.5	1.8	0.7
1 hour	2	20	2.4	0.2		
1 hour	2	40	1.6	0.2		
1 hour	2	80	1.2	0.5		
1 hour	2	100	0.7	0.5		
1 hour	2	200	0.7	0.5		

[a] Average of 4 rats.

control rat was identified earlier to be urea (39). After DDT treatment, the incorporation of orally dosed acetate-1-^{14}C or acetate-2-^{14}C into spot I was increased, which was due to a slight increase of this metabolite formation and a greater decrease in the incorporation of ^{14}C into urea. This effect of DDT on the relative abundance of these two urinary metabolites was both dosage and time dependent and appeared to be more pronounced with acetate-2-^{14}C than with acetate-1-^{14}C (Table 3).

Effect of DDT on the ^{14}C-Urea Formation

Paper chromatography revealed that DDT decreased the formation of ^{14}C-urea from orally administered acetate-1-^{14}C or acetate-2-^{14}C and the effects were directly related to the dosage level up to 100 mg per kg and were inversely related to the time after DDT treatment (Figure 4).

This effect of DDT on the ^{14}C-urea formation may be explained by the fact that most of the nitrogen excreted in mammalian urine is in the form of urea which arises from ammonia and the alpha-amino group of aspartate. This reaction is known to require the expenditure of a large amount of energy (27). In a study of DDT effects on flies, Agosin (1) reported a decrease of both the oxidized and reduced nicotinamideadenine dinucleotide (NAD^+ and NADH) levels in flies receiving a dosage of 0.066 micro-gram DDT per fly. Since

Table 3. Effect of DDT on the Incorporation of Orally Dosed 20 μCi Acetate-^{14}C into Urinary Metabolites

Time Between DDT & Acetate Administration	Number of Rats	DDT Dosage mg/kg	^{14}C Incorporation, Percent of Administered Dose							
			Acetate-1-^{14}C				Acetate-2-^{14}C			
			0-12 Hour Urine		12-24 Hour Urine		0-12 Hour Urine		12-24 Hour Urine	
			I[a]	II[b]	I	II	I	II	I	II
Control	2	0	0.21	2.59	0.00	0.30	0.17	2.03	0.00	0.30
1 hour	2	80	0.23	0.90	0.02	0.50	0.11	0.60	0.06	0.60
24 hours	2	80	0.16	1.50	0.02	0.50	0.50	1.10	0.07	0.40
48 hours	2	80	0.20	2.00	0.00	0.35	0.20	1.10	0.08	0.40
72 hours	2	80	0.22	1.80	0.00	0.50	0.50	1.30	0.20	0.55
1 hour	2	20	0.24	2.15	0.00	0.25				
1 hour	2	40	0.24	1.35	0.07	0.15				
1 hour	2	80	0.23	0.90	0.02	0.50				
1 hour	2	100	0.16	0.60	0.03	0.45				
1 hour	2	200	0.15	0.55	0.02	0.45				

[a] Metabolite x

[b] Urea

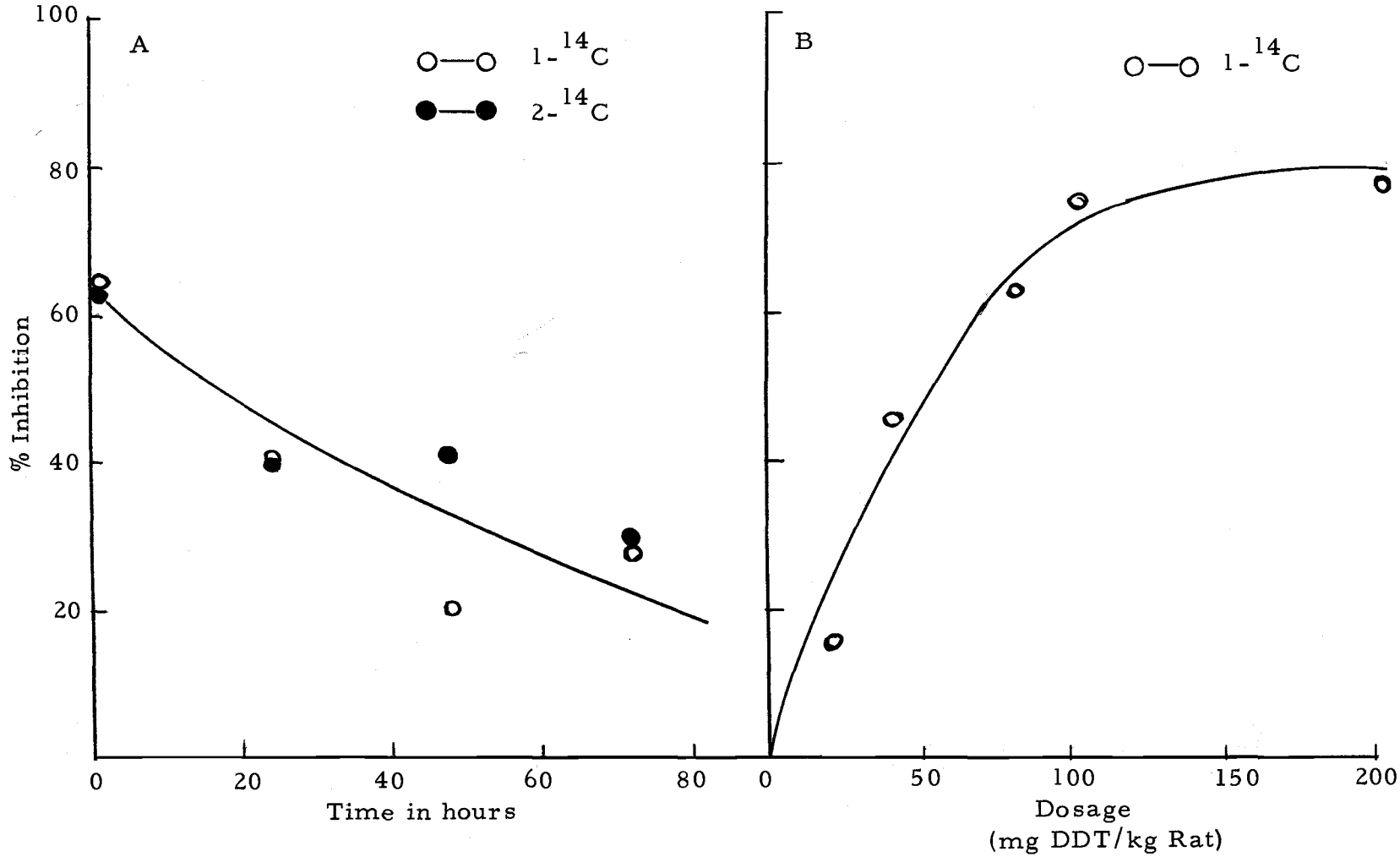


Figure 4. The effect of DDT on the incorporation of radioactivity into urea from labelled acetate.

three molecules of energy-rich phosphate are generated for the oxidation of each NADH through the electron transport chain (12), a decrease in NADH will reduce the quantity of adenosine triphosphate available for the endergonic requirements of urea synthesis. It is also possible that the reduction of urea synthesis in DDT-treated rats could be due to a reduction of ammonia level. It is interesting to see, however, that when acetate-1-^{14}C was injected intraperitoneally into the rat, no signs of disturbance of expired $^{14}CO_2$ elimination pattern, or of the reduction of radioactivity in urine and the inhibition of ^{14}C-urea formation were observed (Table 4). This suggested an alternate explanation for the effects of DDT on acetate metabolism and thus the mode of action of DDT; that is, the effects are primary on the gastro-intestinal tract. Studies on the tissue distribution analysis showed that orally dosed acetate-1-^{14}C accumulated two- to three-fold more in the stomach and intestinal tract of the DDT-treated rats than in those of the control. This probably means that DDT reduced the permeability of the gastro-intestinal tract to the acetate molecule or it interfered with the absorption mechanism by inactivation of the biological active transport system on the membrane surface and thereby prevented rapid elimination.

Table 4. Effect of DDT on the Incorporation of Intraperitoneally Injected 20 μ Ci Acetate-1-^{14}C into Urinary Metabolites.

Time Between DDT & Acetate-1-^{14}C Administration	DDT Dosage mg/kg	C^{14} Incorporation, 0-12 Hour		% of Administered Dose 12-24 Hour	
		Metabolite	Urea	Metabolite	Urea
1 hr.	80	0.21	1.41	trace	0.32
24 hr.	80	0.15	1.78	trace	0.23
48 hr.	80	0.14	1.86	trace	0.21
72 hr.	80	0.15	1.69	trace	0.30
Control	0	0.14	1.50	trace	0.11

Characterization of Urinary Metabolite

The metabolite was separated, isolated and crystallized from cellulose column chromatography (see Method). The chromatographic separation is shown in Figure 5. The metabolite was further purified by sephadex G-10 gel filtration.

Melting Point Determination

Melting point was determined by using the Fisher-Johns melting point apparatus. The metabolite did not give a definite melting point. It charred and decomposed at around 180°C.

Solubility

The metabolite did not dissolve in most of the organic solvents, such as cyclohexane, benzene, ether, chloroform, acetone or 95%

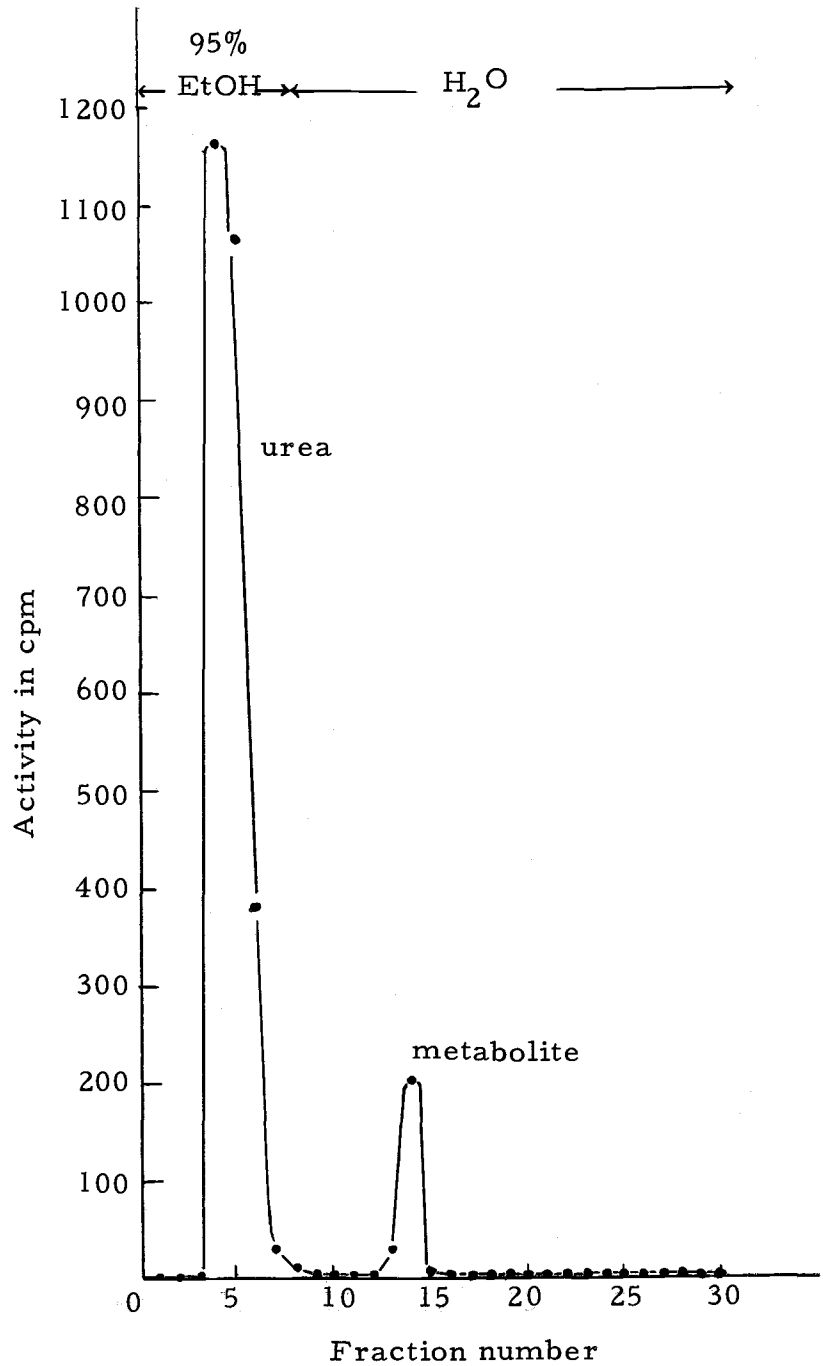


Figure 5. Cellulose column chromatography for the separation of urinary metabolites. Urea was eluted with 95% alcohol and the unknown metabolite, with water. Each fraction equals 5 ml.

ethanol, only dissolved in polar solvents such as water, and slightly dissolved in methanol.

Molecular Weight Determination

The calibration curve of Sephadex G-10 column, packed as described under Method, was constructed by plotting the log molecular weight of the known standards versus the ratio of V_e/V_o (6, 59). Metabolite was calculated to have a molecular weight of approximately 450 from this calibration curve.

Nitrogen Determination

A standard nitrogen calibration curve was constructed by using the known standard ammonium chloride solution, following the method described by M. J. Johnson (25). From the standard curve, metabolite contained approximately 13.5 μg of nitrogen in 200 μg. This gave the metabolite a value of 6.7% nitrogen.

Infra-red Spectra Analysis

A small number of chemicals which were suspected to be closely related to the metabolite had been analyzed by infra-red in an attempt to identify it. These chemical compounds included uric acid, allantoin, alloxan, alloxantin, and urea. From the IR spectra the metabolite resembled urea more than any other compound tested. (Figure 8)

From the above results, the metabolite was very likely a derivative of urea and contained only one nitrogen in its entire molecule. It must be stated, however, that the present investigation failed to

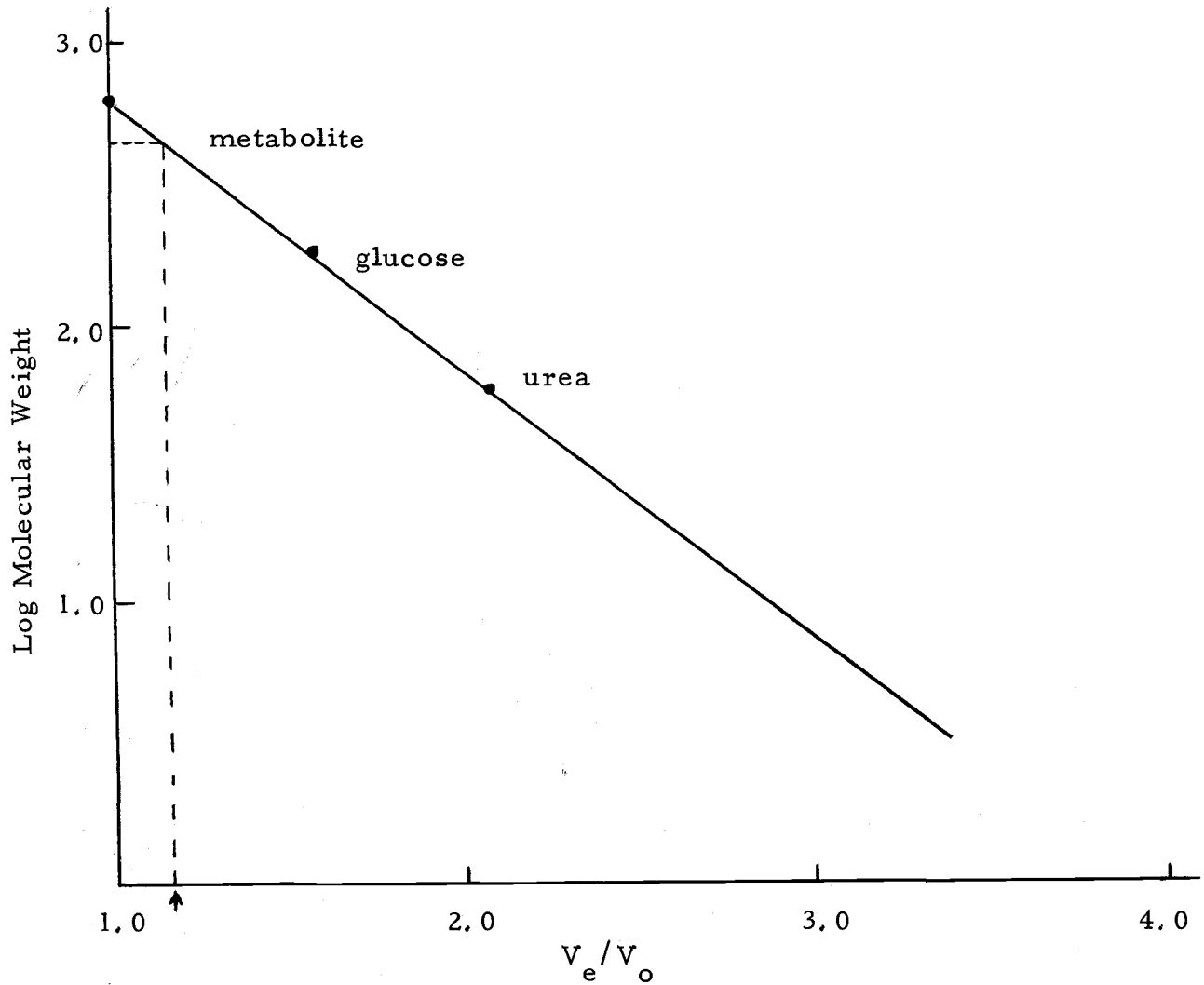


Figure 6. Standard calibration curve of Sephadex G-10 for molecular weight determination.

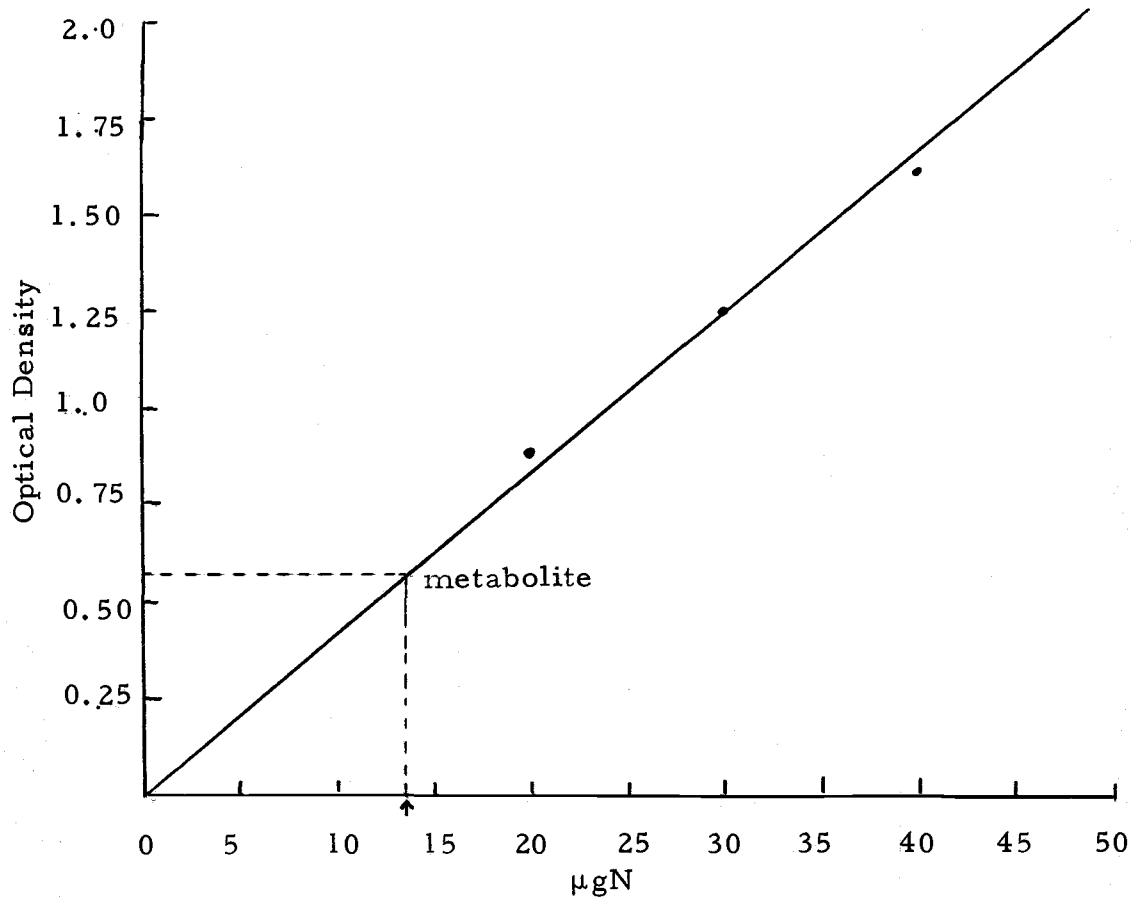


Figure 7. Standard calibration curve for nitrogen determination.

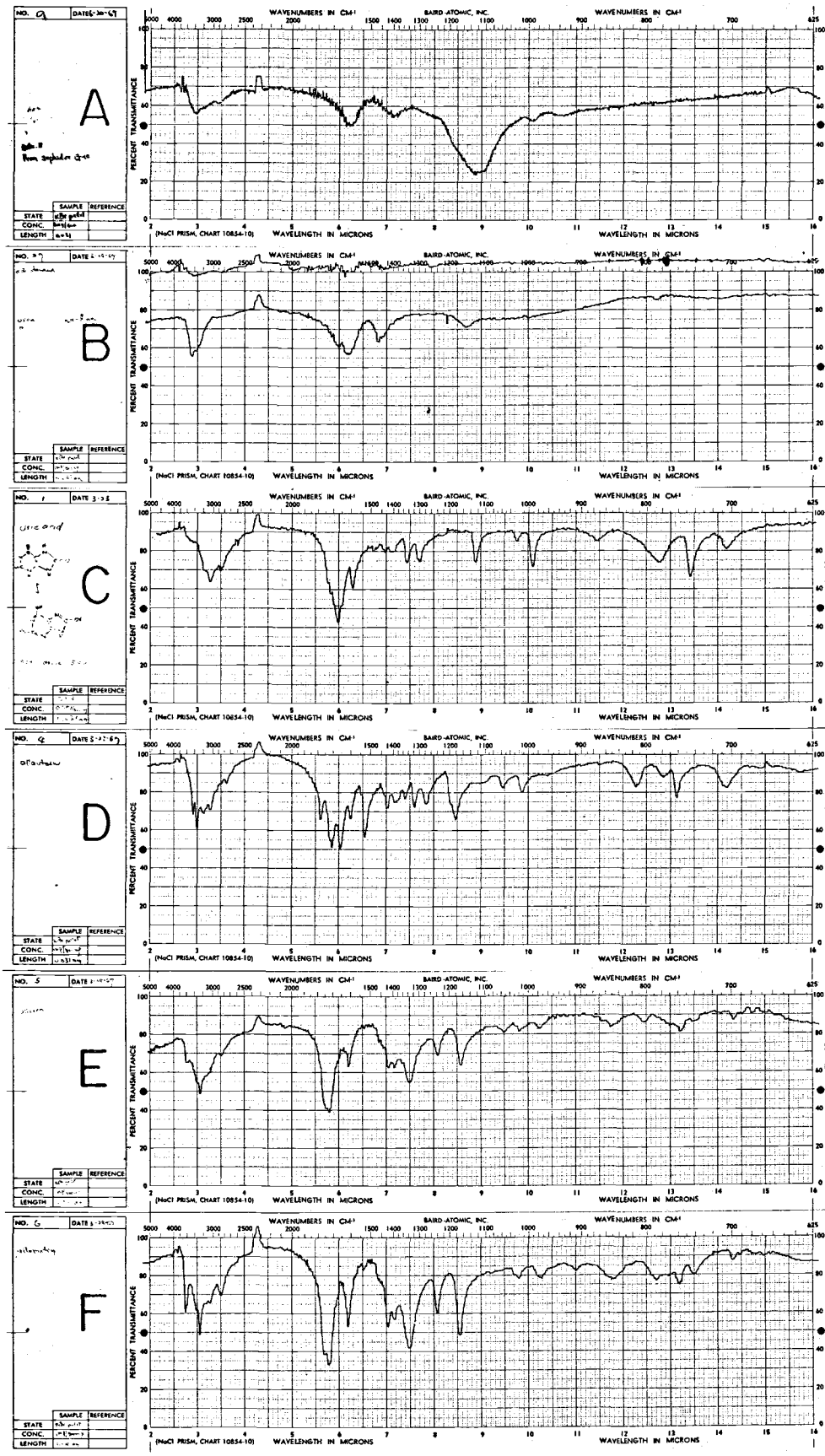


Figure 8. Infra-red spectra of metabolite and some of the chemical compounds.
A. Metabolite. B. Urea. C. Uric acid. D. Allantoin. E. Alloxan.
F. Alloxantin.

reveal the identify of this urinary metabolite.

Effect of DDT on the Active Transport of Acetate-1-^{14}C Across the Intestinal (Jejunum) Wall

From the results of tissue accumulation studies of orally administered acetate-1-^{14}C, it was suggested that DDT might affect the absorption mechanism in the gastro-intestinal tract and thus interferred with the permeability or the active transport of acetate-1-^{14}C across the gastro-intestinal wall. Evidence that intestines are capable of actively transporting some sugars, short-chain fatty acids and amino acids had been reported by many laboratories (5, 15, 20, 49, 61). These investigators used either the conventional loops of intestine or the everted sac technique of Wilson & Wiseman in studying the intestinal transport in vitro. Barry & Smyth (7) have demonstrated the active transfer of acetate and short-chain fatty acids by the rat intestine using the modified sac of everted small intestine of the rat.

In the work presented here, the active transport of acetate-1-^{14}C was carried out according to the procedure described under Method. This relatively simple procedure offered several advantages. It only required a short segment of the rat intestine and thus enabled one to carry out several experiments from a single rat. The solution at the serosal side can be taken out for analysis and refilled quickly at a predetermined time, thus enabling one to study

the rate kinetics. The entire set-up is simple and economical.

The effect of orally administered DDT on the in vitro transport of acetate-1-^{14}C in rat intestine was shown in Figure 9. The intestine taken from the control rats showed a transport of 14.3% of the acetate1-1^{14}C from mucosal to serosal side in two hours time, while under the identical experimental condition, an inhibition of 57% was observed in the intestine taken from the rat which had received 100 mg DDT/kg two hours prior to the dissection (Table 5). Figure 9 also showed that the rates of transport for control and DDT-treated rat intestines were different, and in the case of control, the equilibrium (rate of influx of acetate-1-^{14}C from mucosal surface to the efflux of acetate-1-^{14}C from serosal surface) was reached in 60 minutes, whereas the DDT-treated intestine took almost 100 minutes.

This result supported strongly the foregoing suggested statement, that is, one of the modes of action of DDT is its effect on the in vivo absorption mechanism of the gastro-intestinal tract. Because of its solubility in fats, it is very possible that the lipids of the membrane played an important role in this DDT inhibition of active transport.

Effects of DDT on the Incorporation of Acetate-1-^{14}C into Liver Lipids

The percent of orally administered acetate-1-^{14}C incorporated

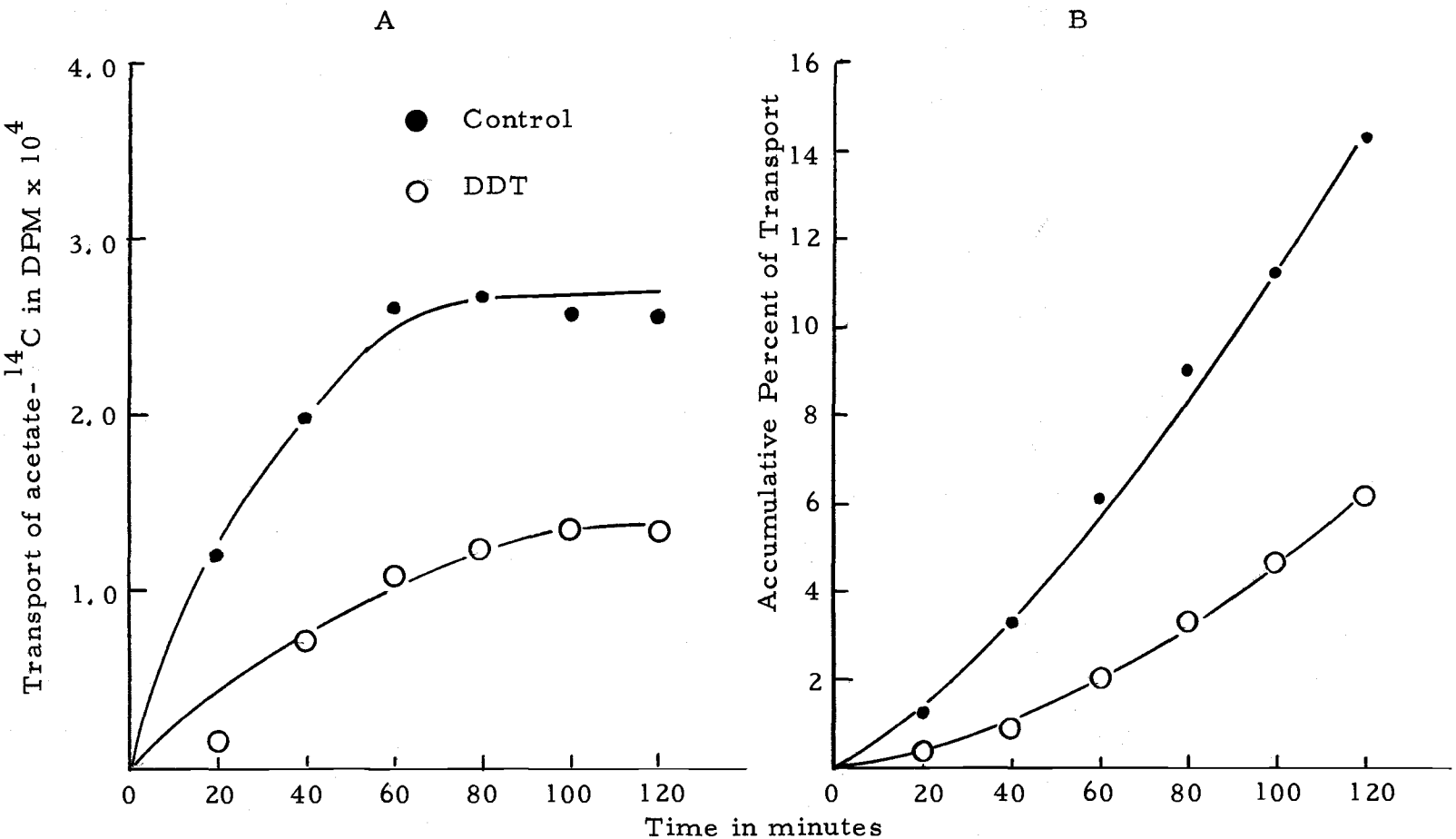


Figure 9. Active transport of acetate-1-^{14}C across the rat intestine for two hours at 25°C.

Table 5. Active Transport of Acetate-1-^{14}C from Mucosal Side of the Rat Intestine to the Serosal Side for Two Hours at 25°C

		Activity in DPM in Mucosal Side		Activity in DPM Incorporated into the Membrane	Combined Activity in DPM Obtained From Serosal Side	% Acetate-1-^{14}C Transported in 2 hrs.	% Acetate-1-^{14}C Oxidized
		Initial	Final				
Control	1	950,000 (21 mμM)	630,000	90,000	142,900	15	9.1
	2	1,200,000 (26.5 mμM)	807,000	133,000	163,000	13.6	8.1
100 mg DDT/kg Two Hours	1	950,000 (21 mμM)	696,000	99,000	49,920	5.3	10.5
	2	900,000 (20 mμM)	673,000	96,000	64,040	7.1	7.5

into liver lipids of rats which had received various amounts of DDT in Wesson oil for four hours was compared with that of control rats which had received Wesson oil only. The results were given in Table 6. Sarett and Jandorf (45) had reported that rats which were chronically intoxicated with oral administration of DDT showed a 25% increase in total lipids, based on the dry weight of liver, and a significant decrease in liver phospholipid and cholesterol based on the percentage of liver total lipid. However, they made no attempt to find out which class of the phospholipids were being affected. The increase of total lipid in rats receiving chronic DDT treatment may be due, in part, to the enlargment of liver size (30% increase according to the authors) since the liver of chronic DDT-treated rats are usually larger than the control.

Table 7 showed that orally administered acetate-^{14}C incorporated significantly less radioactivity into the liver of rats which had received a single oral dose of DDT four hours prior to the administration of the labelled acetate. This was to be expected because DDT had been shown to decrease the absorption of acetate molecule through the gastro-intestinal tract, thus making acetate-1-^{14}C less available to the liver. Table 6 also showed that the incorporation of acetate-1-^{14}C into total lipids of the DDT-treated rats was greatly decreased. However, when the percent of incorporation of acetate-1-^{14}C into the total lipids was calculated based on the

radioactivity in the liver, then Table 7 showed that the total incorporation of acetate-1-^{14}C into liver lipids was increased in the rats which had received DDT treatment. When total lipids were fractionated into neutral and phospho-lipids by silicic column chromatography (Figure 10), it was found that the total incorporation of acetate-1-^{14}C into neutral lipids was increased in the animals receiving DDT and incorporation into phospholipids was decreased. The effects of various amounts of DDT on the incorporation of acetate-1-^{14}C into liver lipids were given in Table 7.

Distribution of Radioactivity in Neutral Lipid Fraction

Neutral lipids eluted from a silicic acid column were concentrated under a stream of nitrogen in a water bath at 45^{o}C and the concentrates were then chromatographed one dimensionally on thin-layer plates (see Method). The results were shown on Table 8. Five radioactive spots were observed and they were identified by comparing them with the known standards. Their Rf values compared favorably with the ones reported in the literature (23). No significant differences were observed in the relative percent distribution of labelled carbon from acetate-1-^{14}C in neutral lipids between the control and 100 mg DDT/kg treated rats except that the radioactivity of monoglyceride in DDT-treated rat liver was a littler lower. But based on the liver radioactivity, DDT-treated rats did show a higher

Table 6. Percent Incorporation of Label in Liver and Liver Lipids from Rats Receiving an Oral Dose of Acetate-1-^{14}C

	Control	100 mg DDT	80 mg DDT	40 mg DDT
Liver	4.5 ± 0.30	2.42	2.90	4.10
Total lipids	2.57 ± 0.27	1.65	1.73	2.59
Nuetral lipids	1.37 ± 0.11	1.08	1.09	1.23
Phospholipids	1.13 ± 0.03	0.52	0.55	0.84

Table 7. Distribution of ^{14}C in Lipids of Rat Liver One-half Hour After the Oral Administration of Acetate-1-^{14}C

	Control	100 mg DDT	80 mg DDT	40 mg DDT
Total lipids	56.4 ± 0.3	68.0	60.3	63.1
Neutral lipids	30.2 ± 0.5	44.5	37.9	41.5
Phospholipids	25.4 ± 1.9	21.3	19.2	20.4

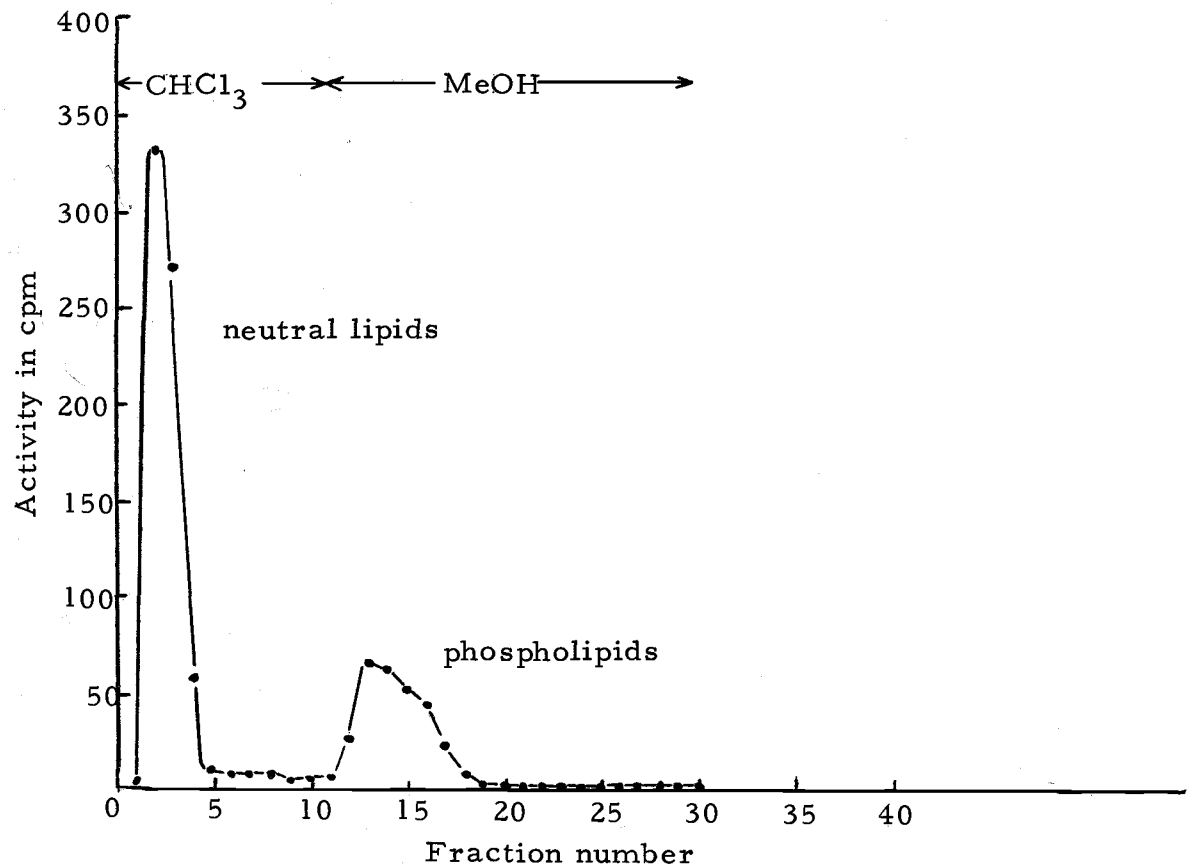


Figure 10. Typical silicic acid column separation of total lipids into neutral and phospholipids.

percentage of incorporation of labelled carbon into neutral lipids. Most of the radioactivity was found in the free cholesterol and triglyceride.

Table 8. Distribution of Percent Radioactivity of Neutral Lipids in Liver Following an Oral Administration of Acetate-1-^{14}C for One-half Hour

Peak	Rf.	Compound	Control	DDT
1	0.00	Monoglyceride	3.81	1.65
2	0.23	Cholesterol	12.00	19.80
3	0.50	Free Fatty Acids	0.39	0.98
4	0.73	Triglyceride	10.08	17.80
5	0.89	Cholesterol Ester	3.02	4.24

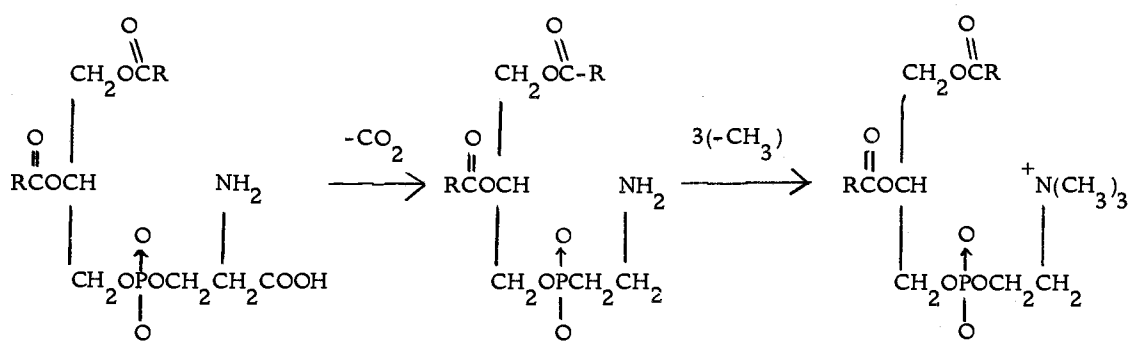
Distribution of Radioactivity in Phospholipid Fraction

After the elution of neutral lipids from the silicic acid column, phospholipids were forced out by methanol. The methanol solution containing the phospholipids was concentrated and chromatographed in a manner similar to the netural lipids. The results were shown in Table 9. Five radioactive peaks were observed and they were identified by comparing them with the known standards, and their Rf values checked well with those in the literature (23). The effects of DDT on the incorporation of acetate-1-^{14}C into liver phospholipids were obvious from Table 9. Most of the radioactivity in the phospholipids of the control rat liver was in phosphatidyl-choline (PC), very

Table 9. Distribution of Percent Radioactivity of Phospholipids in Liver Following an Oral Administration of Acetate-1-^{14}C for One-half Hour

Peak	Rf.	Compound	Control	DDT
1	0.00	Lysophospholipids	8.40	6.81
2	0.25	Phosphatidyl Serine	1.30	10.01
3	0.45	Sphingomyclin	0.84	Trace
4	0.65	Phosphatidyl Choline	14.90	2.70
5	0.92	Phosphatidyl Ethanolamine	Trace	1.62

little in phosphotidyl-serine (PS) and only a trace in phosphatidyl-ethanolamine (PE). When the rats were treated with 100 mg DDT/kg for four hours most of the radioactivity was found to be incorporated into PS and PE while the radioactivity in PC was very much decreased. If one looks closely at the biochemical reactions between these three most common and abundant liver phospholipids, it is immediately obvious that these three compounds are interconvertible through decarboxylation and transmethylation.



The small amount of PE, which was obtained by the decarboxylation of PS, was rapidly converted to PC, the end product, through three consecutive steps of transmethylation. The decrease in radioactivity of PC in the DDT-treated rats could mean that the step of transmethylation was inhibited, but it could also be due to the reduction of radioactivity in choline or free fatty acids or glycerol molecules.

Nature of Radioactivity in Phosphatidyl-serine and Phosphatidyl-choline

The ^{14}C in phosphatidyl-serine, phosphatidyl-ethanolamine and phosphatidyl-choline could either be in the glycerol moiety, free fatty acids moiety, or the individual base moiety. To determine the exact location of this radioactivity, the areas of PS and PC on the thin-layer plates were scraped and extracted with methanol. The extracts, concentrated down to one ml, were hydrolyzed in one percent alkaline methanol for one hour under a reflux. The hydrolyzate was allowed to cool down to room temperature and acidified with 1 N hydrochloric acid. Free fatty acids, which were cleaved during the hydrolysis, were repeatedly extracted out with hexane, and the glycerol-choline or glycerol-serine remained in the methanol phase. After two extractions, all the radioactivity in the hydrolyzate was extracted into the hexane layer. The distribution of the ^{14}C in PS and PC was given in Table 10. It is thus clear that in PS or PC (maybe also PE), it was the free fatty acids which were radioactive, and

little or no radioactivity was incorporated into either glycerol or the bases. Therefore, the decrease of ^{14}C-PC in the DDT-treated rats was not because of the decrease in the availability of ^{14}C-choline or ^{14}C-free fatty acids or ^{14}C-glycerol but rather due to the block in transmethylation and, perhaps, decarboxylation also.

Table 10. Nature of Radioactivity in Phosphatidyl-serine and Phosphatidyl-choline

	PS	PC
DPM in methanol extracts	2040	12300
DPM in methanol extracts after alkaline hydrolysis	1950	12100
DPM in hexane layer	1800	11800
DPM in methanol phase	100	0

SUMMARY AND CONCLUSION

P, p'-DDT given orally to adult female rats, at doses between 40 and 200 mg per kg rat, was shown to have drastic effects on the acetate metabolism. The normal elimination pattern of the expired $^{14}CO_2$ from ^{14}C-labelled acetate was distorted. The rates of elimination, K_I and K_{II}, of the control rats were determined to be 0.52 and 0.033 for acetate-1-^{14}C, 0.40 and 0.013 for acetate-2-^{14}C. When rats received 100 mg per kg for one hour before the administration of acetate-1-^{14}C or acetate-2-^{14}C, K_I changed to a value of 1.08 and 0.42 for acetate-1-^{14}C and acetate-2-^{14}C, respectively. K_{II} was no longer observed; instead, an unsteady rate appeared which fluctuated through the remainder of the experimental period.

DDT decreased the rate of output of $^{14}CO_2$ also. With the control, over 90% of the administered acetate-1-^{14}C or acetate-2-^{14}C was oxidized and recovered as $^{14}CO_2$ in ten hours, whereas only 60-80% was recovered from the rats receiving 100 mg DDT per kg. The animals were able to recover from the intoxication of 100 mg DDT per kg after 72 hours, and 40 mg DDT per kg was found to be the lower limit in order to demonstrate the effect on $^{14}CO_2$ elimination rates.

Orally administered DDT has different effects on the <u>in vivo</u> metabolism of acetate-1-^{14}C and acetate-2-^{14}C in rats, especially

on the second pathway and the incorporation into urinary metabolites, suggesting that the carboxyl and methyl carbons of the acetate molecule go through different metabolic pathways or recycling in the animal body.

The formation of two labelled urinary metabolites, urea and one unidentified compound, was affected by DDT and was dependent upon the dosage level and time of post-administration. An attempt to explain this effect has been made.

No such effects of DDT on $^{14}CO_2$ elimination pattern or the formation of urinary metabolites were observed when labelled acetate was given intraperitoneally, indicating that the active absorption and transport by the gastro-intestinal tract may be involved.

Experiments on intestinal active transport have revealed that DDT decreased the rate of transport of acetate-1-^{14}C across the intestinal wall from mucosal to serosal surface. It is thus concluded that one of the modes of action of DDT is its effect on the absorption mechanism of the gastro-intestinal tract.

DDT reduced the incorporation of acetate-1-^{14}C into liver total lipids. This was because DDT inhibited the absorption and transport in the gastro-intestinal tract and thus made the acetate-1-^{14}C less available to the liver. Based on the dry liver radioactivity, DDT increased the incorporation of ^{14}C into the total liver lipids. This increase in total liver lipid was due to the increase in liver neutral

lipids. However, no significant effect of DDT on neutral lipids was observed. DDT has an effect on liver phospholipids. In the presence of DDT, ^{14}C-phosphatidyl-choline was greatly decreased, while phosphatidyl-serine and phosphatidyl-ethanolamine were increased. Alkaline hydrolysis and subsequent hexane extraction revealed that the radioactivity was in the fatty acids moiety of these phospholipids. It is concluded that the transmethylation reactions between phosphatidyl-ethanolamine and -choline, and to a lesser extent, the decarboxylation reactions between phosphatidyl-serine and -ethanolamine, were affected by DDT.

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