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DIETARY EXPOSURE TO DDT : HORMONAL AND ANTIFERTILITY EFFECTS IN THE RAT

BY.

JOHN G. CLEMENT

A Thesis

Submitted to the Faculty of Graduate Studies through the Department of Biology in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

WINDSOR, ONTARIO, CANADA

1972

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ABSTRACT

Orally administered o,p'-DDT has estrogenic effects on the uterus of immature rats; stimulation of uterine growth is significant, however, only at doses at or above 1000 ppm o,p'-DDT in the diet. At lower doses (100 ppm) both o,p'-DDT and p,p'-DDT reduce the estrogenic response to injected estradiol-17B, probably by stimulating hepatic degradation of the steroid.

Chronic dietary exposure to high concentrations of o,p'-DDT (ca.1000 ppm) over many generations had no effect on fertility, birth weight, sex ratio, or viability of pups. Conversely, low doses of o,p'-DDT (20 to 200 ppm) tended to increase fertility and fecundity but caused a decrease in birth weight and sex ratio; 200 ppm o,p'-DDT increased the number of pups alive at weaning whereas 20 ppm caused a decrease.

p,p'-DDT had no significant effect on fertility, fecundity or sex ratio but decreased the viability of the pups; 500 ppm p,p'-DDT resulted in no offspring reaching weaning age.

Perinatal exposure to high concentrations of o,p'-DDT resulted in decreased fertility and fecundity of F_1 females. Males fertility was not affected. p,p'-DDT had no effect.

Perinatal exposure to DDT induced a hypothyroid condition in animals later in life. Hypothroidism in conjunction with prolonged FSH secretion predisposes the animal to cystic ovaries.

ACKNOWLEGEMENTS

I should like to extend my thanks to Dr. J.E.J. Habowsky and Dr. A.H. Warner, Department of Biology, and Dr. D. Schmidt, Department of Chemistry, at the University of Windsor, for their help on this thesis. I am especially thankful to Dr. A.B. Okey, Department of Biology, University of Windsor, for his guidance during the course of my research and during the preparation of this thesis.

I would like to extend a special thanks to my wife Margaret for her help in the preparation of this thesis and her encouragement during the course of my work.

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INTRODUCTION

DDT is ubiquitous in the ecosystem and such a stable compound that this study was undertaken to assess what physiological effects dietary administration of DDT isomers has on the laboratory rat.

Technical DDT (that which is commercially available) is composed of approximately 80% p,p'-DDT ((1,1,1-trichloro - 2,2-bis (p-chlorophenyl) ethane)) and 15 to 20% o,p'-DDT (1,1,1-trichloro -2- (o-chlorophenyl) -2- (p-chlorophenyl) ethane)), as well as trace amounts of p,p'-DDE ((1,1-dichloro -2,2- bis (p-chlorophenyl) ethylene)).

Recently it has been shown that o,p'-DDT is estrogenic, stimulating premature vaginal opening and an increase in wet weight, dry weight, glycogen, water content, RNA and protein in the uteri of rats and oviducts of chicken and quail; p,p'-DDT has been found to be only weakly estrogenic (Bitman et al., 1968; Cecil et al., 1971; Duby et al., 1971; Levin et al., 1968; Welch et al., 1969; Wrenn et al., 1970). Little information was available in the literature evaluating whether high environmental doses of o,p'-DDT are hazardous. The concentration at which o,p'-DDT produced a significant estrogenic response in a typical dose response test was initially investigated.

Xenobiotics (foreign substances), such as DDT, cause induction of hepatic microsomal enzymes (mixed function oxidases, which are responsible for the metabolism of drugs

and steroids). Induction is manifested by an increase in liver weight and protein (Sanchez, 1967; Morello, 1965) as well as a decrease in plasma half-life of drugs and steroids. Even though o,p'-DDT is metabolized and excreted very quickly and doesn't accumulate in the body, (Bitman et al., 1971b; Feil et al., 1971) is as effective an enzyme inducer as p,p'-DDT. Therefore, o,p'-DDT may act as an antiestrogen. Anitestrogens are substances that act upon target sites to prevent estrogens from expressing their activity.

A number of investigators have found that DDT causes a decrease in fertility and fecundity in laboratory rats and mice (Bernard and Gaertner, 1964; Ware and Good 1967) whereas other investigators found no decrease in fertility or fecundity in animals similary exposed (Duby et al., 1971; Ottoboni, 1969; van Tienhoven and Duby, 1972).

DDT crosses the placental barrier in rats (Backström et al., 1965). Ottoboni and Ferguson (1969) found concentrations of DDT as high as 680 ppm in the milk of lactating dams chronically exposed to DDT. Hypothalamic damage results from exposure to high doses of o,p'-DDT early in postnatal life resulting in a persistant estrus condition at 120 days of age which is characterized by a cornified vaginal epithelium and an anovulatory state. Heinrichs et al. (1971) discovered that neonatal exposure to high concentrations of o,p'-DDT resulted in cystic ovary formation later in life. Therefore, I tested the effects chronic oral exposure to DDT had on fertility and fecundity of progeny perinatally exposed to DDT.

Bakke and Lawrence (1965) discovered that administration of thyroxine (T_4 or tetraiodothyronine) to newborn rats will induce a state of hypothyroidism later in life. DDT and thyroxine are similar in structure (Appendix I); thus resting metabolic rates were measured in order to assess the functional status of the thyroid gland after perinatal exposure to DDT. Hypothroidism in conjunction with human chorionic gonadotropin has been shown to produce polycystic ovaries in rats (Callard and Leathem, 1965).

CHAPTER 1

ESTROGENIC PROPERTIES OF o,p'-DDT IN THE IMMATURE FEMALE ALBINO RAT

DDT is similar in configuration to a synthetic, non-steroidal estrogen, diethylstilbestrol (DES). Welch et al., (1968[a]) discovered that o,p'-DDT present in technical DDT possessed estrogenic activity when injected into immature female rats. p,p'-DDT was found to be only weakly estrogenic. In immature rats o,p'-DDT has produced premature vaginal opening and increases in uterine glycogen, wet weight, water and RNA content similar to those elicited by estradiol (Bitman et al., 1968; Cecil et al., 1971; Duby et al., 1971; Fisher, 1952; Welch et al., 1969; Wrenn et al., 1970). However no experiments have investigated the concentration at which o,p'-DDT, administered orally in the food, produces a significant estrogenic response in the immature female albino rat uterus.

Vaginal Smears

Premature vaginal opening and appearance of cornified vaginal smears are two very sensitive indicators of estrogenic activity. The animals were checked daily for vaginal opening and records kept as to the age when it occurred. Vaginal lavage was performed on the day following vaginal opening using a pasteur pipette with a fire-polished tip. A small amount of distilled water was gently expelled into and aspirated from the vaginal cavity. The water was placed onto a microscope slide and allowed to air dry. The glass slide was placed in methyl alchol for 5 seconds, allowed to dry and stained with Giemsa stain for 15 minutes. Excess stain was rinsed off with water. The slides were dried and examined under the light microscope. During the estrous phase of the reproductive cycle, when the estrogen titre is highest in the blood, large cornified, enucleated epithelial cells are found in the vaginal smear. Body weights were taken daily.

RESULTS AND DISCUSSION

An increase in glycogen content is one of the metabolic changes which estrogen induces in the rat uterus. Bitman et al. (1965) indicated that the uridine diphosphoglucose - glycogen system was responsible for endogenous glycogen synthesis and that phosphorylase "a" was primarily responsible for glycogen breakdown.

Estrogens stimulate an increase in uterine wet weight, dry weight and protein by stimulating hypertrophy and hyperplasia of all uterine tissues concomitant with an increase in uterine amino acid uptake, nucleic acid synthesis and protein content.

Uterine wet weight, dry weight, glycogen and protein were plotted versus the Log_e x dose (Fig. 1). A linear dose - response was obtained over the entire range tested with 3000 ppm of o,p'-DDT not eliciting a maximum response. There was no statistical difference between the control and o,p'-500 ppm group; however, a statistical difference ($p<0.05$) was obtained at the o,p'-1000 ppm level in all parameters tested, except in the case of uterine protein where a statistical difference was not obtained until the o,p'-200 ppm level. No statistical differences in uterine water content were obtained (Table 1).

Diethylstilbestrol caused stimulation of ovarian tissue and growth of follicles in immature female rats. Smith (1961) discovered that the ovarian weight gain produced in intact immature female rats by a small dose of DES was missing in

FIGURE 1

LOG-DOSE / RESPONSE RELATIONSHIP BETWEEN DIETARY o,p'-DDT (ppm) AND UTERINE GROWTH PARAMETERS

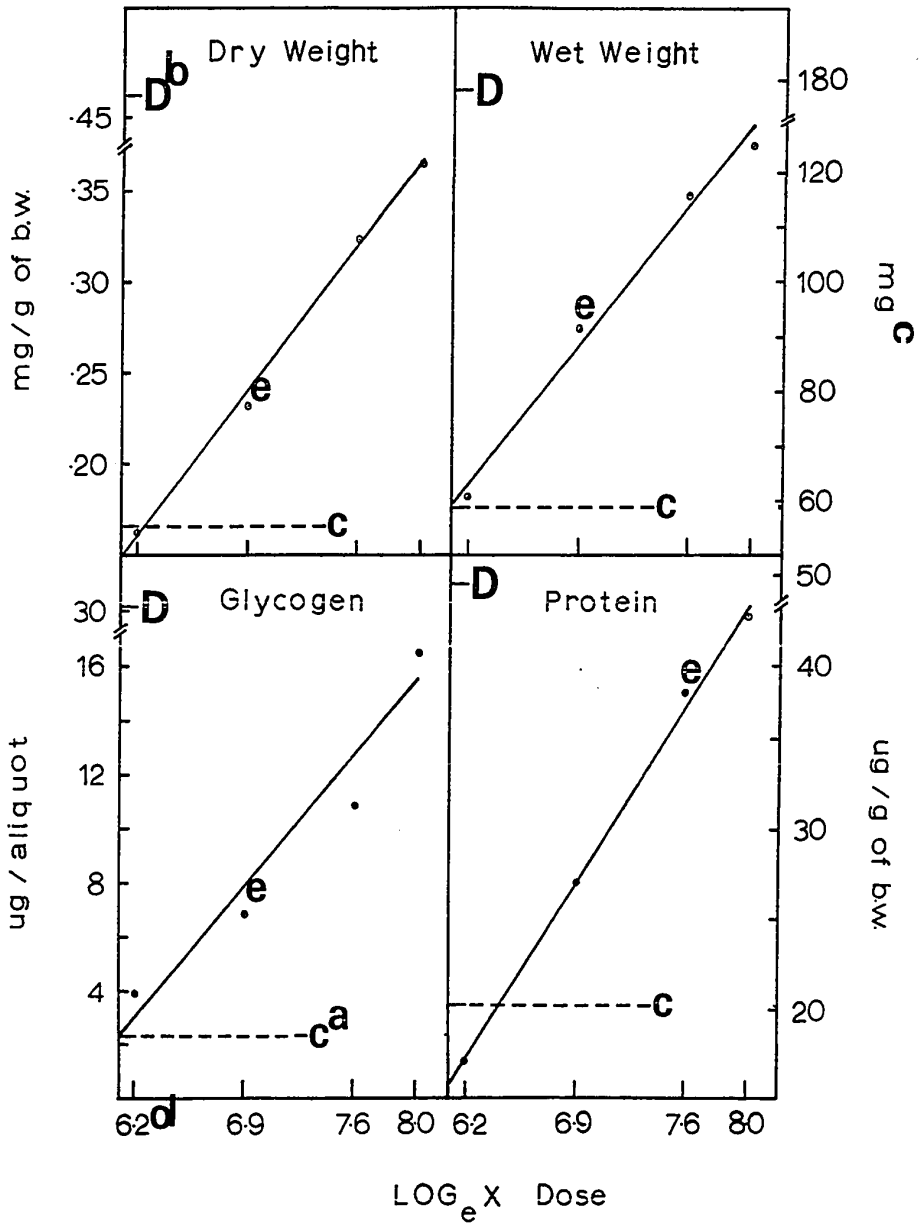
[a] C = untreated control group.

[b] D = positive control group receiving 100 ppb DES in the food.

[c] Although there was a significant depression of body weight at 2000 and 3000 ppm of o,p'-DDT, the linearity of the wet weight curve was not affected when it was expressed as mg/g of body weight.

[d] N = 20 animals for each point except 1000 ppm o,p'-DDT where N = 14.

[e] lowest dose statistically different from controls ($p < 0.05$)


Dry Weight
Wet Weight
Glycogen
Protein
mg/g of b.w.
mg
ug / aliquot
ug / g of b.w.
LOG$_e$ X Dose
6·2
6·9
7·6
8·0
·45
·35
·30
·25
·20
180
120
100
80
60
50
40
30
20
16
12
8
4
D
c
e
a
b
d

hypophysectomized rats. The ovarian weight gain in the intact animals was due to an early release of gonadotropins by the pituitary. Fevold and Fiske (1939) and Bradbury (1947) have also shown that small doses of estrogen increase the output of gonadotropins in rats between the age of 28 and 31 days. It appears that the gonadotropin and estrogen exert a combined influence resulting in increased ovarian weight. As the dietary dose of estrogen (o,p'-DDT) is increased ovarian weight is increased in a linear fashion (Fig. 2). o,p'-500 and 3000 ppm produced ovarian weights statistically different ($p<0.05$) from controls. 500 ppm o,p'-DDT is only mildly estrogenic but possesses the capacity to induce hepatic microsomal enzymes (See below and Fig. 4) resulting in a decrease in the plasma half-life of steroids such as estrogen. Therefore, if endogenous estrogen is metabolized, less ovarian stimulation resulted in a lower ovarian weight as compared with the control group. (Presl et al. (1969) found that the concentration of estradiol in an immature Wistar rat is 0.34 µg of estrogen/ 100 ml of blood.) . The inherent estrogenicity of 500 ppm o,p'-DDT is weak, therefore probably not causing an early release of gonadotropins or direct ovarian stimulation. However, the inherent estrogenic properties of 1000, 2000, and 3000 ppm o,p'-DDT are great enough to "replace" the endogenous estrogen which has been metabolized by producing an early release of gonadotropins as well as stimulating the ovary directly. Therefore, it seems apparent that o,p'-DDT mimics estrogen's effect on the ovary but only at high concentrations.

FIGURE 2

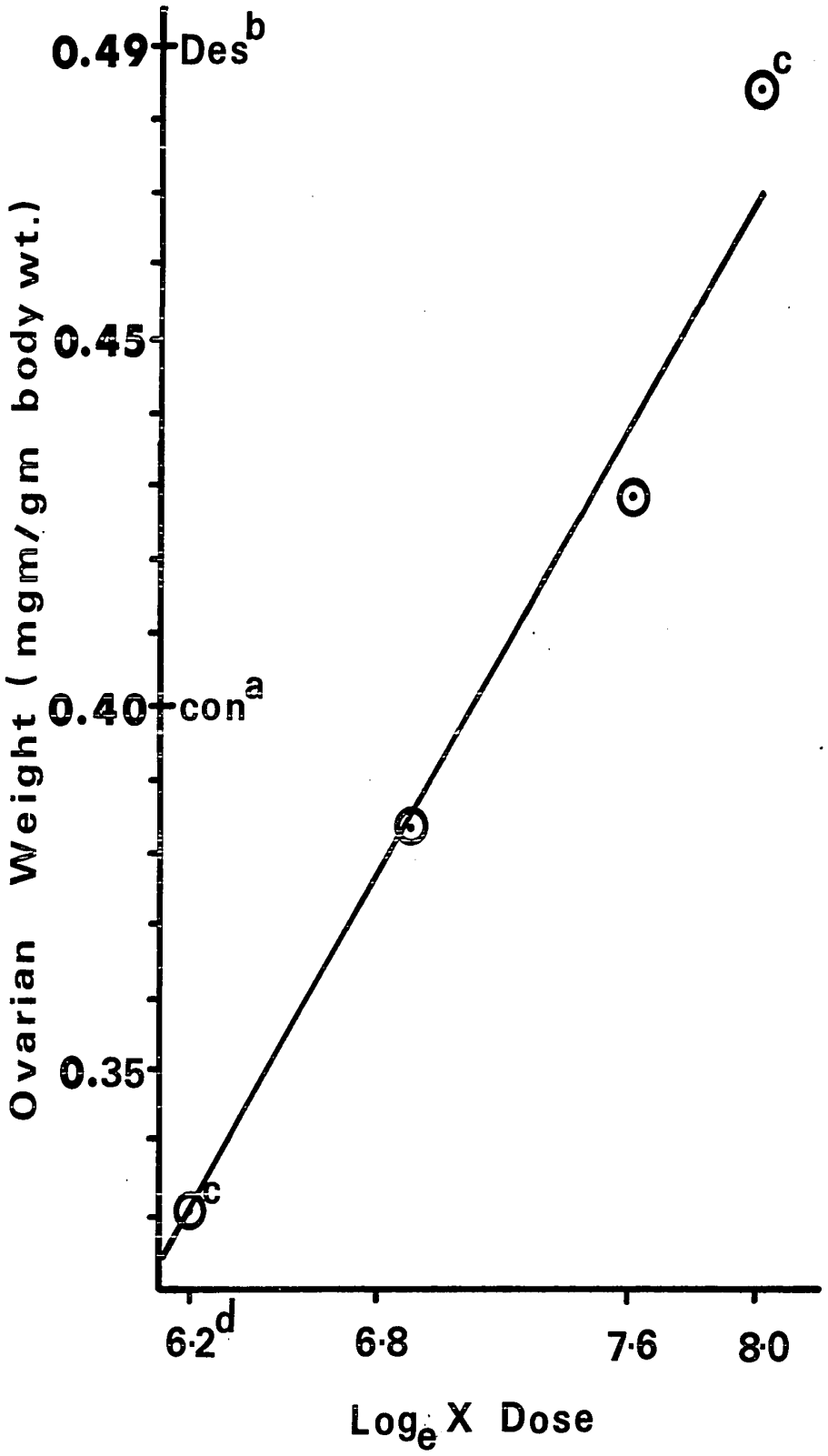
LOG-DOSE / RESPONSE RELATIONSHIP BETWEEN o,p'-DDT AND OVARIAN WEIGHT

[a] Con = untreated control group.

[b] DES (100 ppb) = positive estrogen control group receiving 100 ppb DES in the food.

[c] Statistically different from controls ($p<0.05$).

[d] N = 20 animals for each point except 1000 ppm o,p'-DDT where N = 14.


0.49
Des b
0.45
0.40
con a
0.35
Ovarian Weight (mgm/gm body wt.)
c
c
6·2 d
6·8
7·6
8·0
Log e X Dose

Premature vaginal opening and cornified vaginal smears are two very sensitive indicators of estrogenic activity. o,p'-DDT caused premature vaginal opening in all the treated groups (Fig. 3). On day 29 when the first control vagina opened, 30% of the o,p'-500, 85.7% of the o,p'-1000 and 100% of the o,p'-2000 and 3000 ppm and DES (100 ppb) treated animals had open vaginas. A statistical difference ($p<0.05$) was obtained between the control and o,p'-1000 ppm group. Smears of open vaginas all possessed cornified epithelial cells characteristic of an adult female rat during estrus. The vaginal smear of the control vagina which opened was devoid of cornified epithelial cells but did have a large number of leukocytes characteristic of the diestrous phase of the reproductive cycle when the estrogen secretion is low.

Bitman et al. (1971[a]) demonstrated that o,p'-DDT at dietary concentrations of 50 ppm can induce increased levels of hepatic microsomal enzymes in rats resulting in a reduced duration of action of a standard dose of pentobarbital. Induction is also characterized by an increase in liver weight and microsomal protein. (Sanchez, 1967; Morello, 1965). In preliminary studies using mice treated with o,p'-DDT for two weeks it was noted that the liver to body weight ratio was statistically increased ($p<0.05$) by 500 ppm o,p'-DDT (Fig. 4) suggesting that an increase in hepatic microsomal enzymes had taken place, similar to that which occurs in the rat. In this same preliminary study no statistical differences

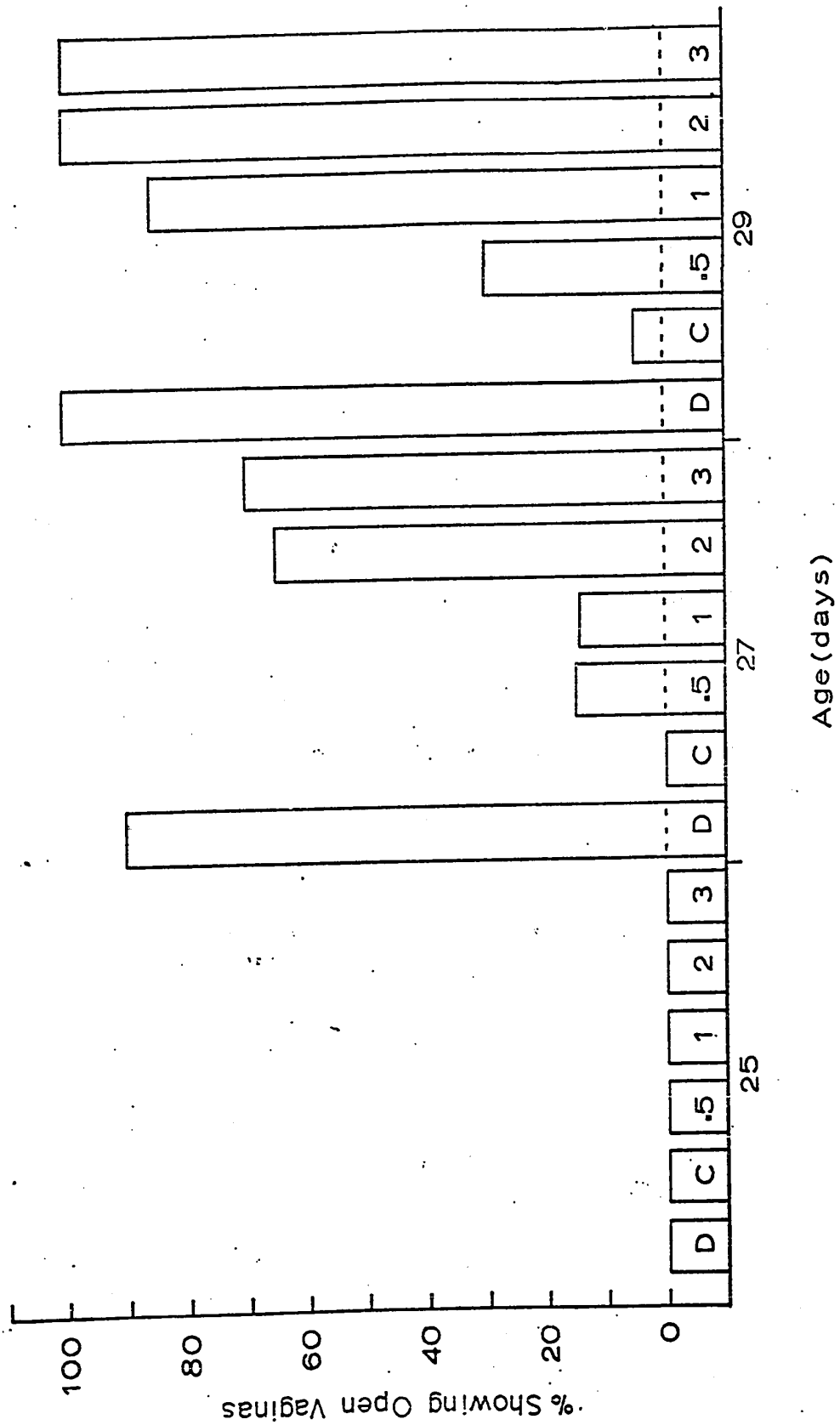
FIGURE 3

PREMATURE VAGINAL OPENING INDUCED IN WISTAR STRAIN RATS BY DIETARY ADMINISTRATION OF o,p'-DDT

[a] D = DES, 100 ppb, positive estrogen control.

[b] C = untreated control

[c] 0.5,1,2,3 = 500, 1000, 2000 and 3000 ppm o,p'-DDT, respectively. N = 20 animals for each group except 1000 ppm o,p'-DDT where N = 14.


% Showing Open Vaginas
0
20
40
60
80
100
D
C
.5
1
2
3
25
D
C
.5
1
2
3
27
D
C
.5
1
2
3
29
Age (days)

11

FIGURE 4

LIVER WEIGHT OF C_3H MICE EXPOSED TO VARIOUS CONCENTRATIONS OF o,p'-DDT IN THE DIET

[a] Con = untreated control group.

[b] DES = positive estrogen control group receiving 100 ppb DES in the food.

[c] Statistically different from controls ($p<0.05$). 1000 and 2000 ppm o,p'-DDT groups were on the diet for 1 week only whereas the other groups received the diets for 2 weeks.

[d] N= 4 to 8 animals / group.

[e] mean $\pm$ standard error.

were obtained with regards to kidney weight demonstrating that o,p'-DDT specifically stimulates some tissues (eg. liver) and not others.

A statistical difference ($p<0.02$) in body weight gain was noted at 30 days in the o,p'-2000 and 3000 ppm groups with o,p'-3000 ppm producing the most marked depression (Fig 5). The estrogenicity of the large concentrations of o,p'-DDT may cause, a depression of the appetite (Gass and Okey, 1968; Hoffman et al., 1970; Meites, 1949), a decreased anterior pituitary secretion of growth hormone (Richards and Kueter, 1941), resulting in a retardation of growth at high dietary doses. No data on food consumption or growth hormone concentration were collected. Therefore either or both of the above mechanisms for body weight depression may be in operation.

From the results of the bioassay it was concluded that o,p'-DDT produces a significant estrogenic response but only at high dietary concentrations (1000 ppm and above). It is unlikely that environmental exposure to DDT constitutes a hazardous exposure to an active estrogen for mammals. o,p'-DDT does not appear to accumulate in the food chain, as other more stable analogues do; reports indicate a lower accumulation of o,p'-DDT in the body (French and Jefferies, 1969; Bitman et al., 1968; Heinrichs et al., 1971; Cranmer, 1972) because o,p'-DDT is converted to hydroxy and methoxy metabolites that are rapidly excreted. (Feil et al., 1971).

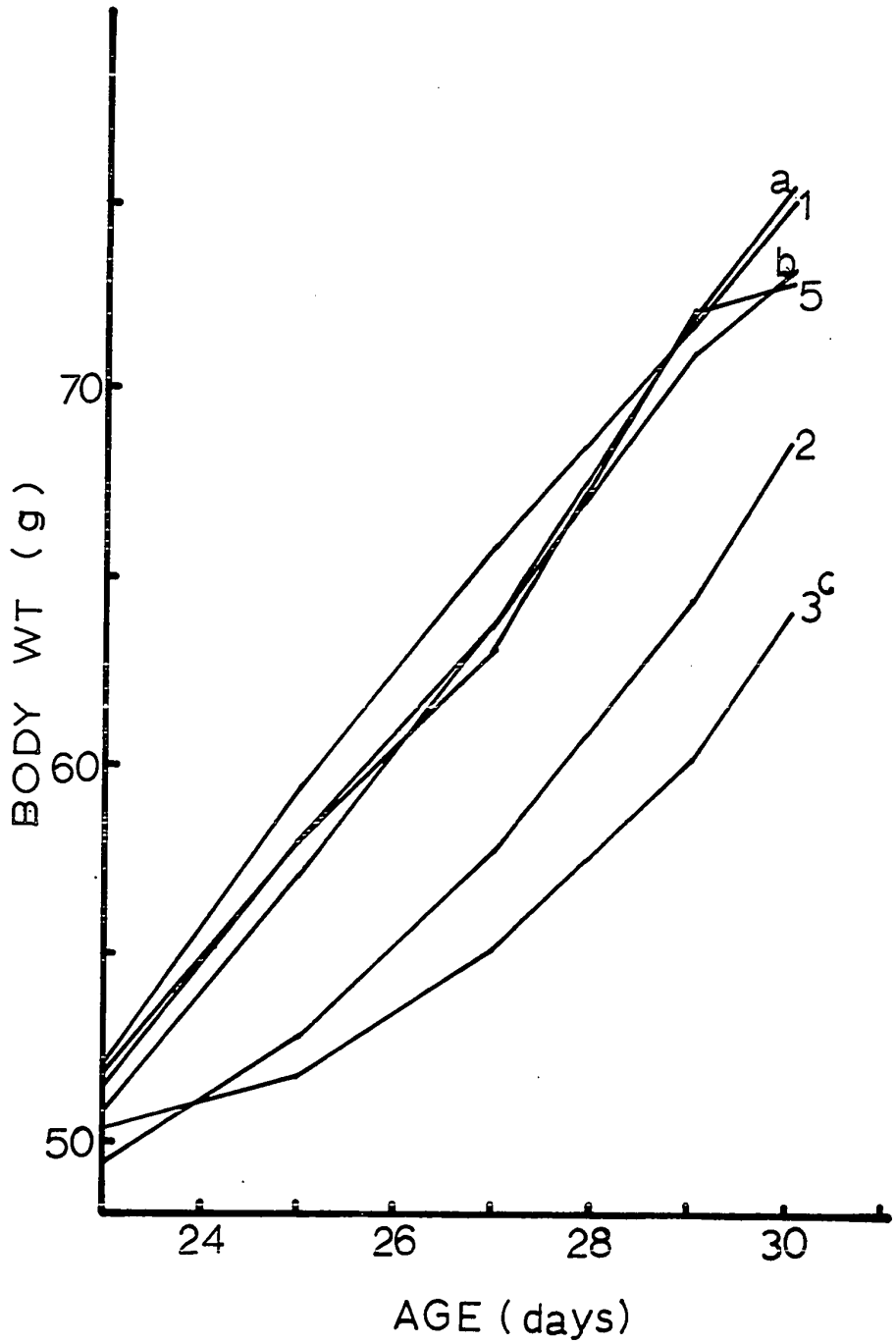
FIGURE 5

GROWTH CURVES OF IMMATURE FEMALE WISTAR STRAIN RATS RECEIVING VARIOUS DIETARY CONCENTRATIONS OF o,p'-DDT

[a] untreated controls

[b] DES, 100 ppb, positive estrogen control.

[c] Dose of o,p'-DDT in ppm $\times 10^{-3}$. N = 20 for each point except o,p'-1000 ppm where N = 14.


a
1
b
5
2
3c
70
60
50
BODY WT (g)
24
26
28
30
AGE (days)

Thus, few animals are likely to ingest estrogenic doses ca. 1000 ppm even in grossly contaminated areas.

SUMMARY

1. 1000 ppm o,p'-DDT produced significant increases in uterine wet weight, dry weight and glycogen whereas 2000 ppm was required to produce an increase in uterine protein.

2. 500 ppm o,p'-DDT produced a decrease in ovarian weight whereas 3000 ppm produced an increase.

3. All doses of o,p'-DDT caused premature vaginal opening. Resulting vaginal smears contained cornified epithelial cells characteristic of an adult female rat during estrus.

4. 500 ppm increased the liver weight but not the kidney weight.

5. 2000 and 3000 ppm of o,p'-DDT depressed body weight.

CHAPTER II

ANTIESTROGENIC PROPERTIES OF o,p'-DDT IN THE FEMALE RAT

The metabolism of various drugs and steroids takes place in the liver by the hepatic microsomal enzyme fraction called the mixed function oxidases. Many drugs such as phenobarbital and pentobarbital (Burns et al., 1963), insecticides such as DDT and chlordane (Conney, 1967; Hart et al., 1963) and polycyclic hydrocarbons such as 3-methylcholanthrene and 3, 4-benzpyrene (Conney et al., 1960) produce an increase in the formation of these mixed function oxidases in rats. This increase in enzyme activity is paralleled by accelerated drug and steroid metabolism in the animal (Conney and Klutch, 1963; Fahim et al., 1971), shortened duration of action of drugs, increased microsomal protein, increased microsomal cytochrome P-450 (Poland et al., 1971), increased amounts of smooth endoplasmic reticulum in the cell (Remmer and Merker, 1965) and increased urinary excretion of ascorbic acid, in rats (Burns, 1965).

A lower K_m (Michaelis constant = the concentration of the substrate which gives half the numerical maximal velocity.) indicates a higher binding affinity of the enzyme for the substrate; it was concluded that steroids may be the natural substrates for the microsomal enzymes (Kuntzman et al., 1965). Chronic administration of drugs or insecticides caused a decrease in the anesthetic action of steroids due to increased metabolism by the hepatic microsomal enzymes (Kuntzman et al., 1965). Therefore, it was demonstrated that steroids are hydroxylated

by the same enzyme systems in the liver microsomes that oxidatively metabolize drugs (Conney et al., 1965; Kupfer, 1968).

Since Hart and Fouts (1963) demonstrated that DDT stimulated hexobarbital metabolism in rats by the induction of hepatic microsomal enzymes, numerous reports have shown that DDT is a potent inducer of liver enzymes (Chadwick et al., 1971; Hoffman et al., 1970; Nowicki and Norman, 1972; Conney, 1967; Kupfer, 1968). In rats 1 mg DDT / kg of body weight significantly increases microsomal enzyme activity.

o,p'-DDT has been shown to possess estrogenic properties but only at very high doses (Chapter I). Bitman et al. (1971[a]) found 50 ppm o,p'-DDT as effective as 50 ppm p,p'-DDT in inducing hepatic microsomal enzymes; thus exhibiting antiestrogenic properties. This suggested that o,p'-DDT had a biphasic action which was dose - dependent. At low doses o,p'-DDT exhibited antiestrogenic properties by increasing the hepatic metabolism of drugs and steroids (Smith et al., 1972; Fahim et al.,1970) at high doses (1000 ppm) o,p'-DDT produced a significant estrogenic effect (Chapter 1). This study was undertaken to investigate what effect DDT pretreatment had on the metabolism of endogenous and exogenous estradiol in the immature and ovariectomized mature female rat.

METHODS AND MATERIALS

Effect of DDT Pretreatment on Hepatic Microsomal Enzymes

Sexually mature Wistar rats (80 to 100 days old) were fed diets containing either 0, 20, 200, or 1000 ppm of o,p'-DDT or 20, 200 or 750 ppm of p,p'-DDT. A sleep-time test using sodium pentobarbital (Nembutal, Abbott Laboratories, Montreal, Canada) was performed following 9 to 10 days exposure to their respective diets to test for the induction of hepatic microsomal enzymes. The sleep time was defined as the time between loss and restoration of the righting reflex following an intraperitoneal injection of Nembutal (35 mg / kg of body weight).

Effect of DDT Pretreatment on Response to Injected Estradiol by Immature Female Rats

Immature Sprague-Dawley rats (Holtzman, Co., Madison, Wisconsin) were fed diets containing either 0, 5, 10, 50, 100, 500, 1000 or 1500 ppm o,p'-DDT or 5, 10 or 100 ppm p,p'-DDT (Aldrich Chemical Co., Lot #100571) from age 21 through 30 days. Between the age of 23 and 30 days half of the animals were given a daily injection of 05 µg estradiol - 17β/0.15 ml of corn oil. Control animals received corn oil only. This dose of estradiol was selected to give a significant but not maximal stimulus to the immature rat uteri. Vaginal opening and body weights were obtained daily. At age 30 days the

rats were sacrificed by cervical fracture, weighed and the uteri analysed as previously described. The experiment was repeated using Wistar strain rats.

Effect of DDT Pretreatment on Response to Injected Estradiol by Mature Ovariectomized Female Rats

Female Sprague-Dawley rats were ovariectomized when 21 to 22 days of age. At age 23 days, the animals were randomly assigned to diets containing either 0, 5, 10, 50 or 500 ppm o,p'-DDT or 5, 10 or 100 ppm of p,p'-DDT for a period of 54 days. During the last week of exposure to DDT the animals received a daily subcutaneous injection of 0.5 µg of estradiol - 17β/0.15 ml of corn oil. The animals were sacrificed by cervical fracture 24 hours after the last injection. The animals were weighed. The uterus and liver were removed, blotted dry and weighed. The uterus was used in the glycogen determination (Appendix 3).

RESULTS AND DISCUSSION

Sodium Pentobarbital Sleep Time

Many drugs, insecticides, and polycyclic hydrocarbons cause an increase in hepatic microsomal enzyme activity resulting in increased oxidation, reduction, hydroxylation and conjugation of drugs, steroids and organochlorine insecticides. These cause a decrease in plasma half life and an attenuated duration of action of drugs and steroids "in vivo".

A sleep time test using sodium pentobarbital was performed on Wistar-strain rats to ascertain how effective o,p'-DDT and p,p'-DDT, administered orally in the food, are as enzyme inducers. From Table 2 it can be seen that enzyme activity is higher in male than female control animals as was observed by other investigators (Gram et al., 1969; Hall et al., 1971; Kupfer, 1970). In female rats, o,p'-1000 ppm was as effective an inducer of microsomal enzymes as 200 and 750 ppm p,p'-DDT with 200 and 750 ppm sleep times not being statistically different ($p > 0.05$) from each other, indicating that maximal enzyme induction took place with 200 ppm. Hoffman et al. (1970) found that administration of tech. DDT at concentrations greater than 750 ppm (equivalent to 600 ppm of p,p'-DDT) caused no further increase in p-nitroanisole metabolism. In the male rat 20 ppm p,p'-DDT is just as effective an enzyme inducer as 200 and 750 ppm p,p'-DDT. However, 1000 ppm o,p'-DDT stimulated the male's liver enzymes to such a degree that the dose which

Table 2. Effect of DDT Pretreatment on the Length of Sodium Pentobarbital[c] Induced Sleep Time.

treatment[e]	females		males	
	time (min)	% of control	time (min)	% of control
control	173.5	-	66.6	-
o,p'-20	146.0	84.1	59.5	89.3
o,p'-200	50.0[d]	28.8	35.0[b]	52.5
o,p'-1000	55.5[a]	32.0	0.0	0.0
p,p'-20	150.5	86.7	21.0[a]	31.5
p,p'-200	54.5[b]	31.4	16.5[a]	24.8
p,p'-750	33.0[b]	19.0	22.0[a]	33.0

[a] Statistically different from controls ($p<0.05$).

[b] Statistically different from controls ($p<0.01$).

[c] 35 mg/kg body weight ip injection of Nembutal (Abbott Laboratories, Montreal, Quebec).

[d] Almost statistically different from controls ($0.1<p>0.05$).

[e] N = 2 to 4 animals / group.

puts the control animal to sleep for 66 minutes resulted in the o,p'-1000 animal not losing the righting reflex.

Furner et al. (1969) found that Sprague-Dawley and Wistar rats respond in similar ways to phenobarbital treatment resulting in increases in microsomal protein, p-nitroanisole, ethylmorphine and hexobarbital metabolism in 100 day old animals.

In mature ovariectomized female rats, 500 ppm o,p'-DDT and 100 ppm p,p'-DDT produced a statistical difference ($p<0.01$) in the liver to body weight ratio (Fig. 16) characteristic of hepatic enzyme induction using large concentrations of DDT (Hoffman et al., 1970).

Effect of DDT Pretreatment on Response to Injected Estradiol by Immature Intact and Mature Ovariectomized Rats.

The purpose of this study was to ascertain in what way DDT pretreatment affected endogenous and exogenous (injected) estrogen metabolism in the female rat. This was tested by observing various uterine growth parameters.

Immature female rats when exposed to o,p'-DDT at low dietary concentrations (10, 50 and 100 ppm o,p'-DDT) possess uterine wet weights which are significantly lower ($p<0.05$) than control animals (Fig. 6, bottom curve). At dietary concentrations above 100 ppm o,p'-DDT, the inherent estrogenic activity of o,p'-DDT stimulates the uterine wet weight to increase and exceed the control value at 1000 and 1500 ppm o,p'-DDT (equivalent to 200 and 300 o,p'-DDT/kg of body weight/rat/day). These results demonstrate that low dietary concentrations of o,p'-DDT stimulate hepatic metabolism of the endogenous estrogen; the inherent estrogenic properties of o,p'-DDT at low dietary concentrations are not great enough to stimulate an increase in uterine wet weight. Above 500 ppm, however, the inherent estrogenicity is great enough to produce an increase in uterine wet weight. Similar results were obtained with Wistar-strain rats (Fig. 7).

Animals on the same DDT diets but receiving 0.5 µg of estradiol/day for seven consecutive days produced similar results; the only difference was that the estrogen response was depressed at dietary concentrations of 500 and 1000 ppm o,p'-DDT with 1500 ppm producing "recovery" towards the estradiol control

FIGURE 6

UTERINE WET WEIGHT IN IMMATURE SPRAGUE-DAWLEY RATS RECEIVING EITHER VARIOUS CONCENTRATIONS OF o,p'-DDT (BOTTOM CURVE) OR VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg ESTRADIOL-17β/0.15 ml CORN OIL (TOP CURVE)

[a] Statistically different from controls ($p<0.05$).

[b] Statistically different from controls ($p<0.05$). This is the dose which has a uterine wet weight closest to the control value. A statistical test could not be performed using the control value because N = 1.

[c] o,p'-0 ppm + estradiol control, N = 1. This value was obtained from the estradiol standard curve. N = 5 or 6 animals / group for all other treatments.

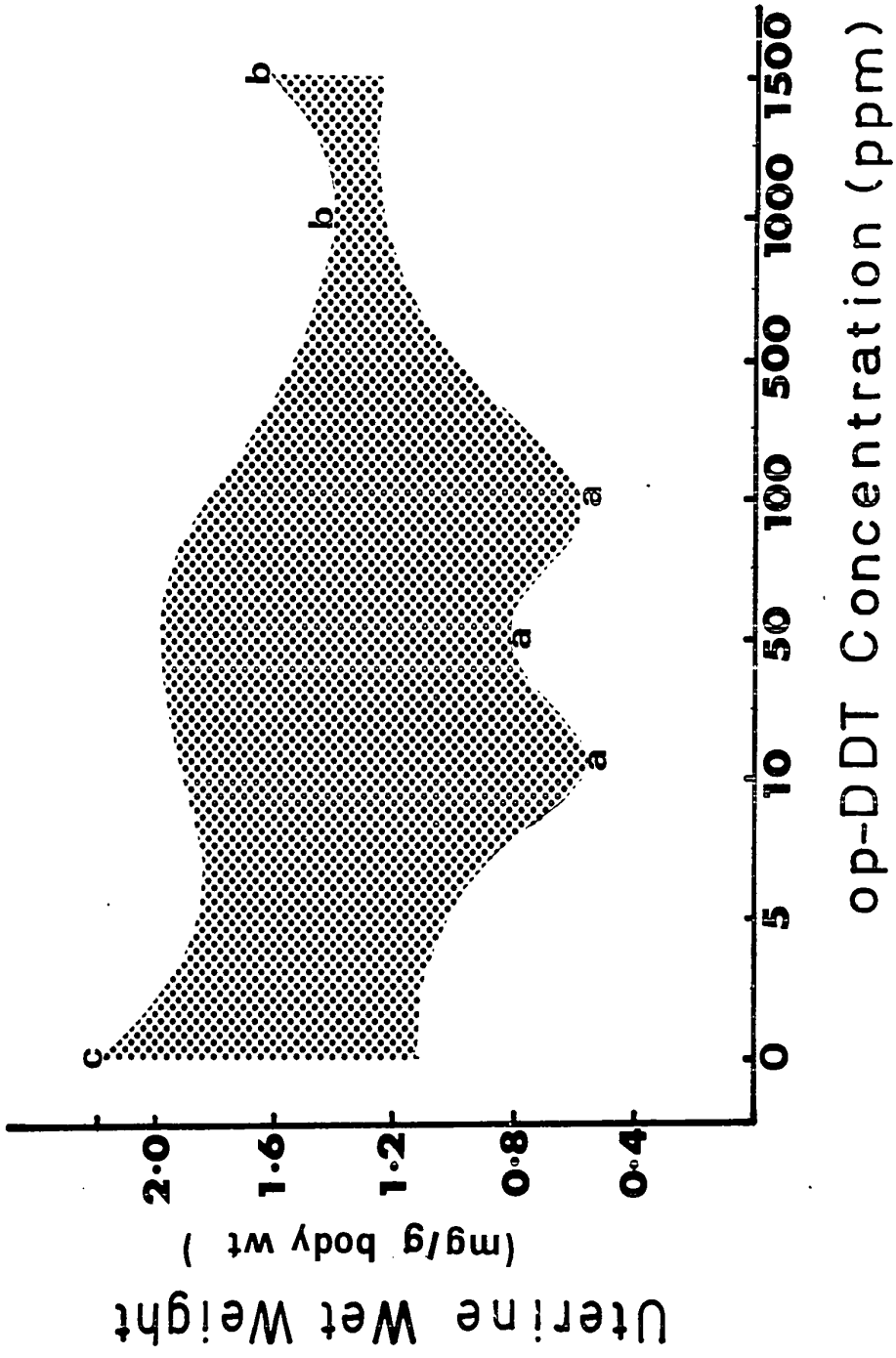
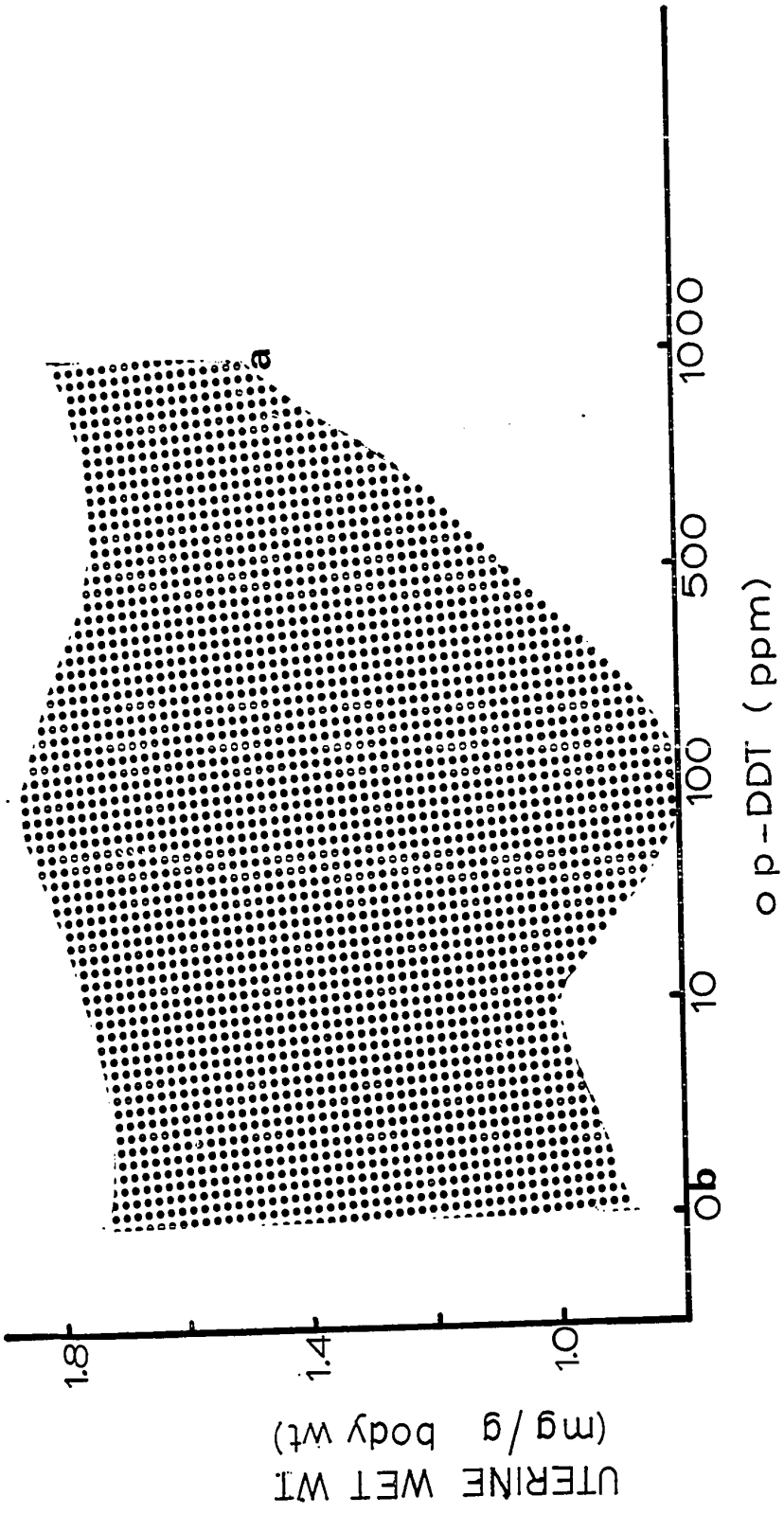

Uterine Wet Weight
(mg/g body wt)
2·0
1·6
1·2
0·8
0·4
0
5
10
50
100
500
1000
1500
op-DDT Concentration (ppm)
a
a
a
b
b
c

FIGURE 7

UTERINE WET WEIGHT OF IMMATURE WISTAR-STRAIN RATS RECEIVING EITHER VARIOUS CONCENTRATIONS OF o,p'-DDT (BOTTOM CURVE) OR VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg ESTRADIOL-17β/0.15 ml CORN OIL (TOP CURVE)

[a] Statistically different from controls ($p<0.05$).

[b] N = 20 animals/group.


UTERINE WET WT
(mg/g body wt)
1.8
1.4
1.0
a
b
10
100
500
1000
o p-DDT (ppm)

value (Fig. 6, top curve). This delay in recovery may be the result of competitive inhibition (o,p'-DDT competes with estradiol for binding sites. These binding sites are small proteins, which are present in the cytoplasm of the cell. Estrogen has to be bound by the receptors in order to elicit a response.); Welch et al (1969) found that pretreatment of female rats with o,p'-DDT reduced the amount of tritiated estradiol - 17β bound by the uterus. With increasing dietary concentrations of o,p'-DDT, increased hepatic metabolism of the exogenous estradiol results, as well as, increased binding of o,p'-DDT by the uterine cytosol receptors. This results in a decrease in the amount of estradiol bound by the uterus which is manifested by a decrease in uterine wet weight. At 1500 ppm the inherent estrogenic activity of o,p'-DDT is great enough to stimulate an increase in uterine wet weight back towards the control level.

Dietary o,p'-DDT caused a reduction in uterine dry weight (Fig. 8, bottom curve) similar to that observed for uterine wet weight reaching a minimum value at 100 ppm. 1500 ppm o,p'-DDT resulted in dry weights statistically different ($p<0.05$) from controls.

In rats receiving dietary o,p'-DDT and an estradiol injection (0.5 µg/day) the opposite effect was noted (Fig. 8, top curve). Here uterine dry weight increased to a maximum value at 100 and 500 ppm o,p'-DDT and then declined to below control values at the higher concentrations (1000 and 1500 ppm). This initial increase isn't significant because the

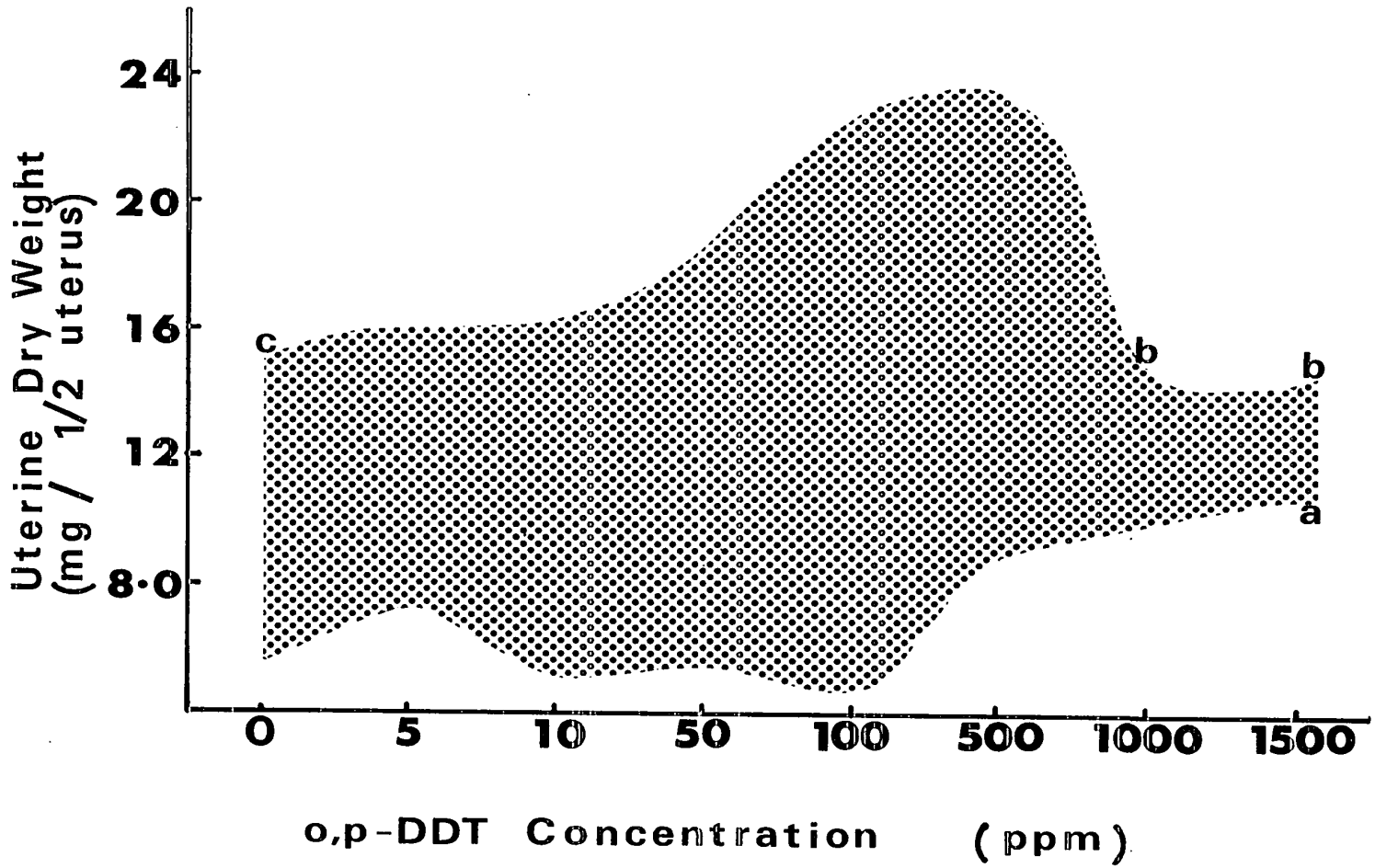
FIGURE 8

UTERINE DRY WEIGHT OF IMMATURE SPRAGUE-DAWLEY RATS RECEIVING EITHER VARIOUS CONCENTRATIONS OF o,p'-DDT (BOTTOM CURVE) OR VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 μg OF ESTRADIOL-17β/0.15 ml CORN OIL (TOP CURVE)

[a] Statistically different from controls ($p<0.05$).

[b] Statistically different from animals receiving the same concentration of DDT without the estradiol injection ($p<0.05$).

[c] o,p'-O + estradiol control, N = 1. This value was obtained from the estradiol standard curve . N = 5 to 6 animals / group for all other treatments.


Uterine Dry Weight
(mg / 1/2 uterus)
24
20
16
12
8·0
c
b
b
a
0
5
10
50
100
500
1000
1500
o,p-DDT Concentration (ppm)

standard deviation within these groups was very large. At high concentrations (1000 and 1500 ppm o,p'-DDT) increasing amounts of o,p'-DDT may be bound to estrogen receptors. The decrease in bound estradiol combined with the fact that the cellular anabolic processes are probably more responsive to low concentrations of estradiol than they are to o,p'-DDT, probably caused the decrease in uterine dry weight noted at 1000 and 1500 ppm o,p'-DDT (Fig. 8, top curve). However, it should be noted that the uterine dry weights for estradiol-treated o,p'-1000 and 1500 ppm groups are statistically different ($p<0.05$) from those animals receiving 1000 and 1500 ppm o,p'-DDT this indicates that a significant amount of estradiol still is present.

Glycogen deposition in the uterus is a very sensitive indicator of estrogenic activity. Low doses (10 and 50 ppm) of o,p'-DDT caused very little glycogen deposition in the intact immature rat uteri (Fig. 9, bottom curve). However, glycogen deposition was increased by concentrations above 100 ppm o,p'-DDT with 1000 and 1500 ppm producing a statistical difference ($p<0.05$). Administration of 100 ppm o,p'-DDT reduced the uterine glycogen response to exogenous estradiol demonstrating the antiestrogenic effect of o,p'-DDT (Fig. 9, top curve). However, glycogen content of the uteri from rats fed 500, 1000 or 1500 ppm o,p'-DDT shows "recovery" toward levels stimulated by estradiol only. This recovery represents the inherent estrogenic activity evoked by high o,p'-DDT concentrations and

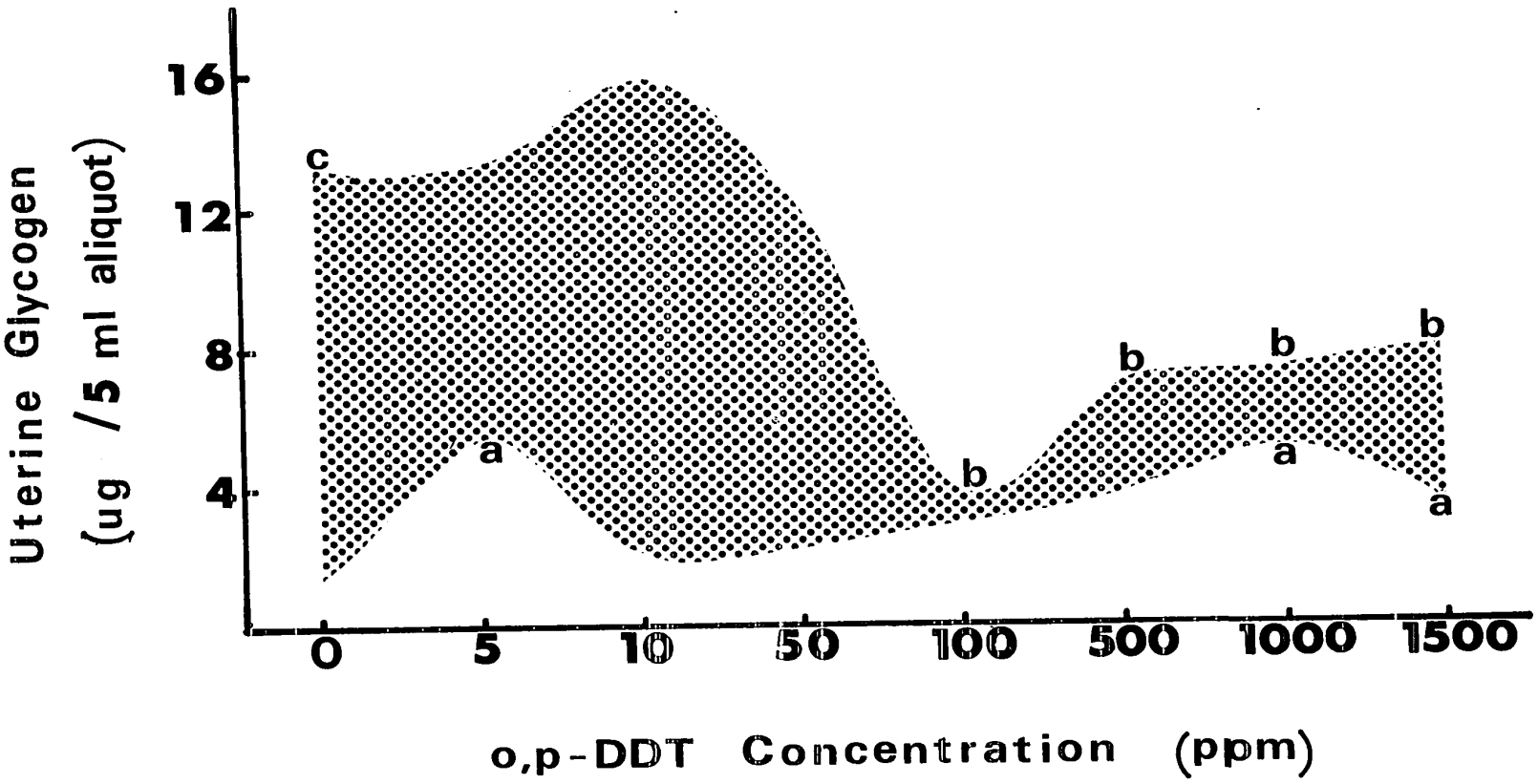
FIGURE 9

UTERINE GLYCOGEN CONTENT OF IMMATURE SPRAGUE-DAWLEY RATS RECEIVING EITHER VARIOUS CONCENTRATIONS OF o,p'-DDT (BOTTOM CURVE) OR VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 μg ESTRADIOL-17β/0.15 ml CORN OIL(TOP CURVE)

[a] Statistically different from controls ($p<0.05$).

[b] Statistically different from 5 ppm o,p'-DDT + estradiol group ($p<0.05$).

[c] o,p'-O + estradiol control, N = 1. This value was obtained from the estradiol standard curve. N = 5 to 6 animals/group for all other treatment groups.


Uterine Glycogen
(ug /5 ml aliquot)
16
12
8
4
c
a
b
b
b
a
b
a
0
5
10
50
100
500
1000
1500
o,p-DDT Concentration (ppm)

not a failure of hepatic enzyme induction (Sleep time data, table 2).

Results obtained with mature ovariectomized rats were very similar to those obtained in intact immature rats. 10 ppm o,p'-DDT reduced uterine wet weight and glycogen to a minimal value (Fig. 10) whereas 50 and 500 ppm o,p'-DDT stimulated the uterus producing an increase in the responses.

p,p'-DDT, a potent inducer of hepatic microsomal enzymes, causes an increased metabolism of exogenously supplied estrogen as evidenced by a decrease in incidence of estradiol stimulated premature vaginal opening (Fig. 11). On day 29 an inverse relationship exists ($r = -.83$: $p > 0.05$) between the number of prematurely opened vaginas and the concentration of dietary p,p'-DDT.

p,p'-DDT produced a statistical difference in uterine wet weight and glycogen when compared with an estrogen treated control (0.5 µg estradiol/day) but failed to cause a similar decrease in uterine dry weight (Fig. 12). This suggests that even though increased estrogen metabolism is taking place, uterine dry weight is very sensitive to small amounts of estrogen or that estrogen isn't the only compound contributing to the increase in uterine dry weight. Increasing the concentration of p,p'-DDT in the diet, beyond 5 ppm didn't cause any further decrease in the estrogenic response in the parameters tested. Note that all the p,p'-DDT + estrogen treated groups uterine growth parameters are still statistically

FIGURE 10

UTERINE GROWTH PARAMETERS OF MATURE OVARIECTOMIZED SPRAGUE-DAWLEY RATS RECEIVING VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg OF ESTRADIOL-17β/0.15 ml CORN OIL

[a] Statistically different from controls ($p<0.05$).

[b] N = 5 to 8 animals / group.

[c] mean ± standard error

[d] 0 ppm o,p'-DDT + an oil injection.

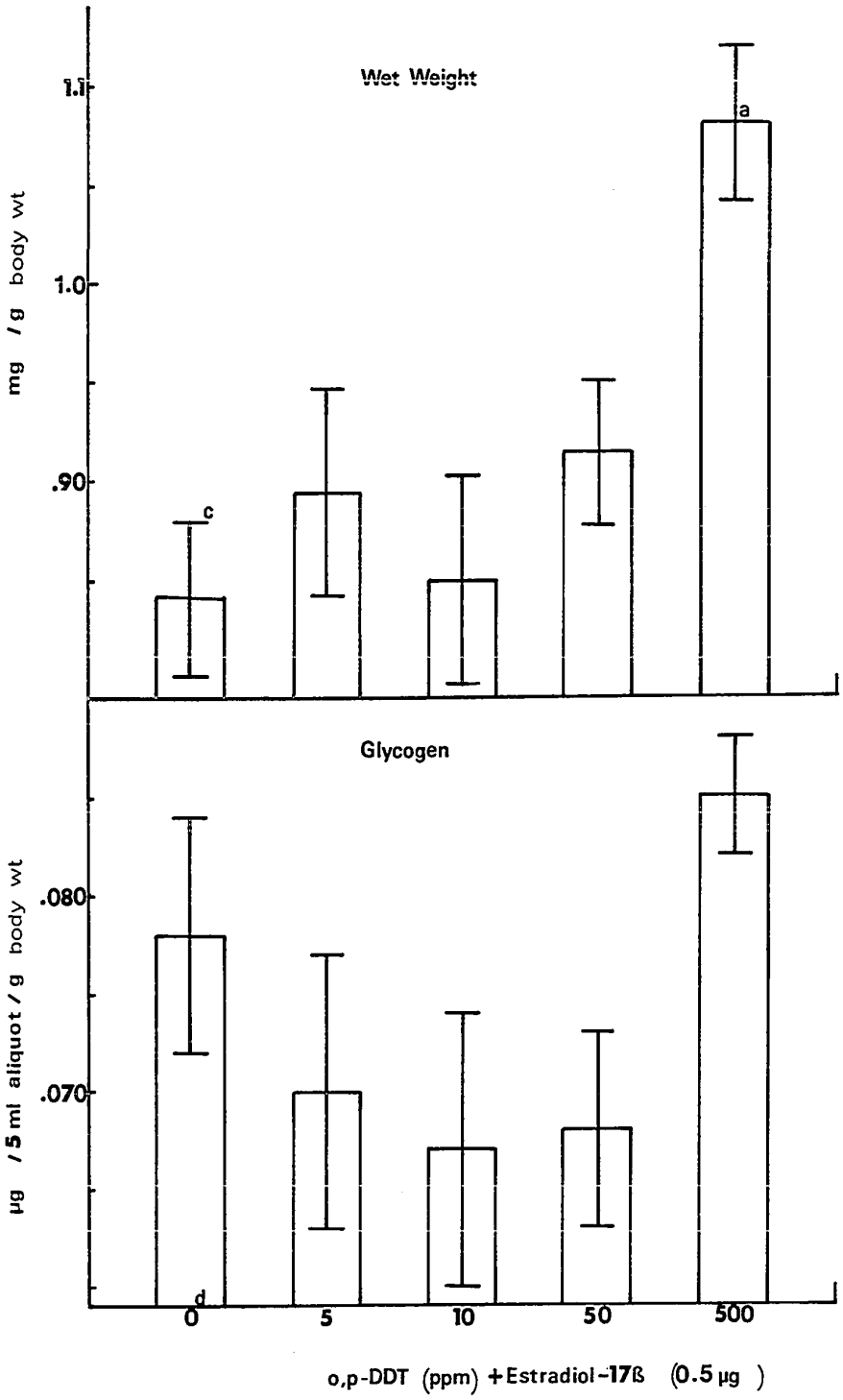

Wet Weight
mg / g body wt
1.1
1.0
.90
a
c
Glycogen
µg / 5 ml aliquot / g body wt
.080
.070
d
0
5
10
50
500
o,p-DDT (ppm) + Estradiol-17ß (0.5 µg)

FIGURE 11

PREMATURE VAGINAL OPENING INDUCED IN IMMATURE SPRAGUE-DAWLEY RATS RECEIVING VARIOUS CONCENTRATIONS OF p,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg ESTRADIOL-17β/0.15 ml CORN OIL

[a] Statistically different from estradiol standard ($p<0.05$).

[b] Concentration of p,p'-DDT (ppm).

[c] N = 6 animals / group.

[d] 0.1 µg[f] estradiol-17β/0.15 ml corn oil/day from day 23.

[e] 1.0 µg estradiol-17β/0.15 ml corn oil/ day from day 23.

[f] standard dose of estradiol used was 0.5 µg. Therefore we can assume that this dose of estradiol would also produce 100 % premature vaginal opening.

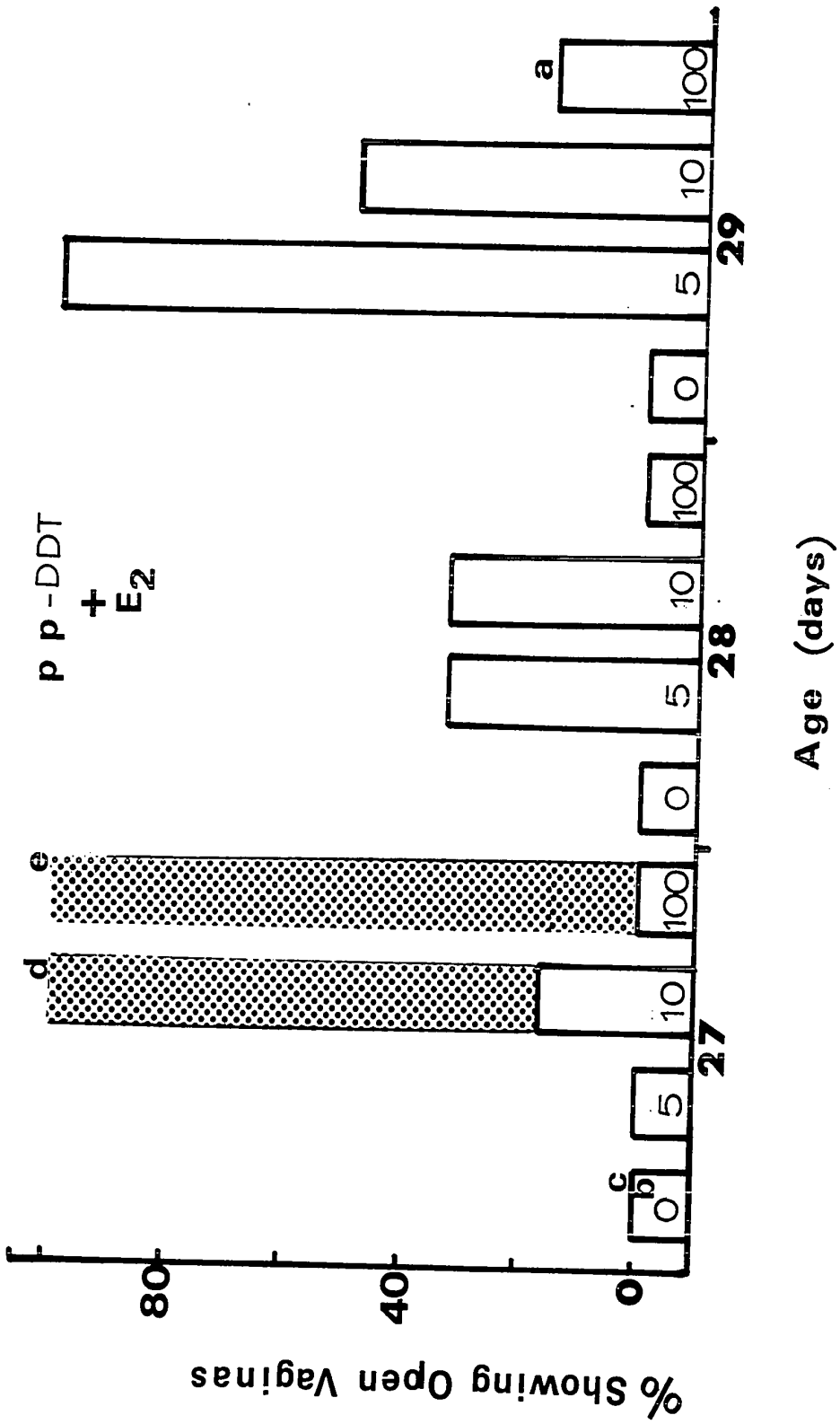

% Showing Open Vaginas
80
40
0
Age (days)
27
28
29
p p-DDT
+
E_2
0
5
10
100
a
b
c
d
e

FIGURE 12

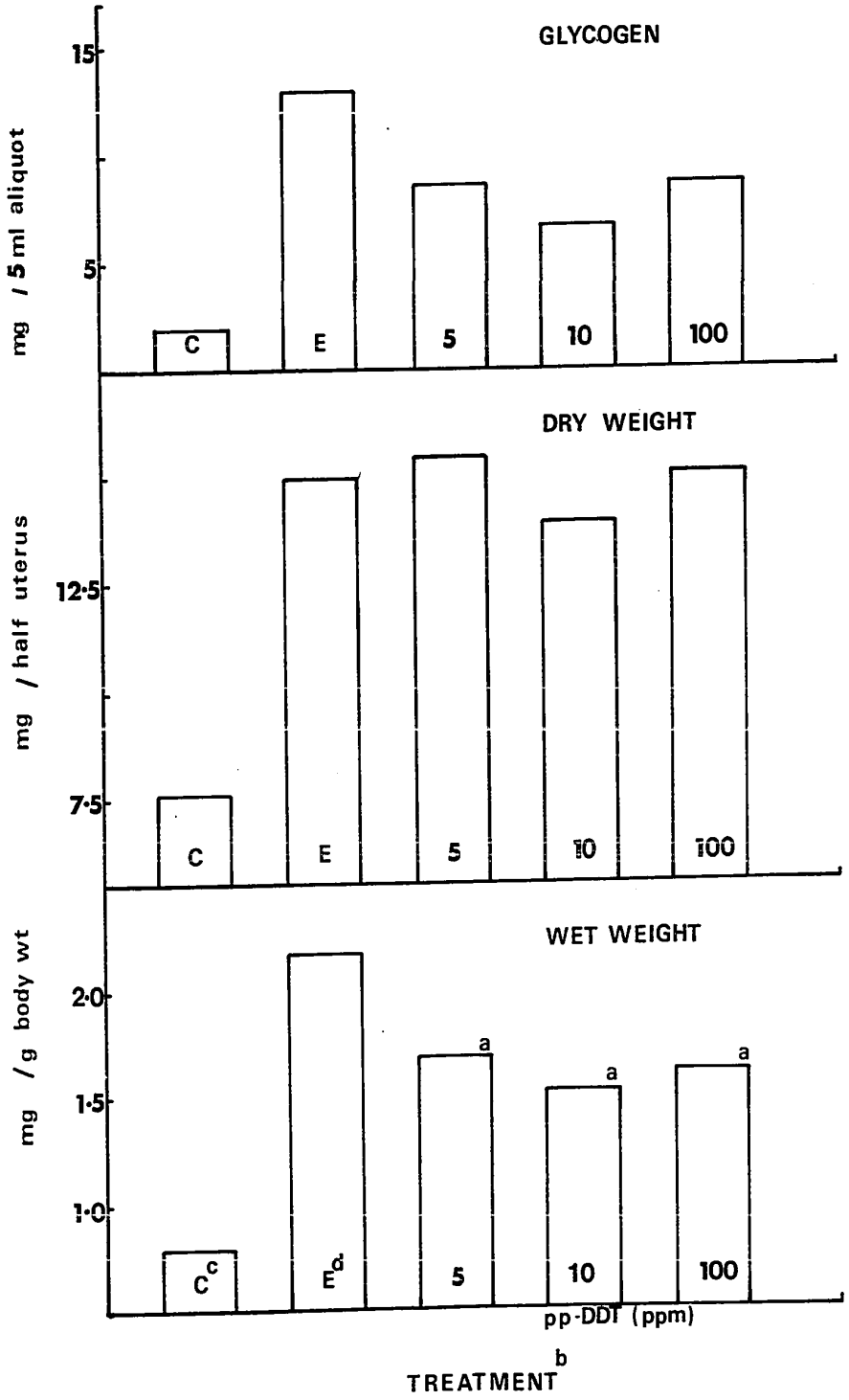
EFFECT OF VARIOUS CONCENTRATIONS OF p,p'-DDT IN ALTERING THE RESRONSE OF VARIOUS UTERINE GROWTH PARAMETERS TO A STANDARD DOSE OF ESTRADIOL-17β IN IMMATURE SPRAGUE-DAWLEY RATS

[a] Statistically different from controls ($p<0.05$).

[b] N = 5 to 6 animals / group.

[c] untreated control

[d] response to a standard dose of estradiol obtained from an estradiol standard curve. N =1.


GLYCOGEN
mg / 5 ml aliquot
15
5
C
E
5
10
100
DRY WEIGHT
mg / half uterus
12·5
7·5
C
E
5
10
100
WET WEIGHT
mg / g body wt
2·0
1·5
1·0
a
a
a
C^c
E^d
5
10
100
pp-DDT (ppm)
b
TREATMENT

different ($p<0.05$) from the oil injected controls. In mature ovariectomized rats p,p'-DDT produced a decrease in uterine wet weight and glycogen as the dose of DDT was increased (Fig. 13) similar to that produced in immature intact females.

Body weight gain (Fig. 14) during exposure to DDT although not statistically different tended to decrease in the animals on high dietary exposure whereas the weight gain in the animals receiving o,p'-DDT and estradiol didn't appear to change. The curve of body weight gain is similar to the uterine wet weight, dry weight and glycogen (Fig. 6, 7, 8, 9).

Only 500 ppm o,p'-DDT produced a statistical difference in body weight in the ovariectomized females (Fig. 15) besides significantly increasing the liver weight. 100 ppm p,p'-DDT produced a significant increase in the liver weight (Fig. 16).

I have shown that o,p'-DDT at concentrations above 1000 ppm, produced a significant estrogenic response. Fahim et al. (1970) found that administration of low concentrations of DDT to sexually mature female rats reduces the effectiveness of endogenous and exogenous estrogen with respect to uterine maintainance. Bitman et al. (1971[a]) demonstrated that in immature rats after 2 days on a diet containing either 50 ppm of o,p'-DDT or p,p'-DDT, the duration of pentobarbital anesthesia was reduced to only half that of controls showing that o,p'-DDT was as effective as p,p'-DDT in stimulating liver enzymes by this test.

Low concentrations of o,p'-DDT ca. 100 ppm displays anti-estrogenic properties due to its ability to increase

FIGURE 13

UTERINE GROWTH PARAMETERS OF MATURE OVARIECTOMIZED SPRAGUE-DAWLEY RATS RECEIVING VARIOUS CONCENTRATIONS OF p,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg ESTRADIOL-17β

[a] N = 4 to 8 animals / group.

[b] mean ± standard error

[c] 0 ppm p,p'-DDT + oil injection.

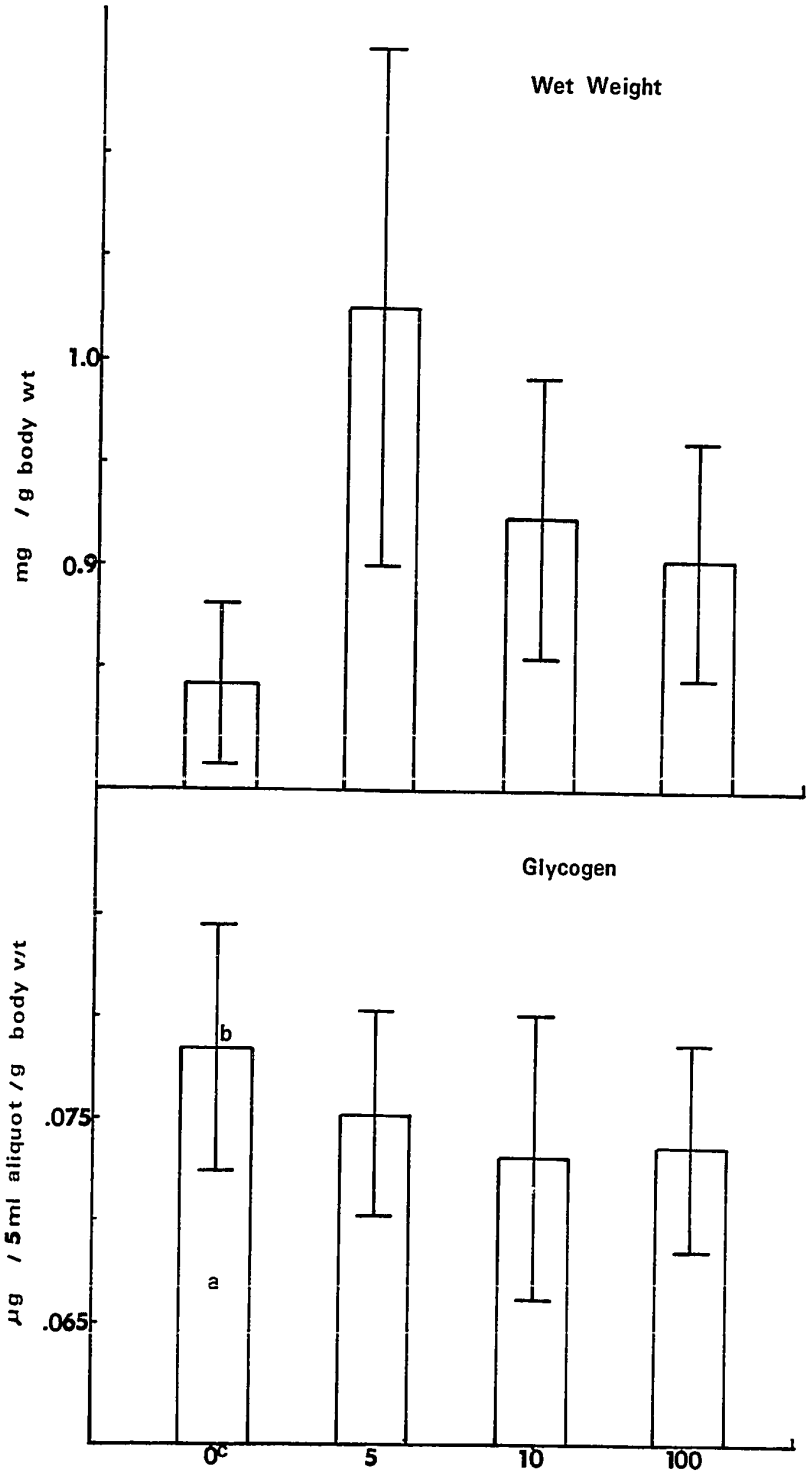

Wet Weight
mg / g body wt
1.0
0.9
Glycogen
µg / 5ml aliquot / g body wt
.075
.065
b
a
0
5
10
100
p,p-DDT (ppm) + Estradiol-17ß (0.5 µg)

FIGURE 14

BODY WEIGHT GAIN OF IMMATURE SPRAGUE-DAWLEY RATS RECEIVING EITHER VARIOUS CONCENTRATIONS OF o,p'-DDT OR VARIOUS CONCENTRATIONS OF o,p'-DDT AUGMENTED WITH A DAILY SUBCUTANEOUS INJECTION OF 0.5 µg ESTRADIOL-17β

[a] o,p'-DDT (ppm) treated animals, N = 6.

[b] o,p'-DDT (ppm) + estradiol (0.5 µg), N = 6.

[c] control animals receiving an oil injection.

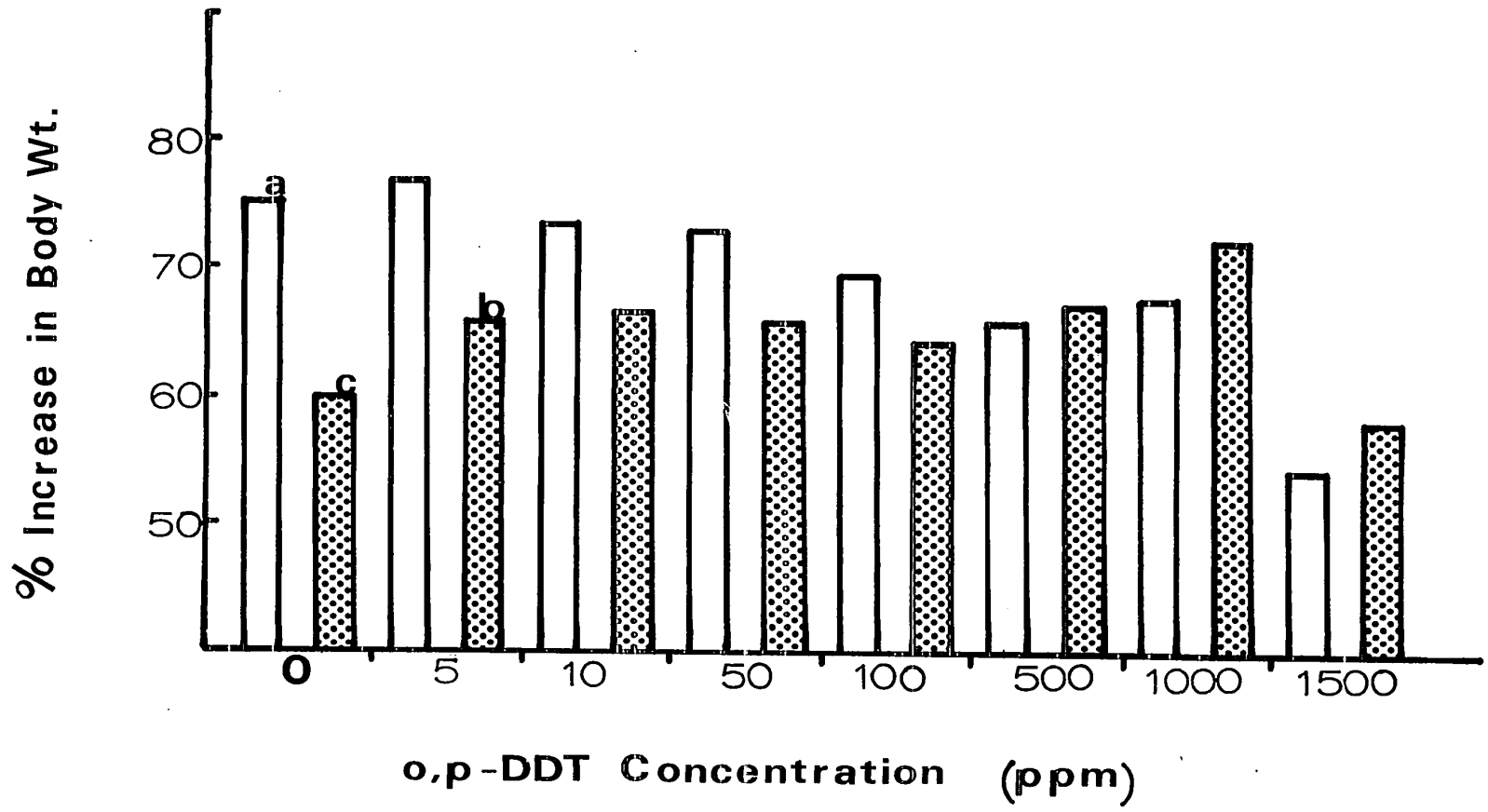

% Increase in Body Wt.
80
70
60
50
a
b
c
0
5
10
50
100
500
1000
1500
o,p-DDT Concentration (ppm)

FIGURE 15

FINAL BODY WEIGHT OF MATURE OVARIECTOMIZED FEMALE SPRAGUE-DAWLEY RATS FOLLOWING 54 DAYS EXPOSURE TO VARIOUS CONCENTRATIONS OF o,p'-DDT AND p,p'-DDT

[a] mean ± standard error

[b] Statistically different from controls ($p<0.01$).

[c] 0.5 µg estradiol-17β/0.15 ml corn oil was injected (sc) daily into all treated animals during the final week of the experiment.

[d] control animals received sc oil injections during the final week of the experiment.

[e] N = 4 to 8 animals / group.

FIGURE 16

LIVER WEIGHTS OF MATURE OVARIECTOMIZED SPRAGUE-DAWLEY RATS FOLLOWING 54 DAYS EXPOSURE TO VARIOUS CONCENTRATIONS OF o,p'-DDT AND p,p'-DDT

[a] mean ± standard error

[b] statistically different from controls ($p<0.01$).

[c] 0.5 µg estradiol-17β/0.15 ml corn oil was injected (sc) into all treated animals during the final week of the experiment.

[d] N = 4 to 8 animals / group.

[e] control animals received sc oil injections during the final week of the experiment.

hepatic metabolism of estrogen. As the concentration of o,p'-DDT is increased, concomitant with its increasing ability to induce hepatic enzymes, is it's inherent estrogenicity. At low concentrations (ca. 100 ppm) the estrogenicity of the o,p'-DDT molecule is low and its affect is not prominent in the bioassays used, however, at higher concentrations the inherent estrogenicity is large enough to produce a significant estrogenic response in uterine wet weight, dry weight and glycogen. The antiestrogenic effect at low concentration and estrogenic effect at high concentrations of o,p'-DDT was noted in both immature rats and in mature ovariectomized female rats; thus the estrogenic and antiestrogenic properties aren't mediated by the ovaries. This is in agreement with similar observations made in rats (Welch et al., 1969) and mink (Duby et al., 1971).

As we previously mentioned, few animals arelikely to be exposed to estrogenic doses of o,p'-DDT even in grossly contaminated areas. Conversely, the antiestrogenic properties of o,p'-DDT, at low concentrations are more deleterious to the animal possibly by altering the endocrine homeostasis resulting ultimately in either a pathological condition or an impaired reproductive performance.

SUMMARY

1. o,p'-DDT is as effective as p,p'-DDT in reducing sodium pentobarbital sleep time.

2. In intact immature female rats, the antiestrogenic properties of low concentrations of o,p'-DDT induced enzymes which degraded endogenous estradiol resulting in a decreased estrogen response; at high doses the inherent estrogenicity of the o,p'-DDT molecule was great enough to stimulate an increase in the estrogen response even though the antiestrogenic properties (increased hepatic microsomal enzyme metabolism) were still present. Similar results were obtained with mature ovariectomized rats indicating that the estrogenic and antiestrogenic properties were not mediated by the ovaries.

3. Body weight gains of intact immature female rats tended to be less than control value at high dietary concentrations of o,p'-DDT; in mature ovariectomized rats only 500 ppm of o,p'-DDT produced a statistical difference in body weight.

4. p,p'-DDT produced a decrease in the estrogen response of uterine wet weight and glycogen but not uterine protein.

5. 500 ppm o,p'-DDT and 100 ppm p,p'-DDT produced increases in liver weight of mature ovariectomized female rats exposed to DDT for 54 days suggesting that hepatic microsomal enzyme induction had taken place.

CHAPTER III

EFFECT OF DDT ON REPRODUCTION IN THE LABORATORY RAT

A number of studies have been performed investigating the effects which DDT has on reproduction in mammals and birds. Ratcliffe (1967) and Jefferies (1967) discovered that DDT causes hormonal disturbances which adversely affects reproduction in birds. Ware and Good (1967) discovered that low dietary concentrations of DDT had no effect on the sex ratio but it did cause a decrease in fertility and an increase in fecundity (litter size) in mice. Bernard and Gaertner (1964) and Cannon and Holcomb (1968) found that 200 and 300 ppm of DDT, when present in the diet, significantly reduced the fertility of mice with no effect on fecundity. However, other investigators found that DDT had no adverse effects on reproduction in rats and mice (Duby et al., 1971; Ottoboni, 1969; Tarján and Kemény, 1969; van Tienhoven and Duby, 1972). Ottoboni (1972) recently reported that low concentrations of tech. DDT increased the reproductive life span of the female rat.

DDT can cross the placenta in rats (Backström et al., 1965), dogs (Finnegan et al., 1949), rabbits (Pillmore et al., 1963) and man (Dénes, 1962; O'Leary et al., 1970) and is present in the maternal milk (Ottoboni and Ferguson, 1969). Sex steroids when administered to the neonatal female rat permanently modifies the hypothalamic regulation of LH secretion possibly by action at the level of the preoptic-anterior hypothalamic area, ultimately impairing reproduction. DDT has been shown to

possess estrogenic and antiestrogenic properties. Heinrichs et al. (1971) discovered that rats injected with high concentrations of o,p'-DDT, during the criticle period (Day 1 to 5 postpartum) sustained hypothalamic damage which resulted in a persistant estrus, anovulatory condition later in life.

Little scientific literature is available concerning the reproductive performance of progeny perinatally exposed to DDT. This study was undertaken to assess what effects various concentrations of o,p'-DDT had on the reproductive performance of rats chronically exposed to DDT and perinatally exposed to DDT, "in utero" (across the placenta) and through the maternal milk.

METHODS AND MATERIALS

Experimental Conditions

Male and female rats of Wistar-strain, 80 to 100 days old on a light regimen of 14 hours of light and 10 hours of dark were randomly assigned to diets containing either 0, 20, 200, or 1000 ppm of o,p'-DDT or 0, 20, 200 or 500[1] ppm of p,p'-DDT prepared as previously described. Temperature of the animal quarters was 72°F and relative humidity 35 to 40%.

Breeding Technique

The mated pairs were caged together throughout the entire experiment to facilitate intensive breeding. This would assure that the female would be exposed to a male during her postpartum estrus, which occurs 12 hours after parturition, thereby producing a maximum number of litters during the experimental period. No record was kept as to the date of conception or gestation time.

Collection of Data

Upon parturition, litter size, weight, sex ratio and mortality were recorded. Offspring were weaned at 21 days, caged individually and fed control food. Body weights were taken at various intervals to assess the effect of DDT on growth. At approximately 80 days of age vaginal smears were taken to ascertain whether the female rats had normal reproductive cycles. The F_1 progeny of treated and control groups were mated when approximately 105 days old to assess the fertility of rats perinatally exposed to DDT.

RESULTS AND DISCUSSION

Effect of Chronic DDT Exposure On Reproductive Performance of Sexually Mature Wistar Rats

The experimental period was approximately 180 days long. During this period all treatment groups produced at least four litters, some produced six (Table 3). Fertility was measured using the Productivity Quotient (P.Q.) which is equal to the number of young produced per 100 days. 20 ppm o,p'-DDT was the only concentration which significantly increased fertility ($p<0.05$); 200 ppm o,p'-DDT had a P.Q. very similar to that of 20 ppm o,p'-DDT but this couldnot be tested statistically because N=1. One mated pair at 200 ppm o,p'-DDT didn't produce any litters throughout the entire experimental period. Upon mating this pair with known breeders it was found that the male was sterile. Vaginal plugs were found indicating he had copulated but no pregnancy resulted. The male was not a proven breeder before the experiment began so it cannot be infered that the sterility of the male was the result of the DDT treatment.

1000 ppm o,p'-DDT produced an overall statistical difference in fecundity (littersize) as compared with the control group (Table 3). Greenwald (1967) found that egg transport in the rat is extremely sensitive to estrogen. Exogenous estrogen (5 µg estradiol cyclopentylproprionate) in the rat caused premature entry of ova into the uterus and then their expulsion "per vaginam". This accelerated ova transport reduced the mean number of implanted embryos from 11.3 to 3.9/pregnant rat. The

Table 3. Fertility and Fecundity (Litter Size)[d] of Female Albino Rats Chronically Exposed to DDT over Many Generations.

treatment	fertility P.Q.[a]	fecundity litter number						
		mean	1	2	3	4	5	6
control	35.1	11.7	12.5	13.0	10.4	11.0	11.0	-
o,p'-20	50.5[b]	12.8	13.5	13.0	18.0[c]	9.0	13.0	10.0
o,p'-200	47.0	14.7	15.0	14.0	18.0[c]	12.0	-	-
o,p'-1000	28.0	6.7[c]	13.0	6.5	7.0[b]	4.5[c]	2.5	-
p,p'-20	47.0	11.8	14.0	15.0	13.5	7.0[c]	13.0	5.0
p,p'-200	33.0	11.5	12.0	16.0	5.0[c]	9.0	9.0	17.0
p,p'-500	34.0	10.0	11.0	12.0	11.0	9.0	9.0	8.0

[a] Productivity Quotient = no. of young produced / 100 days.

[b] Statistically different from controls ($p<0.05$).

[c] Statistically different from controls ($p<0.01$).

[d] Litter size = total no. of young in each litter(male and female; alive or dead).

reduction in litter size at 1000 ppm o,p'-DDT may be the result of the estrogenicity of this large concentration causing accelerated ova transport and expulsion "per vaginam."

Progestrone and small amounts of estrogen are needed in order to precondition the uterine endometrium for blastocyst implantation. It is probable that progesterone is being metabolized by the induced hepatic enzymes resulting in an underdeveloped uterine endometrium which reduces blastocyst implantation. However, since p,p'-DDT, a potent inducer of hepatic enzymes, didn't cause any significant decrease in litter size even at high concentrations, the hypothesis that increased progesterone metabolism decreases blastocyst implantation "may be" discarded.

The ovarian follicles require FSH and LH in order to produce significant quantities of estrogen. When the level of estrogen in the blood becomes high, indicating that the ovarian follicles are fully grown, it acts to prevent a greater release of FSH by the adenohypophysis and to promote an augmented release of LH. At high concentrations o,p'-DDT could possibly affect reproduction at the hypothalamic level by causing a decreased FSH output resulting in a decrease in the number of mature follicles ovulated. From Table 3, it can be seen that the first litter produced by the o,p'-1000 is not significantly different from the control value but for all subsequent litters are significantly different ($p<0.05$) from the control group. The female's ovaries near the time of her

first conception probably had a large number of follicles in various stages of development due to FSH stimulation before and during the early stages of insecticide exposure. The estrogenicity of o,p'-DDT probably caused a decrease in FSH secretion while augmenting the LH release resulting in ovulation. However, following the first litter the FSH secretion is probably chronically depressed by this continuous feedback of high concentrations of o,p'-DDT to the hypothalamus. This produces a decrease in the number of mature follicles being ovulated resulting ultimately in a decreased fecundity.

20 and 200 ppm of o,p'-DDT produced litters which had a higher fecundity than controls however there was no statistical difference between them. 20 and 200 ppm also had fertility rates which were greater than controls (Table 3).

I have previously shown that low doses of o,p'-DDT ca. 100 ppm possess antiestrogenic properties by virtue of increased metabolism of estrogens but possess little inherent estrogenicity. This increased metabolism of estrogens probably causes a failure of the estrogen negative feedback to the hypothalamus which controls gonadotropin secretion from the pars distalis of the adenohypophysis. Another possibility is that the estrogenicity of o,p'-DDT even at low doses may permit it to bind with the estrogen receptors in the hypothalamus thus exerting an antiestrogenic effect in this manner. This reduced binding of estradiol by the hypothalamus results in a prolonged FSH secretion which causes an increased number of follicles to mature and be ovulated.

In general large litters are associated with small young (Murray, 1941). Therefore, in a plot of litter size versus birth weight the least squares regression curve should have a negative slope. However, in this study, no significant relationship between litter size and birth weight was obtained. The predisposition of a mother to cannibalize her young at birth before I recorded the birth weight and litter size, could account for this discrepancy. 200 ppm o,p'-DDT and 20 ppm p,p'-DDT produced statistical differences ($p<0.02$) in overall birth weight (Table 4). There was no significant within treatment group variation. The overall reduction in birth weight at o,p'-200 ppm was due to the reduction in birth weight of litter number three which was very large (18 pups). However, if this litter was left out of calculating the overall mean then the mean was found not to be significantly different from the overall control value.

200 ppm o,p'-DDT produced a significant decrease ($p<0.05$) in the overall sex ratio (Table 5) whereas 20 and 1000 ppm o,p'-DDT and 20, 200 and 500 ppm p,p'-DDT had at least 1 litter with the sex ratio significantly different from the control group. 1000 ppm o,p'-DDT and 500 ppm p,p'-DDT produced a statistical difference ($p<0.05$) in the sex ratio over the experimental period demonstrating within treatment group differences.

All the treated males (except one at 200 ppm o,p'-DDT) sired at least four litters, therefore demonstrating that the

Table 4. Weight of Pups at Birth.[a]

		weight of pups (g)					
		litter number					
treatment	mean	1	2	3	4	5	6
control	6.1	5.9	6.5	6.1	6.0	-	-
o,p'-20	5.6	5.5	6.3	5.7	5.1	5.7	-
o,p'-200	5.2[b]	5.8	6.0	3.6	5.3	-	-
o,p'-1000	5.8	5.5	6.8	-	5.4	6.2	-
p,p'-20	5.3[b]	5.5	5.4	6.0	4.7	4.4	-
p,p'-200	5.6	6.1	6.6	5.2	5.4	5.9	4.6
p,p'-500	5.4	5.2	5.7	5.3	5.3	5.3	-

[a] Weight of pups at birth = $\frac{\text{live weight of litter}}{\text{no. of pups born alive}}$

[b] Statistically different from controls ($p<0.05$).

Table 5. Sex Ratio - % Males at Birth.

		litter number					
treatment	mean	1	2	3	4	5	6
control	50.2	52.6	47.2	57.1	42.2	27.3[b]	-
o,p'-20	45.0	37.1[a]	39.3	58.1	33.3	53.9[a]	60.0[b]
o,p'-200	28.4[a]	26.7[a]	28.6[a]	33.3[a]	25.0[a]	-	-
o,p'-1000	33.3	41.7	54.2[b]	37.5[a]	41.7	16.7[b]	-
p,p'-20	48.3	53.1	49.6	58.5	33.3	53.6[a]	20.0[b]
p,p'-200	47.4	41.7	43.8	40.0[a]	55.6	44.4[a]	58.8
p,p'-5000	49.8	63.6	33.3[b]	18.2[ab]	66.7[ab]	66.7[ab]	50.0

[a] Statistical difference[c] ($p<0.05$) between control values (situated at the top of the collumns) and the treated values within the various litters.

[b] Statistical difference ($p<0.05$) between the observed value of the various litters and the mean value for a particular treatment. Demonstrates within treatment differences.

[c] Statistical tests were performed using χ^2 with Yates correction for continuity.

male reproductive performance is not affected significantly by the DDT treatment.

Higher concentrations of DDT (1000 ppm o,p'-DDT and 200 ppm p,p'-DDT) caused a significant retardation ($p<0.05$) of growth as evidenced by a decrease in body weight at weaning (Fig. 17). Complete data were not available for p,p'-500 because of the large amount of neonatal mortality. Litter growth rates can be used as an estimation of lactational performance. The growth rates of the animals from the high DDT treatments were statistically different from controls. The dams lactational performance may be decreased by an altered endocrine homeostasis such as increased liver metabolism of steroids. 20, 200 and 500 ppm of p,p'-DDT produced statistical differences ($p<0.01$) in the Lactation Index (number of young alive at weaning / number of young alive at 5 days x 100) with 500 ppm of p,p'-DDT resulting in no progeny reaching the weaning age (Table 6). A lactation failure was noted in the p,p'-500 ppm group. The mother took up the lactation stance and the pups were suckling, but no milk was evident in their stomachs. This lactation failure could result from the increased metabolism of steroids causing under-development of the mammary gland; 1000 ppm o,p'-DDT, due to its estrogenicity may inhibit lactation. It is not known whether inhibitory action of estrogen is effected through the pituitary or at the level of the mammary gland or both. Note that litter size had no obvious effect on growth up to weaning age (Fig. 18).

FIGURE 17

GROWTH CHART OF SUCKLING PUPS PERINATALLY EXPOSED TO DDT " IN UTERO" AND THROUGH THE MATERNAL MILK

[a] concentration of o,p'-DDT (ppm), N = 2 to 10.

[b] concentration of p,p'-DDT (ppm), N = 2 to 10.

[c] The litter used for construction of the growth chart had a number of pups which lost weight and were in various states of tremoring. These pups oxygen consumption was measured; see chapter V.

[d] no pups lived any longer than 10 days.

[e] control pups

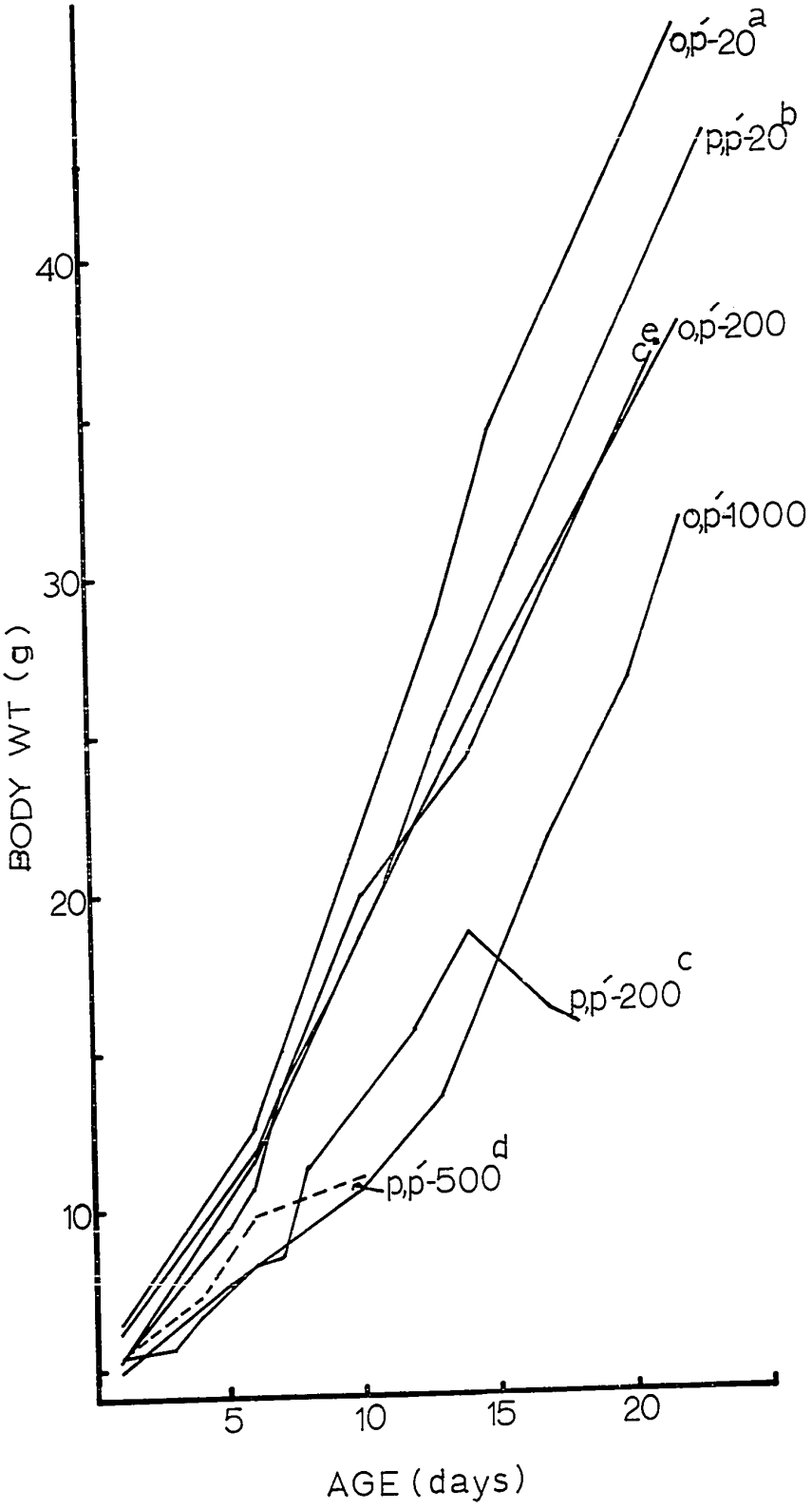

o,p'-20 a
p,p'-20 b
e
c
o,p'-200
o,p'-1000
p,p'-200 c
p,p'-500 d
40
30
20
10
BODY WT (g)
5
10
15
20
AGE (days)

Table 6. Viability of Pups from the F_0 Generation Chronically Exposed to DDT in the Food.

treatment	viability index(V.I.)[a]	lactation index(L.I.)[b]
control	66.75	72.11
o,p'-20	87.50[d]	54.66[c]
o,p'-200	79.16	100.00[d]
o,p'-1000	58.39	81.66
p,p'-20	40.87[d]	40.00[d]
p,p'-200	81.10[c]	37.50[d]
p,p'-500	33.33[d]	0.0[d]

[a] V.I. = no. of pups alive at 5 days postpartum / no. of pups born(alive or dead) ×100.

[b] L.I. = no. of pups alive at 21 days postpartum / no. of pups alive at 5 days ×100.

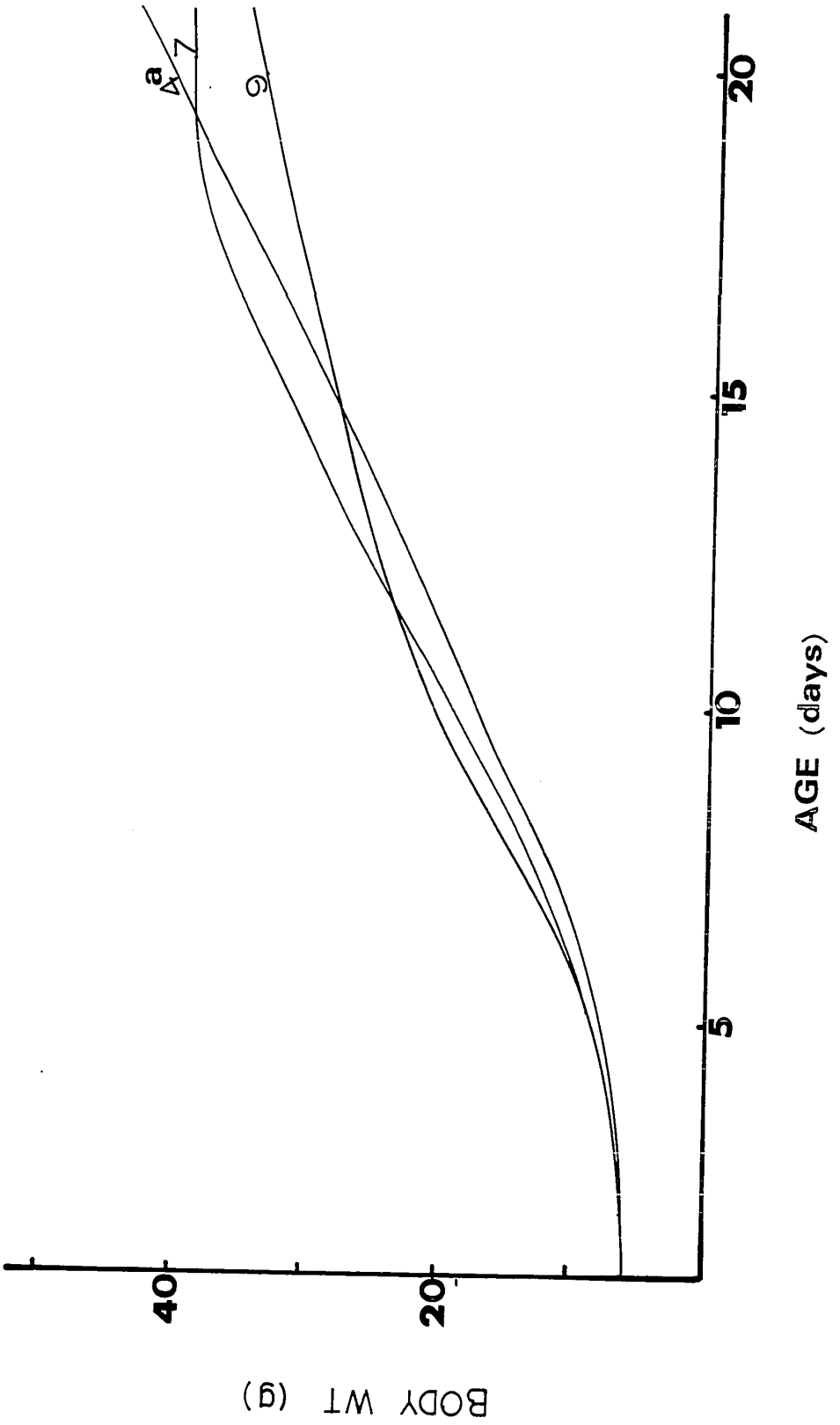
[c] Statistically different from controls ($p<0.05$).[e]

[d] Statistically different from controls ($p<0.001$).

FIGURE 18

EFFECT OF LITTER SIZE ON NEONATAL GROWTH

[a]
litter size


BODY WT (g)
40
20
5
10
15
20
AGE (days)
♂
♀

Upon weaning all animals were fed control food which was nearly free of insecticide contamination[2] for the rest of their lives. Male rats exposed to various concentrations of DDT "in utero" (across the placenta) as well as neonatally through the maternal milk had body weights which were not statistically different from control males at 105 days of age (Fig. 19). The same was true for females (Fig. 19). However a statistical sex difference ($p<0.05$) with males being larger than females was observed in all groups. These results show that the growth depressing effect of DDT during early postnatal life is reversible upon termination of insecticide exposure and suckling.

Liver weights of neonatal animals (age 1 to 10 days) were recorded in order to assess the effect DDT had on the liver weight to body weight ratio (Fig. 20). No statistical difference in liver weight (expressed as mg liver/ g of body weight) were observed except in the p,p'-200 group at 5 days and o,p'-200 group at 10 days ($p<0.05$).

As was previously shown p,p'-DDT produced no statistical differences with respect to fertility, fecundity, birth weight or sex ratio, however p,p'-DDT exposure caused a significant increase in neonatal mortality as evidenced by a decrease in the lactation and viability indices (Table 6). o,p'-DDT, on the other hand, caused an increase in the lactation index (L.I.) as the concentration increased showing that o,p'-DDT doesn't affect lactation at the high concentrations. However, at 20 ppm o,p'-DDT causes initially a significantly higher viability index (V.I.) ($p<0.001$) but a significantly smaller ($p<0.05$) L.I..

FIGURE 19

GROWTH CHART OF F_1 MALE AND FEMALE WISTAR RATS (20 to 105 days) PERINATALLY EXPOSED TO DDT " IN UTERO " AND THROUGH THE MATERNAL MILK

[a] N = 2 to 23 / group.

[b] no statistical differences were found between treated and control males and females, however, there was a statistical difference between sexes ($p<0.05$). The growth curves are those of control male and female rats.

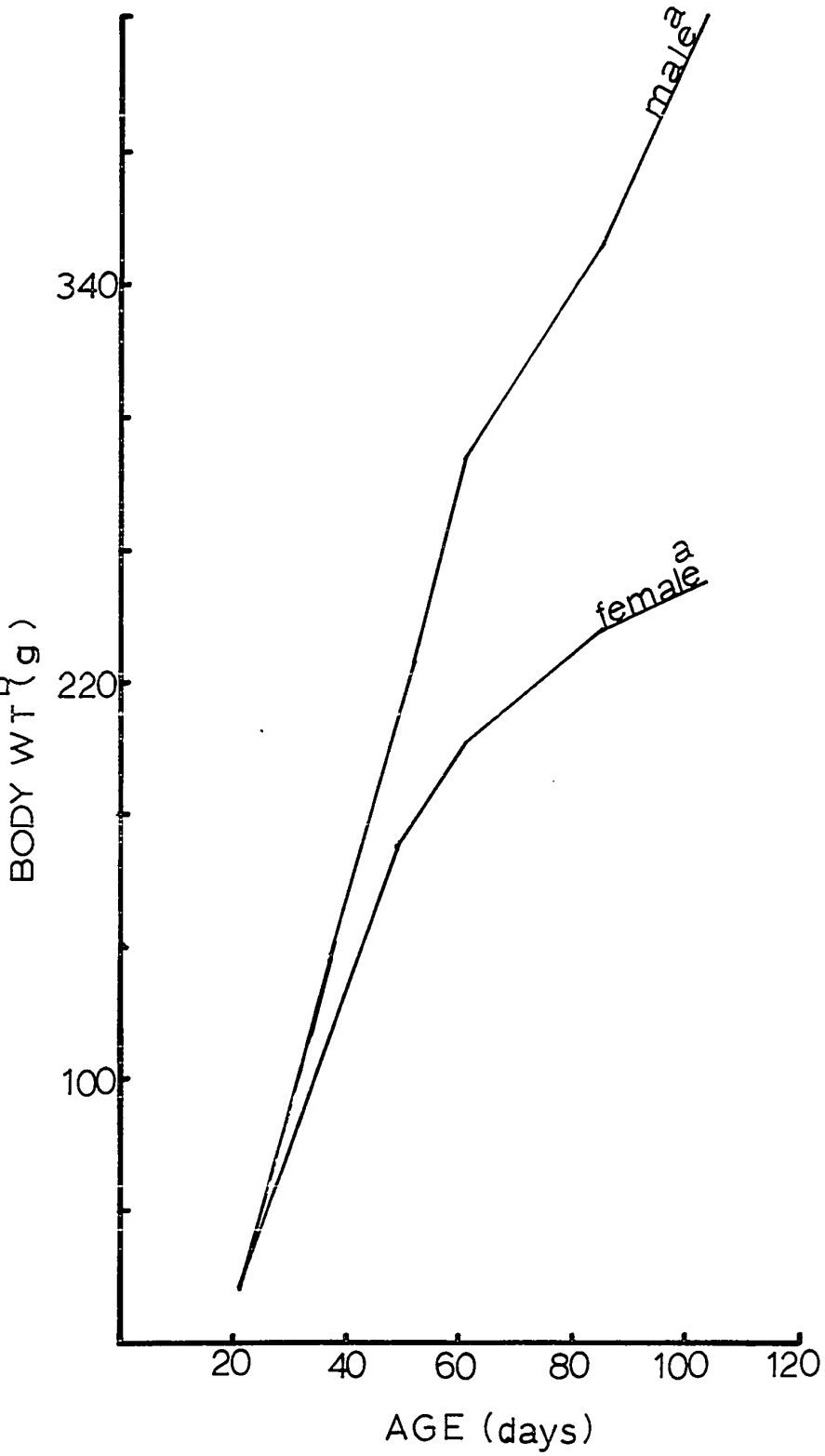

male[a]
female[a]
340
220
100
BODY WT[b] (g)
20
40
60
80
100
120
AGE (days)

FIGURE 20

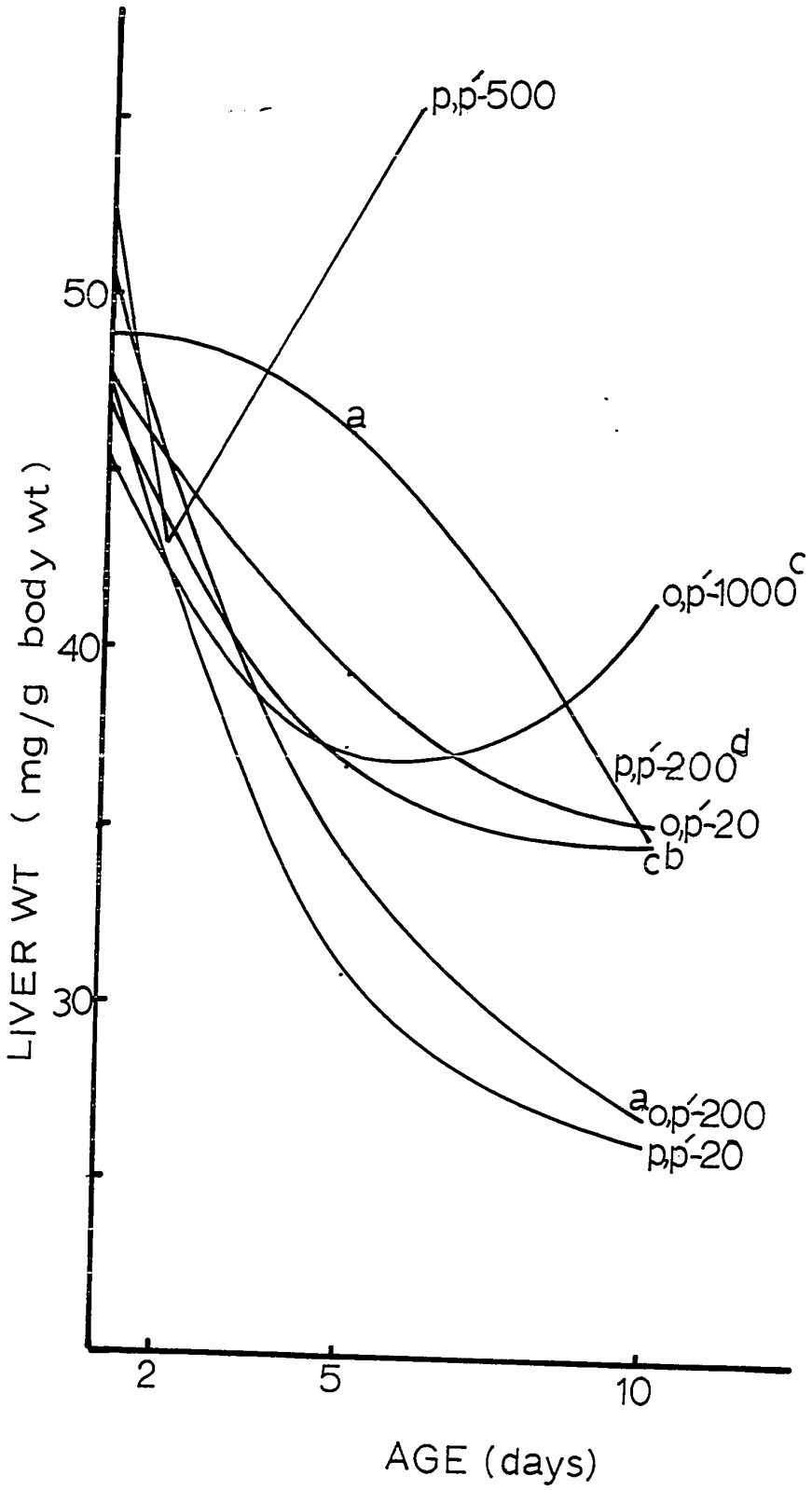
EFFECT OF PERINATAL EXPOSURE TO VARIOUS CONCENTRATIONS OF DDT ON THE NEONATAL LIVER WEIGHT IN WISTAR RATS.

[a] Statistically different from controls ($p<0.05$).

[b] untreated controls.

[c] concentration of o,p'-DDT in ppm.

[d] concentration of p,p'-DDT in ppm.


p,p'-500
50
a
o,p'-1000
c
40
p,p'-200
d
o,p'-20
c b
30
a o,p'-200
p,p'-20
LIVER WT (mg/g body wt)
2
5
10
AGE (days)

Therefore, o,p'-DDT (20 ppm) doesn't produce much neonatal death but it does reduce the lactation performance of the females as evidenced by the L.I. producing a reduction in the number of animals alive at weaning. The reduction in lactation is probably the result of the antiestrogenic properties of low concentrations of o,p'-DDT. The increased hepatic metabolism of estrogens could result in underdevelopment of the mammary glands therefore impairing lactation.

The large amount of mortality present in Wistar rats was not present in Sprague-Dawley rats similarly exposed (Ottoboni, 1969). This may indicate some strain difference with respect to viability of the young.

During the course of this experiment 2 control females and 1 p,p'-200 ppm female died while trying to give birth; 1 control male died from undetermined causes; 1 p,p'-750 ppm female died from DDT toxicity before the diet was reduced to p,p'-500 ppm.

The results demonstrate that chronic environmental exposure to DDT doesn't affect the reproductive performance of the laboratory rat except at high concentrations (ca. 1000 ppm o,p'-DDT). Bernard and Gaertner (1964) and Cannon and Holcomb (1968) found that DDT decreased the reproductive performance of mice. They used continuous lighting in their reproductive studies (continuous lighting induces a state of continuous estrous in females). This sychronized the colony so that the females will be in a receptive state when exposed to males. However, if reproduction is adversely affected you can't be certain whether the treatment or continuous lighting was the cause.

Chronic environmental exposure to p,p'-DDT increased the neonatal mortality. Several investigators have suggested that DDT may have hormonal or antifertility effects resulting in a decrease in small animal and bird populations. However, from the data presented DDT causes an increase in the neonatal mortality, therefore reducing the animal population in this manner.

Reproductive Performance of F_1 Progeny Perinatally Exposed To DDT "In Utero" And Through The Maternal Milk.

Vaginal smears were performed on all female progeny at age 80 days; all females were cycling normally. The neonatally exposed F_1 progeny were mated with F_1 controls, when approximately 105 days old to ascertain whether DDT had any detrimental effect on their reproductive performance (Table 7). o,p'-1000 ppm F_1 progeny produced a statistical difference ($p<0.05$) in fertility and fecundity. Of four o,p'-1000 female F_1 progeny, one produced a litter, one died giving birth and two didn't produce any litters. The two sterile females were sacrificed and the ovaries removed and saved for histological examination. Upon removal the ovaries appeared small and anemic with no haemorhhagic follicles present. One of the ovaries had a definite fluid filled cyst whereas the other's ovaries appeared to have clear follicles which were visible to the eye. The sterility of these two perinatally exposed females demonstrates that the reproductive performance can be decreased possibly due to a cystic ovary condition resulting from hypothalamic damage from concentrations of DDT "in utero" and through the maternal milk. p,p'-20 ppm produced a decrease ($p<0.05$) in birth weight. Significant differences were obtained with respect to the % of litters showing mortaility at birth. However, due to small sample size this data should be interpreted with caution.

All neonatally exposed F_1 males sired litters; high concentrations of DDT during early post-natal life doesn't decrease the male fertility.

Table 7. Reproductive Performance of F_1 Female Progeny Perinatally Exposed to DDT " in utero " (across the placenta) and Through the Maternal Milk.

treatment	N	fertility[a]	fecundity	birth wt. (g)	%males	% mortality[b]
control	14	100	13.6	6.2	46.5	14.4
o,p'-20	1	100	16.0	6.1	50.0	100.0[cd]
o,p'-200	4	100	13.8	5.5	45.2	25.0
o,p'-1000	4[e]	25[c]	3.0	5.9	0.0	0.0[c]
p,p'-20	4	100	13.8	5.5[c]	53.0	50.0[c]
p,p'-200	2	100	15.0	6.0	53.6	0.0

[a] fertility = no. of litters / no. of pairs bred × 100

[b] % mortality = % of total no. of litters in which mortality occurred.

[c] Statistically different from controls ($p<0.05$).

[d] This value should be interpreted with caution because only 1 litter was observed.

[e] N = 4 for fertility but N = 1 for all other parameters.

SUMMARY

1. 20 ppm o,p'-DDT increased fertility; 1000 ppm o,p'-DDT tended to decrease fertility.

2. High concentrations of o,p'-DDT (1000 ppm) decreased fecundity (litter size) probably by one of the following mechanisms:

    1. Accelerated ovum transport and expulsion "per vaginam".
    2. Increased metabolism of estrogen and progesterone resulting in an underdeveloped uterus.
    3. o,p'-DDT produced a decreased FSH secretion resulting in fewer follicles maturing.

3. Low concentrations of o,p'-DDT (20 and 200 ppm) increased fecundity possibly by one of the following mechanisms:

    1. Increased estrogen metabolism may cause failure of the estrogen negative feedback system.
    2. o,p'-DDT may compete with estradiol for binding sites in the hypothalamus producing a failure of estrogen negative feedback system.

4. No relationship between litter size and birth weight was found.

5. 200 ppm o,p'-DDT decreased the number of males born.

6. Male reproductive performance is not affected by various doses of DDT.

7. Large concentrations of DDT cause a growth depression probably the result of reduced lactation.

8. p,p'-DDT didn't produce any significant differences in fertility, fecundity, birth weight or sex ratio but all doses of p,p'-DDT caused an increase in neonatal mortality.

9. Perinatal exposure to high concentrations of o,p'-DDT (1000 ppm) caused a decrease in fertility and fecundity in F_1 female progeny probably due to hypothalamic damage incurred during the criticle period; F_1 males reproductive performance was not affected by perinatal exposure.

CHAPTER IV

EFFECT OF DDT ON THE HEPATIC MICROSOMAL ENZYMES OF PERINATALLY EXPOSED RATS

In fetuses and newborn animals, the activity of the hepatic enzymes required to metabolize and conjugate drugs and steroids is low apparently because of defect in enzyme synthesis, and can be markedly compensated for by treating the animals with various compounds - rats with 3, 4, -benzpyrene and mice with phenobarbitone (Fouts and Adamson, 1959).

Feuer and Liscio (1969) discovered that hepatic enzyme activity is higher in weaned than non-weaned rats. They postulated that elevated concentrations of female gonadal steroids "in utero" (across the placenta) and postnatally through the maternal milk cause a depression of drug metabolism in the fetal and newborn rat.

DDT possesses estrogenic properties and is a potent inducer of liver enzymes.

Does perinatal exposure to DDT "in utero" (across the placenta) or through the maternal milk result in induction of hepatic microsomal enzymes in the neonatal rat? To test this hypothesis a fostering system was devised.

METHODS AND MATERIALS

FOSTERING SYSTEM

Upon discovery of parturition (usually less that 12 hours) the litter of a DDT treated animal was crossed to a lactating control female with young approximately the same age and vice versa for the control litters. The litters were raised to weaning on this foster mother.

If the litters were of similar age the dam accepted them as her own without any problem; however, if the ages of the crossed litters were different (newborns and 2 week old pups) then the litter usually wasn't accepted and high mortality was prevalent.

RESULTS AND DISCUSSION

Liver homogenates were prepared (Appendix 4) and analysed for enzyme activity by the procedure of Kupfer and Bruggeman (1966). However, due to technical difficulties with the procedure no firm conclusions can be drawn concerning the activity of the hepatic microsomal enzymes.

There were no statistical differences ($p<0.05$) in the liver weight of control animals crossed to a treated mother and vice versa (Fig. 21 and 22).

Crossing the litters to foster mothers seemed to cause a decrease in liver weight as compared with the control value no matter which way the fostering took place (Fig. 23). When the DDT treated litters crossed to the control group are compared with a DDT treated group (not crossed) no definite trend was noted. (Fig. 23).

FIGURE 21

EFFECT OF CROSSING LITTERS, FROM CONTROL TO DDT EXPOSED FOSTER MOTHERS, ON NEONATAL LIVER WEIGHT

[a] uncrossed control

[b] control litter crossed to p,p'-DDT (ppm).

[c] control litter crossed to o,p'-DDT (ppm).

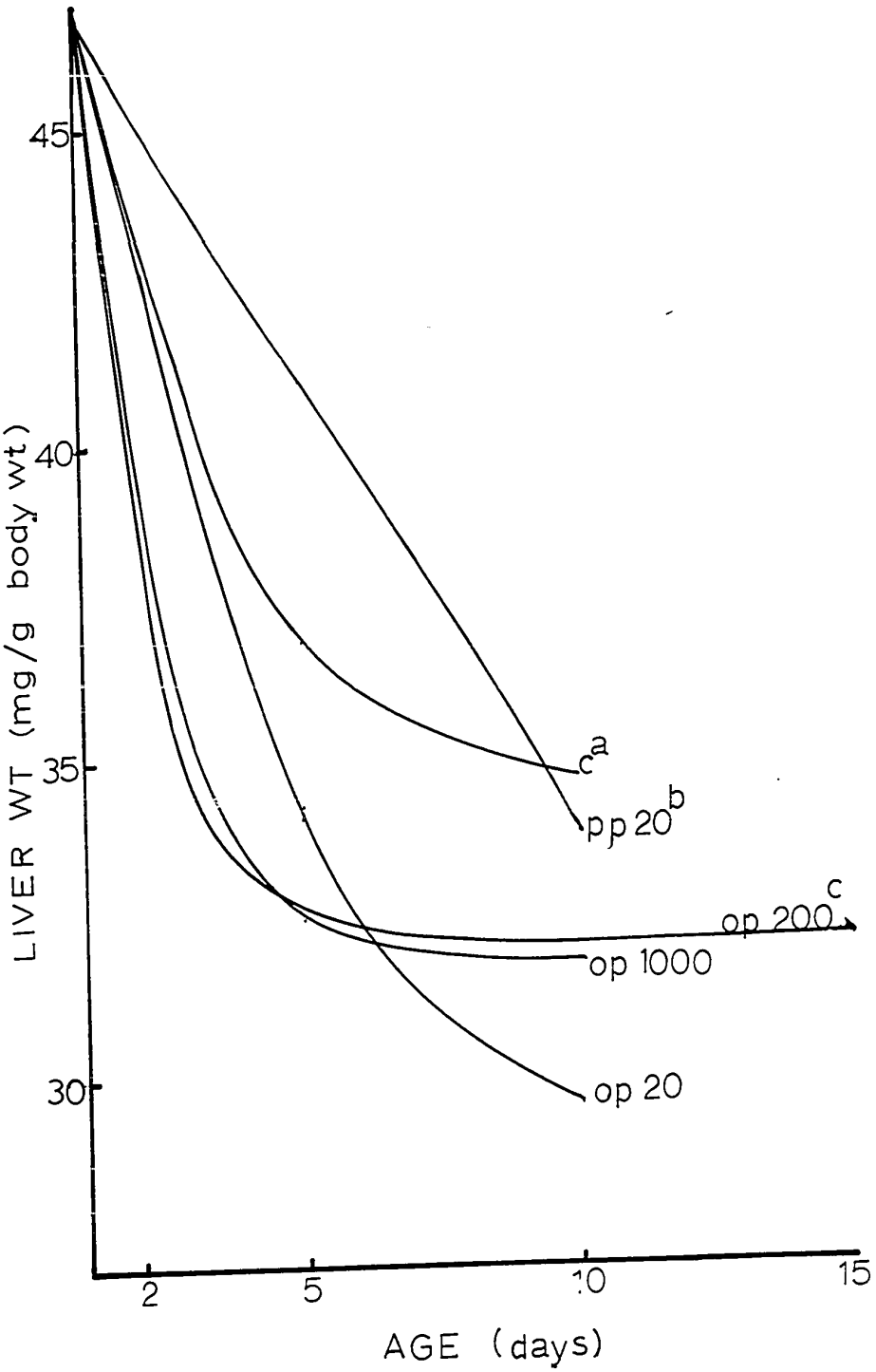

45
40
35
30
LIVER WT (mg/g body wt)
c[a]
pp 20[b]
op 200[c]
op 1000
op 20
2
5
10
15
AGE (days)

FIGURE 22

EFFECT OF CROSSING LITTERS, FROM DDT EXPOSED MOTHERS TO CONTROL FOSTER MOTHERS, ON NEONATAL LIVER WEIGHT

[a] concentration o,p'-DDT (ppm).

[b] concentration p,p'-DDT (ppm).

[c] non-crossed treated litter (treated control).

[d] treated litter crossed to control.

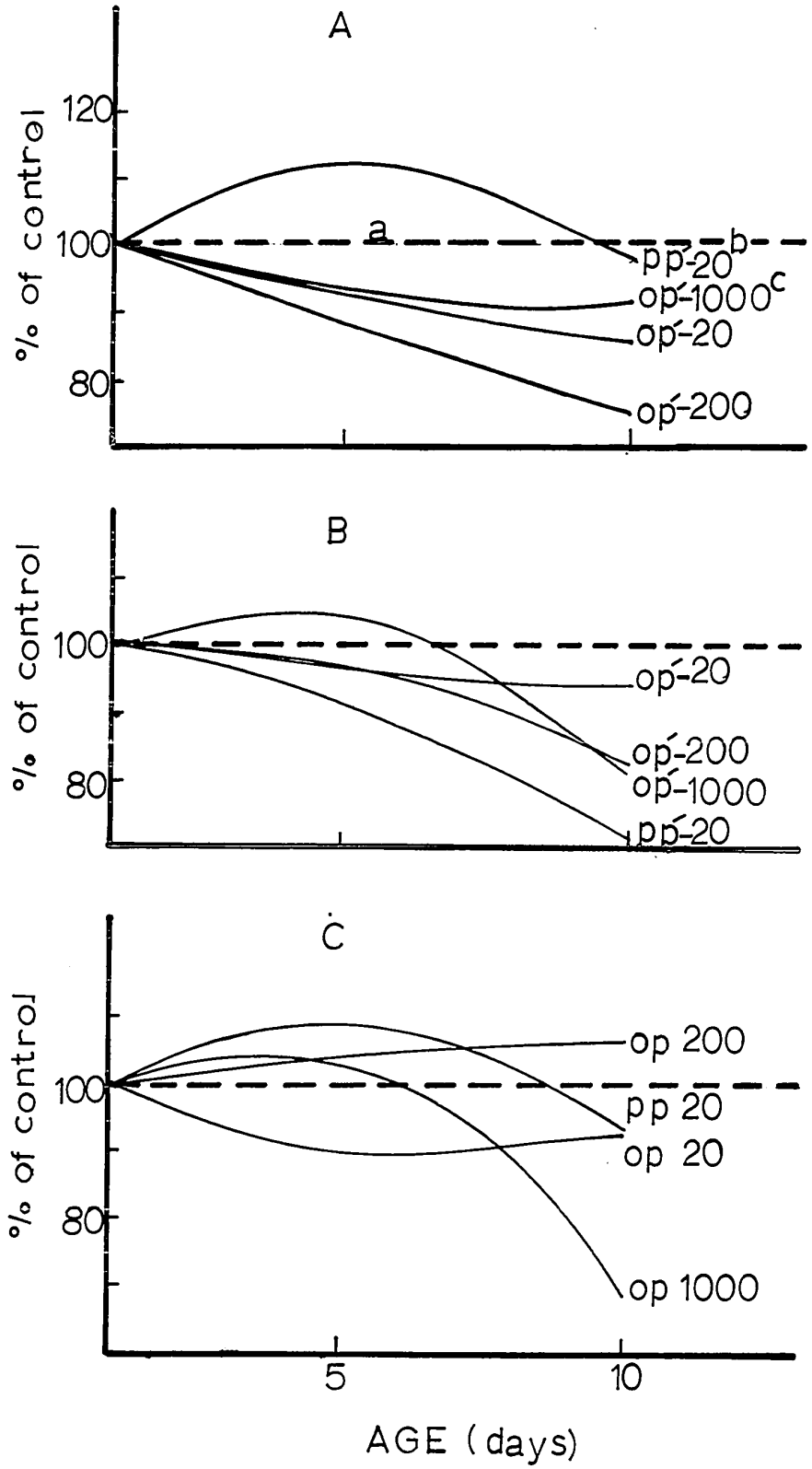
FIGURE 23

EFFECT OF CROSSING ON NEONATAL LIVER WEIGHT AS COMPARED WITH CONTROL LIVER WEIGHT

A
Neonatal Liver Weight. Control litters crossed to treated foster mothers. Values expressed as % of untreated uncrossed control of the same age.

B
Neonatal Liver Weight. Treated litter crossed to control foster mother. Values expressed as % of untreated uncrossed control liver of the same age.

C
Neonatal Liver Weight. Treated litter crossed to control foster mother. Values expressed as % of uncrossed similarly treated liver of the same age.


A
% of control
120
100
80
a
b
pp-20
op-1000
c
op-20
op-200
B
% of control
100
80
op-20
op-200
op-1000
pp-20
C
% of control
100
80
op 200
pp 20
op 20
op 1000
5
10
AGE (days)

CHAPTER V

EFFECT OF PERINATAL EXPOSURE TO DDT ON THE FUNCTIONAL STATUS OF THE THYROID GLAND

Little information is available in the scientific literature concerning the effect which DDT has on the thyroid gland in the rat.

Jefferies and French (1969) reported that p,p'-DDT produced an increase in thyroid weight and a reduction in colloid content of the follicles in pigeons. Liver weights were increased as well. They concluded that these results indicated either a hypo - or - hyper - functioning gland. (No precise statement could be made because the metabolic rate had not been measured). Fregly et al. (1968) concluded that increased thyroid weights in rats fed o,p'-DDT were symptoms of hypothyroidism.

Bakke and Lawrence (1965) discovered that administration of thyroxine to neonatal rats will induce a state of hypothyroidism later in life. Ottoboni and Ferguson (1969) found that milk obtained from lactating dams during early stages of lactation (first 3 days postpartum) contained greater concentrations of DDT than milk obtained at later stages of lactation. The high concentrations of p,p'-DDT (up to 750 ppm) in the milk during the criticle period of hypothalamic differentiation could possibly have some effect on hypothalamic organization of the neonate. DDT and thyroxine are similar in structure (Appendix 1); thus oxygen consumption

and resting metabolic rates[3] were measured to assess the functional status of the thyroid gland.

METHODS AND MATERIALS

OXYGEN CONSUMPTION AND CALCULATION "RESTING METABOLIC RATE" (RMR)

Between the ages of 16 and 18 days and 30 to 32 days oxygen consumption was calculated using the method of Ozburn and Morrison (1965). The animals were placed in the apparatus for an initial period of 45 minutes. This was the acclimation period. During preliminary studies it was found that the oxygen consumption during the first 45 minutes was significantly greater than the oxygen consumption during the second 45 minute period (Table8).

There was no statistical difference in oxygen consumption due to sex during either the first or second 45 minutes test period. Oxygen consumption and RMR were calculated irrespective of sex and using the readings from the second 45 minute test period. The animal's "resting metabolic rate" ($Cal/hr/m^2$) was calculated using the following equation:

$$\text{R M R } (Cal/hr/m^2) = \frac{\dfrac{\text{Vol. } O_2 \text{ (l) x } 1.333 \text{ (B.P. - W.V.P.) x } 273}{} \text{ x } 4.825}{\text{Body Surface Area } (m^2)}$$

Table 8. Oxygen Consumption During the Initial Acclimation Period as Compared to the Test Period.

sex	O_2 consumption (ml/min/kg) acclimation period	test period
M[b]	235.44	179.87[a]
F[c]	227.99	182.08[a]

[a] Statistically different ($p<0.01$) from reading during the acclimation period.

[b] N = 8 to 10 animals

[c] N = 7 to 9 animals

Where

O_2 vol. = the volume of water displaced into the buret.

1.3333 = conversion factor to give litres of O_2 / hour.

B.P. = Barometric pressure at time of oxygen consumption measurement (mm H_g).

W.V.P. = Water vapour pressure (mm H_g) at the temperature of the respirometer chamber.

Temp. = temperature of respirometer chamber(^0C)

4.825 = the average caloric value of oxygen in Cal/ 1 O_2

$$\text{Body surface area } (m^2) = \frac{W^{0.667} \times 7.42}{100}$$

Where W = Weight in kg

7.42 = conversion factor (Klieber, 1947)

100 = 100 square decimeters

RESULTS AND DISCUSSION

Oxygen Consumption

Oxygen consumption data were collected for most treatment groups in order to assess what effect o,p'-DDT had on the thyroid gland. For 16-18 day old animals the oxygen consumption of the o,p'-20 and 200 ppm diets was statistically different ($p<0.05$) from the control value of 242.1 ml/min/ kg of body weight (Fig. 24). Oxygen consumption studies were also performed on 16-18 day old animals exhibiting whole body tremors (Fig. 24). These animals with severe tremors (p,p'-20 and p,p'-200 ppm) had statistically different ($p<0.05$) oxygen consumption rates than similarly treated animals not exhibiting tremors. Possibly these animals had less food stuffs to oxidize. Animals in a severe state of tremoring didn't eat any food; these animals died the day following measurement of oxygen consumption. Another possibility is that the DDT is acting as a goitrogen, promoting an increase in thyroid weight but blocking thyroxine synthesis. This would induce a hypothyroid state in the animal resulting in a decrease in oxidative metabolism and heat production producing a lower metabolic rate. Therefore, the tremors observed may result from the animal being cold (due to the hypothyroid condition) and not due to DDT toxicity.

Oxygen consumption studies performed on animals 30 - 32 days old produced no statistical differences ($p>0.05$) when compared with controls.

FIGURE 24

OXYGEN CONSUMPTION (IRRESPECTIVE OF SEX) OF PROGENY PERINATALLY EXPOSED TO DDT " IN UTERO " AND THROUGH THE MATERNAL MILK

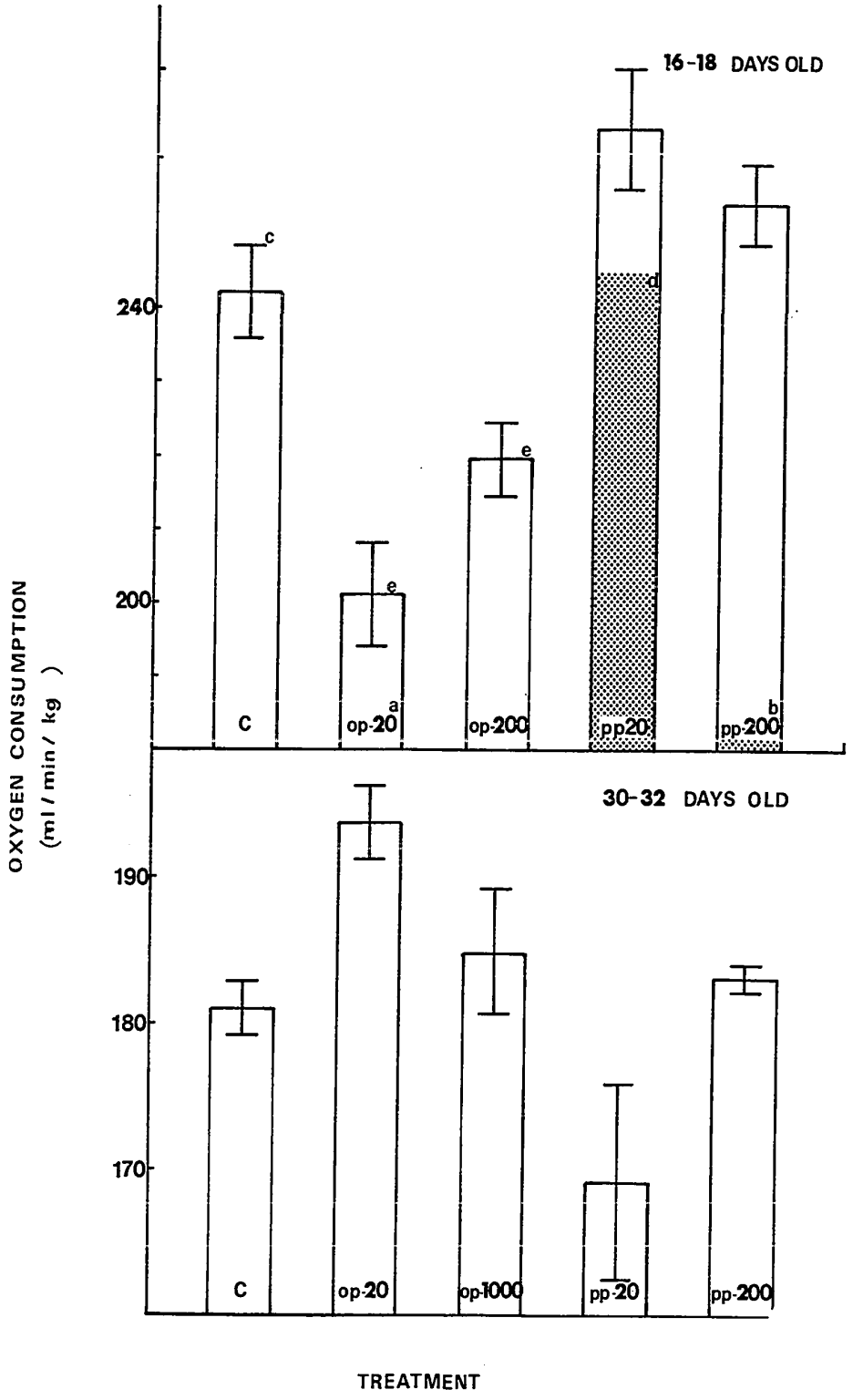
[a] concentration of o,p'-DDT (ppm).

[b] concentration of p,p'-DDT (ppm).

[c] mean ± standard error.

[d] oxygen consumption of animals in a severe state of tremors.

[e] statistically different from controls ($p<0.05$).


16-18 DAYS OLD
240
200
c
e
e
d
b
a
C
op-20
op-200
pp20
pp-200
30-32 DAYS OLD
190
180
170
C
op-20
op-1000
pp-20
pp-200
OXYGEN CONSUMPTION
(ml / min / kg)
TREATMENT

Resting Metabolic Rate

When body surface area (m^2) was calculated (Methods and Materials) and resting metabolic rate determined, a statistical difference ($p<0.05$) was obvious at 32 days of age (Fig. 25). All the neonatally exposed offspring had a lowered resting metabolic rate at age 30 - 32 days which was not apparent at 16 - 18 days of age, showing the animals to be hypothyroid (Fig. 25).

The mechanism for causing hypothyroidism in DDT treated animals is probably similar to that discovered by Bakke and Lawrence (1966). They injected the neonatal animal with thyroxine which produces a hypothyroid condition in the animal later in life.

Ottoboni and Ferguson (1969) found large concentrations of DDT in the milk of lactating rats exposed to DDT in their food. Therefore DDT, due to its similarity in structure with thyroxine, induced a state of hypothyroidism in the rats so exposed. Callard and Leathem (1965) found that a hypothyroid state predisposed female rats to cystic ovaries. Heinrichs et al. (1971) presented evidence which showed that the hypothalamus was damaged by high concentrations of o,p'-DDT during early postnatal life. This impairment of the hypothalamus prevents the pituitary gland from secreting adequate quantities of LH. These cystic ovaries found by Heinrichs et al. (1971), following neonatal injections of large concentrations of o,p'-DDT are probably the result of the induced hypothyroid state as well as a prolonged FSH secretion.

FIGURE 25

RESTING METABOLIC RATE (IRRESPECTIVE OF SEX) OF PROGENY PERINATALLY EXPOSED TO DDT " IN UTERO " AND THROUGH THE MATERNAL MILK

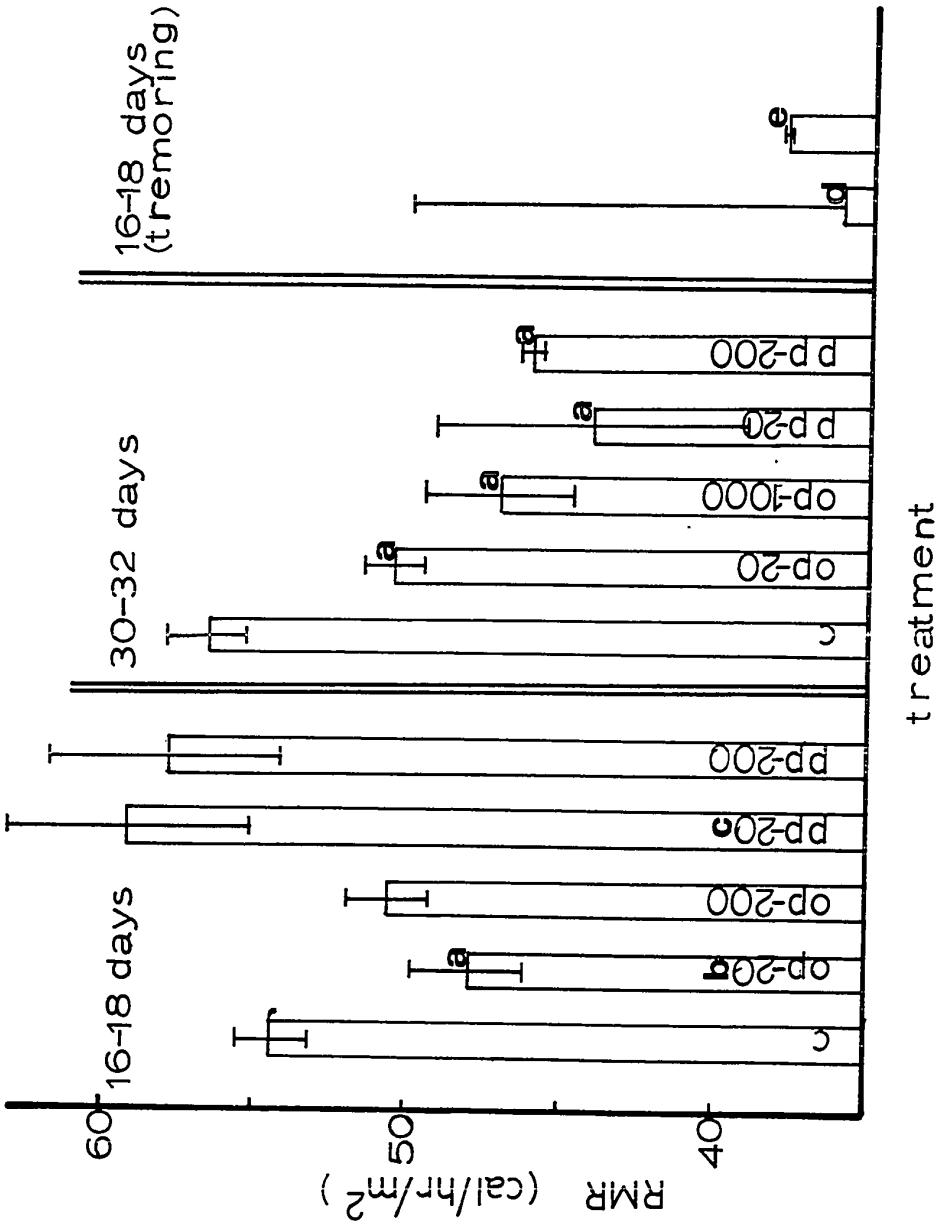
[a] statistically different from control ($p<0.05$).

[b] concentration of o,p'-DDT (ppm).

[c] concentration of p,p-DDT (ppm).

[d] almost statistically different from similarly exposed animals and controls ($0.1<p<0.05$).

[e] statistically different from similarly exposed animals without tremors as well as the control group ($p<0.05$).


RMR (cal/hr/m2)
60
50
40
16-18 days
c
f
OP-20
a
OP-200
PP-20
c
PP-200
30-32 days
c
OP-20
a
OP-1000
a
PP-20
a
PP-200
a
16-18 days
(tremoring)
d
e
treatment

SUMMARY

1. Perinatal exposure to 20 and 200 ppm o,p'-DDT produced a decrease in oxygen consumption when the animals are 16 - 18 days old; tremoring animals had lower metabolic rates than animals similarly exposed. No differences in oxygen consumption were apparent at 30 - 32 days.

2. Animals 30 - 32 days old, perinatally exposed to DDT all possessed RMRs lower than controls (hypothyroid state); 20 ppm o,p'-DDT produced a decreased RMR in 16 - 18 day old animals.

3. The hypothyroid condition predisposes the females to cystic ovaries.

FOOTNOTES

1. The p,p'-500 ppm group initially was fed a diet containing 750 ppm p,p'-DDT for a period of 10 days. This high concentration of p,p'-DDT caused tremors in all animals and the death of 1 female. On day 11 of the experiment the concentration of p,p'-DDT was reduced to 500 ppm; the tremors stopped.

2. Commercial laboratory ration contains 0.06 to 0.10 ppm DDT with approximately 50% of this being in the form of p,p'-DDE (Ottoboni and Ferguson, 1969). DDT is very slowly metabolized to DDE in mammals. The metabolite has a similar inducing capacity but stays in the organism much longer (Remmer et al., 1968).

3. The metabolic rates measured were not basal because food consumption wasn't regulated before the test was performed.

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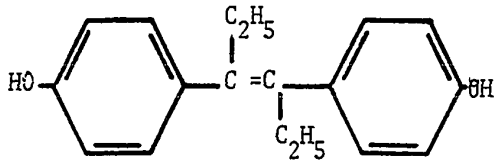
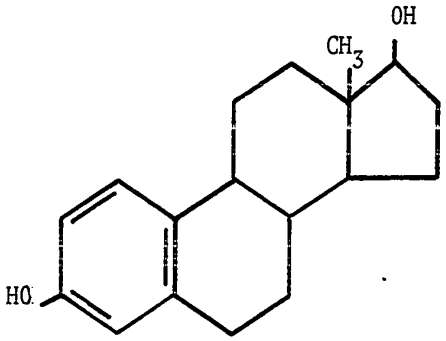
APPENDIX 1

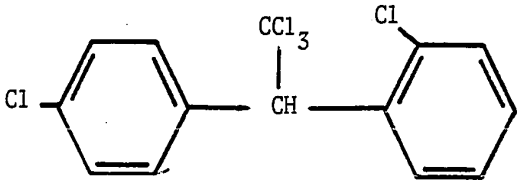
Diethylstilbestrol (α,α'-diethylstilbenediol)



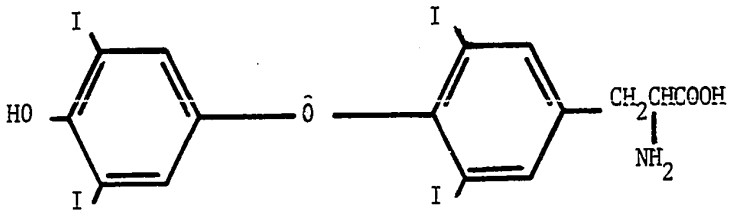
Estradiol- 17β ((estra-1,3,5(10)-triene-3,17β-diol))



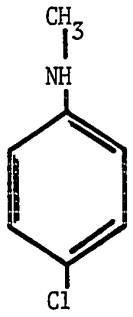
o,p'-DDT ((1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl) ethane)).



Thyroxine (3,5,3',5'-tetraiodothyronine)



P-chloro-N-methylaniline



APPENDIX 2

Determination of Uterine Protein from Dehydrated Uteri

the Lowry Procedure (1951).

Solution A: $Na\ CO_3$ - 20 gm
$Na\ OH$ - 4 gm

Mix these two and then add NaK tartrate. If all compounds are added together preciptate may form.

Na K tartrate 0.2gm

in a total volume of 1000 ml water

Solution B: $Cu\ SO_4\ .5H_2O$ ——— 0.5 gm. in 1000 ml dist. H_2O.

Solution C: 50 ml. of Solution A + 1 ml Solution B.

(This is made up just before use.)

The dehydrated tissue was placed in 0.3 ml of 1 N NaOH for 5 minutes in a boiling water bath. After it cooled in an ice water bath 2.0 ml 5% TCA was added to precipitate out the proteins. This precipitate was spun down at 1500 x g for 10 minutes. The supernatant was discarded and the pellet was resuspended in 2.0 ml hot TCA to remove nucleic acids and spun down. The protein pellet was redissolved in 1.0 ml of 0.1N NaOH. To this was added 5 ml of solution C. The mixture was shaken and heated for 10 minutes at 40°C in a water bath. The tubes were removed from the bath and 0.5 ml of freshly prepared Folin reagent was added and mixed immediately. (The Folin reagent has a half life of only eight seconds, therefore, if the mixture isn't mixed immediately the colour won't develop properly giving fortuitous results). After waiting 30 minutes the optical density of the tubes was determined in

a spectrophotometer at a wavelength of 660 nm set to 100% with a reagent blank containing 1 ml of water instead of sample. If the optical density of the sample was beyond the machine's range the sample was diluted with solution C and the appropriate correction for determining the amount of protein present was made.

Note: Pretreatment of protein for 5 minutes in 1 N Na OH at 100°C has almost no effect on colour reaction.

APPENDIX 3

Determination of Uterine Glycogen Using the Anthrone Procedure (Seifter, 1950).

The entire uterus was quickly excised, blotted of excess luminal fluid and weighed on a torsion balance. The uterus was divaricated through the cervix with the left half being digested in 3 ml of KOH at 100° C for 5 minutes and then placed in an ice water bath at 0-4°C where it was kept until the time of glycogen analysis (ca. 1 week). These tubes were then placed in a boiling water bath for an additional 15 minutes removed and placed in an ice water bath. (This additional heating period ensured that all the tissue was digested.) The entire contents of the tubes were then transfered to a 50 ml volumetric flask and diluted to the mark with distilled water, and thoroughly mixed. A 5 ml aliquot was then drawn off and placed in another test tube. A reagent blank using 5 ml of distilled water (in place of the sample) and a glucose standard (20 µg/ml) were also included. These tubes were then submerged in an ice water bath and 10 ml of 0.2% Anthrone reagent was added and mixed immediately. (Anthrone reagent is prepared by dissolving 320 mg of anthrone in 160 ml of 95% H_2SO_4.) The tubes were then placed in a boiling water bath for an additional 10 min.. ((The sulphuric acid and heating causes dehydration of the sugar to a furfural derivative (see formula below) which then condenses with anthrone to form a blue coloured compound.)) The tubes are then transferred

to an ice water bath and read in a spectrophotometer at a wavelength of 620 nm which has been set to 100% transmission with the reagent blank. The amount of glycogen is then calculated using the following equation;

$$\mu g \text{ glycogen/ 5 ml aliquot} = \frac{50 \times U}{1.11 \times S}$$

where

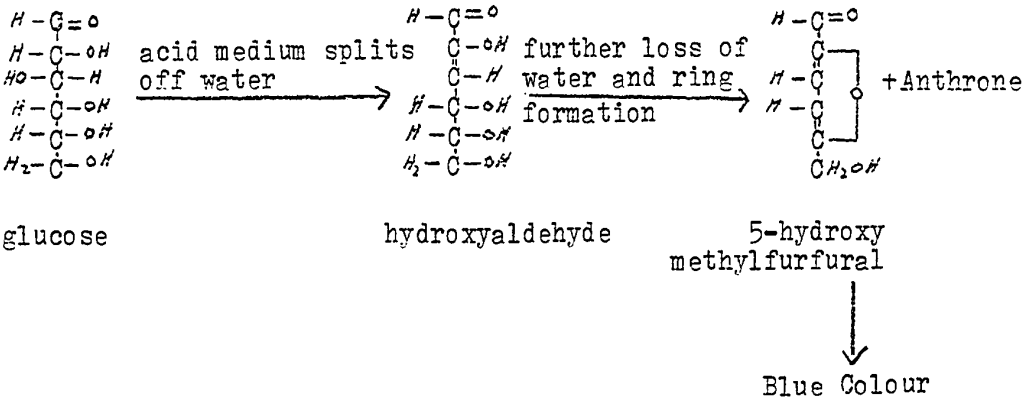
50 = Total volume of sample after dilution.

U = Optical density of unknown.

S = Optical density of standard.

1.11 = is a factor for the conversion of glucose to glycogen.

Reaction Mechanism



(Hexoses yield hydroxymethylfurfural whereas pentoses yield furfural derivatives.)

APPENDIX 4

Measurement of hepatic microsomal enzyme activity using the demethylation of P-chloro-N-methylaniline (Kupfer and Bruggeman, 1966).

The liver was excised, rinsed, blotted dry and weighed on a torsion balance. The liver was then homogenized in 14 volumes of 1.15% KCL and centrifuged at 9,000 Xg for 20 minutes in the cold. This removes the nuclei and mitochondria. The supernatant was then stored at -10°C until the time of enzyme assay (anywhere from 1 to 5 mo.)

To a 25 ml erlenmyer flask in an ice water bath the following constituents were added to a final volume of 2.2 ml:

1.0 ml of 9,000 x g supernatant (equivalent to 0.667 g of original liver.)

0.1 ml of nicotinamide adenine dinucleotide phosphate (1.6 µM).

0.2 ml $MgCl_2$ (30 µM)

0.2 ml glucose-6-phosphate (12.5 µM)

0.2 ml glucose-6-phosphate dehydrogenase (10 I.U.)

0.2 ml phosphate buffer (100 µM)

0.2 ml nicotinamide (20 µM)

0.1 ml P-chloro-N-methylaniline (3.0 µM)

The entire contents of the flask were then incubated in an atmosphere of air in a shaking water bath at 37°C for 20 min.. A parallel incubation was carried out in the absence of PCMA but containing 0.1 ml of water

Reaction was terminated by the addition of 3.0 ml of p-dimethylaminobenzaldehyde (PDAB ; 20 mg/ 1.0 ml 1 N H_2SO_4), to the flask.

Reaction mixture was transferred to a centrifuge tube and centrifuged at 9,000 x g for 30 min. in the cold (0-4°C).

The supernatant solutions were decanted off and allowed to stand at room temperature. The tubes were read 2 hours after the addition of PDAB in a spectrophotometer at 440 nm. A reagent blank (containing no substrate or product) is used to set the machine to 100% transmission.

Amount of p-chloroaniline (PCA), the product of the reaction, in sample is determined from a PCA standard curve.

The PCA standard curve was linear from 0 to 1.0 µM (Fig. 26).

A time-course reaction with a fresh liver preparation yielded 19 times more PCA at 20 min. than the frozen liver preparation (Fig. 27). This demonstrates that prolonged freezing (3 mo.) destroys any enzyme activity which may be present. However, freezing for short periods of time (ca. 2 week) results in no significant loss of activity (Fig. 28).

FIGURE 26

PCA STANDARD CURVE

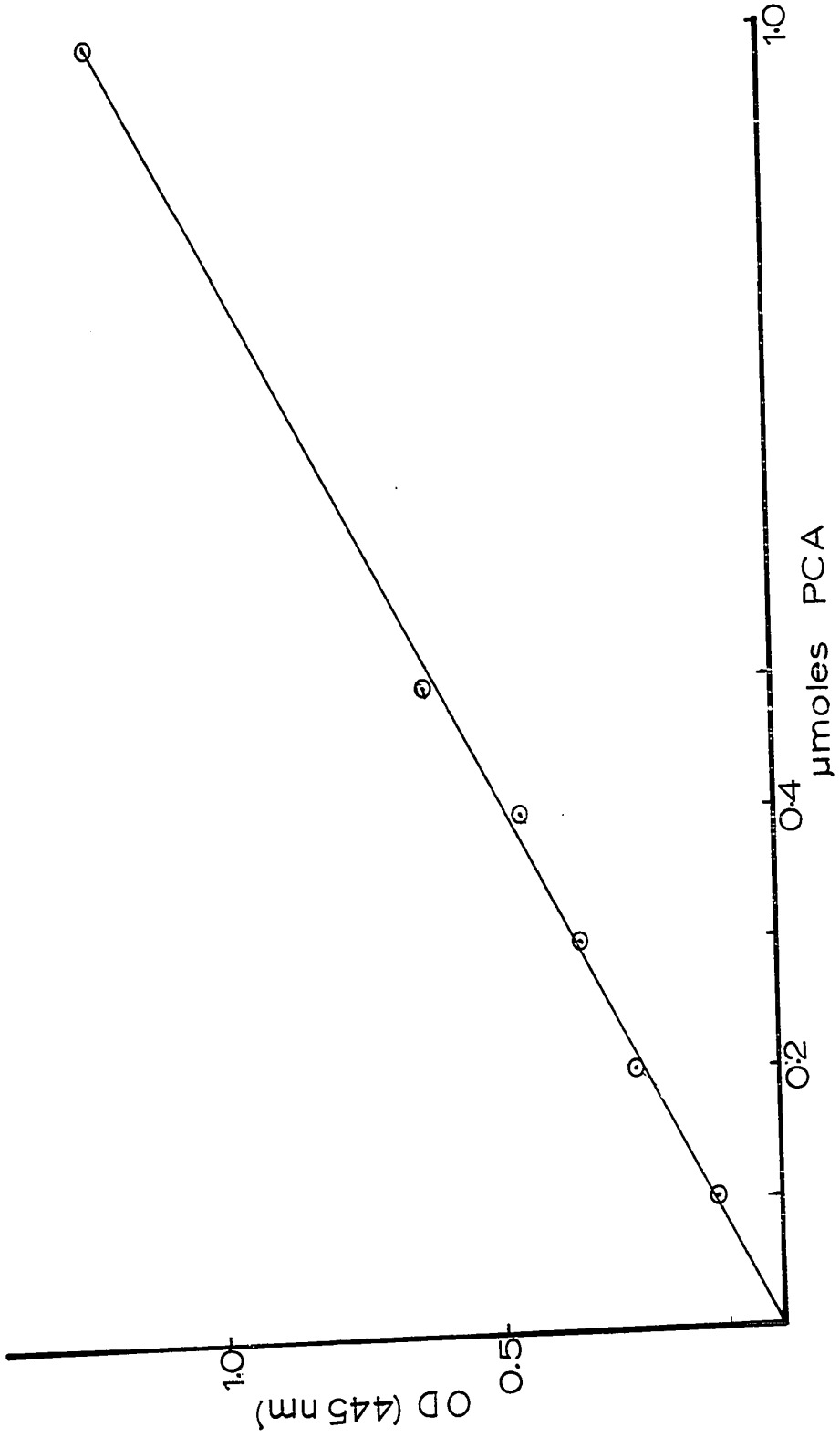

OD (445 nm)
0.5
1.0
0.2
0.4
1.0
µmoles PCA

FIGURE 27

TIME-COURSE REACTION USING UNFROZEN AND FROZEN LIVER SAMPLES

[a] frozen liver sample.

[b] unfrozen liver sample.

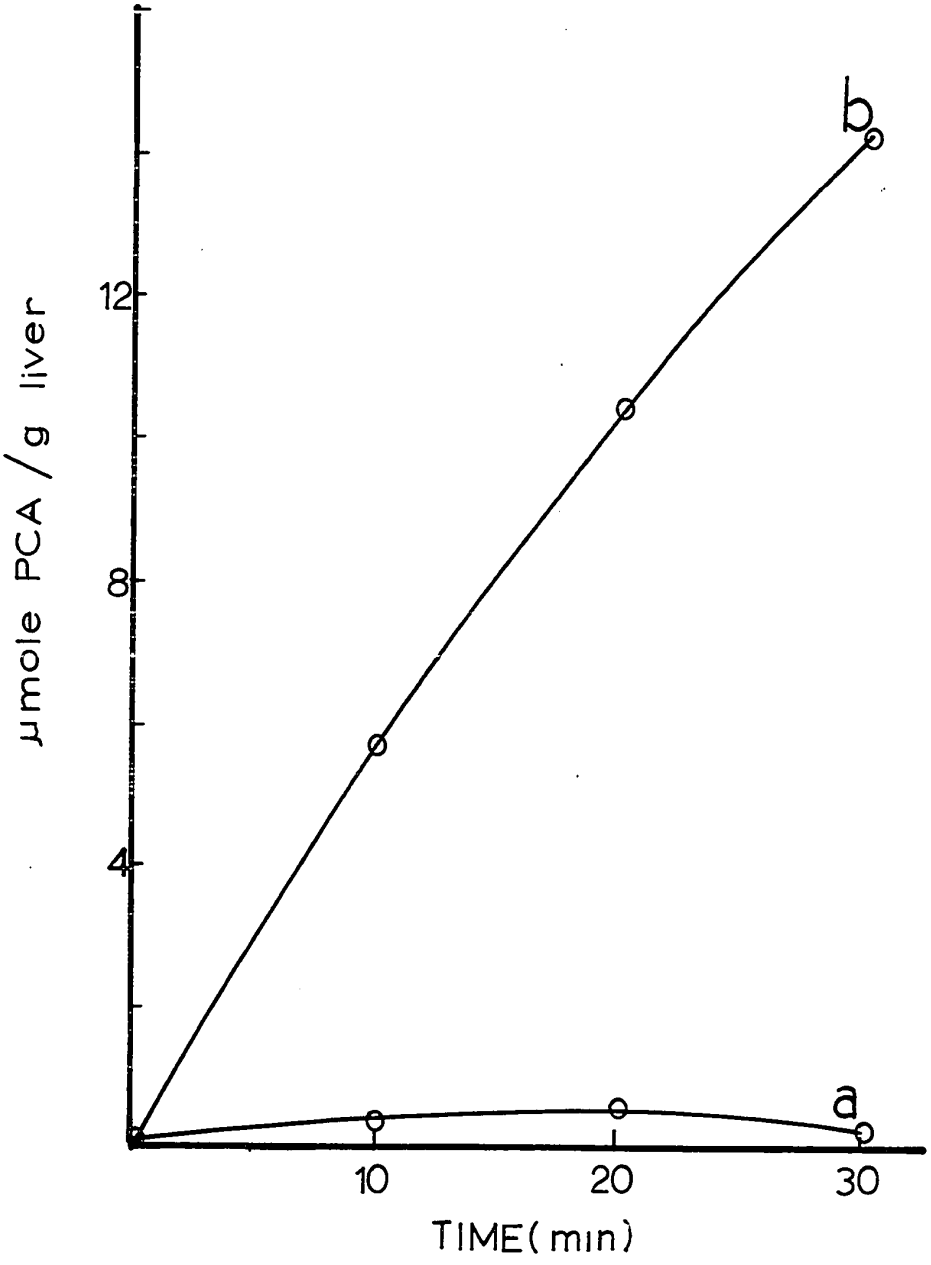
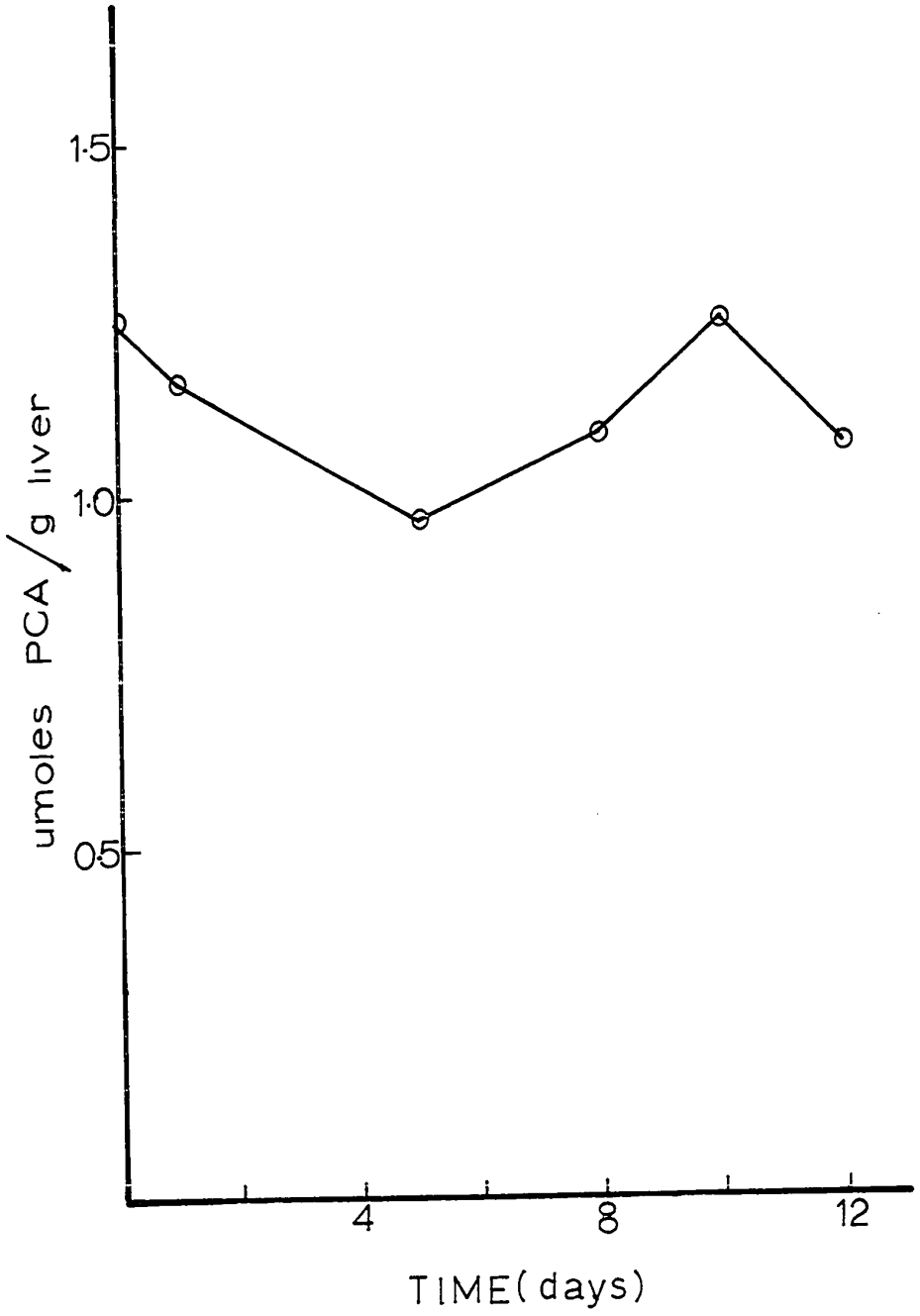

μmole PCA /g liver
12
8
4
b
a
10
20
30
TIME(min)

FIGURE 28

LOSS OF ENZYME ACTIVITY IN A FROZEN LIVER PREPARATION WITH TIME


1·5
1·0
0·5
umoles PCA/g liver
4
8
12
TIME(days)

APPENDIX 5

Conversion[h] of DDT Administered, to the Immature Female Rat, Which Causes an Estrogenic Response, into a Daily Exposure Per Animal.

DDT Isomer	Concentration Administered	Route	Strain of Rat	mg o,p'-DDT/ 50 mg rat/ day	Reference
o,p'-DDT	4 mg/rat	sc[a]	NS[b]	4.00	Bitman et al. (1968)
o,p'-DDT	1 mg/kg b.w.	ip[c]	S-D[d]	0.05	Welch et al. (1969)
tech DDT	5 mg/kg b.w.	ip	S-D	0.25	" "
o,p'-DDT	10 mg/rat	sc	NS	10.00	Cecil et al. (1971)
o,p'-DDT	1000 mg/kg food	oral[e]	NS	10.00	" "
o,p'-DDT	1 mg/rat	inj[f]	S-D	1.00	Duby et al. (1971)
o,p'-DDT	1 mg/rat[g]	sc	S-D	10.00	Heinrichs et al. (1971)
o,p'-DDT	5 mg/kg food	oral	W[g]	0.05	Clement & Okey, in press
o,p'-DDT	1000 mg/kg food	oral	W	5.00	" "

[a] sc = subcutaneous injection

[b] NS = not specified

[c] ip = intraperitoneal injection

[d] S-D = Sprague-Dawley rats

[e] oral = in the food

[f] inj = type of injection was not specified.

[g] rats were newborns weighing ca. 5g.

[h] 0.05 kg rat eats ca. 5 g of food per day.

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