ANALYSIS OF THE DISTRIBUTION OF C^{14}-DDT IN MICE AND ITS EFFECTS UPON HEPATIC MITOCHONDRIAL RESPIRATION

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INTRODUCTION

In recent years much research has been undertaken on the effects of chemical pollutants used by man to help him control his environment. Of all the contaminants, the chlorinated hydrocarbon insecticides are probably more widely distributed than any other synthetic chemical (Wurster, 1969b). Of the chlorinated hydrocarbons, DDT (1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane) is the most common.

DDT was originally synthesized by a German chemistry student, Othmar Zeidler, in Strasbourg in 1874. In 1938 a Swiss chemist, Paul Muller, re-synthesized DDT and discovered its insecticidal action (Leary, 1946). His studies showed that it was effective aginst a number of insects including lice. As World War II broke out, the Swiss offered DDT to the American Army as a potential lousicide.

Throughout history, disease has followed closely the footsteps of war. Like the "Four Horsemen," war is always closely associated with Pestilence--and that Pestilence, despite the biblical version, is more likely to be astride a mosquito than a horse (Leary, 1946). DDT proved to be the answer, a potent weapon against disease carrying insects. The record of disease control established by the American armed services in the war is testimony to the effectiveness of DDT. Its profound effect is evidenced by the return of thousands of men who would otherwise have died somewhere of infectious disease.

Following the end of the war, DDT was made available to the public and was called "the perfect answer" to the bedbug problem as well as most

all other types of lice. Its dramatic effect was manifested by the virtual elimination of malaria from the United States which had had a yearly average of 4,000 deaths and 4,000,000 cases. DDT also proved to be effective in controlling almost all mosquito borne diseases such as yellow fever, sleeping sickness, filariasis, plague and typhus.

Agriculturally DDT was shown to protect crops against a wide variety of insects. DDT rapidly came into use against grasshoppers, bollworms, beetles and practically all other crop pests. It was used to protect tobacco, cotton, and most other commerical crops. It is important to note that DDT emerged during a time of great need and for a time served its master gallantly. It was only after years of research and experimentation that the sword of DDT was double-edged.

Researchers have shown DDT to have high toxicity, great persistence, and a variety of side effects. The residues of DDT seem to be everywhere, in soils never treated with insecticides, in birds and seals that never leave the Antarctic even though DDT has never been used on that continent, and in most other animals and probably all humans. DDT, like the other chlorinated hydrocarbon insecticides, has great biological activity that isn't limited to insects, but includes most all animals and even some plants. It forms suspensions in water and air and tends to adsorb to particulate matter. In this manner it can be transported in the air and come down in the precipitation. DDT is very stable and may remain toxic long enough to affect non-target organisms. DDT is extremely insoluble in water while remaining highly lipid-soluble. Its partition coefficient therefore favors the accumulation of DDT in living tissues. These characteristics are responsible for the worldwide contamination of the food chains which results in increasing concentration up the chain with the highest concentration in the carnivores (Wurster, 1969a).

It is generally agreed that the primary effects of DDT are upon the nervous system, both in vertebrates and invertebrates. The direct toxic action of DDT on the nervous system apparently involves the formation of a complex between DDT and some component of the nerve axon, which at sufficient concentrations of DDT leads to hyperactivity of the nerve, repetitive firing, tremors, and death of the organism (O'Brien, 1967).

It has been shown with radioactive potassium that DDT greatly increases the potassium ion permeability of cockroach nervous tissue (O'Brien, 1967). DDT has been suggested to compete for calcium ions at the nerve synapses in cockroaches (O'Brien, 1967). Many of the typical symptoms such as hyperactivity of the nerve and repetitive firing and tremors, can be induced by subnormal calcium concentrations both in isolated nerves and whole organisms. It has been shown in crustaceans that there is a inverse effect between the calcium ion concentration and the effect of the DDT (Wurster, 1969a). Increased calcium ion concentrations protect the nerve from DDT and decreased calcium increased the susceptibility. It would appear that DDT competes with the calcium ion and interferes with the stabilization of the axon membrane by the calcium.

It was found in 1945 that the treatment of dogs for calcium deficiency by injections of calcium gluconate affected DDT toxicity (Leary, 1946). If the calcium was given before the DDT, it prevented the appearance of the characteristic DDT symptoms. If given afterwards it shortened their duration and enabled the animals to survive.

DDT and dieldrin have been linked to declining populations of sparrow hawks. Diets of DDT and dieldrin affected reproduction by reducing the thickness of the eggshells (Porter and Wiemeyer, 1969). Similar effects have been observed in eagles (Wurster, 1969a).

Residue tests in pheasants following chronic exposures to DDT indicate the increased presence of DDT in egg shells and yolks (Hunt et al., 1969). It was observed that DDT residues can appear in the yolk within one day of the first exposure. Large amounts of DDT in excess of 1000 ppm were observed in some eggs. The analysis of the tissues indicated that the DDT in the brain can reach lethal concentrations. Residue levels thus have some value in diagnosing the cause of death (Hunt et al., 1969). Bird kills from acute poisoning usually result in more male deaths than female, which would be expected since in females the estrogen raises their blood calcium concentration and thus provides increased protection (Wurster, 1969a).

Nerve endings in the rat brain are sensitive to DDT. The DDT selectively inhibits the action of adenosine triphosphatase (Matsumura and Patil, 1969). The toxicity of DDT may result then from its ability to disrupt the transport mechanisms of sodium and potassium ions in the nervous system. The Na^+, K^+, and Mg^{2+}-adenosine triphosphatases are known to be present in biological membranes and are believed to contribute to the active transport of Na^+ and K^+. There is a component of this enzyme system which specifically binds with C^{14}-DDT (Matsumura and Patil, 1969). This indicates the possible involvement of an ATPase in DDT poisoning.

Certain compounds have the ability to stimulate the livers of various animals to produce drug-metabolizing enzymes. Various insecticides have been shown to stimulate hepatic enzyme induction. DDT markedly increases the activity of drug metabolizing enzymes in liver microsomes (Conney, 1967). The action of the enzymes is not restricted to drugs but includes several normal body substrates such as steroid hormones. These insecticide-induced, hepatic microsomal steroid hydroxylases cause hydroxylation of estrogens

and progestational steroids which results in the decreased action of testosterone, estradiol, estrone, and progesterone (Conney, 1967). Relatively small amounts of dieldrin and DDT can increase the rate of metabolism of steroids in pigeons (Peakall, 1967). Dietary levels of 2.5 ppm of DDT have induced microsomal enzymes in rats (Wurster, 1969a).

The induction of hepatic enzymes by the chlorinated hyrocarbons may play a significant role in the decreasing reproduction of certain birds. Abnormally thin egg shells with consequent breakage of the eggs has been implicated. One possible mechanism by which DDT affects eggshell thickness is thought to be the induction of liver enzymes which decrease the estrogen level in the blood. Estrogen controls the deposition of calcium in bone and thus a deficiency of estrogen results in a decrease in the calcium reserve necessary for healthy egg production (Peakall, 1970). Another possible mechanism is the inhibition of carbonic anhydrase, which is an enzyme in the bird shell-forming gland necessary for the delivery of calcium to the eggshell in the oviduct (Peakall, 1970).

It has been suggested that DDT may inhibit some of the endocrine glands necessary in hormone balance and thus upset the mechanism for egg production (Bitman, 1969). Decreased estrogen levels resulting from the induction of liver enzymes by DDT have resulted in delayed breeding by birds which reduces the chances for reporductive success (Peakall, 1970).

DDT also induces the formation of enzymes which metabolize male hormones such as testosterone to polar metabolites which could further enhance the probability of reproductive failures by their failure to acquire secondary sex characteristics (Conney, 1967).

Phenobarbital and chlordane cause morphological changes in the livers as well as enzyme induction (Fouts and Rogers, 1964). The level of activity

of hepatic drug metabolizing enzymes can be associated with the structure of the smooth endoplasmic reticulum. Electron micrographs revealed that phenobarbital and chlordane cause a marked proliferation of the smooth-surfaced endoplasmic reticulum in the hepatic cell (Fouts and Rogers, 1964).

Low level feeding of various chlorinated hydrocarbons cause cytoplasmic alterations in various rat tissues (Ortega et al., 1957). Abnormalities were observed in the fatty inclusion bodies and lipospheres. Both DDT and dieldrin have been shown to cause morphological changes in the livers of rats (Kimbrough et al., 1971). Electron and light microscopy reveal an increase in smooth endoplasmic reticulum and atypical mitochondria. When fed DDT, the livers of rhesus monkeys display an alteration of sinusoidal and hepatic cell permeability (Durham et al., 1963). Liver function did not appear to be impaired, even at dietary concentration of 5000 ppm, although the monkeys developed the typical symptoms of DDT poisoning. Characteristic cytoplasmic inclusions were observed in those animals fed 200 ppm.

Tissue analysis of pheasants following exposure to various dietary regimens of DDT reveal residue levels in whole blood, fat, reproductive organs and eggs (Hunt et al., 1969). Residue in fat appeared to be influenced by the length and level of exposure to DDT. Levels as high as 11,000 ppm were found in a male that was fatally poisoned. The ability of the blood to transport DDT was evidenced by the large amount of DDT deposited in eggs (1000 ppm). Liver residues tended to reflect recent exposure whereas fat residues reflected chronic exposure to DDT. Fecal samples indicated that the DDT was absorbed from the pelleted diets in excess of 95 percent whereas absorption rates from corn oil did not exceed 25 percent.

Tissue storage analysis of monkeys fed DDT indicated no significant differences between sexes (Durham et al., 1963). However, it was noted that monkeys appeared to be unable to convert DDT to DDE. The average daily recovery of DDT and known metabolites in feces and urine accounted for 24.8 percent of the daily intake. DDA was the primary metabolite.

DDT residues have been found in human adipose, liver, blood, kidney, and brain tissue following autopsy analysis (Morgan and Roan, 1970). Higher concentrations of DDE, a metabolite of DDT, have been observed in the whole blood of premature babies (O'Leary and Davies, 1970). This suggests the transplacental passage of DDT.

Measurements of human serum DDT concentrations are capable of reflecting changes in oral DDT intake (Apple et al., 1970). Human serum DDT concentrations are related to environmental DDT exposure and are subject to changes in the external environment (Perron, 1969).

Recent studies on the distribution of C^{14}-DDT in the lobster have shown the highest activity in the hepato-pancreas, with the activity of the heart, muscle and green gland starting high and then decreasing (Guarino and Call, 1971). The distribution and excretion of C^{14}-DDT in the flounder indicate that following intravenous injection, the DDT is rapidly cleared from the plasma (Pritchard, 1971). After 15 minutes, 22 percent of the dose was present in the liver which decreased to 6 percent in one week. The other tissues followed the same pattern. The intra-arterial administration of DDT in Squalus acanthias found initial distribution to all tissues and then storage in organs with high fat concentrations (Dvorchik, 1971). The liver contained approximately 50 percent of the dosage in one hour and essentially 100 percent could be accounted for by 48 hours.

Studies of the distribution of radioactivity in pregnant rats and fetal tissues after administration of C^{14}-labeled insecticides showed that

after 4 hours, the liver, spleen, adipose, and blood had the highest activity. The liver concentration continually decreased whereas the brain activity decreased rapidly and then gradually increased. The activity of adipose tissue continued to increase (Wheeler and Strother, 1971).

This evidence indicates that DDT is metabolized and distributed in a highly efficient manner. When this investigation was initiated, the purposes were to (1) study the distribution of DDT in various organs of the mouse, (2) analyze the relative concentrations of DDT residues in these structures, and (3) to determine the effect of DDT upon hepatic mitochondrial respiration.

MATERIALS AND METHODS

Experimental Animals

The animals used in this study were young adult albino mice between 60 and 90 days in age. The average weight of the males was 26.7 grams and females was 23.0 grams. The parental stock was originally obtained from Kansas State University.

Fractional centrifugation techniques

Animals were sacrificed by means of a sharp blow on the head with a steel rod. The abdominal cavity was quickly opened and the animal allowed to bleed for about 30 seconds by cutting one of the major blood vessels. The liver was removed and rinsed in ice-cold isolation medium (0.25M sucrose), blotted and weighed to the nearest 0.001 gm. A sample of the liver weighing approximately 0.200 gm was placed in a tared 50 ml beaker and dried in an oven at 80°C for 24 hours to determine the percent water present in each sample. The remaining portion of the liver was placed in ice-cold isolation medium, 4 ml of medium for each gram of tissue, and cut into small pieces. This tissue was then homogenized with cooling for 2 minutes with a Potter-Elvehjem homogenizer (Plate 1). The homogenate was centrifuged in the refrigerated centrifuge (International Equipment Co., Model 13-2) at 600 x g (2000 rpm) for ten minutes at 4°C to remove cell debris. The supernatant was decanted and centrifuged at 9000 x g (10,500 rpm) for 20 minutes. The pellet was then resuspended in fresh isolation medium, in a volume equal to the decanted supernatant. The suspension was recentrifuged at 9000 x g for 15 minutes. The supernatant was discarded and the

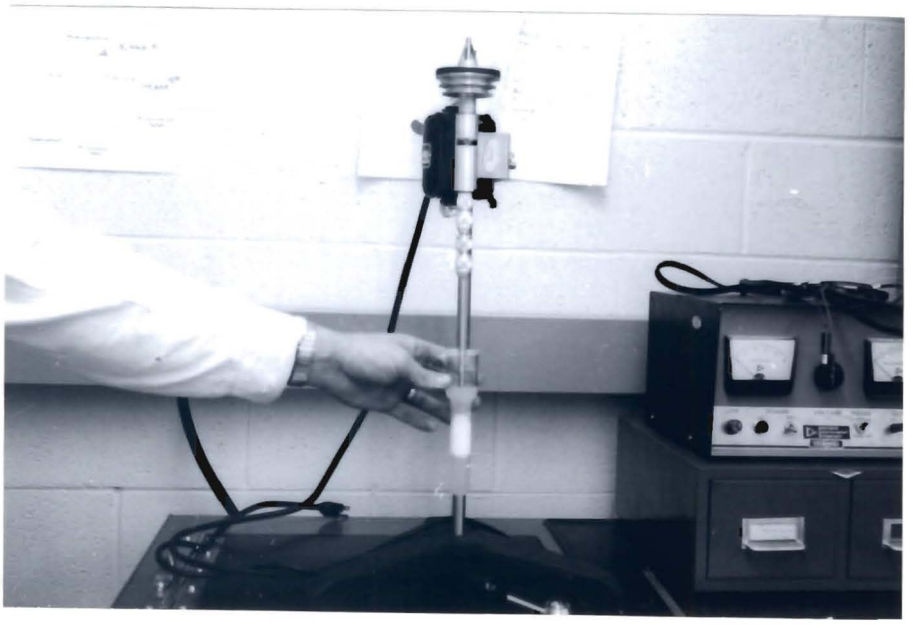


Plate 1: Technique for homogenizing the liver tissue.

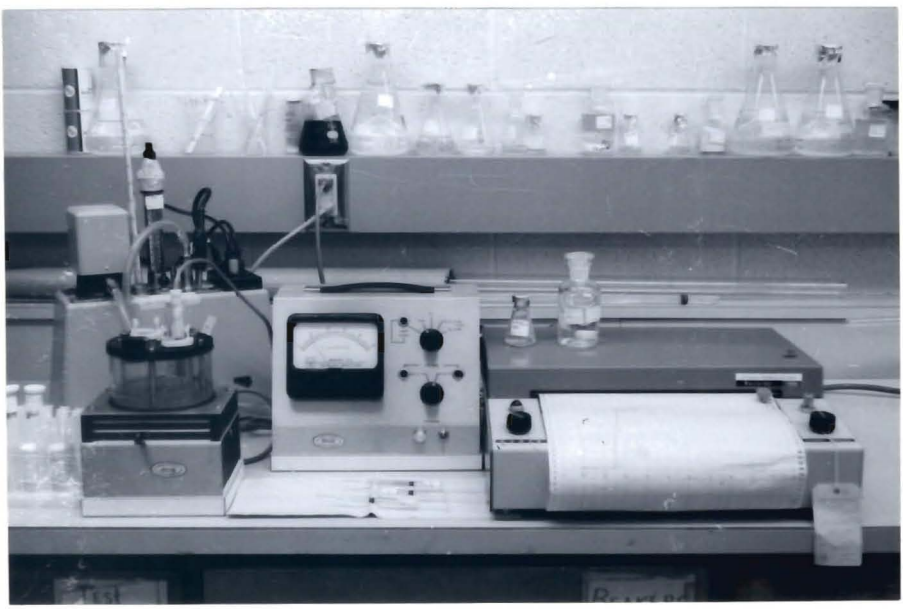


Plate 2: Oxygen polarograph apparatus and recorder.

washed mitochondria resuspended in about 5 ml of cold isolation medium and keptrefrigerated for 24 hours.

Protein Determination Procedure

The relative concentration of the mitochondrial samples was determined by the amount of protein present in each sample. The protein determination was by the Biuret method. Standard solutions of bovine serum albumin were prepared and aliquots of each mitochondrial solution were placed into colorimeter cuvettes containing distilled water and 1% deoxycholate. Biuret reagent was then added to the tubes and allowed to stand for 20 minutes. The absorbancy of the solutions was measured at 540 nm on a Bausch and Lomb Spectronic 20 colorimeter. The known protein concentrations were used to prepare a standard curve from which the protein concentration of the mitochondrial suspensions could be calculated. The volumes of the mitochondrial suspensions were adjusted to contain an average protein concentration of 8 mg protein per ml of homogenate.

Reagents

For isolating the hepatic mitochondria, an isolation medium was used which consisted of 0.25 M sucrose in distilled water. A reaction medium which consisted of 0.1 M sucrose, 0.02 M KCl, 0.003 M Na_2HPO_4, 0.005M Tris buffer adjusted to a pH of 7.5 with HCl, was utilized in the examination of the mitochondrial respiration.

Due to the extremely low solubility of DDT in water, 1.2 ppb (Wurster, 1969b), it was necessary to dissolve the DDT in ethanol. A 10 mM DDT solution was prepared in 95 percent ethanol which was designated as the standard stock solution and dilutions of the stock solution were prepared. The concentration of the standard was determined by the following calculations:

MW DDT = 354

1 M = 354 gm DDT/l
1 mM = .354 gm DDT/l
10 mM = 3.54 gm DDT/l = 354 mg DDT/100 ml
354 mg DDT/100 ml = 3.54 ug/ul
50 ul of 10 mM DDT = 50 ul x 3.54 ug/ul
= 177 ug DDT injected/50 ul
Final Volume in reaction bath = 2.6 ml

177 ug/2.6 ml = 68 ug/ml = 68 ppm DDT = .92 uM DDT

Therefore, the concentration of the DDT stock solution when injected into the system according to procedure was 68 ppm. Dilutions of the standard were carried out to study the effects of desired concentrations.

0.1 M D-gluconic acid (Ca salt) was prepared in distilled water and 50 ul samples were injected into the system.

Oxygen Polarograph Techniques

The mitochondrial respiration and the effects of DDT were determined by means of the oxygen polarograph (Plate 2; YSl Model 53 Biological Oxygen Monitor, Yellow Springs Instrument Company, Inc., Yellow Springs, Ohio). The oxygen polarograph is a device for measuring oxygen uptake in biological systems. In this study, the polarograph measured the rate at which oxygen was removed from the mitochondrial system and allowed the analysis of the manipulated variables affecting the rate.

A Hitachi Perkin-Elmer Recorder model 159 was attached to the polarograph to provide continuous monitoring of the system. The polarograph was standardized at 100 percent oxygen solubility with aerated water and the recorder synchronized with the oxygen monitor. In all experiments, 2.2 ml of reaction medium and 0.3 ml of the mitochondrial suspension were added to the sample chamber and allowed to equilibrate to the temperature of the water bath for at least three minutes. The temperature of the water bath was maintained at 37.4°C which was determined as the average

body temperature of the experimental animals by means of a rectal thermometer. Once an endogenous rate had been determined, 50 ul of substrate (1M Na succinate) was added to the system resulting in the exogenous rate of approximately 16 percent per minute. Aliquots of 50 ul of DDT were added to the system resulting in a final volume of 2.6 ml in the sample chamber.

The sample chambers were rinsed carefully following each trial with 95 percent ethanol followed by rinses with distilled water.

When using DDT, care was taken to wipe clean the electrode face with a tissue to avoid getting a cumulative effect with the DDT residue.

Contamination checks were made of each mitochondrial suspension for bacteria and other organisms by using 1 M glucose as the substrate. Mitochondria, unlike bacteria, mold is unable to utilize the glucose. This was an excellent method of confirming the purity of the suspension.

Preparation of Isotopes

C^{14}-DDT with a specific activity of 23.9 mCi/mM (Amersham/Searle Corporation, Arlington Heights, Illinois) was used to analyze the distribution of DDT in mice. The radioactive compound was originally obtained in a Pyrex glass break-seal type ampoule with the C^{14}-DDT dissolved in benzene to reduce decomposition. The top of the ampoule was cut off and the C^{14}-DDT benzene solution decanted into a 10 ml glass vial using a thin glass rod. The ampoule was then rinsed three times with 2 ml volumes of absolute ethyl alcohol and the washings added to the vial. Corn oil was added to the vial to give a desired final concentration of 5 uCi C^{14}-DDT per 0.1 ml corn oil. The vial was then placed in a steam bath overnight which evaporated off the benzene and ethanol leaving a C^{14}-DDT corn oil solution of the desired concentration. The vial was then sealed and

stored at 10°C until used. The final concentration of the radioisotope was 5 uCi DDT/0.1 ml = 2.09 uM/ml = 740 ug/ml.

Injection Procedure

To study the distribution of C^{14}-DDT in mouse tissue, 24 albino mice were divided by sex into two groups of twelve each. The mice were weighed and removed from food for a period of twelve hours prior to the injection. The C^{14}-DDT was administered intragastrally with a 1 cc syringe to which a four inch length of intradermal plastic tubing had been attached (Plate 3). It was necessary for an assistant to help in this process as the accurate injection of the desired concentration of radioisotope is extremely crucial. Each mouse was allowed to chew on a wooden applicator while the stomach tube was pushed down his esophagus into the stomach. The C^{14}-DDT was administered at a dosage of 2 uCi C^{14}-DDT per twelve grams of body weight at an equivalent of 2.5 ppm DDT per mouse.

Following injection of the radioisotope, the mice were placed in screen-bottomed battery jars to collect the urine. At sequential time intervals, 4, 8, 12, 24, 48 and 72 hours, the mice were sacrificed. The final test sample included two mice of each sex for each time period.

Collection of Tissue Samples

Following the distribution interval for the C^{14}-DDT after injection, the mice were killed by chloroform. The animals were then secured to a dissection board and the abdominal skin of the animal was excised exposing the abdominal muscles. Using a scalpel, a sample of the abdominal adipose tissue was taken and placed on weighing paper. The abdominal cavity was then opened and a sample of the mesenteric adipose tissue taken and added to the previous sample. Liver, brain, kidney, adrenal gland, heart, lung, spleen, and muscle samples were also taken.

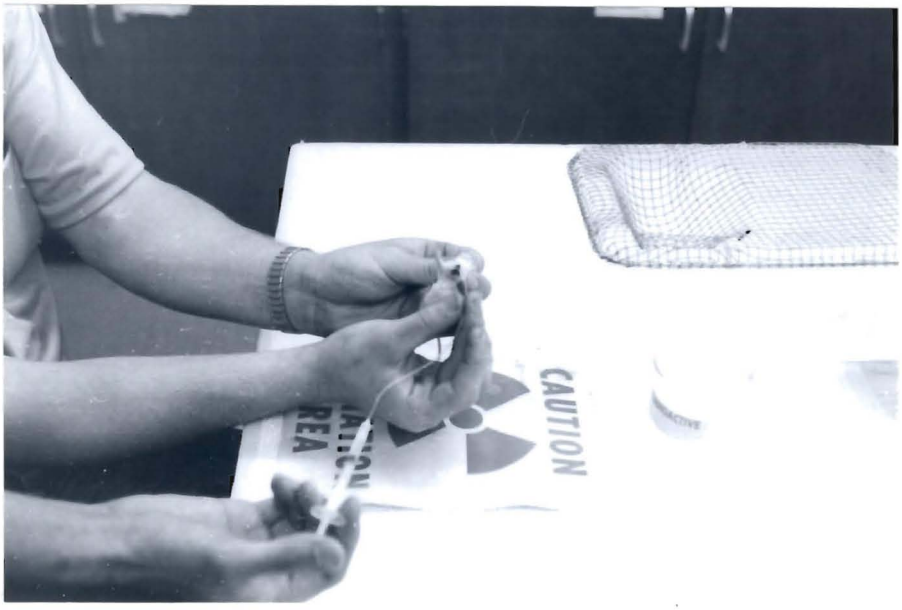


Plate 3: Intragastral injection of C^{14}-DDT via stomach tube.

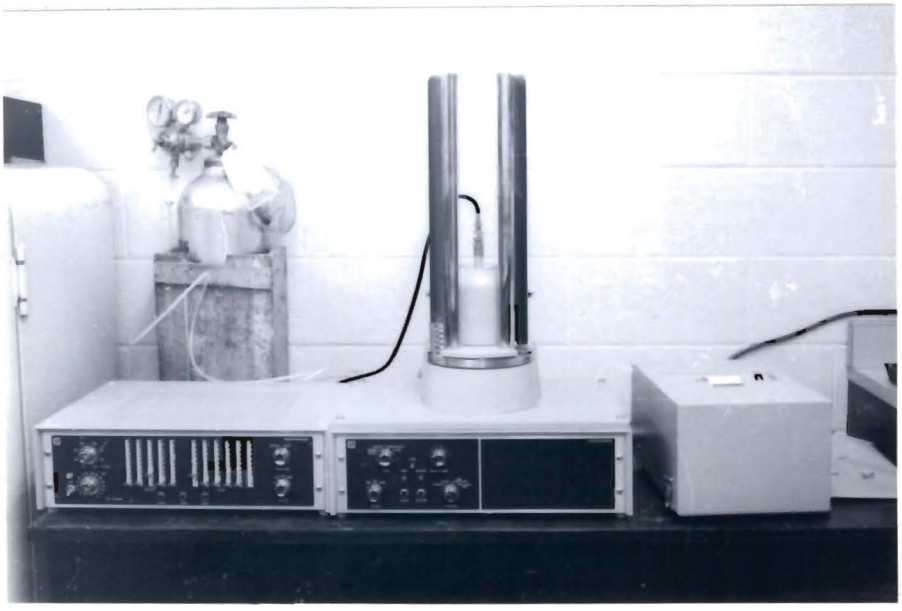


Plate 4: Radioisotope counter

These were rinsed in 0.25 M sucrose to remove as much of the residual blood as possible, blotted and weighed to the nearest 0.001 gm using a Mettler H-8 balance. The tissues were then wrapped in aluminum foil and frozen until they were analysed.

The dry fecal pellets were removed from the battery jars and the jars rinsed twice with 25 ml aliquots of chloroform:methanol (2:1) and then combined for analysis.

Two uninjected mice of each sex were sacrificed and samples of the desired tissues taken, placed in tared beakers, weighed, and dried in an 80°C oven for at least twelve hours. The tissues were then reweighed and the percent water for each tissue calculated. This water determination was then used as the basis for the calculations of the specific activity of each tissue.

Treatment of Radioactive Tissues

For radioactive analysis, each tissue sample was treated by Chase's procedure, which involved digestion of the sample with 5 ml of HNO_3 per gram of tissue for twelve hours and then diluted with distilled water to a final volume of 10 ml (Chase, 1955).

This procedure was used for all tissues except those with high fat concentrations. Following digestion with concentrated HNO_3, the oil in the adipose sample forms a layer and attempts were made to separate the solution into two phases using isoamyl alcohol. However, this technique results in excessive bumping and several samples were lost. The lipid analysis was eventually accomplished by transferring the HNO_3 solution to test tubes and then neutralizing with concentrated NaOH. This procedure results in a visual indication of neutrality. When the pH nears neutrality, the HNO_3 solution, turns from its normal dark yellow to a reddish tan. When neutral, a 5 ml aliquot of hexane was added to the

HNO_3 solution, shaken vigorously, centrifuged, and the hexane layer removed. This was repeated with two more hexane aliquots and the hexane layers combined and stored at 10°C until analyzed. The fecal pellets were pulverized in a mortar and pestle and the C^{14}-DDT extracted with methanol for 24 hours. The methanol was then collected and stored. The urine pools of $CHCl_3$:MeOH were used directly as sample solutions.

Appropriate diutions of the tissue digest samples were placed in aluminum planchets (Amersham/Seale Corporation, Illinois), and dried under an infrared lamp. The dilution of the sample was varied in order to obtain a planchet with a thin layer of residue.

Radioisotope Counting

After the samples had been placed into the planchets and dried, they were assayed radiochemically. The planchet radioisiotope counter (Plate 4) consisted of a gas-flow detector (Nuclear-Chicago Corporation, model 470), a decade scaler (Nuclear-Chicago Corporation, model 8703), and an automatic planchet sample changer (Nuclear-Chicago Corporation, model 1042). The counter had been standardized and was operated at 1200 volts.

The sample planchets were counted three times each for three minutes. These results were averaged to determine the specific activity (counts per minute per gram of dry weight) of each sample. Blank planchets and control planchets containing dry nonradioactive HNO_3 dilutions were counted to determine the background radioactivity for the planchets.

Results

Mitochondrial Respiration

A major problem faced by many investigators working with *in vitro* systems is contamination of the system by unknown organisms. The mitochondrial suspensions were checked for contamination by the addition of glucose to the system. This constant check provided a simple and efficient test for purity. All mitochondrial studies were conducted at 24 hours.

Analysis of the effects of various ethanol solutions was necessary since the ethanol was the carrier for the DDT solutions. Ethanol exhibited no effect upon the mitochondrial system.

The effect of DDT upon the respiration was tested by systematic dilutions of the stock solutions. It is apparent that DDT has a dramatic inhibitory effect at very low concentrations (20 ppm). The effect of DDT upon the Q_{O_2} is a dosage dependent response (Fig. 1). The semilogarithmic plot shows that the effect is exponential which would suggest the binding of DDT with some unknown factor (Fig. 2).

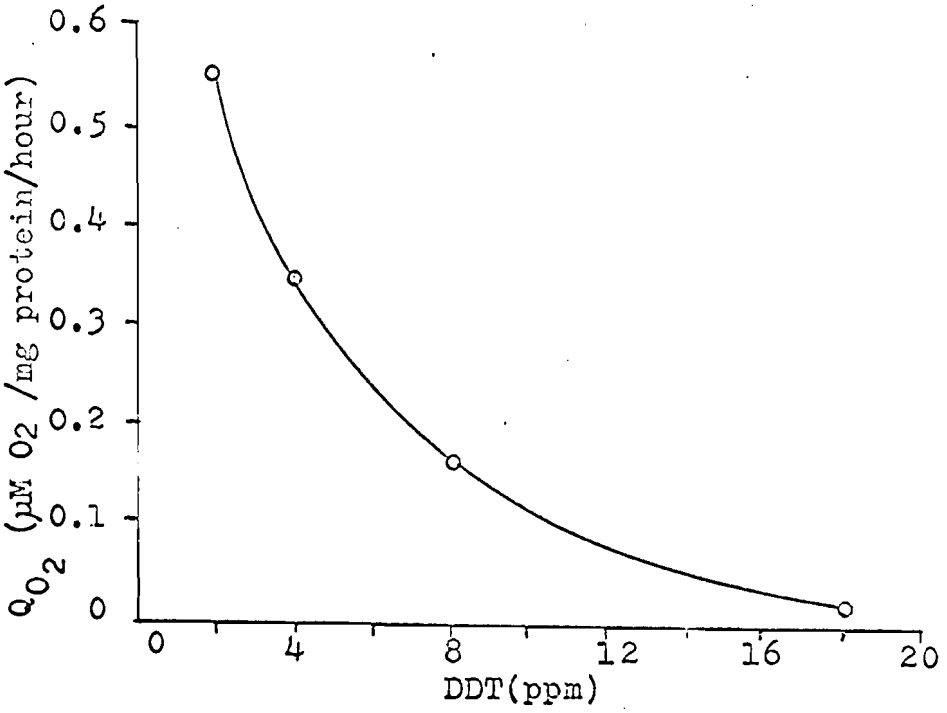
Attempts to neutralize the effect of the DDT with calcium gluconate were unsuccessful. It also was ineffective in protecting the mitochondria from the effects of DDT.

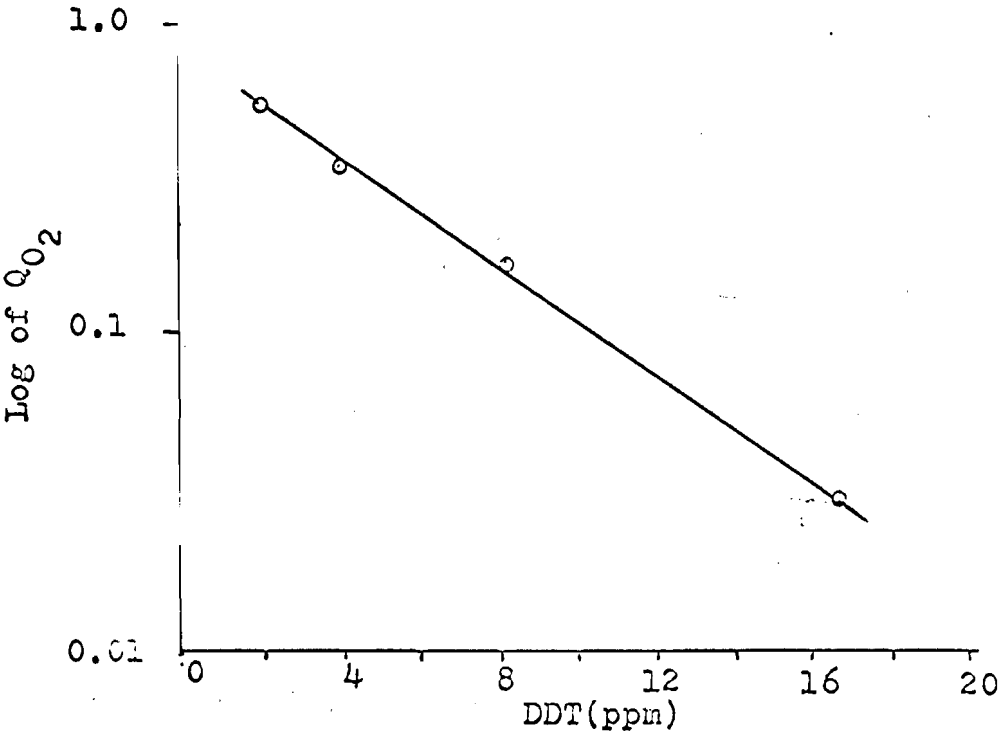
Analysis of C^{14}-DDT Distribution

The tracing of the radioisotope through the mouse indicated that the DDT is rapidly absorbed from the intestine and distributed throughout the body.

Figure 1: Effect of DDT upon hepatic mitochondrial respiration in mice.

Figure 2: Semilogarithmic plot of the effect of DDT upon hepatic mitochondrial respiration.


0.6
0.5
0.4
0.3
0.2
0.1
0
Q_{O_2} (µM O2 /mg protein/hour)
0
4
8
12
16
20
DDT(ppm)


1.0
0.1
0.01
Log of Q_{O_2}
0
4
8
12
16
20
DDT(ppm)

The results from analysis of the liver indicate that the DDT reaches its peak concentration in both sexes by 12 hours and then the amount of DDT decreases (Fig. 3). The decrease probably indicates redistribution to other parts of the body.

The brain concentrations indicate that there is a relatively high initial concentration at four hours which rapidly decreases to a plateau (Fig. 4). Wheeler and Strother, (1971) found that the concentration of methylcarbamate insecticides in the brains of pregnant rats rapidly decreased and then gradually increased.

The muscle tissue had a peak activity at eight hours which then gradually decreased (Fig. 5).

The adipose tissue had a high initial activity with a probable plateau being achieved by 12 hours (Fig. 6). These results were obtained from the female mice as the problem of phase separation resulted in the loss of the samples from the male animals.

The heart had a high initial activity which decreased and then gradually increased (Fig. 7). The obvious question is whether the activity was in the heart itself or in the blood.

The activity of the kidney remained relatively stable (Fig. 8). The differences noted between sexes at 24 hours is probably an artifact resulting from one very low male sample.

The activity of the spleen decreased gradually for 48 hours and then increased sharply (Fig. 9). The sharp increase may be due to the redistribution of the DDT indirectly to the spleen via the blood or lymph.

The lung peaks at 12 hours and then appears to gradually decrease (Fig. 10). A plateau appears to be established by 48 hours.

The examination of the distribution according to sex reveals no significant differences. The comparative study of the tissues, however,

Figure 3: Distribution of C^{14}-DDT in the liver.

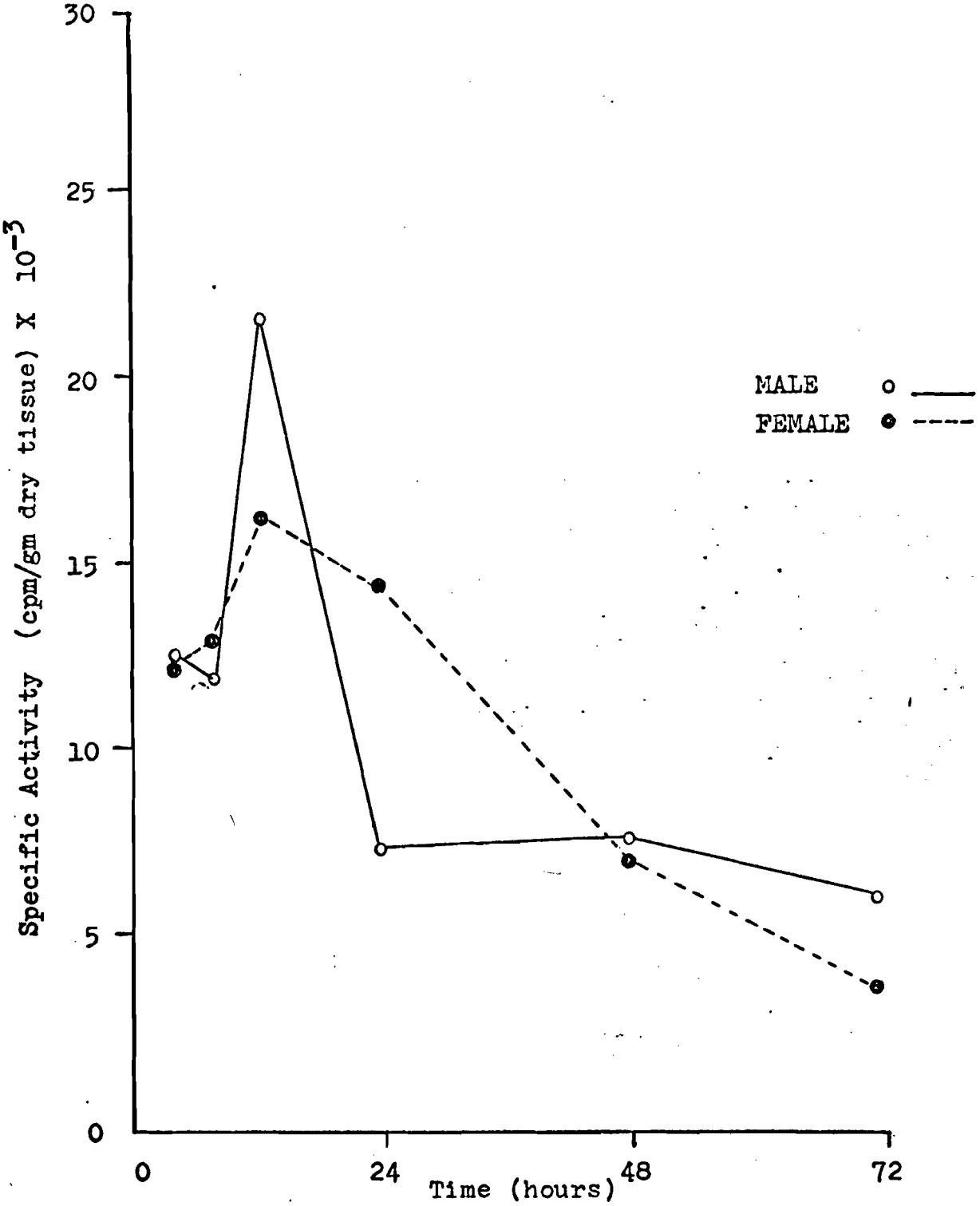

30
25
20
15
10
5
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
0
24
48
72
Time (hours)
MALE
FEMALE

Figure 4: Distribution of C^{14}-DDT in the Brain.

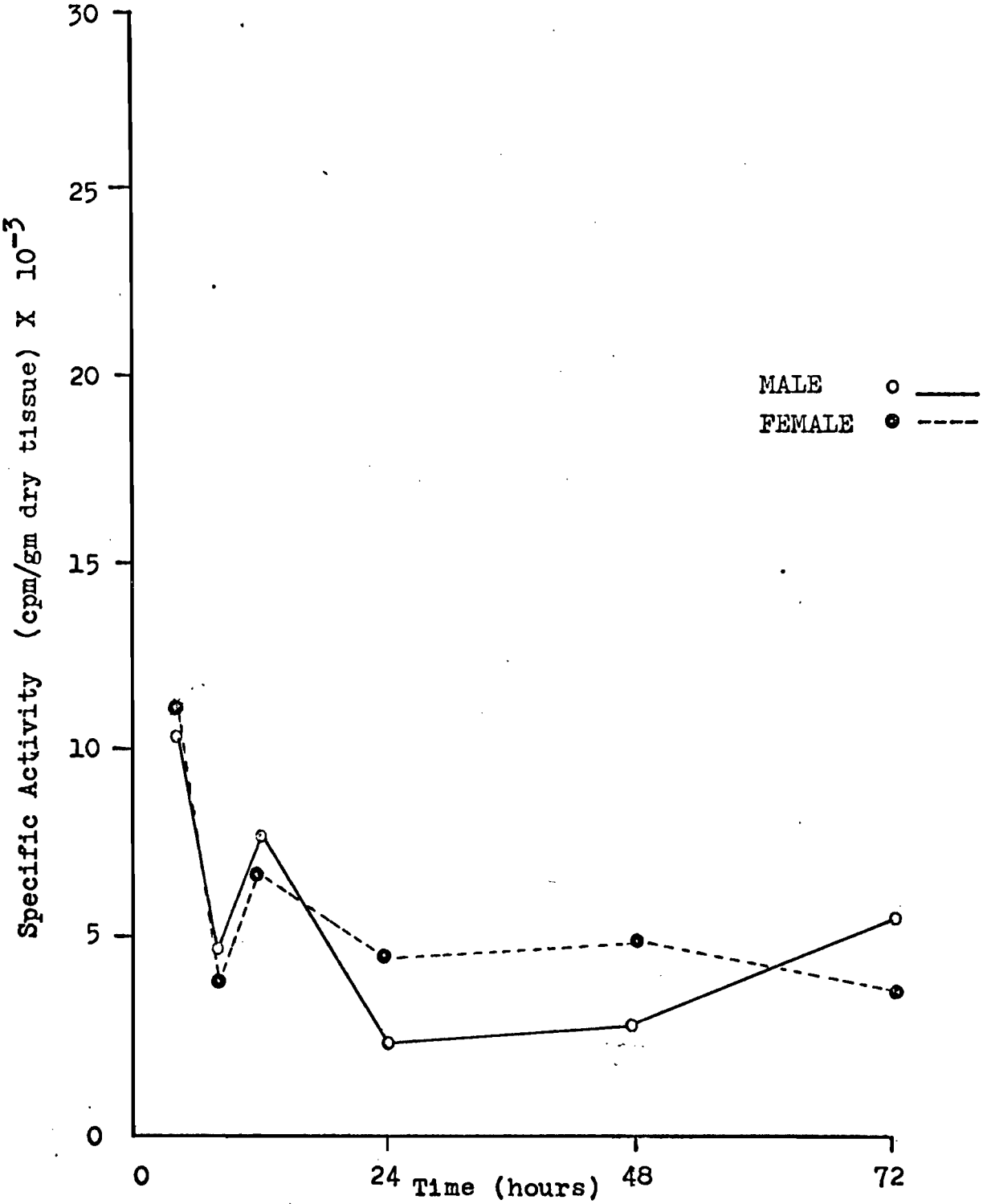

30
25
20
15
10
5
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 5: Distribution of C^{14}-DDT in the muscle.

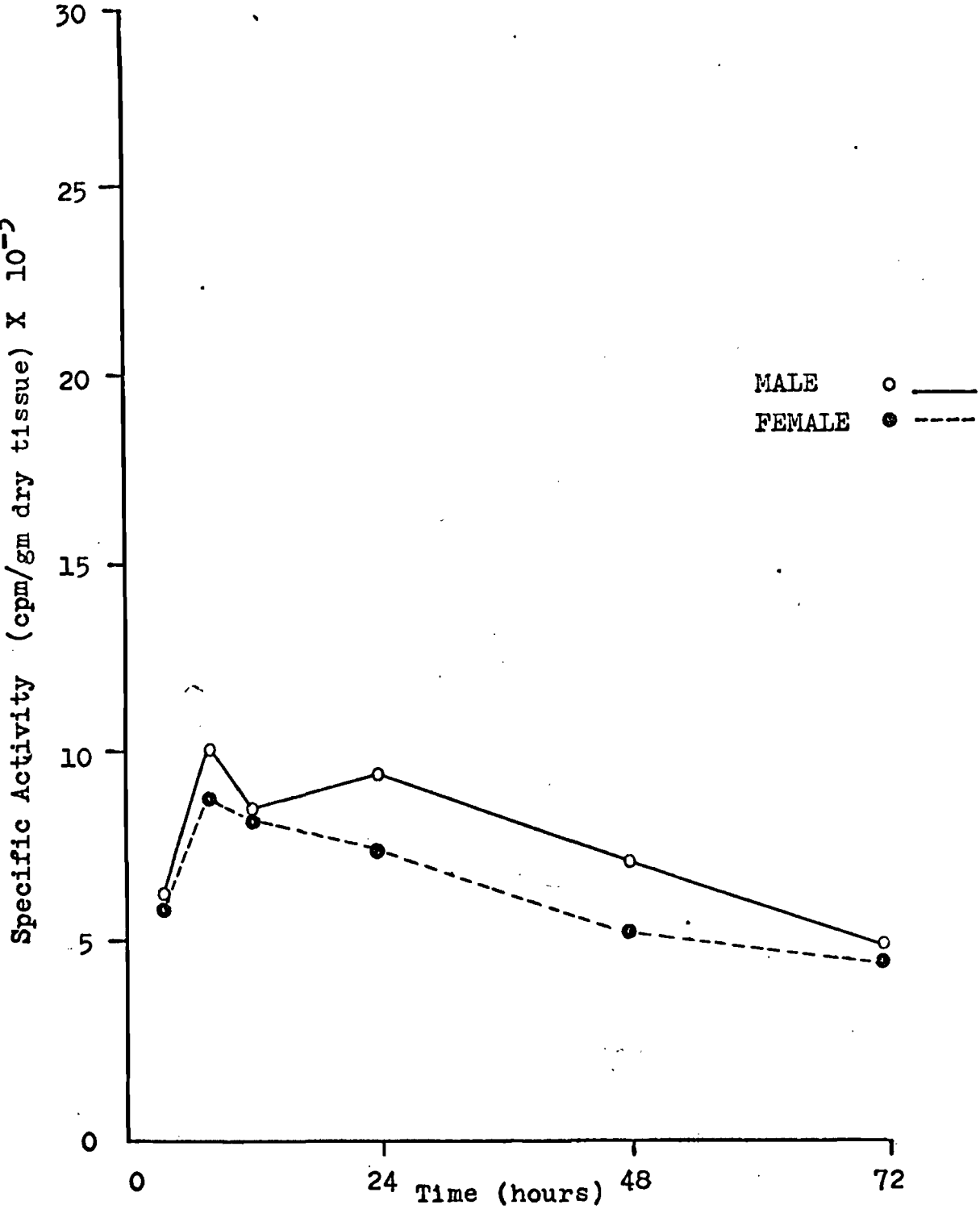

30
25
20
15
10
5
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 6: Distribution of C^{14}-DDT in the adipose tissue

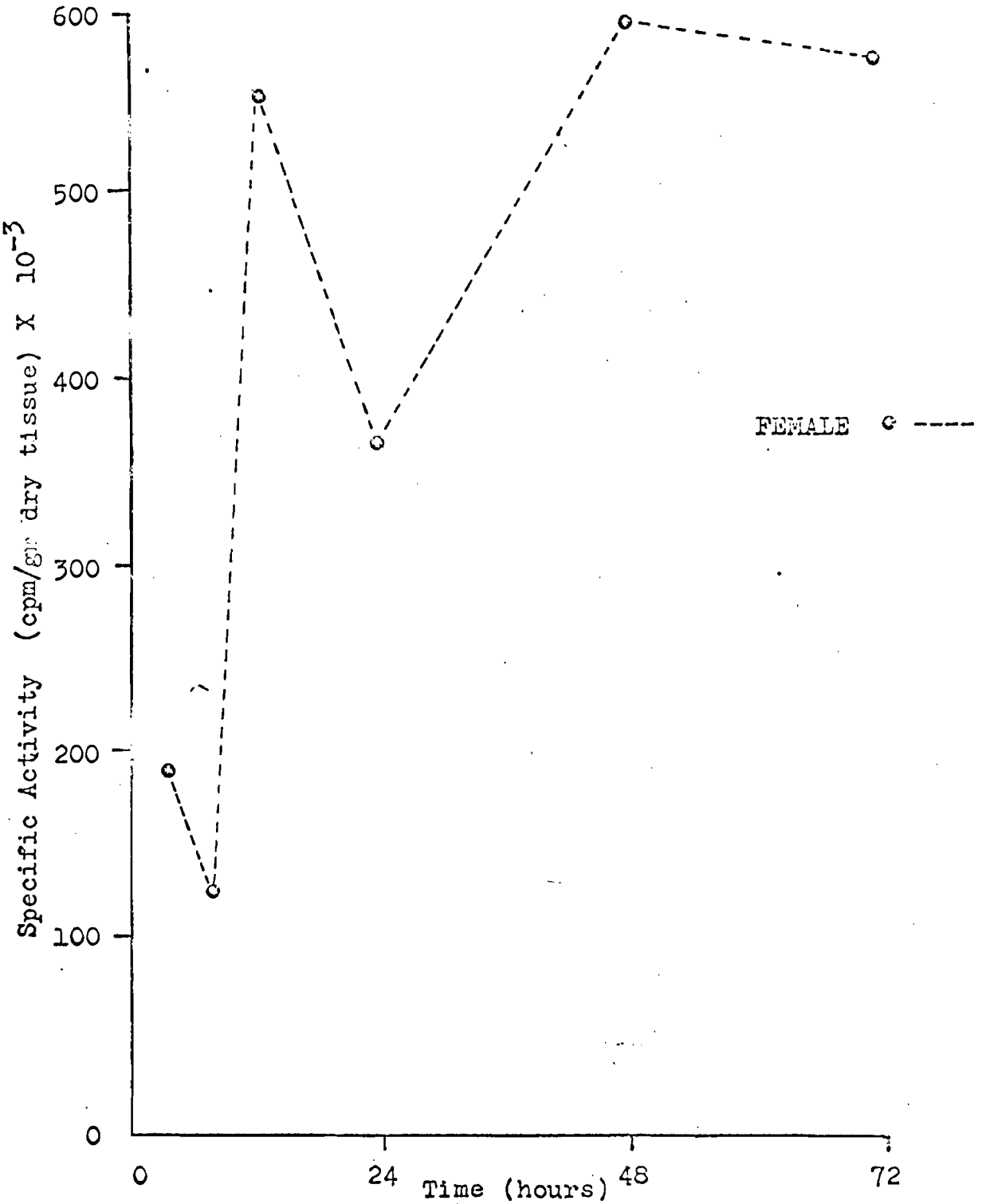

600
500
400
300
200
100
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
0
24
48
72
Time (hours)
FEMALE

Figure 7: Distribution of C^{14}-DDT in the heart.

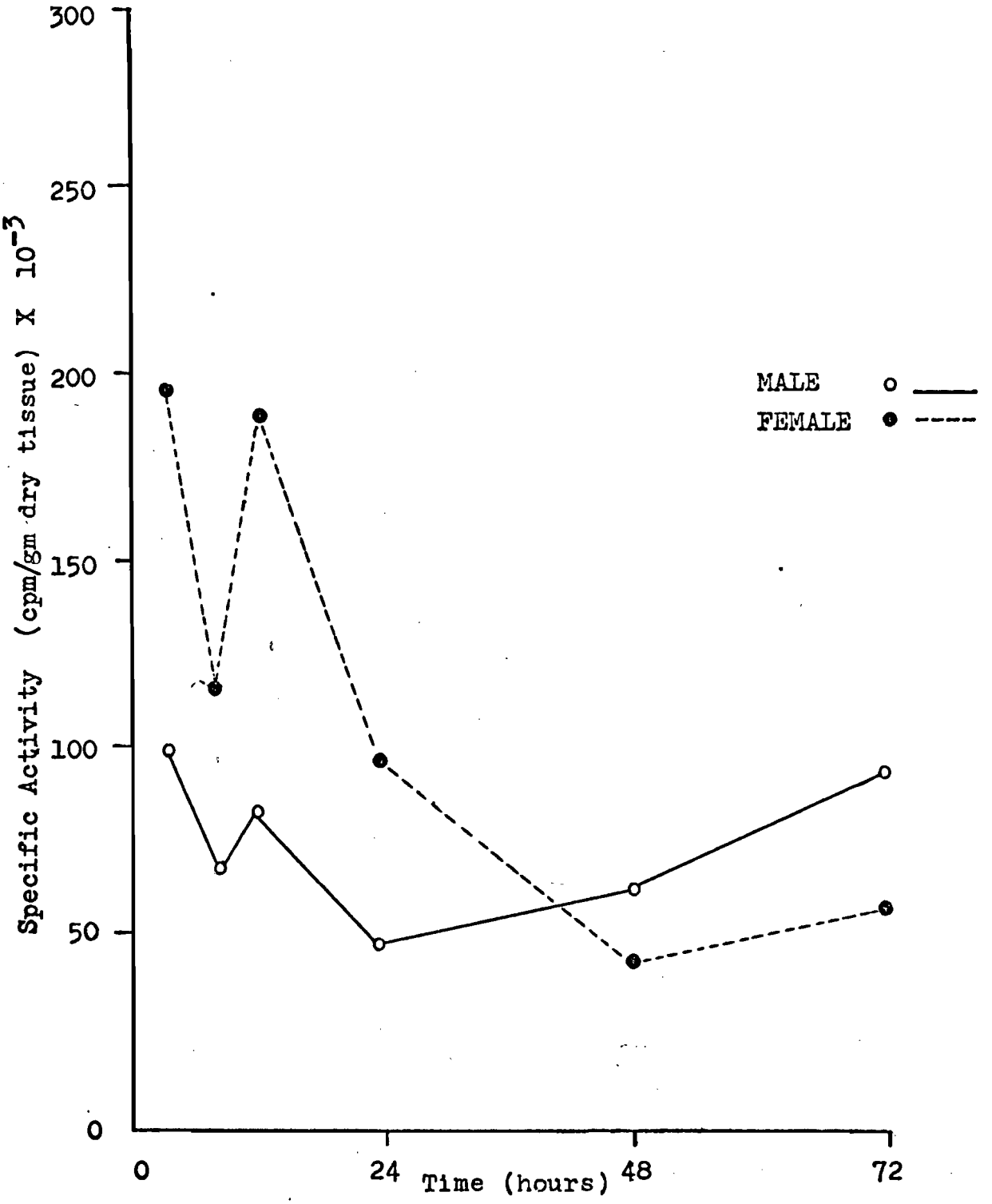

300
250
200
150
100
50
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 8: Distribution of C^{14}-DDT in the kidney.

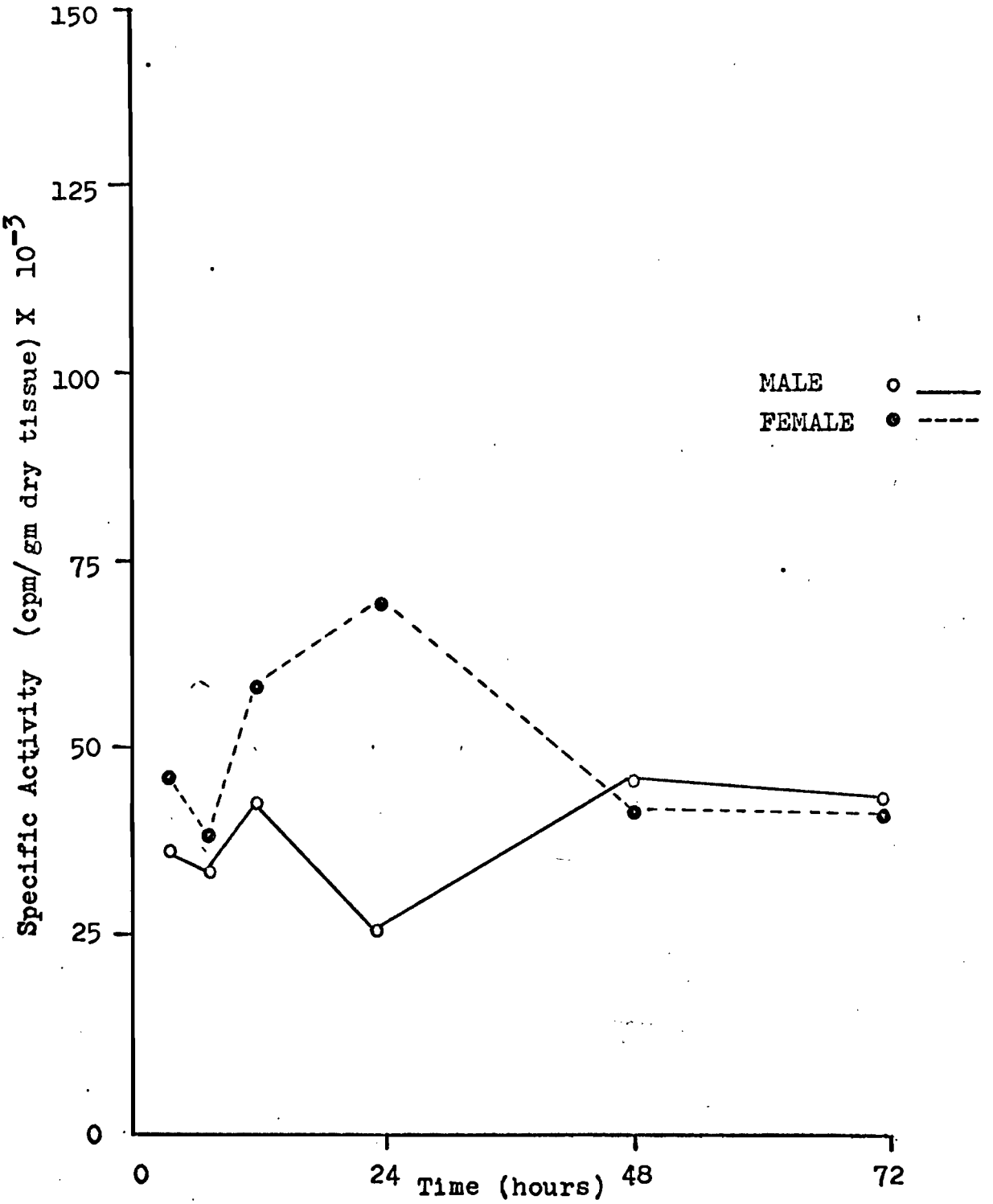

150
125
100
75
50
25
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 9: Distribution of C^{14}-DDT in the spleen.

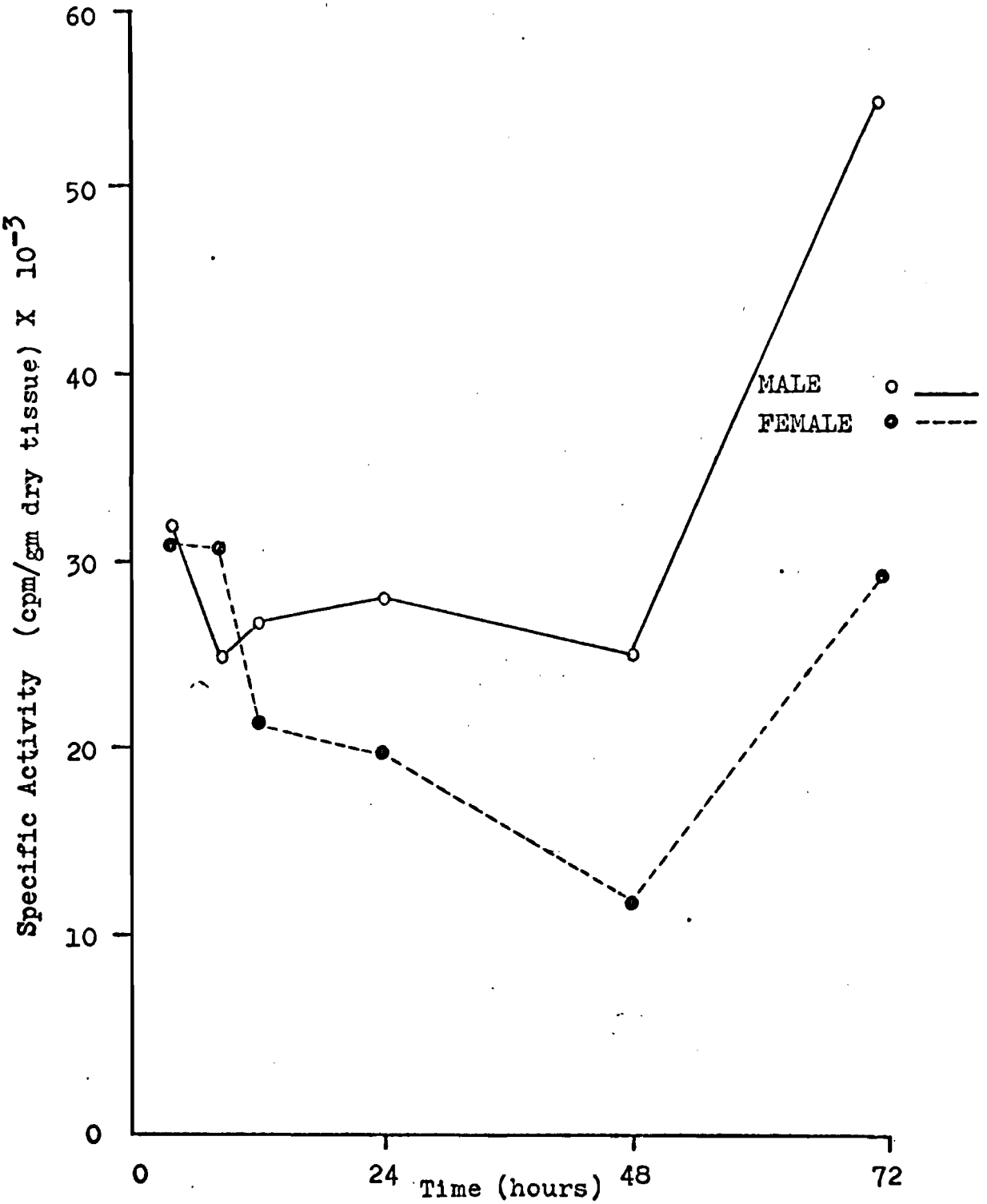

60
50
40
30
20
10
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 10: Distribution of C^{14}-DDT in the lung.

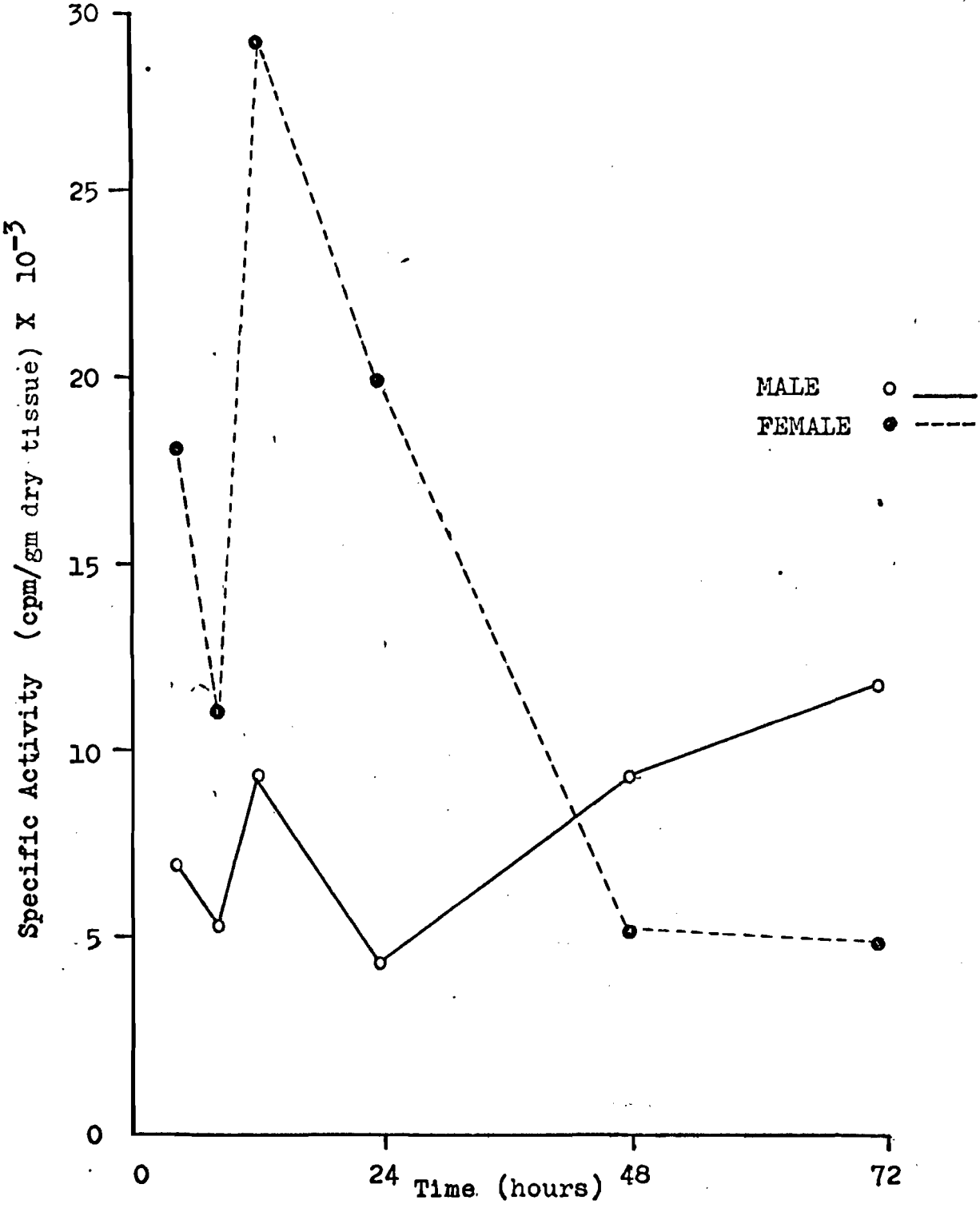

30
25
20
15
10
5
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
MALE
FEMALE
0
24
48
72
Time (hours)

Figure 11: Distribution of C^{14}-DDT in male mice.

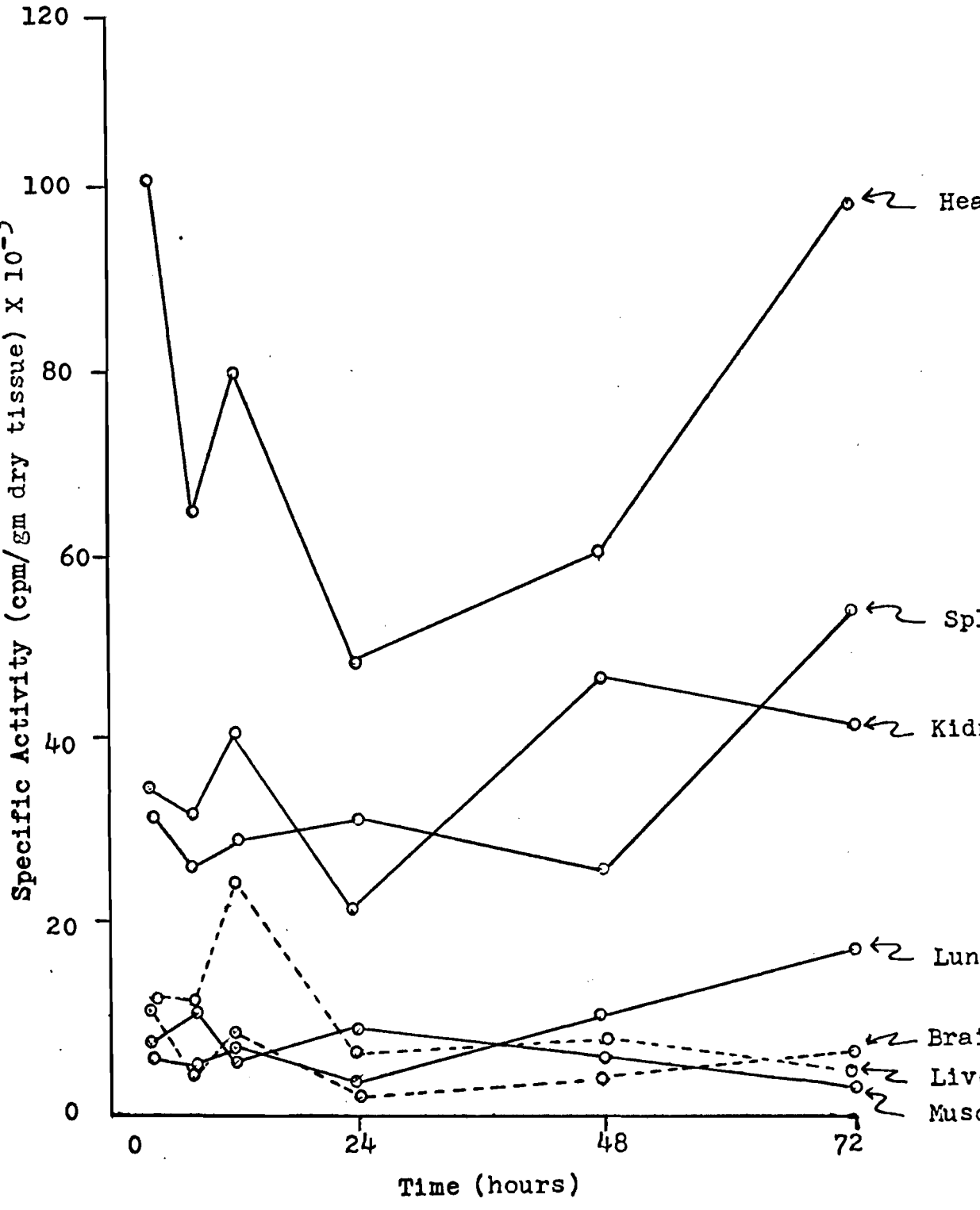
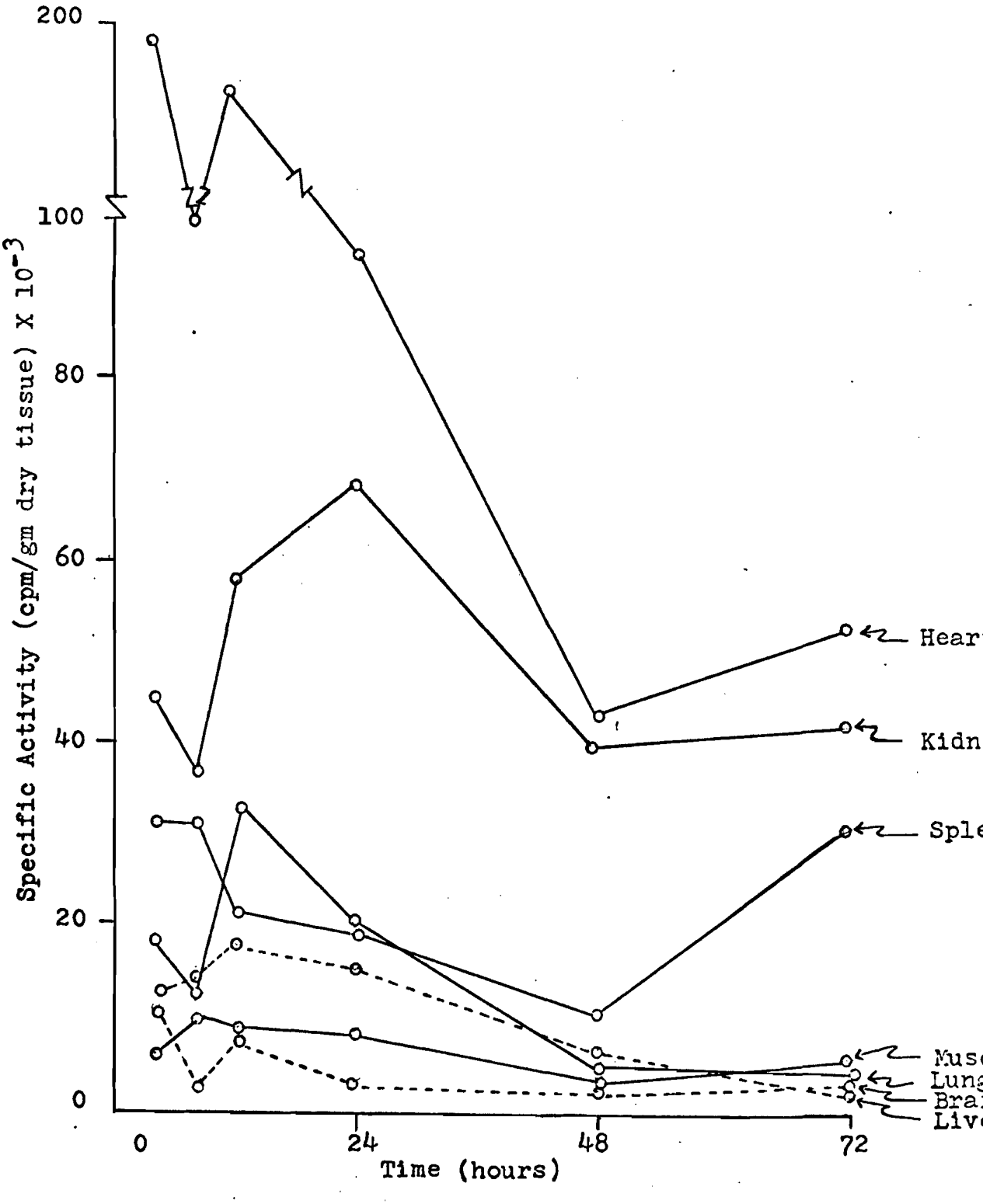

120
100
80
60
40
20
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
0
24
48
72
Time (hours)
Hea
Spl
Kid
Lun
Bra
Liv
Mus

Figure 12: Distribution of C^{14}-DDT in female mice.


200
100
80
60
40
20
0
Specific Activity (cpm/gm dry tissue) X 10^{-3}
0
24
48
72
Time (hours)
Hear
Kidn
Sple
Musc
Lung
Brai
Live

illustrates that the highest activity is found in the adipose with the heart, spleen and kidney having much higher activities than the other organs. It appears that the highest activity is generally found in those organs with the largest blood supply. In some instances there appears to be an inverse relationship between the highly vascular organs and the release of DDT from the other organs (Fig. 11, 12). The exception is the liver which may be explained by the fact that the portal vein was cut to drain the blood from the animals. The correspondingly high adipose activity suggests that the C^{14}-DDT is probably being redistributed throughout the body for fat storage.

The analysis of the urine revealed that by 72 hours seven percent of the injected activity had been excreted. This figure is likely too low as the urine was not removed from the bladders. The fecal samples indicated high activity but the data are inadequate since fecal material from the intestines of sacrificed animals was not analyzed.

These results indicate that the C^{14}-DDT is initially distributed throughout the body to the major tissues. These tissues appear to release the DDT back into the circulatory system where it or its metabolites are redistributed to either the adipose tissue for storage or the urine for excretion.

Discussion

The action of DDT upon the respiratory rate of the hepatic mitochondria appears to be a dosage dependent response. DDT drastically effects respiration at very low concentrations. The evidence indicates that an enzyme is inactivated by binding with DDT; however, the mode of action remains unknown. Although calcium gluconate has been shown to relieve whole animals of DDT poisoning (Wurster, 1969a; Leary, 1946), calcium gluconate does not appear to protect mitochondria from DDT effects. Further studies using fresh mitochondria may provide more information concerning the mode of action.

Temperature changes are known to influence the effect of DDT on cockroaches and rats (O'Brien, 1967). Further studies should explore the possible effects of temperature changes upon the inhibition of mitochondrial respiration. A more sophisticated search for possible enzymes to neutralize the DDT may reveal the DDT complex believed formed at the nerve axon (O'Brien, 1967). Related neurophysiological work may provide valuable information on the possible action of DDT (Fitch, 1971).

The distribution of the C^{14}-DDT in mice indicates that the DDT is rapidly distributed throughout the body. The work of Guarino and Call (1971) with the lobster indicates that highest activity is found in the hepato-pancreas with decreasing activity in the heart, muscle and green gland.

Other studies indicate the rapid distribution of an intravenous injection of C^{14}-DDT to the liver where it evidently is metabolized and redistributed (Pritchard, 1971). Other mammals appear to store DDT in the

adipose tissue. Human adipose tissue has been demonstrated to rapidly store DDT and then reach a plateau concentration (Perron, 1970).

Conditions which tend to suddenly reduce the total body lipid have been shown to cause characteristic DDT poisoning in animals fed chronic amounts of DDT (Hunt et al., 1970).

Studies with pregnant rats have shown the continued increase in activity in the adipose tissue (Wheeler and Strother, 1971). It would appear from this data that the DDT is eventually being deposited in the adipose tissue.

The high specific activity found in the heart may possibly be due to its highly vascular nature. Those organs with large blood supplies seem to exhibit generally high specific activities. Further studies should include the analysis of blood samples. Effective methods for determining DDT and DDT metabolites in blood serum have been developed (Dale et al., 1970). Blood analysis could provide detailed data concerning the distribution of DDT when used along with tissue analysis for the various DDT metabolites. As the DDT is distributed throughout the body, an inverse relationship would be expected between the various organ concentrations and blood concentrations.

This research indicates the need for a systematic investigation of the possible ditribution pathways of an orally administered dosage of DDT (Fig. 13). It is the recommendation of this investigator that future work include the analysis of the blood for distribution through the systemic circulation of both DDT and its primary metabolites and controlled studies of the excretion of metabolites in both the feces and urine.

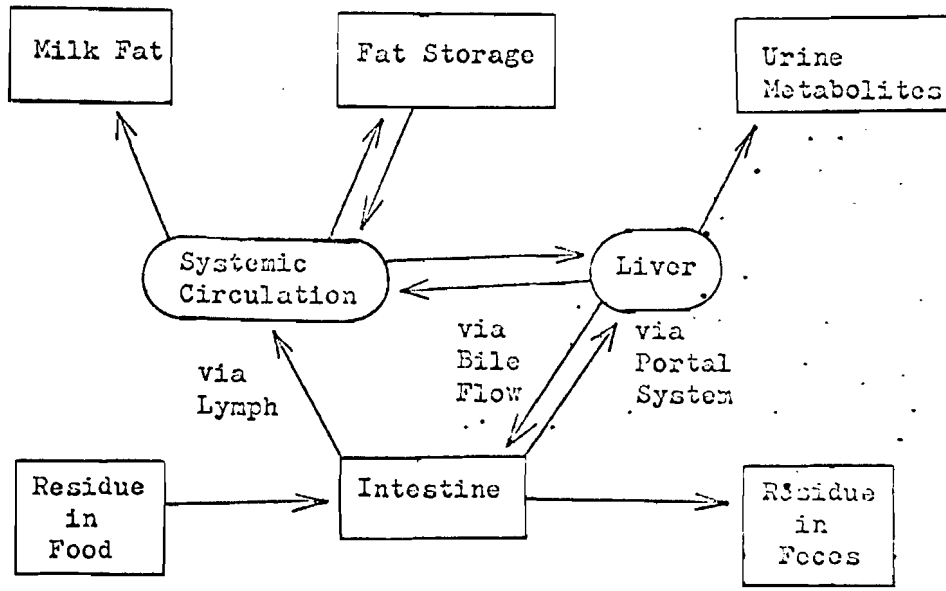


Figure 13: Possible distribution pathways of an orally administered dosage (Modified from Street, 1965).

Summary

Although DDT has been widely used and studied, relatively little is known of its mode of action in cases of DDT poisoning or the manner in which DDT is metabolized within the body.

The effect of DDT upon the hepatic mitochondrial respiration and the distribution of C^{14}-DDT in mice were investigated and the results were as follows:

1. DDT inhibits mitochondrial respiration *in vitro* at very low concentrations.
2. DDT appears to be rapidly distributed to the major organs of the body and eventually stored in the adipose tissue or excreted by the urine.

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