AN ABSTRACT OF THE THESIS OF

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Title DEVELOPMENT OF PROGENY OF MICE GIVEN DDT OR PARATHION DURING GESTATION

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Attempts were made to study the effect of parathion and DDT on the postnatal development of mice. Parathion, DDT or corn oil (control) was administered to gravid mice during the first, second, and third trimesters of pregnancy. Thus, nine groups of offspring were obtained. Four different techniques, Audiogenic Seizure, Conditioned Avoidance Response, Open Field, and Electroshock Seizure threshold were used to study the effect of the insecticides on the offspring at different ages, 16 to 30, 30 to 36, 60 to 66, and over 70 days of age, respectively.

The offspring of gravid mice given parathion or DDT exhibited high incidence of sound induced seizures on 20 days of age, whereas the offspring from the corn oil treated mothers showed high incidence on 18 days of age. Therefore, the insecticides delayed the maturation of the nervous system by two days. Similar results were obtained in the groups of offspring of pregnant mice given parathion or

DDT during the first, second, and third trimesters. Hence, these insecticides affected the maturation of the nervous system regardless of the stage of pregnancy at which administered. The high incidence of seizures in all of the groups of offspring had passed by 28 days of age. The insecticides did not influence the percent incidence after 20 days of age.

There were no differences between the parathion and corn oil groups on the acquisition of a Conditioned Avoidance Response, Open Field behavior and Electroshock Seizure threshold. This may indicate that prenatal parathion, regardless of the trimester of administration, had lost its influence on the nervous system of the offspring by the time they were 30 days of age. The offspring of mice given DDT during the second or third trimester were slower to acquire a Conditioned Avoidance Response than the offspring of mice given only corn oil. All the groups of offspring (60 - 66 days old) of mothers given DDT or corn oil during the first, second or third trimester exhibited similar behavior in the Open Field. The Electroshock Seizure thresholds for all of the groups of offspring (over 70 days old) were essentially the same. This may indicate that the influence of DDT on the nervous system had disappeared by the time the offspring were two months old.

There were no differences in litter size between all nine groups of offspring. Abortion at full term occurred in two pregnancies of

the group of gravid mice given DDT during the third trimester. This may indicate that the insecticides influence the prenatal as well as the postnatal development of the offspring. The weight gain of all of the groups of offspring from parathion or DDT treated mothers was less than that of the groups from corn oil treated mothers. By 60 days of age, the average body weight, and the general appearance of the mice of all groups were similar.

DEVELOPMENT OF PROGENY OF MICE GIVEN DDT OR PARATHION DURING GESTATION

by

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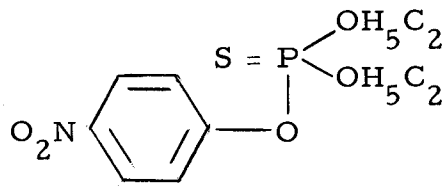
GENERAL INTRODUCTION

Pesticides (economic poisons) are a diversified class of chemical products used to kill or repel insects (insecticides, fumigants, repellents), destroy or repel rodents (rodenticides), control weeds (herbicides) and prevent or modify plant disease (fungicides and certain bacteriocides). They are of value for improving crop production, preserving foods and increasing man's freedom from pest annoyance. They also have virtue in eradicating insect and rodent vectors of disease and removing unwanted vegetation that harbors insects, blisters the skin, or produces unwanted types of pollen (95).

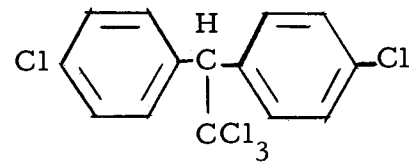
As chemical protectants against pests of economic and medical importance, pesticides are occasionally the sole practical means for the successful management of pest infestations. More frequently, they serve as invaluable adjuvants that supplement more cumbersome but thoroughly effective non-chemical control measures such as the use of exclusion devices (screening, ratproofing, protective clothing), the elimination of breeding areas (the drainage of stagnant bodies of water, mechanical weed and brush removal) and sanitary measures (proper disposal of garbage, excreta and other wastes). With growing frequency the chemicals used in agriculture are also

finding a place in medicine as therapeutic agents (parasiticides, fungicides, cholinergic agents), and as tools of research in unraveling the details of physiological processes (neurology, endocrinology, biochemistry) (95).

Man's welfare is materially affected in a multiplicity of ways by insects, e.g., his body may be attacked, his food despoiled, and his diseases contracted in their feeding. Most of his stored possessions and the house in which he lives are subject to insect damage. Field crops, livestock, fruit, timber and other agricultural products are also involved. Thus the most widely used branch of pesticides in daily life is the insecticides. In man's struggle against his insect enemies, thousands of organic chemicals have been evaluated for potential insecticidal action and several hundred are in commercial use (67). By definition, an insecticide is a substance or mixture of substances used to kill and thus to control insects and related arthropods. Chemically, the insecticides are classified into many classes. The two most well known classes and also the most widely used are organic phosphates and the chlorinated hydrocarbons. Parathion (diethyl-p-nitrophenyl thiophosphate) and DDT (dichlorodiphenyltrichloroethane) are the prototype compounds of these two classes.



Parathion



DDT

The organic phosphate, parathion, and the chlorinated hydrocarbon, DDT, are a "necessary evil" for the human being. They are necessary because they protect man's welfare from his insect enemies and evil because they are killers to man and other useful organisms. Hence, they are agents with two phases, useful and dangerous phases. Many scientists all over the world have studied extensively to increase the usefulness and to decrease the harmfulness of parathion and DDT, and to bring them and many other related compounds under complete control by man.

Studies on the percutaneous absorption of parathion and its metabolic product, paraoxon, have been done by Fredricksson and co-workers (41). They studied the ability of the skin of man, cat, rabbit, and rat to hydrolyze or otherwise metabolize parathion by utilizing the Warburg technique and paper chromatography. They showed that parathion was not hydrolyzed or transformed into paraoxon by the skin of any of the species tested. The distribution of P^{32} labeled parathion within the excised skin from man, rat, rabbit, and cat following topical application for various periods of

time also was investigated by Fredricksson (40). He chose two different approaches, (a) determination of the radioactivity in 25 consecutive cellulose tape strips from the surface of human skin, (b) autoradiography of skin sections with the use of four different techniques. He found that parathion penetrated into hair follicles and sebaceous glands to some extent, but he concluded that this was not necessarily the main route of absorption. He also showed increasing activity below the epidermal layers and thus transepidermal absorption is likely. The body distribution of P^{32} labeled parathion was further investigated by Fredricksson and Bigellow (39), by means of an autoradiograph technique applied to sections of whole mice. They found that the material was absorbed very slowly from a subcutaneous deposit, that the level of radioactivity in blood was low during the whole period of observation and that the labeled materials accumulated in various organs and tissues. They found that the highest activity appeared in the salivary glands and cervical brown fats. Also, they showed liver, kidneys, and adipose tissues contained high activity and indicated fairly high activity in gastric and intestinal walls, thyroid, spleen and lungs. They found less activity in the central nervous system, musculature, and bone marrow. The labeled material was mainly excreted by the kidneys and not in the bile or in the intestinal mucosa.

The effect of oral and dermal administration of parathion on

the ruminant animals was determined by Andersen and Karlog (6). They incubated in vitro rumin fluid with 40 mg parathion per milliliter and found a rapid reduction of the nitro group of the parathion to an amino group. After oral administration to two Jersey cows of 1 mg and 10 mg parathion per Kg respectively, no signs of poisoning were observed and no inhibition of cholinesterase was detected. They found in the urine 97% of the excreted metabolities, conjugated p-aminophenol and 3% p-nitrophenol. Neither parathion, nor the metabolites paraoxon, aminoparathion, p-nitrophenol or p-aminophenol could be detected in milk samples. After dermal application of 1 mg/Kg parathion, there was inhibition of the cholinesterase activity in the blood and development of mild poisoning. The deposit in the coat was exclusively of parathion. In the urine p-nitrophenol was the only metabolite found. They did not find either parathion or metabolites in the milk samples.

The biochemical damage of mitochondria due to parathion intoxification was investigated by Comba and Mor (19). These investigators injected albino rats with parathion, 120 mg/100 g of body weight, and killed them 15-25 minutes later. They studied oxidative phosphorylation, ATPase and succino-oxidase activities on the liver homogenates. The oxidative phosphorylation was rapidly decreased and P/O ratio lowered. ATPase activity did not change significantly. Succino-oxidase activity in 0.88M sucrose

increased significantly but did not change in 0.25M sucrose; this was attributed to the swelling of mitochondria. They suggested that the alterations of mitochondria produced by parathion are related not only to the inhibition of acetylcholinesterase but to many other factors.

Parathion activation by liver of aquatic and terrestrial vertebrates had been studied by Potter and O'Brien (80). They studied liver slices from seven terrestrial and six aquatic animals. They found parathion is converted to paraoxon. Although there are similarities between the liver systems which activate parathion and which degrade drugs such as aminopyrine and phenacetin, liver slices of aquatic vertebrates can activate parathion but cannot degrade the drugs aforementioned. The relation between parathion and metabolism of vitamin A in the rat had been investigated by Philips (77). He showed that feeding a diet containing 10 p.p.m. parathion resulted in no detrimental effect on the degree of liver storage of vitamin A and similarly parathion did not adversely affect the rate of decrease of hepatic vitamin A stores.

In 1958 Iuchi (58) studied the distribution of parathion splitting substances in various organs. He reported that blood serum showed the greatest parathion splitting activity, the liver, the lungs, and the bone marrow were also important tissues which decomposed parathion; there was no significant parathion splitting activity in the

brain and heart. He indicated that blood (plasma) plays the greatest role in the detoxification of parathion with liver being second in importance. Iuchi found no similarity in tissue distribution between the parathion splitting substances and cholinesterase or alkaline phosphatase. The alkaline parathion splitting substance is not identical either with alkaline phosphatase or cholinesterase. Adebahr (2) studied 25 cases of death due to acute poisoning with parathion. He indicated that severe changes in the kidneys could always be noted. There is rapid appearance of the poison in the kidney with the point of attack in the renal tubules. He also reported obstruction of the external respiration and hypotension resulting from blocking of the acetylcholinesterase. Sassi and co-workers (87) reported that in acute poisoning with parathion, total lipids and fat-fatty acid increased, phospholipids decreased; subacute poisoning produced significant increase of the total lipids and fats-fatty acids, a decrease of the phospholipids and an increase of cholestrol. They suggested that inhibition of the esterase or the oxidation-reduction system and lesions of the internal organs are responsible for this change.

5-Hydroxytryptamine of rats' brains had been determined by Schawabe and co-workers (89) after poisoning with organophosphates. No significant difference from those of control animals appeared. They indicated also that organophosphates were without effect

in vitro or in vivo on the monoaminoxidase activity of the brain.

The effect of small quantities of parathion on rats in long lasting experiments had been investigated by Mlodecki (71). He reported that LD_{50} for rats was 12.9 mg/ Kg in water and 33.75 mg/ Kg in oil solution for parathion preparation administered per os. He reported also that 12-1/ 2 months feeding of fodder containing 2 mg/ Kg parathion resulted in a lower cholinesterase activity, arrested growth, increased mortality and inability to reproduce.

Gerhon and Shaw (46) reported that 14 men and 2 women exposed for ten years to organophosphorous insecticides showed schizophrenic and depressive reactions. In Egypt, Salama and co-workers (86) studied the action of parathion on CNS. They demonstrated that parathion exerts its effect on spinal synapses but it has no direct effect on the muscle. They reported too that the gradual fall in blood pressure shows that parathion has a peripheral action on the vessels.

The effect of parathion on pancreatic secretion has been investigated by Paradisi and Cavazzuti (76) in rats. They injected a single non-fatal toxic dose of parathion I. P. and provoked histological alterations in the pancreas, manifested by swelling of the cells in the first 24 hours. They suggested that the effects are not due to vagal stimulation of the pancreas, but that alterations of some enzymes (especially esterase and lipase) are mainly responsible for

these time-dependent morphological alterations. Montaldo (72) considered the liver alteration after parathion poisoning; the endoplasmic reticulum of the hepatocytes was profoundly altered after acute parathion poisoning; extensive dilation of the vesicles conferred a sieve-like appearance; the membranes appeared fragmented and degranulated. He indicated also that mitochondria showed membrane lesions and an accentuated crystal breakdown amounting almost to an homogenization of mitochondrial content. Thereafter he suggested that parathion acts on phospholipid-protein structure characteristic of all membrane.

Acetylcholinesterase inhibition was applied as a method for detecting organophosphate residue and related compounds in milk by Bean and Hankinson (11). They reported that the level of parathion recovered from milk in their investigation was 0.008 p.p.m. (γ / ml) by this method. Parathion and paraoxon appear in milk in extremely low levels even when very high levels of parathion are fed, but the amino derivatives are the metabolites that are secreted into milk. Fortunately they are of extremely low toxicity (21).

DDT is the abbreviated name for the various mixtures of para, paraprime (P, P') and ortho, paraprime (O, P') isomers of dichlorodiphenyltrichloroethane. There are 45 possible isomers of dichlorodiphenyltrichloroethane, the P, P' isomers being the most active as insecticides (94).

Stiff and Castillo (93) reported that DDT occurs in the bile of rabbits dosed orally with DDT. However, no DDT was obtained when they examined the same tissue and fluids, including bile, by their xanthydrol-potassium hydroxide-pyridine colorimetric method. This discrepancy is explainable on the basis that the xanthydrol method will not give a color reaction with any of the known metabolites of DDT, i.e., DDE and DDA. Neal and von Oettingen (75) recognized as early as 1946 that DDT was excreted in the bile in undetermined amounts. A study of DDT derived products occurring in rat feces and bile, following the injection of DDT, by Jensen et al. (60) showed that the major products are complexed forms of DDA in both bile and feces. In addition they concluded that bilary excretion is responsible for almost all of the DDT metabolites in feces.

Alekseyeva (5) investigated the toxicity of DDT after external application to rabbits. He applied DDT (chlorophenothane) in oil to the skin of rabbits and detected that it penetrated through intact skin and remained there for 35 days. Also he showed that in doses of 1.7-3.1 g/Kg DDT caused acute toxic manifestations with damage to the central nervous system, liver and kidneys. Following repeated application of DDT in oil to skin, an increase in blood leukocytes was noted. Dale et al. (30) investigated excretion of DDT in starved rats. He found that mobilization of body fat during starvation resulted in an increased concentration of DDT derived

material in plasma, brain, liver, kidneys, urine, and feces. The increased excretion was inadequate to prevent an increase in concentration of DDT-derived material in the body. Also he indicated that when sufficiently great, the augmented concentration of DDT in the brain associated with starvation was correlated with the occurrence of signs of poisoning. The effect of DDT on primates was studied by Durham et al. (35). They fed monkeys a diet containing up to 200 p.p.m. of DDT and noted no clinical signs of illness, but with 5000 p.p.m. of DDT the monkeys developed tremor, convulsion, and other signs of DDT poisoning. The maximum storage of DDT in body fat was achieved after six months of feeding. They indicated that the monkeys stored very little or no DDE.

Hukuhara et al. (55) indicated that no significant changes occurred in the metabolic activity of adipose tissue of rats fed DDT. Also they mentioned the ability of adipose tissue to accumulate large amounts of DDT without any measurable effect on the tissue enzymes that render protection for other organs.

Kartashova and Kartashova (61) indicated that DDT is transferred from the maternal organism to the embryo. About 80% of the DDT applied on the animal skin is excreted in milk as intact molecules and toxic amounts accumulated in organisms which used the milk. The relation between DDT and metabolism of vitamin A and carotein in the rat was mentioned by Philips (78). He indicated

that feeding over 10 p.p.m. of DDT decreased the utilization of orally administered carotein and decreased liver storage of vitamin A. Also he showed that the storage of vitamin A was reduced when a diet containing both DDT and carotein or DDT and vitamin A was fed.

Schulke (88) treated male albino rats with DDT orally and studied the irritability of their skeletal muscle. He found that 300 mg DDT/Kg body weight reduced the irritation threshold of the muscle to galvanic stimulation and lead to a significant decrease of the rheobase and other factors. The changes in chronaxie did not furnish any significant indications. After neurotomy the author ascertained a decreased irritability of the muscle which was not influenced by a dose of DDT or 100 mg/Kg. Involvement of the spinal cord and peripheral nerves in DDT poisoning of albino rats was investigated by Shankland (90). He found that the DDT tremor is produced in the caudal parts of the spinal cord.

Tobias et al. (103) studied the effect of acetylcholine and related compounds on DDT-intoxicated and normal flies, cockroaches, crayfish, frogs, and rats. He indicated that there is no significant difference between both groups. Tinsley (102) investigated the activity of liver glucose-β-phosphate dehydrogenase after injection of DDT. His observation that DDT inhibits glucose-β-phosphate dehydrogenase suggests that in vivo effects could result

from direct interaction of DDT or closely related metabolite with this enzyme. His hypothesis is substantiated by preliminary experiments which establish the presence of significant amounts of such compounds in the tissue fractions from DDT fed rats used for the enzyme assay (32).

The effects of DDA (dichlorodiphenylacetic acid), a metabolite of DDT, on rabbit intestinal motility in vitro, on the patellar reflex, and on choline acetylase system were studied by Bleiber et al. (13). They indicated that DDA inhibited the pendular activity of the rabbit's small intestine at minimal bath concentration of 10^{-4}M, and that DDA completely blocked rhythmic activity and depressed intestinal tone. The activity could be restored by washing or by exposure to 10^{-8}M acetylcholine, $5X10^{-8}$M physostigmine, or $2X10^{-8}$M TEPP. The induction of peristaltic activity by physostigmine or TEPP was partially inhibited or completely blocked by prior treatment with DDA. They injected DDA directly to the lumbar spinal cord in a coconut oil emulsion in doses between 0.4 and 1.8 mg and facilitated the patellar reflex response; doses larger than 2 mg depressed the patellar reflex. Also they showed that when the patellar reflex was depressed, spontaneous activity could be momentarily restored by acetylcholine or acetylcholine + TEPP similarly injected; diphenylacetic acid and diphenylamylacetic acid, known choline acetylation inhibitors, produced effects similar to those of DDA. Thereafter

they interpreted the effect of DDA on intestinal motility on the basis of the acetate - activating step.

Datta et al. (31) indicated in their research with rats that feeding p, p' DDT to the rats resulted in conversion to p, p' DDD [1, 1-dichloro-2, 2-bis (p-chlorophenyl) ethane] in the liver and this conversion does not require the intermediate formation of p, p DDE.

Distribution of C^{14} DDT in pregnant mice determined by whole body autoradiography has been investigated by Backstrom et al. (9). They showed that the oral dose was well absorbed and distributed in tissues such as depot fat, liver, intestines, kidneys, mammary glands and urinary bladder; a considerable activity formed in the gall bladder, brain and spinal cord especially in the gray matter; a moderate concentration in the blood, lungs, spleen, adrenal, and corpora lutea. Also they indicated that DDT passed the placenta freely with the highest concentration in the fetus found in the liver and fat. New-born mice that suckled for a few days showed a distribution of DDT similar to that of their mothers with high uptake in liver and fat tissue. The animals given DDT per os showed the same distribution of radioactivity as the animal injected intramusculary. They mentioned that since the brain contains little neutral fat, this might explain low uptake in the brain of DDT. DDT is excreted in the milk from animals fed a diet containing DDT. The effect of DDT on the liver carboxylesterase and vitamin A utilization of mother

rats and their young had been extensively investigated by Read et al. (82). They reported the result of DDT (1000 p. p. m.) added to the diet of female rats bred to male receiving the same diet; liver carboxylesterase and liver and kidney vitamin A levels were measured in dams and their young at parturation and weaning. They indicated that DDT did not cause any adverse effect on the breeding performance of the adult or on the vitamin A store of the new born or weaned rats. But vitamin A stores of the dams were reduced by pesticides. Liver carboxylesterase increased greatly between birth and weaning and remained almost constant thereafter.

Luckens and Davis (66) studied the sensitivity of bats to DDT. They indicated 40 mg/Kg of body weight is lethal to this animal and concluded that bats appear to be far more sensitive to DDT than any other mammal yet tested. Hart and Fouts (53) proved that DDT stimulates hepatic drug metabolism. They reported that there were stimulatory effects of the insecticides, technical chlordane and γ-chlordane on hepatic microsomal drug metabolism. Related insecticides mentioned produced significant decrease in hexobarbital sleeping time at 1, 3, 8, and 15 days after single I. P. injections in mice. They reported too that acute I. P. injections of DDT, 125 mg/Kg did not alter sleeping time. DDT, 500 p. p. m., was mixed in the diet of male rats, drug metabolizing enzyme activity was measured after two weeks, one and two months on the diet. Significant increase

in the rates of metabolism of several drugs was seen at these times. Hexobarbital sleeping times were determined in all rats 16 to 18 hours prior to sacrifice. Shortened sleeping times paralleled increases in hexobarbital metabolism in vitro. They also reported that chronic feeding of DDT caused a proliferation of smooth surfaced endoplasmic reticulum (SER) in rat liver and that hepatic drug metabolizing enzymes are primarily localized in SER. Then they suggested that chronic stimulatory effect of DDT may result from proliferation of SER and consequent increases in drug metabolizing enzymes.

The preceding survey of literature indicates that the insecticides parathion and DDT are absorbed by the skin and digestive tract mucosa, penetrated liver, kidneys, central nervous system, and interfered with the functions of these tissues. DDT passed through the placenta into the embryo and accumulated in fetal organs similar to that of the mother. Besides this, parathion and DDT and their active metabolites are secreted with the milk during the lactation period and given to the offspring.

This review of the literature indicates to us that much work has been done by many pioneers in pharmacology and toxicology about the effect of parathion or DDT on mammals. However, not enough study has been done to demonstrate the effects of these insecticides on the offspring that receive these agents through the

placenta and through milk secretion. We believe it is very important to extend this kind of work and to participate with other scientists to carry out this research further. For this reason we studied the postnatal development of the offspring of mice who received DDT or parathion during different stages of pregnancy.

GENERAL PROCEDURE

Adult female and male albino mice (CF #1 strain) obtained from Carwarth Farms were used in the experiments. One male and five females were placed in private cages for breeding. The vaginal plug method was used to determine pregnancy. Each pregnant mouse was separated and housed in a private cage (gallon can with sawdust bedding).

Technical DDT and technical parathion were dissolved in corn oil for use in the experiments. Preliminary work had been done on matured non-gravid mice to determine the amount of DDT or parathion dissolved in corn oil to be given orally to mice without abnormal changes of the animals. It was found that 3 mg per Kg body weight of parathion and 2.5 mg per Kg body weight of DDT were the most suitable amounts to be used in our research.

Three mg of parathion or 2.5 mg of DDT were dissolved in five milliliters of corn oil. Thus, five milliliters solution of DDT or parathion or plain corn oil per Kg body weight were orally administered to the pregnant animal. Every pregnant animal received at least three injections, once every two days during one trimester of the gestation period. The pregnant mothers were divided randomly into nine groups. Every group numbered from five to eight animals. Each of the first three groups was injected orally with DDT,

parathion or corn oil respectively during the first trimester. Each of the second and third three groups were injected with DDT, parathion or corn oil at second and third trimester in like manner.

The mothers gave birth in individual cages and the litter sizes were recorded (indicated in the Appendix). Two mothers given DDT at third trimester gave premature litters. The young were not disturbed until 16 days of age when testing for sound-induced seizure susceptibility began. All the young were weaned at 30 days of age. Nine randomly selected groups of ten mice each (from drug and control groups at first, second, and third trimester) were reserved for avoidance training which began at 30 days of age.

The offspring were subjected to a sound stimulus (door bell suspended in a glass box) and the number of audiogenic seizures in each group of the nine basic groups as well as the degree of seizure response of each mouse (running, clonic, or tonic seizure) from the drug and control groups were reported. The body weight of the animals in each group also was measured. The test continued for 16 days, once every two days.

For avoidance training each mouse was subjected to 16 consecutive avoidance-conditioning trials per day in the avoidance conditioning apparatus. The number of conditioned responses and the body weight for each group of animals were reported during this test. The test continued for a week.

At 60 to 70 days old each mouse from the 18 groups of offspring (nine groups that passed through audiogenic seizure and the other nine groups that passed through avoidance conditioning response) was subjected to an Open Field Test for three minutes daily. The number of squares traversed and the number of defecations given by each animal in each group were reported for every trial. The body weight was reported for each animal. The Open-Field Test continued for seven days.

At 70 to 90 days of age all the animals from the nine basic groups were shocked by an electric stimulus to determine their minimal electroshock-seizure threshold. The electroshock procedure employed ear-clip electrodes to avoid restraint of the animals. All of the animals were tested on the same day, and the electroshock seizure thresholds calculated for each of the nine groups of offspring.

AUDIOGENIC SEIZURE

Introduction

The first indication that sound might induce seizures in animals seems to have been reported by Mead in 1762 (22). Dice in 1935 (34) was the first to study the effect of the auditory stimuli on mice and called the abnormal response "epilepsy." Morgan and Waldman, 1941, (73) suggested the phrase "audiogenic seizure" to describe the convulsive behavior induced by sound. Audiogenic seizures may consist of wild running, clonic, or tonic convulsions which resemble those produced by other stimuli. The latency of audiogenic seizures is the time from the beginning of the sound stimuli to the onset of the wild running. The subject of audiogenic seizures had been reviewed by Finger (42) and by Bevan (12).

Audiogenic seizures are accompanied by changes in the electroencephalogram which are very similar to those associated with epileptic seizures in man and the seizures induced in non-epileptics by metrazol and electric shock (74, p. 598). In 1942, Lindsley and co-workers (64) observed that auditory stimulation of rats restrained in a holder produced no significant changes in the electroencephalogram and also no seizures. In unrestrained rats which failed to exhibit seizures, auditory stimulation likewise elicited no significant changes in the electroencephalogram.

Humphrey and Marcuse (56) measured the changes in heart rate in rats associated with audiogenic seizure. These changes differ considerably from one seizure to another, but the heart rate usually increases or decreases prior to seizure, is markedly elevated during the convulsion and drops to a very low level in the comatose state.

Audiogenic seizure appears to be a satisfactory technique to test the effect of drugs on the animal behavior. The effects of drugs on the incidence of audiogenic seizures has been studied with either one or two aims in mind, (1) to discover the neurophysiological mechanisms and (2) to ascertain the effectiveness of drugs used to alleviate epilepsy in man, which has much in common with the convulsive aspect of audiogenic seizures.

Some neurophysiological and neuropharmacological characteristics of audiogenic seizures in susceptible mice had been reported by Swinyard and co-workers (96). Their data indicated that the maximal seizure induced by supramaximal electroshock, metrazol and sound are remarkably similar. They reported the maximum incidence and severity of the seizure by the twenty-second day of age after which the incidence of maximal seizures decreased with increased age and weight. The same authors also showed that audiogenic seizure susceptible mice differ in at least two ways from non-susceptible mice. First, the oscillator mechanism of maximal

audiogenic-seizure mice is more sensitive to discharge by electric current than that of the non-susceptible animals. Second, seizure spread is more easily evoked in the maximal audiogenic seizure-susceptible mice.

The relationship between 5-HT, norepinepherine in the brain and audiogenic seizure had been investigated by Picchioni and co-workers (79). They indicated that reserpine increased the severity of audiogenic seizures which persisted up to 96 hours. Although audiogenic convulsions had returned to normal (clonic convulsion) at 192 hours, 5-HT and norepinephrine levels were still markedly lowered. Iproniazid increased 5-HT and norepinephrine and reduced the incidence of audiogenic seizure at 6 and 15 hours. At 48 hours the convulsant effect was no longer significant, although 5-HT and norepinephrine remained elevated beyond this time. They reported too that reserpine-iproniazid, at five hours decreased 5-HT, norepinephrine and incidence of audiogenic seizure were gone at 24 hours. The effect of audiogenic seizure pattern persisted for 48 hours and norepinephrine was still lowered at 96 hours. Iproniazid-reserpine increased 5-HT and decreased norepinephrine and incidence of audiogenic seizure at 24 hours. The effect on audiogenic seizure was gone at 24 hours, however, the effect on 5-HT and norepinephrine persists up to 96 hours. Their study indicated that the actions of reserpine and iproniazid on audiogenic seizure in rats

are independent on total brain levels of 5-HT and norepinephrine. Behavioral effect of prenatal drug administration in rats has been investigated by Werboff et al. (105). They found that reserpine and 5-HTP increased the susceptibility of the offspring to seizures produced by audiogenic stimuli.

Castellion and Swinyard (23) studied maturation and reproducibility of audiogenic seizure in two strains of audiogenic seizure susceptible mice, Frings (F) and O'Grady (O). They showed that it appeared one to three days earlier in F than in O mice, and in the following sequence for F mice, running or clonic activity by day eight, and maximal seizures (MS) by day 13. The sequence for O mice was running or clonus by day nine and MS by day 16. All animals ultimately developed MS but peak incidence for F (96%) and O (86%) mice occurred at day 20 and 24 respectively.

Numerous investigators have attempted to explain the mechanism of audiogenic seizure. Ginsburg and co-workers (47, 48, 49, 50) had reported that many substances, all related to Kreb cycle and/or to energy turn over mechanisms are involved in audiogenic seizures and cannot be separated from either the seizure itself or the inheritance of susceptibility.

Abood and Gerard (1) demonstrated that both energy degradation, as indicated by the lowered ATPase, and energy synthesis, as indicated by lowered P/O ratios and decreased specific activity of

high energy phosphates are significantly depressed in audiogenic seizures mice during the susceptible period.

Chai (25) reported that the thyroid activity in 30-day old DBA mice (most susceptible age) is twice that of non-susceptible strain. Meier (68) has indicated that a high pituitary function is correlated with audiogenic seizure susceptibility in a strain of DBA mice.

Coleman (28) found decreased phenylalanine hydroxylase activity and increased amounts of phenylacetic acid in DBA mice as compared to non-susceptible strains. The reduced phenylalanine hydroxylase activity results in inhibition of tyrosine production and in formation of abnormal breakdown products from the accumulated phenylalanine. These products are inhibitors of decarboxylase reactions, including these concerned with the production of serotonin and gamma-aminobutyric acid, compounds thought to be involved in normal brain metabolism.

Castellion (22) reported that the appearance of audiogenic seizures in rats coincided with the mylination of some long nerve tracts of the subcortex. Furthermore the beginning formation of connection between subcortical areas and the cerebral cortex were found to be directly related to the onset of sound-induced convulsions.

Millichap (69) had indicated that the development and reproducibility of full tonic-clonic seizures in rats could be correlated with the level of carbonic anhydrase activity in the brain. In later

reports Millichap and co-workers (70) discovered that the susceptibility of rats to maximal electroshock seizures was not only correlated to carbonic anhydrase activity, but to the distribution of water, electrolyte concentrations, and total carbon dioxide content of the brain. Gelhorn and Ballin (45) reported that in older and adult rats, that a reduction in susceptibility to seizures occurred with increasing age. Frohlich and Mirsky (43) reported that newborn rats are more susceptible to acid fuchsin than adult rats because the blood-brain barrier is not completely developed at birth, that the permeability of the blood-brain barrier decreases with increased age of the animal, and that theophylline increases the permeability of the blood-brain barrier to the dye in adult rats. Clark and Sarkaria (27) supported the preceding results with mice.

Pylkko and Woodburry (81) indicated that typical maximal seizures produced by administration of strychnine in rats did not appear until the twelfth to the sixteenth day of age. The seizure patterns in rats showed no marked alteration from 16 days to several months of age. Millichap (69) indicated that picrotoxin and pentylenetetrazol, like electroshock, did not induce the mature maximal seizure pattern until after the twenty-first day. Since strychnine acts predominantly on the spinal cord and electroshock affects mainly the higher centers in the central nervous system, these workers suggest that the spinal cord matures more rapidly

than do the supraspinal components of the central nervous system.

The latency for onset of audiogenic seizures (time from the beginning of the sound stimuli to the onset of a convulsion) has been considered by Wilson (22) to indicate severity and/or susceptibility. He considered a short latency indicative of more severe seizure and/or an increased susceptibility to sound. Some workers, as Kim and Kim (62), have employed total seizure duration as measure of seizure severity. They consider that an increase in seizure duration is characteristic of more severe seizures. Other workers, Tedeschi et al. (101) consider seizure severity in terms of the ratio of duration of tonic hindleg flexion (F) to the duration of tonic hindleg extension (E). Thus, an increase in F/E ratio is characteristic of less severe seizure, whereas a decrease in this ratio is characteristic of more severe seizure.

From the preceding review of literature it is clear that the mechanism of audiogenic seizure is not completely understood. Nevertheless, it was believed important to use the audiogenic seizure as a tool to study the development of animal behavior. This would allow the study of the effect of prenatal parathion or DDT on postnatal development of the offspring by comparing the audiogenic seizures and the latencies that appeared in DDT or parathion groups with that of the control groups.

Procedure

Nine different groups of offspring were obtained from pregnant mothers treated with parathion, DDT or corn oil at first, second, or third trimesters as described in General Procedures. Beginning at 16 days of age individual offspring was subjected to sound stimuli for 60 seconds. Latency of each seizure, the number of audiogenic seizures in each group, as well as the degree of seizure response (running, clonic, or tonic) were reported. Artificial respiration was used in case of respiratory failure as a result of occurrence of a full maximal audiogenic seizure. All of the animals were weighed during each experiment. The apparatus was built from a glass box (12 x 21 x 8 inches) in which one side (8 x 12 inches) could be opened. A door bell was used as the source of sound stimulus. The bell was connected with a three volt battery and a knife-switch. The open side of the box was closed with a heavy plastic sheet during the test. The results were analyzed by an electronic computer using a Mann-Whitney Test for latency and X^2 for audiogenic seizures (91).

Results

Since the data obtained concerning the different types of seizures (running, clonic or tonic) were not sufficient for statistical analysis and to compare the different groups of animals, they were

combined to indicate the presence or absence of any seizure activity (approximately 50% of all the seizures exhibited were maximal). The data for the incidence of audiogenic seizures for the nine groups of animals are shown in Tables 1, 2, and 3 and the average latency of their seizures is shown in Tables 4, 5, and 6.

There are some differences in the incidence of seizures between the control and treated groups at first, second or third trimester.

With the first trimester there was significant difference between the incidence of seizures of the control group (84.60%) and the DDT group (59.10%) on 18 days of age. Hence the high incidence of seizures (at least 80%) appeared earlier in the control group, 18 days of age, than in DDT groups, 20 days of age. There was also a significant difference between the incidence of seizures of the control (84.60%) and the parathion group (66.61%) on 18 days of age. The high incidence of seizure appeared earlier in the control group, 18 days of age, than in the parathion group, 20 days of age.

At second trimester, there were significant differences in the incidence of seizures between the control and DDT groups on 18, 20, and 22 days of age, (100.00%), (100.00%), (100.00%), and (79.41%), (82.10%), (84.40%) respectively. Hence, the high incidence of seizures appeared earlier in the control group, 18 days of age, than

Table 1. The percentage of the offspring of mice treated with DDT, parathion or corn oil (control) during the first trimester that showed seizures at different ages.

Age (Days)	Corn Oil % Seizures	Corn Oil Animals Tested	DDT % Seizures	DDT Animals Tested	Parathion % Seizures	Parathion Animals Tested
16	0.00	39	0.00	22	9.30	43
18	84.60	39	59.10	22	66.61*	42
20	82.10	39	90.90	22	73.80	42
22	89.11	37	81.80	22	85.70	42
24	83.30	36	90.50	21	82.50	40
26	86.10	36	90.50	21	87.10	39
28	60.00	35	80.00	20	65.71	38
30	50.00	34	70.00	20	57.91	38

* Significantly different from corresponding control ($P<0.05$)

Table 2. The percentage of the offspring of mice treated with DDT, parathion or corn oil (control) during the second trimester that showed seizures at different ages

Age (Days)	Corn Oil % Seizures	Corn Oil Animals Tested	DDT % Seizures	DDT Animals Tested	Parathion % Seizures	Parathion Animals Tested
16	4.00	25	10.21	39	0.00	35
18	100.00	25	79.41*	39	57.10*	35
20	100.00	25	82.10*	39	82.31*	34
22	100.00	25	84.20*	38	91.11	34
24	92.00	25	84.20	38	81.80	33
26	84.00	25	80.51	36	70.00	30
28	76.00	25	71.40	35	55.51	27
30	56.00	25	41.11	34	44.00	25

* Significantly different from corresponding control ($P<0.05$)

Table 3. The percentage of offspring of mice treated with DDT, parathion or corn oil (control) during the third trimester that showed seizures at different ages.

Age (Days)	Corn Oil % Seizures	Corn Oil Animals Tested	DDT % Seizures	DDT Animals Tested	Parathion % Seizures	Parathion Animals Tested
16	3.30	30	0.00	18	6.70	15
18	83.30	30	38.81*	18	38.81*	15
20	80.00	30	83.30	18	73.30	36
22	72.40	29	87.50	16	88.80	36
24	75.81	29	81.21	16	84.80	33
26	71.40	28	81.21	16	86.20	29
28	75.00	28	68.71	16	91.30	23
30	75.00	28	43.71	16	69.50	23

* Significantly different from corresponding control ($P<0.05$)

Table 4. The average latencies of seizures for offspring of mice treated with DDT, parathion or corn oil (control) during the first trimester.

Age (Days)	Average Latencies of Seizures (seconds) Corn Oil	DDT	Parathion
18	8.51	10.07	7.17
20	9.81	5.70	6.41
22	8.81	6.94	6.47
24	9.63	8.47	5.56
26	8.03	7.68	8.35
28	12.40	7.18	12.20
30	11.57	9.78	9.13

Table 5. The average latencies of seizures for offspring of mice treated with DDT, parathion or corn oil (control) during the second trimester.

Age (Days)	Average Latencies of Seizures (seconds) Corn Oil	DDT	Parathion
18	8.12	7.20	10.44
20	8.26	7.56	8.25
22	8.34	7.39	8.75
24	8.08	7.65	9.85
26	11.36	8.03	10.59
28	10.78	11.52	19.66
30	11.00	12.42	22.50

Table 6. The average latencies of seizures for offspring of mice treated with DDT, parathion or corn oil (control) during the third trimester.

Age (Days)	Average Latencies of Seizures (seconds) Corn Oil	DDT	Parathion
18	5.64	7.71*	13.63*
20	6.22	9.33	12.61*
22	5.65	8.66	14.09*
24	8.42	9.26	10.18
26	7.84	9.42	10.52
28	6.47	11.33	14.40*
30	10.00	13.50	10.28

* Significantly different from corresponding control ($P<0.05$)

in the DDT group, 20 days of age. There was significant difference between control and parathion groups in the incidence of seizure on 18 and 20 days of age, (100.00%), (100.00%) and (57.10%), (82.31%) respectively. Thus the high incidence of seizures appeared earlier in the control group, 18 days of age, than in the parathion group, 20 days of age.

At third trimester, there was significant difference in the incidence of seizure between the control group (83.30%) and the DDT group (38.81%) on 18 days of age. The high incidence of seizures appeared earlier in the control group, 18 days of age, than in the DDT group, 20 days of age. There was significant difference in incidence of seizure, between the control group (80.00%) and the parathion group (38.81%) on 18 days of age; this high incidence of seizure appeared earlier in the control group, 18 days of age, than in the parathion group, 20 days of age.

The data for the means of latencies shown in Tables 4, 5, and 6 for control, DDT or parathion groups illustrate several points. At first and second trimesters, there was no significant difference between the means of latencies of control, DDT or parathion groups. At third trimester, there was significant difference in the average of latencies on 18 days of age between the control group and the DDT group, (5.64) and (7.71) respectively. There were also significant differences in the average of latencies on 18, 20, 22, and 28 days

of age between the control group and the parathion group, (5.64), (6.22), (5.65), (6.47) and (13.63), (12.61), (14.09), (14.40) respectively. In general, at third trimester, the means of latencies for the parathion or the DDT group were longer than that of the control group at different ages.

Discussion

In all three stages of pregnancy, there were significant depressions by DDT or parathion on the incidence of audiogenic seizures, i.e., the insecticides delayed the high incidence of seizure for two days. These effects, therefore, are indications for the passage of the insecticides through the placenta and/or milk secretion from the mother to the offspring in the first, second and third trimesters. These facts support the results of Casida (21), Kartashova and Kartashova (61) and Backstorm and co-workers (9). The depressing action of these insecticides on the growth during the experiment, as shown in the Appendix, is another supporting evidence for the results of Casida (21), Kartashova and Kartashova (61) and Backstorm and co-workers (9). The depressing effects of DDT were more clear in the early days of testing than the later days. This might be due to high concentration of DDT in the animal's tissue in the early stages than the late stage of the audiogenic seizure tests due to the continuing loss of insecticides in feces and

urine excretion from the animals (39, 6, 19). Since DDT passes through the placenta (9) and is secreted with the milk (61) the depressing effects of DDT on the audiogenic seizure and growth might be caused by the amount of DDT and its metabolites through both pathways. But it is possible that DDT passed through milk has more effect on the growth of offspring than DDT passed through the placenta, because the rate of growth of offspring was faster after weaning than before. The increased latency of seizure by DDT appeared only at the third trimester. This might be due to a larger amount of DDT delivered to the offspring from the mother treated with DDT during the third trimester than the first and second trimester where a large quantity of DDT received during the first and second trimesters had been lost through mother excretion (61).

Parathion is metabolized and excreted within a few days after administration to the animal (8, 44). Thus, the offspring of parathion treated mothers during the first and second trimester got no parathion in the milk during the lactation period. But there is a possibility that the offspring of mothers treated with parathion during the third trimester received parathion and its metabolites when they were one day old only. For this reason the action of parathion was mainly prenatal and not postnatal. The depressing action of parathion on the incidence of audiogenic seizure and the growth and the increase in latency of audiogenic seizure might be

due to its direct action on the fetus after passing the placenta; or due to its action on the mother and/ or the placenta which affected the metabolic activity of the fetus indirectly. Parathion and its metabolites are well known anticholinesterase agents. It is possible that parathion and its metabolites passed through the placenta and depressed the fetal cholinesterase activity which needs many weeks to recover. Hence, cholinesterase inhibition might have affected the metabolic activity of the fetus and thereafter affected the postnatal development of the offspring. If parathion and its metabolites did not pass the placenta, it is possible that depression of the cholinesterase of the pregnant mothers affected indirectly the postnatal development of the offspring. Besides these hypotheses, it is also possible that parathion has unknown effects on the placenta itself which will affect the postnatal development of the offspring. Finally, two or all the mentioned possibilities might have occurred and affected the postnatal development of the offspring.

Since the seizure incidence seems dependent on the maturity of the central nervous system (23), the appearance of high audiogenic seizure earlier, in the control group, than in the DDT or the parathion group at first, second, and third trimesters might be indications for delay in the maturation of the central nervous system of the drug-treated groups.

CONDITIONED AVOIDANCE RESPONSE

Introduction

The conditioned avoidance response has been used extensively to test the learning ability of animals. In this technique, animals are trained to respond to an auditory or visual stimulus such as a buzzer or light (conditioned stimulus, C.S.). When the animal fails to respond to this C.S., or warning, it receives a punishment, usually an electric shock (unconditioned stimulus, U.S.). The animal may escape the punishment by performing some arbitrary response known as the conditioned response (C.R.) such as climbing a pole, crossing a barrier, etc.

Ader and Clink (4) studied the effect of chlorpromazine on the acquisition and extinction of an avoidance response in rats. They mentioned that animals under the influence of chlorpromazine are significantly inferior to control animals in acquiring the avoidance response. An increase in the dosage of chlorpromazine increases the number of trials required to attain the acquisition criterion. Chlorpromazine also decreased resistance to extinction.

The effect of ribonucleic acid on conditioned avoidance response was investigated by Cook and co-workers (29). They reported that acquisition of behavioral responses motivated by shock was enhanced in rats chronically treated with yeast ribonucleic acid

(RNA) and resistance to extinction was greater in rats so treated than in control. Hyden and Egyhazi (57) reported that during learning, an increase in synthesis of neuronal RNA was found and that the nuclear RNA changed composition with an increased adenine to uracil ratio. The same authors studied also the RNA content and base ratios of cortical neurons in rats.

Tapp and Markowitz (100) studied the effect of infant handling on avoidance learning, brain weight and cholinesterase activity. They reported that handling increased ventral-cortex and subcortical weights and decreased subcortical cholinesterase content. No differences in avoidance conditioning were observed.

Werboff and co-workers (105) investigated the behavioral effect of prenatal drugs in rats. They gave pregnant albino rats daily injections of reserpine, iproniazid, 5-HTP, BAS or sterile water during the second trimester of pregnancy. They indicated that greater susceptibility was found in the experimental offspring in either the maze learning or conditioned behavior situation.

It has been reported that rats which differ genotypically and phenotypically in maze learning ability do not differ significantly in brain weight nor in the ratio of body to brain weight (74, p. 331).

Most of these reports in the literature indicate that CAR is a good technique to test the animal's learning ability. Since insecticides might have some effect on the development of the central

nervous system, CAR is used to compare the learning ability of the offspring of treated and control animals.

Procedure

Nine randomly selected groups of ten mice each from the parathion, DDT and the control offspring (General Procedure) were reserved for avoidance conditioning which began at 30 days of age. (The mice were also weaned at 30 days of age.)

For avoidance training, a shuttle box (12 x 8 x 4 inches) was divided into two compartments by a low barrier. Each compartment had a grid floor through which a 40 volt shock could be delivered to the animal's feet. A mouse was placed in one compartment, and the trial began with a warning buzzer for five seconds. A mouse that crossed to the opposite side of the barrier during the buzzer period (conditioned response) would avoid the electric shock that followed the buzzer and stimulated crossover to the opposite non-electrified compartment (unconditioned response). Buzzer and shock were terminated with a crossover and a 20 second rest period followed before another trial began. Each mouse was subjected to 16 consecutive avoidance conditioning trials per day and the number of conditioned responses noted. The experiment continued for seven consecutive days. The body weight of the animals was measured daily during the test. The data were analyzed by the Mann-Whitney Test (91).

Results

The data obtained from testing the offspring from parathion, DDT and corn oil treated groups are shown in Tables 7, 8, and 9.

At first trimester, there were no significant differences in the acquisition of conditioned avoidance responses.

At second trimester there were significant differences in the acquisition of a conditioned avoidance response between control and DDT groups on 32 days of age (8.90) and (4.45), on 33 days of age (10.54) and (6.66), on 34 days of age (10.72) and (7.33), and on 35 days of age (13.00) and (9.44) respectively. In general, animals under the influence of DDT appeared inferior to control animals in acquiring the avoidance response at different ages.

At third trimester, there were significant differences in the acquisition of conditioned avoidance responses between the DDT and the control groups on 32 days of age (8.11) and (4.00), on 35 days of age (13.77) and (9.22), and on 37 days of age (15.11) and (11.50) respectively. Animals under the influence of DDT were clearly inferior to control animals in acquiring the avoidance response.

Discussion

There were depressant influences of DDT on the acquisition of avoidance responses and the growth of the different groups of the

Table 7. Acquisition of a conditioned avoidance response (CAR) of offspring of corn oil (control), DDT and parathion treated mice during the first trimester.

Age (Days)	Mean Number of CAR		
	Corn Oil	DDT	Parathion
31	1.40	2.37	2.90
32	4.20	3.00	3.72
33	8.60	8.62	9.81
34	9.90	10.12	9.63
35	10.50	11.87	11.81
36	12.30	11.75	11.18
37	13.40	12.12	13.09

Table 8. Acquisition of a conditioned avoidance response (CAR) of offspring of corn oil (control), DDT and parathion treated mice during the second trimester.

Age (Days)	Mean Number of CAR		
	Corn Oil	DDT	Parathion
31	4.18	2.63	2.55
32	8.90	4.45*	6.12
33	10.54	6.66*	8.37
34	10.72	7.33*	10.50
35	13.00	9.44*	10.75
36	11.81	11.77	11.37
37	13.27	12.66	10.62

* Significantly different from corresponding control ($P<0.05$)

Table 9. Acquisition of a conditioned avoidance response (CAR) of offspring of corn oil (control), DDT and parathion treated mice during the third trimester.

Age (Days)	Mean Number of CAR		
	Corn Oil	DDT	Parathion
31	3.88	3.20	3.91
32	8.11	4.00*	8.16
33	9.66	6.22	12.09
34	11.66	7.55	12.90
35	13.77	9.22*	12.36
36	13.66	9.50	13.72
37	15.11	11.50*	13.20

* Significantly different from corresponding control ($P<0.05$)

offspring at second and third trimester. The influence of DDT on the acquisition of a conditioned avoidance response (CAR) was not evident with the first trimester. These different results might be due to different concentrations of DDT in the animal tissues when administered during different stages of pregnancy. The concentration of DDT in animal tissues successively decreased from the third trimester to the first trimester due to greater loss of DDT from mothers exposed earlier to DDT than those exposed later because of metabolic excretion through urine and feces (60, 61). Thus the effect of DDT on CAR was gradually diminished from the third trimester group to the first trimester.

The brain uptake of DDT is less than liver, intestine and kidney, etc. (9). Hence, the growth of the offspring from DDT treated mothers during the first, second and third trimesters (see Appendix) was depressed during the experiment, but it is possible that DDT concentrations in the brain of the offspring of the first trimester treated mother was not enough to cause any effect on CAR.

Parathion had no effect on CAR of any group of offspring during the testing period. Thus, it is possible that the prenatal effect of parathion on the central nervous system disappeared when the offspring of first, second and third trimesters parathion treated mothers were one month old. The depressive action of parathion on the growth of offspring from mothers treated with parathion

during the first, second and third trimesters might be due to its inhibition of activity of cholinesterase of the fetus. The other possibility is that the parathion inhibition of the cholinesterase of the mother caused some kind of growth depression on its offspring. And finally, both of these possibilities mentioned might have occurred and affected the growth of the offspring.

OPEN FIELD TEST

Introduction

The Open Field Test of Hall (51) has been used for many years to study behavior and it is generally accepted as giving a valid measure of activity and emotionality in animals. The principle of the test is that the novel situation produces exploration, ambulation, rearing, defecation, urination, and displacement behavior like preening.

In the Open Field Technique the animal is placed on a smooth flat surface with limited dimensions and divided into uniform equal units, usually squares. The number of squares traversed by the animal during a specific period of time and the number of defecations or other activity exhibited by the animal are observed. The number of squares traversed by an animal is an indication of his activity and the number of defecations is considered an indication of his "emotionality."

The test has been used to study the effect of drugs on animal behavior. Broadhurst et al. (16) using two stimulants, pipradrol and ephedrine, and two depressants, amobarbital and methylpentynol, found that only pipradrol had any significant effect on Open Field behavior, i.e., an increase in ambulation. Ryall (85) reported that either reserpine or chlorpromazine at a dose level which caused

drowsiness produced a reduction in all measured categories of behavior, while prochlorperazine reduced ambulation, rearing and preening without producing drowsiness. Meprobamate, methylpentynol and pentobarbital, on the other hand, modified behavior only at dose levels which caused ataxia. Janssen et al. (59) showed that after either chlorpromazine or haloperiodol there was a decrease in ambulation, rearing and defecation. Haloperiodol was about 50 to 100 times as active as chlorpromazine in its effects on the two former activities but only slightly more potent in its effect on defecation.

Brimblecombe and Green (14) investigated the effects of monoamine oxidase inhibitors on the behavior of rats in the Open Field. With a series of aralkylhydrazines, they noticed a correlation between the monoamine oxidase inhibitory powers of these compounds and their effectiveness in producing changes in the behavior of normal untrained rats. The effect of psychotropic drugs on Open Field behavior in rats has been reported by Brimblecombe (15). He found significant differences in emotional defecation after administration of psychotomimetic drugs.

Young (108) administered epinephrine or chlorpromazine to neonatal rats and studied the effects on later emotionality and learning. He reported that there were behavior changes in locomotion and defecation in the Open Field and that there were changes in speed

and accuracy of learning a simple maze problem. Pregnant albino rats injected daily with reserpine, iproniazid, 5-HTP, BAS (the benzyl analogue of serotonin, 1-benzyl-2-methyl-5-methoxytryptamine) or sterile water during the second trimester of pregnancy were studied by Werboff and co-workers (105). They reported increased activity and increased emotionality in the experimental offspring on the Open Field Test. Werboff and Havelena (106) indicated that activity and emotionality decline with increasing age and that females exhibit higher activity and emotionality scores than males.

Stress alters Open Field behavior as reported by Candland (20) who noted specifically that stress significantly increased emotionality as indicated by greater defecation in the Open Field. The effects of behabioral stress on mice during pregnancy, and on the behavior of their offspring, are mimicked by epinephrine injection of mice during pregnancy as reported by Liberman (20). Hydrocortisone and norepinphrine also produce behavioral changes in the offspring. Similar results were obtained in chicks hatched from injected eggs. He suggested that epinephrine can directly on the developing embryo to produce change in behavior.

The treatment of the mother is a very important factor which affects the behavior of its offspring. Denenberg and Whimbey (33) reported that behavior of adult rats was modified by the experience their mothers had as infants. Not only the handling of future mothers during infancy, but also the handling of pregnant mothers

affect the behavior of their offspring. Ader and Colkin (3) reported that the offspring of handled pregnant mothers were generally less emotional than those of non-handled ones.

It would seem from the preceding discussion that the Open Field Test is a useful technique to measure the emotionality and activity of animals. Since the emotionality and activity of an animal has a direct relation with the central nervous system, and parathion or DDT poisoning provokes symptoms related to the central nervous system, the author believed that the Open Field Test would be a good tool to study the prenatal effect of parathion or DDT on the postnatal development of the offspring, by comparing the emotionality and activity of the DDT or parathion treated mice with a control group.

Procedure

The apparatus consisted of a rectangular box with an open top. The dimensions of this box were 21 x 21 x 10 inches. The floor was painted white and was divided into 3 x 3 inch squares by black lines. The lighting was uniform from over head.

Eighteen groups of animals prepared as described below were used in this experiment when the animals were 60 to 66 days of age. Each animal was weighed and subjected to this test for seven consecutive days, once every day for a three minute period. The number of squares traversed and the number of defecations of each

animal during the three minute trials were reported.

Effect of Electric Shock on Behavior

The nine groups of progeny reserved for avoidance conditioning response obtained from DDT, parathion or corn oil treated mothers (as described in General Procedure) were tested in the Open Field Test separately and compared with similar groups subjected only to the audiogenic seizure test and not to the avoidance conditioning response test. This part of the experiment should enable us to see if there is any effect of electric shock on behavior. The number of squares traversed and the number of defecations for each mouse were reported and the results analyzed by the Mann-Whitney Test (91).

Effect of DDT and Parathion on Behavior

Nine groups of offspring obtained from DDT, parathion or corn oil treated mothers(as described in General Procedures) were employed. Members of each group had passed through the audiogenic seizure test or the avoidance response test. The number of squares traversed and the number of defecations were reported and analyzed by the Mann-Whitney Test (91).

Results

Effect of Electroshock on Emotionality and Activity

The data in Tables 10 through 18 represent the results from the nine groups of animals that had passed through the avoidance conditioning test and had been exposed to foot electroshock (as described in General Procedure) and compared with animals from the same group that had not passed through the avoidance response test before testing them for Open Field behavior.

The data of the effect of electroshock on activity and emotionality shown in Tables 10 through 18 indicate, in general, that electric shock increased the activity of the groups of animals tested in Open Field. Tables 10 and 11 showed that electroshock caused a significant increase in the animal's activity for many days in the corn oil groups of first and second trimester. Tables 13 and 15 showed that electroshock caused a significant increase in activity in parathion groups of first and third trimesters. Tables 17 and 18 showed that electroshock caused significant increase in activity in DDT groups of second and third trimesters. There was no clear effect of electroshock on emotionality except on 60 days of first trimester shocked DDT group and on 64 and 66 days of age of second trimester shocked DDT group as indicated in Tables 16 and 17 respectively. Electroshock in both cases depressed significantly

Table 10. The average number of squares traversed and the number of defecations by shocked and non-shocked offspring obtained from mothers treated with corn oil at first trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecation	Squares	Defecation	Squares
60	5.00	116.40*	3.40	32.10
61	2.80	111.10*	2.80	60.30
62	3.40	158.80*	1.80	60.90
63	2.60	145.50*	3.60	55.00
64	1.50	145.50*	2.70	55.50
65	3.10	145.00*	2.60	97.70
66	1.80	144.00*	2.00	90.80

* Significantly different from the corresponding control ($P < 0.05$)

Table 11. The average number of squares traversed and the number of defecations by shocked and non-shocked offspring obtained from mothers treated with corn oil at second trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecation	Squares	Defecation	Squares
60	1.00	141.30*	1.72	87.63
61	2.00	130.10	2.54	102.90
62	1.40	132.70*	1.63	68.63
63	1.10	125.40	2.00	94.00
64	0.90	136.40*	1.63	78.72
65	0.60	133.10	1.36	100.72
66	1.80	164.40	2.36	72.36

* Significantly different from the corresponding control ($P < 0.05$)

Table 12. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with corn oil at third trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecation	Squares	Defecation	Squares
60	1.72	163.90	3.66	124.66
61	1.72	127.63	2.33	112.11
62	1.63	146.63	3.33	110.55
63	1.36	134.00	2.33	150.33
64	0.90	145.18	1.88	126.55
65	2.00	139.36	3.66	111.66
66	1.90	149.90	2.44	115.22

Table 13. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with parathion at first trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	1.27	130.81*	0.81	50.63
61	1.72	124.27	2.18	80.00
62	1.63	135.90*	1.45	82.09
63	1.45	139.72	2.36	101.36
64	1.81	117.54	1.72	88.72
65	1.90	123.27*	1.63	82.45
66	2.00	130.72	1.90	82.90

*Significantly different from corresponding control ($P < 0.05$)

Table 14. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with parathion at second trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	2.40	148.40	2.20	104.60
61	1.20	119.60	1.60	134.90
62	1.20	100.40	1.50	95.10
63	2.00	118.60	1.40	98.80
64	2.20	110.60	1.20	133.20
65	1.60	126.00	1.60	86.40
66	1.40	178.60	1.40	50.63

Table 15. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with parathion at third trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	1.25	145.87*	1.36	81.36
61	0.87	165.50*	1.18	74.63
62	1.62	140.25*	1.81	83.27
63	1.62	162.12*	0.81	96.36
64	1.75	146.50	2.27	97.81
65	1.25	123.87	2.54	106.36
66	1.37	117.62	1.54	114.00

*Significantly different from the corresponding control ($P < 0.05$)

Table 16. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with DDT at first trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	2.16*	91.00	3.41	115.33
61	3.50	83.00	1.33	91.33
62	2.83	78.00	2.18	101.63
63	2.00	89.00	3.63	70.36
64	1.50	106.00	2.18	97.18
65	2.33	112.50	3.45	75.00
66	2.50	146.50	3.09	90.54

* Significantly different from the corresponding control ($P < 0.05$)

Table 17. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with DDT at second trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	0.37	128.50	1.00	96.60
61	1.12	93.50	1.00	76.10
62	2.00	116.62	3.00	77.44
63	0.62	138.50*	1.55	82.33
64	1.00*	118.37	3.33	74.44
65	0.50	82.75*	2.00	37.11
66	1.12*	129.62	4.11	76.88

* Significantly different from the corresponding control ($P < 0.05$)

Table 18. The average number of squares traversed and the number of defecations given by shocked and non-shocked offspring obtained from mothers treated with DDT at third trimester.

Age	Electrically Shocked		Non-Shocked	
(Days)	Defecations	Squares	Defecations	Squares
60	2.28	145.42*	3.85	78.14
61	3.14	122.71	2.57	88.71
62	3.71	164.42*	2.14	82.57
63	2.42	156.00*	2.28	113.00
64	2.28	125.42	3.85	113.42
65	1.85	137.00*	4.00	109.00
66	1.42	149.28*	3.00	122.57

* Significantly different from the corresponding control ($P < 0.05$)

the emotionality of animals.

Effect of DDT or Parathion on Emotionality and Activity

This part deals with the effect of DDT or parathion on all of the offspring obtained from mothers treated with DDT, parathion or corn oil at different trimesters of pregnancy, regardless of the kinds of treatment which the offspring received before the test. Both DDT and parathion groups are compared with the corn oil group in Tables 19, 20, and 21.

At first trimester there was a significant difference between emotionality of the control (4.20) and the parathion group (1.04) on 60 days of age. There was a significant difference in activity of animals between the control (74.25) and the DDT group (107.20) on 60 days of age. At second trimester there was significant difference between emotionality of the DDT group (1.05) and the control group (2.28) on 61 days of age. At third trimester there was significant difference in emotionality of the DDT group (3.07) and the control group (1.35) on 64 days of age.

Discussion

Electroshock increased the number of squares traversed by offspring in three minute trials in the Open Field Test in comparison with the non-shocked animals (control). These increases in

Table 19. The average number of squares traversed and the number of defecations given by offspring of mice who received DDT, parathion or corn oil (control) during first trimester.

Age	Corn Oil		DDT		Parathion	
(Days)	Defecation	Squares	Defecation	Squares	Defecation	Squares
60	4.20	74.25	3.00	107.20*	1.04*	90.72
61	2.80	85.70	2.05	88.77	1.95	102.13
62	2.60	109.85	2.41	93.58	1.54	109.00
63	3.10	100.25	3.05	77.23	1.90	120.54
64	2.10	100.50	1.94	100.29	1.77	103.13
65	2.85	121.35	3.05	88.23	1.77	102.86
66	1.90	117.55	2.88	110.29	1.95	105.81

* Significantly different from the corresponding control ($P<0.05$)

Table 20. The average number of squares traversed and the number of defecations given by offspring of mice who received DDT, parathion, or corn oil (control) during second trimester.

Age	Corn Oil		DDT		Parathion	
(Days)	Defecation	Squares	Defecation	Squares	Defecation	Squares
60	1.38	113.19	0.72	110.77	2.26	119.20
61	2.28	115.85	1.05*	83.83	1.46	129.80
62	1.52	99.14	2.52	95.88	1.40	131.20
63	1.57	108.95	1.11	108.76	1.60	102.93
64	1.28	106.19	2.23	95.11	1.53	102.73
65	1.00	116.14	1.29	58.58	1.60	130.80
66	2.09	116.19	2.70	101.70	1.40	117.13

* Significantly different from the corresponding control ($P<0.05$)

Table 21. The average number of squares traversed and the number of defecations given by offspring of mice who received DDT, parathion, or corn oil (control) during third trimester.

Age	Corn Oil		DDT		Parathion	
(Days)	Defecation	Squares	Defecation	Squares	Defecation	Squares
60	2.60	146.25	3.07	111.78	1.31	108.52
61	2.00	120.65	2.85	105.71	1.05	112.89
62	2.40	130.40	2.92	123.50	1.73	107.26
63	1.80	141.35	2.35	134.50	1.15	124.05
64	1.35	136.80	3.07*	119.42	2.05	118.31
65	2.75	126.90	2.92	123.00	2.00	113.73
66	2.15	134.30	2.21	135.92	1.47	115.52

* Significantly different from the corresponding control ($P<0.05$)

activities of shocked animals were in most cases significant as indicated in Tables 10, 11, 13, 15, 17, and 18.

The usefulness of electroshock in the treatment of patients with mental depression has been known for some time and may help to explain the increased activities of the electroshocked mice in our experiments. Our results confirm the finding of Broadhurst and co-workers (16), who reported that the stimulant drugs caused increased activity of animals tested in the Open Field Test.

The effect of electroshock on emotionality was very little as compared with activity. The emotionality results might indicate that there is no important effect of electroshock on emotionality of animals, or the time of testing animals, three minutes, was not long enough to collect enough data about emotionality of animals to show the electroshock effect on it. The effect of DDT or parathion on emotionality, activity and growth (see appendix) of offspring from DDT or parathion treated mothers during first, second and third trimesters was minor and had no biological value. These findings indicate that the prenatal effects due to parathion had disappeared when the offspring from parathion treated mothers during first, second, and third trimesters became two months old. The results indicate also that the concentration of DDT delivered into the offspring of DDT treated mothers during first, second, and third trimesters was very low in the animal tissues when they were two months old and

no longer had an effect. Thus, the offspring of DDT or parathion treated mothers did not show different results as compared to the offspring of corn oil treated mothers during the experiment.

MINIMAL ELECTROSHOCK SEIZURE THRESHOLD

Introduction

Convulsions produced by electrical stimulation of the brain have been studied extensively in mammals. The primary aims are to throw light upon seizure mechanism and to observe behavioral consequences. The convulsive pattern differs to some extent with the intensity and duration of the current and with the place and mode of application. Most of the recent investigators have utilized eye electrodes or clips applied to the ears.

Maximal Electroshock Seizure threshold measures the intensity of the stimulus required to evoke a maximal seizure (characterized by a hind leg tonic-extensor component) in 50% of the animals. Minimal Electroshock Seizure threshold measures the intensity of the stimulus required to evoke a minimal seizure (three to five seconds of sustained clonus) in 50% of the animals. The latter threshold is often employed to estimate brain excitability.

The relation of brain excitability to brain γ-aminobutyric acid (GABA) concentration had been investigated by Woodbury and Verandakis (107) in rats under various conditions. They found that adrenalectomy had increased brain excitability as measured by electroshock seizure threshold and decreased brain GABA concentration. Diphenylhydantion in intact rats slightly decreased

brain excitability and slightly but not significantly increased GABA concentration. In adrenalectomized animals diphenylhydantion markedly decreased brain excitability and significantly increased brain GABA concentration. Acetazoleamide significantly increased brain GABA concentration and decreased brain excitability. In intact rats, deoxycorticosterone acetate (DCA) decreased brain excitability and slightly increased brain GABA concentration. In adrenalactonized rats, DCA markedly decreased brain excitability and markedly increased brain GABA concentration. In intact rats, cortisol increased brain excitability but did not alter brain GABA concentration. In adrenalactomized rats, cortisol increased brain excitability to a greater extent and markedly decreased GABA concentration. Hypoxia (7.5% CO_2) increased brain excitability and decreased brain GABA concentration. In contrast, hypoxia (75% CO_2) decreased brain excitability and increased GABA concentration. Carbon dioxide (12.5, 50%) decreased brain excitability and increased brain GABA concentration. Their data indicated that brain excitability varies inversely with brain GABA concentrations.

Swinyard and co-workers (99) reported that restraint of mice significantly lowered the threshold for seizures produced by a.c. electroshock, low frequency electroshock, and I.V. pentylentetrazol. They reported that in adrenalectomized mice, restraint had no such lowering effect on the seizure threshold. They suggested that the

increase in brain excitability caused by restraint is the effect of endogenously released epinephrine on the central nervous system. Castellion and Swinyard (23) reported that the development and reproducibility of seizure were not altered by previous auditory or electroshock stimulation.

Seizure susceptibility increased in rats maintained at high altitude as indicated by Castilo and Timiras (24). They suggested this increase was due to depression of the inhibitory action of the higher nervous system centers on the lower centers. It is possible to modify the pattern of electroshock seizure by psychopharmacologic drugs as indicated by Fink and Swinyard (38). Hyponatremia decreased electroshock seizure threshold, Brown (18); hypernatremia increased electroshock seizure threshold as indicated by Swinyard and co-workers (98).

Essig and Flanary (36) reported that repeated electroshock increased electroshock seizure threshold and Swinyard and co-workers (97) showed that hydration decreased electroshock seizure threshold. Besides all these factors which modify electroshock seizure threshold, Rosenthal and Timiras (84) indicated that x-irradiation decreased electroshock seizure threshold, and Rosenblum (83) discovered that light deprivation decreased electroshock seizure threshold.

Swinyard and co-workers (96) reported that the electroshock

seizure threshold in audiogenic seizure susceptible O'Grady mice was significantly lower than that of audiogenic seizure non-susceptible CF #1 mice. They observed that O'Grady mice are more resistant to the threshold lowering effects of hyponatremia than are CF #1 mice. They showed too that there was no significant difference in the minimal or maximal pentylenetetrazol seizure threshold of O'Grady or CF #1 mice. Hamburg and Essman (52) indicated that there was no significant difference in electroshock seizure threshold between audiogenic seizure susceptible mice and non-susceptible ones.

This review of literature indicates that drugs and many other factors affect electroshock seizure threshold. As previously postulated, prenatal DDT or parathion might affect the postnatal development of the central nervous system of the offspring. Thus it was thought that determination of minimal electroshock seizure threshold may be a useful technique to study the effect of DDT or prenatal parathion on the postnatal development by comparing the minimal electroshock seizure threshold of parathion or DDT groups with the control group.

Procedure

Nine different groups of progeny obtained from DDT, parathion, or corn oil treated mothers (as described under General

Procedure) were used in the experiment. The animals in each group were 70 to 90 days of age.

Minimal electroshock seizure threshold was determined in the animals by measuring the intensity of unidirectional current (six pulses per second, 0.2 millisecond pulse duration, three second stimulus duration) required to evoke a minimal seizure ("stun" response, or three to five seconds of continued minimal clonic activity) in 50% of the mice. The electrical stimuli was delivered by a Grass Stimulator (Model S4G) according to the parameters reported by Brown and co-workers (17). The electroshock procedure employed ear-clip electrodes as indicated by Chen and co-workers (26), with long leads which were connected to the ear of the mouse after moistening the ear canal with 0.9% sodium chloride solution. Since the brief physical restraint lowers seizure threshold as reported by Swinyard and co-workers (99), care was exercised to measure seizure threshold only while the animals were unrestrained (tested 30 seconds after attaching electrodes). The results are statistically analyzed by the method of Litchfield and Wilcoxon (65).

Results

The results of the electroshock seizure threshold tests for the nine groups are shown in Table 22. The condition and appearance

of all offspring from gravid mice given DDT, parathion, or corn oil appeared the same.

Table 22. The minimal electroshock seizure threshold for offspring of gravid mice given DDT, parathion, or corn oil (control) during pregnancy.

	Minimal Electroshock Seizure Threshold (volt)		
Stage of Pregnancy	Corn Oil	DDT	Parathion
First trimester	95 (89-100)	97 (92-101)	90 (85-95)
Second trimester	92 (88-96)	90 (85-96)	88 (83-93)
Third trimester	82 (71-96)	94 (89-99)	95 (90-100)

The data indicated no significant difference between any of the groups for first, second, and third trimesters.

Discussion

Parathion is metabolized and excreted within a few days after administration in the animals (8, 44). It has been indicated previously that the prenatal effect of parathion had disappeared from the offspring of parathion treated mothers during first, second, and third trimesters by the time they were two months old. Likewise, no significant differences in the brain excitability of all groups of offspring from parathion treated mothers and that of the control groups were found in the experiments. On the other hand, DDT is

metabolized and excreted for months, as reported by Dale and co-workers (54), and Hayes and co-workers (30). As pointed out already the concentration of DDT in the tissues of offspring from DDT treated mothers during the first, second, and third trimesters was low at the age of two months and probably caused no important biological effects. Therefore, the low concentration of DDT in the tissues of the offspring had little effect in these experiments and the brain excitability of all the groups of offspring from DDT treated mothers was not different from that of the control groups.

GENERAL DISCUSSION

The investigation presented herein pointed out that the offspring from DDT or parathion treated mothers showed different incidences of audiogenic seizure than the offspring from corn oil treated mothers (controls). In general all the control offspring showed high incidence of seizure on 18 days of age and the offspring from DDT or parathion treated mothers showed high incidence on 20 days of age. It has been reported that the appearance of audiogenic seizures in animals coincided with the mylination of some long nerve tracts of the subcortex. Furthermore, the beginning formation of connections between subcortical areas and the cerebral cortex was found to be directly related to the onset of sound induced convulsion (22). Thus parathion or DDT delayed the maturation of the central nervous system of the offspring from DDT or parathion treated mothers two days in comparison to the offspring from corn oil treated mothers. Delaying the maturation of the central nervous system by the insecticides for two days is a considerable time in comparison with the gestation period of mice, only 21 days. Many investigators have reported that in older and adult animals, a reduction in susceptibility to seizure occurred with increased ages (34). In this investigation, also, all offspring from DDT, parathion, or corn oil treated mothers showed decreased seizure incidence with increased age.

In general, the high incidence of seizures of all the different groups of offspring had passed by the time they were 28 days of age. The delay of maturation of the central nervous system by DDT is due to its effect on the offspring through the placenta and/or milk secretion (61, 9). The molecular weight of parathion is 291.27. Anselimo (7) reported that chemicals which have molecular weights, no more than 350 can pass through the placenta easily. Thus, parathion probably passed through the placenta into the fetus of parathion treated mothers. Since parathion is metabolized and excreted after administration into the animals within a few days (8, 44), the delay of the central nervous system maturation of the offspring from parathion treated mothers was caused by the prenatal parathion only.

DDT had an influence on the acquisition of an avoidance response by the offspring when given to mothers during the second and third trimesters only. The brain uptake of DDT is low compared to liver and many other viscera (9). Therefore, it is possible that the brain of the offspring of DDT treated mothers during second and third trimesters picked up more DDT than the offspring of DDT treated mothers during first trimester, or else the brain was more susceptible to its effects at the later time. This might be due to larger amounts of DDT delivered to the offspring of DDT treated mothers during second and third trimesters via the placenta and milk secretion than to the offspring from DDT treated mothers at

the first trimester. Parathion had no influence on the acquisition of the avoidance response of all groups of offspring from parathion treated mothers; this might be due to the disappearance of prenatal influence of parathion on the offspring by the time they were one month old.

Previous electric shock increased the activity of the mice in the Open Field Test perhaps due to the stimulation of the central nervous system by the electric stimulus. As already indicated, the prenatal effect of parathion on the central nervous system of offspring from parathion treated mothers disappeared by the time they were one month old, and also there was no effect of parathion on the activity, emotionality, and the minimal electroshock seizure threshold of these offspring when they were older than one month. As previously pointed out, DDT lost its effect on the central nervous system of the offspring from DDT treated mothers during the first trimester when they were one month old, and also had no biological effect on the emotionality, activity, and the minimal electroshock seizure threshold of all groups of offspring from DDT treated mothers when they were two months old or more.

DDT and parathion depressed the growth of the offspring from DDT or parathion treated mothers from one day old through 60 days of age (see Appendix). This depression might be due to the direct toxic effect of the insecticides on the normal metabolic activity of

these offspring, because DDT or parathion had been delivered into the offspring from the DDT or parathion treated mothers via the placenta and/or milk secretion (7, 8, 9, 61). The weight, the condition, and the appearance of all the groups of offspring from DDT, parathion, or corn oil treated mothers by the age of 60 days were similar, probably because the insecticides had lost their effect on these animals by the time they reached this age. DDT caused premature birth in DDT treated mothers during the third trimester. This might be due to DDT stimulation of the hypothalamus which caused indirect stimulation to the pituitary gland and thereafter caused early release of oxytocin or it might be due to uterine contraction caused by the DDT itself.

SUMMARY AND CONCLUSION

Mice (CF #1 strain) were used in the studies presented herein. The offspring of DDT, parathion, or corn oil treated mothers during first, second, or third trimester were subjected to different techniques in order to study the effect of the insecticides administered during gestation on the postnatal development of the offspring. The results of these studies are presented in Tables 1 through 22. Audiogenic seizure, conditioned avoidance response, open field, and minimal electroshock seizure threshold tests were used when the offspring were 16 to 30, 30 to 37, 60 to 66, and 70 to 90 days of age respectively. The body weights were measured from 16 to 66 days of age. The data pointed out that the offspring of DDT or parathion treated mothers showed high incidence of seizures on 20 days of age but the offspring of corn oil (control) treated mothers showed high incidence of seizures on 18 days of age, i.e., the insecticides delayed the maturation of the central nervous system two days, a period approximately equal to 10% of the gestation period of mice. The high incidence of seizures in all groups of offspring from DDT, parathion, or corn oil treated mothers had passed by 28 days of age. Since the incidence of seizures was still fairly high when the offspring were 30 days of age, it is suggested that the audiogenic seizure test should be continued until no audiogenic seizure appears.

The administration of DDT or parathion to gravid mice did not affect the percent incidence of audiogenic seizures of their offspring but they delayed the peak time of seizure incidence in comparison with the control groups. The insecticides influenced the maturation of the central nervous system of the offspring regardless of the stage of pregnancy in which they were administered to the gravid mice. This fact suggests the pregnant animals should not be exposed to these insecticides under any circumstances. The prenatal effect of parathion on the central nervous system had apparently disappeared by the time the offspring from parathion treated mothers were one month old. Thus, there were no effects for parathion on the conditioned avoidance responses (CAR), activity, emotionality, and minimal electroshock seizure threshold of these offspring.

The prenatal and postnatal effect of DDT on the offspring from DDT treated mothers on the central nervous system was gone by the time the offspring were about two months old. Thus, there was some influence by DDT on the CAR (tested at 30 days of age) and no influence on the emotionality, activity, and the minimal electroshock seizure threshold of the offspring (tested after 60 days of age). These results indicate that the chronic effect of the insecticides is probably not only proportional to their concentration but also proportional to their half life. DDT or parathion depressed the growth of offspring from DDT or parathion treated mothers as shown in

the Appendix. The depressant effect of these insecticides on the growth of the offspring had greatly diminished by the time they were 60 days old. Thus, the condition, appearance, and weight of all the groups of offspring were about the same by 60 to 66 days of age. DDT caused premature birth in DDT treated mothers during the third trimester as shown in the Appendix. These facts pointed out that the insecticides affect different systems of the animals differently.

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APPENDIX

APPENDIX

A. Average growth of offspring obtained from mothers treated with parathion, DDT, or corn oil (control).

1. First Trimester

Age (Days)	Body Weight (Grams) DDT	Parathion	Corn Oil
16	4.72	5.03	5.93
18	4.89	6.26	6.64
20	4.01	5.98	7.41
22	5.63	6.54	8.03
24	6.01	7.22	10.82
26	5.90	7.56	9.86
28	6.12	8.00	10.93
30	7.58	8.44	11.91
31	9.22	9.39	11.93
32	11.96	10.79	12.78
33	12.62	11.20	13.68
34	13.18	11.90	14.60
35	13.15	12.88	15.45
36	13.50	13.43	16.88
37	14.15	14.79	18.88
60	24.41	21.19	24.05
61	22.61	21.64	24.03
62	23.60	21.83	24.15
63	24.13	22.66	23.97
64	23.90	21.88	24.43
65	24.27	22.11	24.72
66	24.55	23.00	24.01

2. Second Trimester

Age (Days)	Body Weight (Grams) DDT	Parathion	Corn Oil
16	4.89	3.94	5.71
18	5.30	5.02	6.68
20	5.65	5.93	7.87
22	5.96	6.42	8.41
24	6.22	6.92	9.17
26	6.19	7.59	10.25
28	7.31	8.88	11.26
30	8.07	9.00	12.45
31	10.88	10.11	12.90
32	11.86	11.09	14.07
33	11.36	12.43	15.06
34	11.69	13.09	16.18
35	12.31	13.25	17.04
36	12.94	13.81	17.89
37	13.70	14.50	18.66
60	22.92	23.35	23.88
61	22.50	24.37	23.76
62	23.06	23.40	24.68
63	23.42	23.54	25.37
64	23.23	23.45	24.59
65	23.29	23.45	24.62
66	23.72	23.45	25.10

3. Third Trimester

Age (Days)	Body Weight (Grams) DDT	Parathion	Corn Oil
16	4.62	5.53	6.43
18	5.59	5.71	7.46
20	5.02	5.94	8.49
22	5.60	6.51	9.59
24	5.87	7.19	12.50
26	6.42	8.21	11.28
28	7.70	8.85	13.92
30	8.35	9.89	13.58
31	10.27	9.70	16.03
32	11.27	11.47	17.61
33	13.94	12.65	17.77
34	13.69	12.81	18.42
35	13.77	13.34	18.89
36	15.37	14.25	19.50
37	15.98	14.55	20.08
60	22.11	24.01	24.00
61	22.52	23.85	24.15
62	22.05	23.89	24.20
63	23.09	24.72	24.65
64	22.58	24.34	24.12
65	22.98	24.74	24.85
66	23.30	25.07	24.32

B. Litter size of mothers treated with DDT, parathion, or corn oil (control) at different stages of pregnancy.

1. First Trimester

DDT	Parathion	Corn Oil
9	10	7
10	8	9
10	9	10
12	2	10
10	7	10
13	12	9
	12	
	7	
	12	

2. Second Trimester

DDT	Parathion	Corn Oil
11	10	6
9	12	9
10	8	9
11	10	9
9	9	12
9	9	7
9	11	12
10		8
6		

3. Third Trimester

DDT	Parathion	Corn Oil
8	8	11
11	11	10
12*	10	8
9	10	5
9*	8	3
	8	6
	12	9
		13

* Premature offspring