1985

The Effects of in Utero Chlordecone Exposure on Receptor-Coupled Phosphatidylinositol Turnover in Developing Rat Brain

Kristine D. Dahl
Loyola University Chicago

THE EFFECTS OF IN UTERO CHLORDECONE EXPOSURE ON RECEPTOR-COUPLED PHOSPHATIDYLINOSITOL TURNOVER IN DEVELOPING RAT BRAIN

by

Kristine D. Dahl

A Dissertation Submitted to the Faculty of the Graduate School of Loyola University of Chicago in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

August

1985

ACKNOWLEDGMENTS

I would like to take this opportunity to thank all those who aided me during my stay at Loyola.

To my advisor, Dr. Michael Collins, who willingly ventured into a new avenue of research. His guidance and patience have been incalculable.

To Dr. Mary Manteuffel for her helpful discussions and her donation of protein deficient offspring.

To Dr. Daniel Selivonchick for attempting to teach me lipid biochemistry.

To Deb Pronger and Bhi Cheng for their friendship and invaluable assistance with chromatography methodology.

To my family who have supported me without reservation in whatever endeavor I have attempted.

Finally, I would like to dedicate this dissertation to Jo Ann Hartline, without whose help and support I would never have made it through these final stages.

VITA

Kristine Dahl is the daughter of Edward Dahl and Gwendolyne (Bohlman) Dahl. She was born on May 26, 1953, in Janesville, Wisconsin.

Her elementary and secondary education was obtained in the public schools in Janesville, Wisconsin, where she graduated in 1971.

In August, 1971, she entered the University of Wisconsin in Madison, Wisconsin. In August, 1973, she was awarded a state scholarship and transferred to Edgewood College, Madison, Wisconsin. She graduated in May, 1975, with the degree of Bachelor of Science with a major in biology and a minor in chemistry.

From January, 1979, to October, 1981, she was a research assistant to Dr. Samuel Epstein at the University of Illinois School of Public Health. In March, 1981, she entered the University of Illinois School of Public Health and was awarded a NIOSH traineeship in toxicology. She graduated in June, 1983, with the degree of Master of Science in toxicology.

In October, 1981, she became a research assistant to Dr. Michael Collins at Loyola University Medical Center,

Maywood, Illinois. In July, 1982, she was granted a basic science fellowship in the Department of Biochemistry at Loyola University Medical Center. In March, 1984, she was recommended for the Julia Hoffman Award for Outstanding Achievement in Science.

In September, 1984, she accepted an NIH postdoctoral fellowship in reproductive medicine with Dr. Aaron J. W. Hsueh at the University of California-San Diego, La Jolla, California. She will begin her work there in September, 1985.

PUBLICATIONS:

Iqbal, Z. M., Dahl, K. D., and Epstein, S. S. (1980). Role of NO_2 in the biosynthesis of nitrosamine in mice. Science 28: 1475.

Iqbal, Z. M., Dahl, K. D., and Epstein, S. S. (1981). Biosynthesis of DMN in DMA treated mice following exposure to NO_2. J. Nat. Cancer Inst. 67: 137.

ABSTRACTS:

Collins, M. A., Dahl, K. D., Nijm, W. A., and Major, L. F. (1982). Evidence for homologous families of dopamine (DA) and serotonin (5HT) condensation products in cerebrospinal fluid (CSF) from monkeys. Abst. Soc. Neurosci. 8: 277.

Dahl, K. D., Selivonchick, D. P., and Collins, M. A. (1984). Chlordecone and synaptosomal phospholipids (PL) labeling in developing rat. Abst. Soc. Neurochem.

Dahl, K. D., and Collins, M. A. (1985). Brain muscarinic receptor density and affinity are reduced in rats exposed to chlordecone in utero. Abst. FASEB.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

LD_{50}	lethal dose 50
GC-EC	gas chromatograph-electron capture
GC-MS	gas chromatograph-mass spectroscopy
HPLC	high performance liquid chromatography
TLC	thin layer chromatography
ng	nanogram
ug	microgram
mg	milligram
g	gram
kg	kilogram
ul	microliter
ml	milliliter
nmol	nanomole
umol	micromole
mmol	millimole
uM	micromolar
mM	millimolar
M	molar
ppm	parts per million
Kcal	kilocalorie
nm	nanometer
P	inorganic phosphorus
w	weight

vol	volume
Ci	Curie
X g	times gravity
SPM	synaptic plasma membrane
PPIs	polyphosphatidylinositols
TPI	triphosphatidylinositol
DPI	diphosphatidylinositol
PI	phosphatidylinositol
PA	phosphatidic acid
PE	phosphatidylethanol amine
PS	phosphatidylsereine amine
PG	phosphatidylglycerol amine
DAG	diacylglycerol
CC	carbamylcholine
NA	noradrenaline
DA	dopamine
5-HT	serotonin
HA	histamine
DOPAC	3,4-dihydroxyphenylacetic acid
HIAA	hydroxyindoleacetic acid
BSA	bovine serum albumin
HCl	hydrochloric acid
Na_2SO_4	sodium sulfate

EDTA-KCl	ethylenediamine tetraacetic acid-potassium chloride
ATP	adenosine triphosphate
mAChR	muscarinic acetylcholine receptors
QNB	quiniclinidyl benzilate
PPO	2,5-diphenyloxazole
POPOP	1,4-BIS(5-phenyloxazol 2-yl) benzene
DHBA	3,4-dihydroxybenzoic acid

LIST OF TABLES

LIST OF FIGURES

CONTENTS OF APPENDICES

CHAPTER I

INTRODUCTION

Chlordecone is a polycyclic chlorinated hydrocarbon insecticide that was used in the control of leaf-eating insects. This insecticide has been implicated as being responsible for neurological signs, liver damage, and sterility in male factory workers employed in a plant where the agent was manufactured (Cohn et al., 1978). Further investigation showed that the James River, which drained the Chesapeake Bay, was extensively and seriously contaminated. Several investigators (Cohn et al., 1978); Cannon et al., 1978; Sanborn et al., 1979; Taylor et al., 1978) have discovered adverse effects on central nervous system (CNS) function such as tremor, headaches, abnormal elevation of cerebral spinal fluid pressure, and disturbances of vision in other chlordecone exposed workers. Similar CNS disturbances have been induced experimentally in rodents by chemical means (Chang-Tsui and Ho, 1980; Reiter et al., 1977; Deitz et al, 1979; Tilson et al., 1980).

The mechanism of chlordecone neurotoxicity has been attributed to inhibition of mitochondrial and synaptosomal membrane-bound sodium-potassium ATPases. Desaiah et al.

(1980) demonstrated that chlordecone induced a neurotoxic effect on a synaptosomal event, but this neurotoxicity could be attributed to other related synaptosomal membrane phenomena.

Several investigators (Eroschenko and Place, 1977; Good et al., 1965) have observed blocked or impaired reproductive function in chlordecone-treated birds and rodents. High doses of chlordecone produce overt teratogenic effects and a variety of toxic manifestations. These include low fetal weight (Chernoff and Rogers, 1976; Chernoff et al., 1979), reduced postpartum survival (Chernoff et al., 1979), developmental abnormalities involving skeletal, ureterogenital, and central nervous systems (Chernoff and Rogers, 1976) and in multigenerational studies, decreased reproductive capacity as adults (Chernoff et al., 1979). Despite these studies of teratogenic effects at high doses in adults, there appear to be no reports of biochemical studies on neurotoxicity from in utero exposure. Given that chlordecone is used extensively in developing countries, such studies would have considerable merit.

Therefore, the objective of this research is to examine the effects of maternal chlordecone exposure on receptor mediated events in developing rat brain. It is

proposed that CNS development in the presence of a lipophilic chlorinated insecticide such as chlordecone results in a significant change in the extent of neurotransmitter stimulated synaptic membrane events. To investigate this hypothesis, this research will determine whether receptor-coupled events such as phosphatidylinositol turnover, muscarinic and dopaminergic receptor binding and endogenous biogenic amine levels are altered by *in utero* exposure to chlordecone and whether these changes, if any, persist after clearance of the insecticide.

CHAPTER II

LITERATURE REVIEW

Environmental Impact of Pesticides

Environmental Teratogens

There is speculation about the magnitude of chemical involvement in birth defects and reproductive failure. To date, only about 25 chemicals are known to be teratogenic in humans, compared to more than 800 in laboratory animals (Vernadakis, 1982). It is not known if this discrepancy is due to a greater resistance of humans to the agents or to inability to pinpoint the source of teratogenicity in humans. The potential danger is great, however, because almost 2000 new chemicals are introduced into the environment each year (Vernadakis, 1982).

Many factors play a role in human fetal susceptibility to a teratogenic agent. The time of administration during the pregnancy is probably one of the most important factors. Chemical or drug exposure during the first 17 days of embryonic life (the period of fertilization and implantation) usually results either in death of the embryo or total recovery without defects. Exposure from days 18 to 55 (the embryonic period) corresponds with the period of organogenesis and presents the greatest risk of birth

defects. From 56 days until term (the fetal period) the process of organogenesis is complete; however, growth retardation or functional deficits (brain damage) may result from exposure to a teratogen during this stage of development (Berlovki et al., 1983). The age and nutritional state of the mother, the route of administration of the chemicals, and the size and number of doses also affect fetal susceptibility.

Several mechanisms exist to reduce fetal exposure to environmental pollutants. The fetus is protected by the placenta and maternal detoxification mechanisms. The placenta allows only a small fraction of most chemicals to reach the fetus. Transfer across the placenta decreases with increasing molecular weight, increasing electrical charge, and decreasing lipid solubility.

The dose reaching the placenta via maternal circulation is further reduced by detoxification by the liver, excretion by the kidneys and in the bile, and binding to plasma proteins (which makes transplacental transfer more difficult). Peak circulating concentrations are further decreased by deposition of toxicants into maternal storage depots, for example, insecticide deposits in adipose tissue.

Some researchers believe that variation in the dose of chemicals that reach the fetus, as determined by the above factors, could explain why a particular chemical causes birth defects in one animal species but not in another (Bungay and Dedrick, 1979).

Assessment of Toxicity

The expression "acceptable daily intake" has become part of the terminology concerning the assessment of the toxicity of pesticide chemicals, and is extensively used to denote either a concept or a figure. The concept is based on the widely accepted fact that all chemicals are toxic, but that their toxicity varies markedly, not only in nature, but also in the amount that is required to produce signs of toxicity. The figure, when applied to pesticide chemicals, provides an indication of the amount that might be ingested daily in food, even over a lifetime, without appreciable risk to the health of the consumer. The figure is derived from the experimental data in laboratory animals and/or appropriate observations in humans available at the time of evaluation.

Although the evaluation of toxicological data rests on expert judgment, the type of information required for the assessment of toxicity of a pesticide represents a problem which is not easily solved. In general, the type of

information and the procedures which generate it should be designed to estimate the maximum no-effect dose.

The following types of information are usually necessary: (a) Biochemical aspects, including the kinetics of absorption, tissue distribution, and excretion; biological half-life; effect on enzymes; and metabolism. (b) Special studies--carcinogenicity, mutagenicity, neurotoxicity, potentiation, reproduction, teratogenicity, etc. (c) Acute toxicity--LD_{50} and other studies mainly involving single dosings in several species of experimental animals. (d) Short-term studies, which generally include classical sub-acute 90-day toxicity tests. These studies generally extend from the time of weaning of an animal to the time of sexual maturity, usually 3 months in rodents and 1-2 years in dogs or monkeys; (e) Long-term studies--usually 80 weeks in mice and 2 years in rats. Multigenerational studies may belong in this category depending on the duration of the treatment received by each generation. (f) Observations in humans, consisting mainly of observations in individuals having had occupational or accidental exposure (Vettorazzi, 1975).

Chlorinated Hydrocarbon Insecticides

The major classes of pesticides have been grouped as "nonpersistent," "moderately persistent," or "persistent"

(Harris, 1969). "Persistence times" reflect the time required for 75 to 100 percent diappearance of pesticide residues from the site of application. Nonpersistent pesticides have persistence values of 1 to 12 weeks; moderately persistent pesticides, 1 to 18 months; and persistent pesticides 2 to 5 years. Persistence varies with environmental conditions.

The chlorinated hydrocarbon insecticides such as aldrin, dieldrin, endrin, heptachlor, mirex, and chlordecone are prime examples of persistent pesticides. Studies with this group have elucidated several important concepts in environmental toxicology. First, persistence is not a desirable attribute as originally believed. Second, the transport and disposition of persistent pesticides is affected by physical and biological processes which occur from the micro to the global scale. Third, high lipid solubility combined with chemical and biological stability can lead to biological magnification of pesticide residues (Goring, 1967).

Persistence is primarily a function of physiochemical properties of substances. In addition, the adsorption/desorption process is one of the most important factors controlling the fate of pesticides in soils (Bailey and White, 1970). Chlorinated hydrocarbon insecticides are

highly soluble in lipids and most organic solvents, but have low water solubilities and relatively low vapor pressures. Studies of the absorption of chlorinated hydrocarbons on various soils are difficult because of their low water solubility. However, studies show that adsorption of the hydrophobic chlorinated hydrocarbons depends strongly on the presence of soil organic matter. Once adsorbed, these chemicals do not readily desorb (Weber, 1972). Two implications are readily apparent: (1) such compounds will not leach or diffuse in soil and (2) transport into the hydrosphere from contaminated soils will be through erosion of soil particles or sediment, not by desorption and dissolution. When chlorinated hydrocarbons are poorly adsorbed, as in sandy soils, the vaporization loss will be significant as compared to that in soils with higher organic matter.

Volatilization of pesticides into the atmosphere from water and soils is also a transport route. The volatility of a chemical from a soil or from water is a function of its vapor pressure, but the actual vaporization rate depends on several environmental parameters. Temperature, soil properties, soil water content, and other physiochemical properties such as water solubility and degree of adsorption affect the vaporization rate of pesticides from soil.

Degradation of the chlorinated hydrocarbons is quite slow as compared to other classes of insecticides, and in soil and water it is mainly due to the action of microorganisms. To a lesser extent, chemical reactions and photochemical reactions degrade the chlorinated hydrocarbons under certain conditions. The "caged" compounds such as mirex and chlordecone undergo very little detectable degradation (Lichtenstein and Schulze, 1960).

The bioaccumulation of the chlorinated hydrocarbons is well documented by environmental residue data (Edwards, 1970). Bioaccumulation ratios relate organism residues to environmental residue levels and are higher in aquatic ecosystems as opposed to terrestrial ecosystems (Acree et al., 1963). The processes involved in bioaccumulation are quite complex due to the population fluctuations, food web relationships, metabolic capabilities of various species and numerous other ecologic considerations. However, the physiochemical parameters of lipid solubility, low water solubility, and chemical stability, which characterize the chlorinated hydrocarbons, appear to be most important in bioaccumulation of organic pesticides.

Chlordecone was used extensively for a variety of agricultural pest control purposes and in many public health disease control programs worldwide. High levels of the

compound were applied over wide areas. The result of this heavy usage was the widespread contamination of many of the components of the environment with residues of chlordecone that are likely to persist for many years. Very low levels of the insecticide, only marginally detectable in some cases, exist in the air, atmospheric dust, and rainwater. From there the compound is transported to the soil and water ecosystems where it becomes available for a variety of organisms. These residues are then bioaccumulated, with man sitting atop the food chain as the ultimate consumer. Although direct toxicity to humans has not been documented as a result of the accumulation of chlordecone, the thin eggshells and reproductive failures of birds and chronic toxic effects on fish served as the warning signs that led to restriction of the use of this chemical.

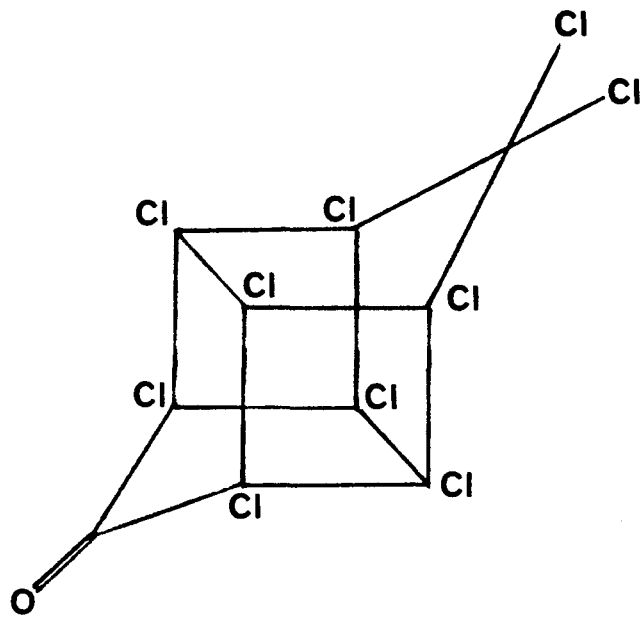
Toxicity of Chlordecone

Chemistry and Analytical Methods

Chlorodecone (Decachloro octahydro-1,3,4-metheno-2,4-cyclobuta [cd] pentalen-2-one) is an odorless, colorless, crystalline solid, synthesized by derivatization of hexachlorocyclo-pentadiene in the presence of sulfur trioxide followed by hydrolysis to form a ketone (Figure 1). Used as an insecticide, it is added to bait and other inert

Figure 1

THE STRUCTURE OF CHLORDECONE



material to control the banana root borer, tobacco wineworm, and domestic insect pests. It is also an ingredient in about 55 commercial pesticide formulas in the United States and other countries. It has recently come under intense scrutiny from a methodological and toxicological viewpoint because of the discovery in 1975 of environmental contamination by this compound near its place of manufacture in Virginia.

The discovery of the persistence and widespread distribution of chlordecone has resulted in tremendous interest in the development of analytical methodology for the compound. Few articles on analytical methodology for chlordecone appear in the literature. Other articles pertain to the evaluation of insecticidal properties of chlordecone derivatives (Gilbert et al., 1966) and elucidation of the structure of chlordecone or similar compounds (McBee et al., 1956, Griffen and Price, 1964; Eaton et al., 1960). Gilbert et al. (1966) prepared numerous alcohol and amine adducts of chlordecone in a search for compounds possessing insecticidal activity. Brewerton and Slade (1964) presented a procedure for the analysis of chlordecone residues on apples, utilizing gas chromatography with electron capture (GC-EC). They reported 0.2 nanograms (ng) as being readily detected and measured;

however, most of the chlordecone levels reported in other papers have been in the parts per million (ppm) range (Moseman et al., 1977). Determination of chlordecone in milk from cows fed treated feed was reported to be quantitative at 0.005 ppm (Smith and Avant, 1967). Cleanup of samples with oleum and dilute base was followed by GC with microcoulometric detection.

The choice of solvents is critical in the extraction, cleanup and analysis of chlordecone. Also, unless great care is taken to exclude water from solvents, the compound exists in solution as a hydrate. Aliphatic hydrocarbon solvents such as hexane are poor solvents for this pesticide; methanol, acetone and benzene are better solvents. Acetone has been reported to react with chlordecone under 18 hours of reflux (Hodgson et al., 1978). At ambient temperatures methanol apparently reacts with chlordecone to form the hemiketal. Combined gas chromatography-mass spectrometry (GC-MS) indicates that the hydrate and hemiketal forms revert to chlordecone in the injection port of a gas chromatograph at temperatures above 180 degrees centrigrade.

Due to the polar nature of chlordecone, it is difficult to remove it from substrate using nonpolar extraction solvents such as petroleum ether or hexane.

Several investigators have found that the most effective extraction solvent systems are methanol-benzene (1:1) and toluene-ethyl acetate (1:3).

Pharmacokinetics

Absorption

Chlordecone is readily absorbed (>90%) from the gastrointestinal tract. This has been established in rats consuming chlordecone in maternal milk (Kavlock et al., 1980) or when given as a single dose of 40 mg/kg in corn oil by gastric intubation (Soine et al., 1981). The absorbed chlordecone rapidly establishes an equilibrium of distribution among most tissues. The equilibrium was achieved within 24-48 hours following a single dose to rats regardless of whether chlordecone was given intravenously, by gastric intubation, or by intraperitoneal injection (Kavlock et al., 1980). A similar pattern of tissue distribution was found in mice fed a diet containing 40 ppm of chlordecone for many months (Huber, 1965). Quantitative measurements of chlordecone absorption through the skin or by inhalation have not been reported.

Distribution

Cohn et al. (1978) inspected the distribution of chlordecone in the tissues of factory workers. From these studies it was evident that there was an unusually high

concentration of chlordecone in blood as compared to that in adipose tissue, the ratio being 1:7. This ratio is similar to that reported by Egle et al. (1978) in rats two weeks or 26 weeks following a single dose of chlordecone (Table I). The high blood to fat ratio for the distribution of chlordecone contrasts markedly to the partitioning of DDT (Morgan and Roan, 1974), polybrominated biphenyls (Landrigan et al., 1977), and other lipophilic environmental chemicals (Robinson and Hunter, 1966) which are hundreds of times less concentrated in blood than fat. It is generally believed that the coefficient of distribution of hydrophobic chemicals among tissues including blood parallels the lipid content of the tissues (Lindstrom et al., 1974). Hence, the disproportionately high concentration of chlordecone in blood might be explained by the fact that chlordecone hydrate may be more water soluble than other organochlorine pesticides. However, from unsuccessful attempts to detect unbound chlordecone in plasma, it has been concluded that the "free" plasma chlordecone, if present at all, is less that 1% of the total chlordecone in blood (Soine et al., 1981). Therefore, it has been hypothesized that chlordecone may be specifically associated with plasma proteins in preference to blood lipids (Skalsky et al., 1979). Soine et al. (1981) found that when chlordecone was incubated with

Table I

DISTRIBUTION OF RADIOLABELED CHLORDECONE IN THE MALE RAT (EGLE ET AL., 1978)

Tissue	Liver	Fat	Cerebellum	Cerebrum
Day 1	233±15.2	134±4.0	62.8±1.6	57.5±3.6
Day 3	198±17.9	90±12.7	55.5±3.4	48.2±3.2
Day 7	180±20.2	134±17.9	48.2±2.9	43.8±1.3
Day 14	128±5.5	34±2.6	19.0±0.8	18.5±1.4
Day 28	85±8.3	13±1.4	8.6±0.9	8.8±1.0
Day 49	39±2.7	9±1.0	5.0±0.4	4.8±0.5
Day 84	18±0.9	1.1±0.2	1.1±0.1	0.9±0.1
Day 182	3.6±0.1	0.4±0.2	0.2±0.0	0.2±0.0

Data represent the mean ± the standard error.

human, rat, or pig plasma, more than 75% of the chemical was associated with the albumin and high-density lipoprotein fractions, whereas these same fractions from human plasma contained only 24% of an endogenous, hydrophobic substance, namely, cholesterol. Moreover, of the total chlordecone or cholesterol associated with human lipoproteins, the high-density lipoprotein fraction prepared by either ultracentrifugation or by heparin-manganese precipitation followed by agarose gel electrophoresis contained 53% of the chlordecone, but only 22% of the cholesterol. Whereas most organochlorine pesticides are associated with the lipid rich fractions of plasma through nonspecific, hydrophobic interactions (Skalsky et al., 1979), specific binding of chlordecone by albumin and high-density lipoproteins offers an attractive explanation for the enormous amounts of this hydrophobic chemical in such an aqueous fluid as blood.

A second distinctive feature of chlordecone distribution in man (Cohn et al., 1978) and rats (Egle et al., 1978) is that among all sampled tissues, the highest concentration is found not in body fat, although this might be expected based on the distribution of most lipophilic compounds, but in the liver. Rats given a single dose of chlordecone established within 48 hours a stable partition ratio of chlordecone between fat and blood of 13:1, whereas

the ratio of the initial concentration of chlordecone in liver and blood was 28:1. The fat to blood concentration ratio remained unchanged for 26 weeks thereafter, despite the fact that the concentration of chlordecone in fat declined by a factor of 362. In contrast, the concentration in liver declined less rapidly during this study (by a factor of only 65) so that the liver to blood concentration ratio rose progressively during the 26 week study to a final value of 119:1. This finding was confirmed in mice chronically fed chlordecone (Huber, 1965) and in the fetuses of pregnant chlordecone-treated rats (Kavlock et al., 1980). In both studies the highest concentration of chlordecone among all sampled tissues was found in the liver. There is no explanation for the preferential sequestration of chlordecone in liver. Hence, it may be concluded that the disproportionate accumulation of body chlordecone in the liver is due to binding of chlordecone by constituents of liver membranes, presumably proteins or lipids (Arias et al., 1980).

Excretion

The major route of elimination of chlordecone is in the stool. This has been confirmed in humans (Cohn et al., 1978), rats (Egle et al., 1978), mice, and monkeys (Fariss, 1980). Only minimal amounts of chlordecone appeared in the

urine of these species even when tritiated-chlordecone tracer studies were carried out (Egle et al., 1978) (Table II). An exception to this generalization may be lactating females. Chlordecone has been detected in samples of breast milk from women living in southern states of the United States (Smith and Avant, 1967). The environmental source of chlordecone in this region is presumed due to extensive use of mirex, which may contain chlordecone as a contaminant or which may undergo photodegradation to form chlordecone (Carlson et al., 1976). Chlordecone promptly (within 4 days) appeared in the milk of cows started on chlordecone-containing diets ranging from 0.25 to 5.0 ppm (Smith and Avant, 1967). After 60 days, the levels of chlordecone in the milk of the cows on these diets rose to 20 and 320 ng/ml, respectively, and then declined to barely detectable levels after resumption of a normal diet for 83 days. It has been estimated that female rats may excrete as much as 52% of an acutely administrated dose of chlordecone in milk during the lactation period (Kavlock et al., 1980). This may be compared to male rats which excreted 33% of a single dose in stool during a similar situation (Egle et al., 1978).

Table II

RADIOACTIVITY IN FECES AND URINE AFTER TREATMENT WITH RADIOLABELED CHLORDECONE, 40 MG/KG (EGLE ET AL., 1978)

Days after Treatment	Cumulative Excretion	
	Feces (%)	Urine (%)
1	12.7±2.8	0.533±0.36
2	15.6±2.6	0.582±0.36
3	18.9±2.5	0.603±0.36
4	21.7±2.1	0.622±0.35
5-6	27.1±2.1	0.667±0.36
7	29.5±2.1	0.688±0.36
8-13	33.1±2.1	
14-20	37.8±2.0	
21-28	46.0±1.8	
29-35	50.5±1.7	
36-42	54.1±2.0	
43-49	57.4±2.0	
50-56	59.9±2.0	
57-63	61.9±2.0	
64-70	63.4±2.0	
71-77	64.5±2.0	
78-84	65.5±2.0	
85-91	66.3±2.5	
92-98	67.0±2.5	
99-105	67.5±2.4	
106-112	67.9±2.5	
113-119	68.2±2.4	
120-126	68.5±2.4	
127-133	68.8±2.4	
134-140	69.0±2.4	
141-147	69.2±2.4	
148-154	69.4±2.4	
155-161	69.5±2.4	
162-168	69.6±2.4	
169-175	69.7±2.4	
176-182	69.8±2.5	

Data represent the mean ± the standard error.

General Toxic Effects

There are no reports of death in humans exposed to chlordecone. Chlordecone-poisoned workers lacked such constitutional symptoms as fever, chills, sweating, or fatigue. However, in 10 of 23 cases, there was prominent weight loss (as much as 60 pounds in four months) despite a normal diet (Cohn et al, 1978).

The susceptibility of laboratory animals to the lethal effects of a single oral dose of chlordecone is similar between males and females (Larson et al., 1979; Gaines, 1969), but varies among species. The LD_{50} for rabbits (71 mg/kg) is lower than that for rats (126 mg/kg), dogs (250 mg/kg), or chicks (480 mg/kg) (Sherman and Ross, 1961). All rats receiving diets of 50 ppm of chlordecone or more died within 6 months. Mice receiving diets containing 80 ppm of chlordecone or higher died within 32 days (Huber, 1965). When given daily doses of 50, 25, or 10 mg/kg, 90% of the animals died within 5, 9, or 24 days, respectively (Huang et al., 1980). The acute and cumulative LD_{50} for chlordecone in mice are in the same range. The mechanism of death in these studies was not ascertained. Chronic dermal contact with chlordecone produced minimal toxicity in rats (Gaines, 1969). In several studies (Curtis and Mehendale, 1979; Cannon and Kreb, 1979) of adult rats maintained for

many months on diets containing more than 10 ppm chlordecone, significant weight loss was noted. Depressed growth has been observed in pregnant rats given as little as 2 mg/kg/day of chlordecone (Chernoff and Rogers, 1976), in mice fed 40 ppm (Huber, 1965), in mice treated with 10 mg/kg/day (Ho et al., 1981), in laying hens fed 75 ppm (Naber and Ware, 1964), and in quail fed 300 ppm (McFarland and Lacy, 1969). "Hypermetabolism" manifested by increased food and oxygen consumption was reported in rats (Larson et al., 1979) and mice (Huang et al., 1980).

Neuromuscular Toxicity

The sine qua non of chlordecone intoxication is an irregular, nonpurposive waking tremor involving the extremities, head and trunk (Taylor et al., 1978). Also present in the chlordecone poisoned worker was opsoclonus, an unusual oculomotor disorder consisting of chaotic eye movements causing blurred vision. In some patients who complained of headaches, spinal fluid pressure was elevated, and three patients had frank pseudo-tumor cerebri due apparently to impaired capacity to absorb cerebrospinal fluid (Sanborn et al., 1979). Neuropsychiatric abnormalities included irritability, memory disturbances, exaggerated startle response and, in one case, visual and

auditory hallucinations (Taylor et al., 1978). No evidence of dysfunction of muscle or peripheral nerves was adducted, and muscle biopsies of five patients were histologically normal.

The prompt appearance of tremors in rats (Larson et al., 1979; Curtis and Mehendale, 1979; Cannon and Kimbrough, 1979; Egle et al., 1979; Desaiah, 1981), chicks (Sherman and Ross, 1961), Japanese quail (McFarland and Lacy, 1969), and mice (Huang et al., 1980) receiving chlordecone has been well documented. Also, like patients, chlordecone-treated rats and mice exhibited hyperexcitability and an exaggerated startle response. These rats were especially susceptible to chemically invoked seizures (Huang et al., 1980). A strict departure from the symptoms noted with chlordecone poisoned workers is the observation of severe muscle weakness following a single dose of chlordecone to chicks (Sherman and Ross, 1961) or rats (Cannon and Kimbrough, 1978). Although muscle weakness has been ascribed to effects of chlordecone as a competitive inhibitor of muscle lactate dehydrogenase (Hendrickson and Bowden, 1975), the myopathic effects of chlordecone in rats appeared to be permanent, increasing at a time when there was both a decline in chlordecone levels in muscle and a disappearance of neurotoxic manifestations (Cannon and Kimbrough, 1978).

Liver Toxicity

In most of the chlordecone-poisoned workers, the liver and spleen were enlarged; chemical tests of liver function, including clearance of sulfobromopthalein (BSP) from plasma, was normal; histologic examination of liver biopsies revealed nonspecific findings, including minimal fatty metamorphosis, focal proliferation of sinusoidal cells, hyperglycogenation of nuclei, increased residual bodies, branched mitochondria with paracrystalline inclusions, and accumulation of smooth endoplasmic reticulum (Guzelian et al., 1980). The last observation suggests that the hepatic microsomal drug metabolizing system (cytochrome P-450-dependent monooxygenase system) was induced. In line with this finding, workers displayed an accelerated clearance of antipyrene from the blood as compared to normal subjects. Furthermore, the workers had abnormally high levels of glucaric acid, a substance which is reported to be a derivative of hepatic endoplasmic reticulum (Hunter et al., 1972). However, none of the workers had elevated serum gamma-glutamyl transpeptidase activity, an enzyme proposed as a marker for the induction of hepatic drug metabolizing enzymes in man (Whitfield et al., 1973; Rosalki et al., 1971). It should be noted that these effects of chlordecone on hepatic functions associated with the endoplasmic

reticulum may reflect an adaptive response on the liver rather that "hepatotoxicity" per se.

Similar to the effects of chlordecone in human liver, treatment of rats (Ishikawa, 1978), quail (Eroschenko, 1974; 1980), mice (Huber, 1965), or dogs (Larson et al., 1979) with chlordecone increased the size of the liver relative to the total body weight. Also similar were histopathologic examinations of the livers from chlordecone-treated rats (Chu et al., 1980), quail (Eroschenko and Wilson, 1975), or mice (Hewitt et al., 1979) which revealed only such nonspecific changes as fatty infiltration, pleocytosis, and focal lymphoid aggregates. There was no evidence of fibrosis, cholestasis, or significant hepatocellular necrosis.

The hepatic drug metabolizing system is induced in rats treated with commercially available chlordecone. Chlordecone treatment increased the concentration of the drug binding hemoprotein, microsomal cytochrome P-450, the level of NADPH-cytochrome C reductase activity, and the activity of cytochrome P-450 dependent oxidation of pentobarbital (Mehendale et al., 1977; 1978). Pentobarbital sleeping time was reduced by chlordecone pretreatment of rats (Mehendale et al., 1978). It has been concluded that chlordecone resembles the prototype inducer, pentobarbital,

because of the profile of its effects on the hepatic microsomal monooxygenases, and because chlordecone binds to cytochrome P-450 (Ebel, 1980). However, it should be noted that commercially available chlordecone contains an impurity that is reported to be solely responsible for the "phenobarbital-like" induction in aryl hydrocarbon hydroxylase activity in genetically responsive mice (Nebert et al., 1977). To resolve the question of induction of cytochrome P-450 by chlordecone, Guzelian (1980) administered a single oral dose of highly purified chlordecone (>99.9%, based on GC-MS) to male rats (40 mg/kg) or gerbils (20 mg/kg) and found the concentration of hepatic microsomal cytochrome P-450 increased an average of 150% and 259%, respectively.

Toxic Effects In The Endocrine And Reproductive Systems

There were no overt clinical manifestations of endocrinologic toxicity among 28 of the chlordecone poisoned male workers except for complaints of decreased libido in seven patients. All of the patients had normal serum levels of follicle stimulating hormone, luteinizing hormone, and testosterone (Anderson et al., 1976). Nevertheless, only eight patients had normal sperm count, and in only one of these eight was the concentration of chlordecone in blood

greater than 1000 ng/ml (Cohn et al., 1978). In all cases, low or absent sperm numbers were associated with an abnormally low percentage of motile sperm. Histologic examinations of testicular biopsies in two patients revealed arrest of sperm maturation (Anderson et al., 1976), a finding that suggests a potentially reversible lesion. In 12 of 13 cases where it was possible to make repeated examinations of sperm in a given individual over several years, there was an increase in the concentration of motile sperm coinciding with a fall in blood levels of chlordecone (Cohn et al., 1978). Several years have elapsed since the chlordecone was removed from the body tissues of these men. Although no comprehensive study of reproductive function has been carried out, several individuals have since fathered children. No information is available regarding the effects of chlordecone on reproductive function in women.

Blocked or impaired reproductive function in chlordecone-treated birds and rodents has been abundantly documented. Chronic consumption of diets containing even small amounts of chlordecone by bobwhite quail (1 to 25 ppm) (Dewitt et al., 1962), by pheasants (5 to 10 ppm), by laying hens, or by Japanese quail (200 ppm) (Eroschenko, 1977; 1978; 1979) reduced the number of eggs produced. Morever, these eggs were smaller than normal, had reduced eggshell

thickness and strength, hatched less frequently, and produced defective chicks with decreased survival rates (Eroschenko, 1977; 1978). Similarly, feeding chlordecone to inbred mice (5 or 10 ppm) (Good et al., 1965) or rats (25 ppm) (Cannon and Kimbrough, 1979) reduced the number and size of the litters produced. The defect was primarily associated with females and could be reversed within several months upon resumption of a normal diet (Mehendale, 1977). Overt teratogenic effects are observed at higher doses of chlordecone. An increased number of offspring exhibited a variety of toxic manifestations such as low fetal weight and reduced postpartum survival (Chernoff et al., 1979). Chernoff and Rogers (1976) observed developmental abnormalities involving the skeletal, ureterogenital, and central nervous system. In multigenerational studies, decreased reproductive capacity was seen in adults (Good et al., 1965).

The consensus view of all these investigators is that chlordecone impairs reproduction by mimicking the effects of excessive estrogens. For example, testicular atrophy was reported in chlordecone-treated rats (Larson et al., 1979) and quail (Eroschenko and Wilson, 1974). Gross and histologic examinations of testes taken from chlordecone-treated quail (McFarland and Lacy, 1969) showed a congeries

of findings (reduced size, decreased spermatogenesis, and abnormalities of the seminiferous tubules) similar to those produced by estrogen administration to birds. Chlordecone treatment also produces a variety of "estrogen-like" effects on the female reproductive system. In immature quail or chicks, chlordecone treatment caused premature development of the oviduct, manifested grossly by an increase in oviduct weight, and histologically by the presence of cilia, cellular proliferation and differentiation, formation of tubular glands, and secretory activity. Examination of the treated quail ovaries revealed fewer eggs and impaired follicular development (McFarland and Lacy, 1969). In immature rats, chlordecone produced an increase in uterine growth, a persistent vaginal estrous, anovulation, and the disappearance of corpus lutea from the ovaries (Gellert, 1978). The ultramicroscopic changes in the cell lining of the oviduct, vagina, and uterus of these rats was characteristic of those produced by estrogenic steroid hormones.

It has been suggested that the estrogenic effects of chlordecone may be exerted indirectly through the hypothalamic pituitary system by prolonging release of follicle stimulating hormone and preventing release of luteinizing hormone (Huber, 1965). However, it should be

noted that these studies were carried out prior to the advent of sensitive and specific immunochemical techniques for measuring pituitary hormones. There is now strong evidence that chlordecone exerts its estrogenic effects directly. When incubated with explants of chick oviduct *in vitro*, chlordecone, like estradiol, induced *de novo* synthesis of specific egg white proteins and increased the number of specific mRNA sequences encoding for these proteins (Palmiter and Mulvihill, 1978). These effects could be prevented by tamoxifen, an antiestrogen that competitively inhibits binding of estradiol to its specific cytoplasmic receptors (Palmiter and Mulvihill, 1978). Moreover, there was competition between estrogen and several preparations of highly purified chlordecone for binding to nuclear (Hammond *et al.*, 1979) and cytoplasmic (Bulger *et al.*, 1979) estradiol receptors. Chlordecone stimulated transfer of the cytoplasmic estradiol receptor into the nucleus and stimulated synthesis of the progesterone receptor (Hammond *et al.*, 1979). These are two processes for which the estrogen receptor is thought to be indispensible. Despite the fact that chlordecone was found to be 10,000 times weaker than estradiol in its biological effects or in its affinity for the estrogen receptor (Palmiter, 1978), it is possible that persisting in tissues

in high concentrations, chlordecone may produce local adverse estrogenic effects on the reproductive system (Hammond et al., 1979). This hypothesis would account for the selectivity and reversibility of estrogen-like effects of chlordecone on the testes of the poisoned workers.

Neurotoxicity

The mechanism of chlordecone neurotoxicity has been attributed to inhibition of mitochondrial and synaptosomal membrane bound sodium-, potassium- ATPase(s). Research groups at the University of Mississippi have worked sedulously to document this property of chlordecone using various tissue sources for the enzyme. These include brain from untreated or chlordecone-treated rats (Desaiah, 1981; Mishra et al., 1980; Desaiah et al., 1980a), mice (Ho et al., 1981; Desaiah et al., 1980b), and catfish (Desaiah and Koch 1975); muscle from the heart (Desaiah, 1981) or extremeties (Mishra et al., 1980) in rodents; liver from chlordecone-treated rats (Curtis and Mehendale, 1979); or isolated perfused rat liver preparations (Desaiah et al., 1977; Mehendale, 1977). The extent of the inhibition of sodium-, potassium-ATPase and oligomycin-sensitive magnesium ATPase activities in brain synaptosomes prepared from chlordecone-treated rodents was directly proportional to the concentration of chlordecone in the brain (Ho et al., 1981)

and was proportional also to the degree of tremor produced in the animal. Furthermore, several investigators (Desaiah, 1981; Mishra et al., 1980) have demonstrated inhibition of this enzyme activity in vitro was blocked in a dose-dependent fashion by chlordecone. Added chlordecone produced a greater inhibition of the enzyme than did an equal amount of mirex, a compound that produces no tremors in vivo (Desaiah et al., 1980b), and yet is structurally similar to chlordecone. Finally, the inhibition of ATPase activity can be reversed if bound chlordecone is removed by washing (Desaiah, 1981) or by addition of anti-chlordecone antibodies (Mishra et al., 1980). The Mississippi research group believes that neurotoxicity may be due to an inhibition of synaptosomal membrane ATPase(s) by chlordecone, resulting in blocked cellular uptake and storage of such neurotransmitters as catecholamines (Desaiah, 1980). Others have suggested that chlordecone inhibits a step in rat brain mitochondrial energy production prior to the magnesium ATPase reaction (End et al., 1979). This results in loss of the capacity of mitochondria to maintain normal rates of calcium ion uptake, leading to an increase in cytoplasmic calcium ion. Depolarization of the neuronal membrane would ensue, promoting release of neurotransmitters (Carmines et al., 1979). Since the toxic

manifestations of chlordecone bear little resemblance to those of cyanide, a classical mitochondrial poison, nonspecific loss of cellular energy seems an unconvincing explanation for chlordecone toxicity. On the other hand, competitive, reversible inhibition of ATPase linked processes by chlordecone could explain (a) the reversibility of neurotoxicity associated with decreasing concentrations of chlordecone in human tissues (Cohn et al., 1978; Taylor et al., 1978); (b) the inhibition of cerebrospinal fluid uptake in humans which is thought to be dependent upon sodium, potassium ATPase (Sanborn et al., 1979); and (c) the decrease in brain content of neurotransmitters in experimental animals treated with chlordecone (Ho et al., 1981).

Chang-Tsui and Ho (1980) suggested that one of the actions of chlordecone in the CNS is on neurotransmitter and receptor interaction. Decreases in tritiated spiroperidol binding to striatal preparations were observed in rats fed as little as 10 ppm chlordecone (Seth et al., 1981). In the same studies higher doses depressed tritiated muscimol and tritiated quiniclidinyl benzilate binding in cerebellum. Binding affinities returned to normal within 30 days following cessation of treatment. Rats exposed to 6 ppm chlordecone perinatally and then subjected to footshock

stress at 80 days of age were found to have reduced dopamine (DA) levels in the caudate nucleus; however, no evidence of altered DA turnover was observed in associated measurements of DOPAC levels (Rosecrans et al., 1982). The same studies found increases in serotonin turnover in all brain regions examined.

Therefore, several investigators have demonstrated that chronic exposure of adults to chlordecone produced pronounced, but apparently reversible, effects on membrane protein content and transmitter-receptor sites in several brain regions. Chang-Tsui and Ho (1980) discovered that gestational and lactational dosing with this agent caused decreased dopamine receptor binding intensity that was detected 18 days after cessation of maternal dosing. These changes were in the opposite direction to those caused by dosing of adult rats. A tendency of a toxic agent to cause opposite receptor changes in the brain of gestationally or neonatally exposed animals relative to adult exposure has also been observed in the case of acrylamide (Agrawal and Squibb, 1980). These data confirm the general concept of the distinctive susceptibility of young animals to toxicants and the possible tendency for early toxic insult to be less reversible than the corresponding phenomenon in the adult. The sensitivity of the immature brain to environmental

agents acting over a long period of time may be greater than its susceptibility to acute damage by stroke or other anoxic episodes.

Carcinogenicity

The National Cancer Institute sponsored a carcinogenesis bioassay of technical grade chlordecone (>98% purity) in Osborne-Mendel rats and B6C3F1 mice (National Cancer Institute, 1976). The initial doses were found to be too high and had to be reduced during the study because of excessive mortality. The four groups of animals, male rats, female rats, male mice and female mice, ultimately received a "low" (8, 18, 20 or 20 ppm, respectively) dose or "high" (24, 26, 23 or 40 ppm, respectively) dose for 80 weeks. This was followed by resumption of a normal diet for either 32 weeks (rats) or 10 weeks (mice). There were no hepatocellular carcinomas in control rats, whereas the incidences in high dose rats were 7% for males and 22% for females, both significantly increased. The incidences of hepatocellular carcinomas in female mice receiving the low dose (52%) or high dose (47%) groups were significantly higher than in controls (0 of 40 mice). Male mice receiving the low dose (81%) or high dose (88%) had a significantly higher incidence of liver tumors as compared to matched

controls (32%). Moreover, the time to detection of the first hepatocellular carcinoma was significantly shortened in all groups of treated mice. Metastases of the hepatocellular carcinomas were not observed, and there were no significant increases in extrahepatic tumors. From these studies it was concluded that chlordecone was a liver carcinogen in rats and mice. Reuber (1978; 1979), in reinterpreting the histologic material available from this bioassay and from studies of rats fed 1 ppm or 5-25 ppm (Larson et al., 1979) of chlordecone for 12-24 months, had reached the same conclusions. In addition to carcinomas, other investigators (Cueto et al., 1978; Reuber, 1978) observed hepatocellular noncancerous proliferative lesions accompanied by fatty infiltration and degeneration.

The mechanism underlying production of liver cancer by chlordecone is unknown. Although most agents that are carcinogenic in rodents also produce mutations in short term tests in animals or in vitro, chlordecone, like some other organochlorine insecticides, proved to be negative when mutagenicity was assayed using the Ames test (Schoeny et al., 1979). Chlordecone also proved to be negative as a mutagen when tested for enhancement of unscheduled DNA synthesis in primary cultures of adult rat hepatocytes (Williams, 1980; Probst et al., 1981). It has been

suggested that chlordecone is a promoter. "Promotion" relates to a current concept of experimental liver carcinogenesis which involves a multistage process. First, preneoplastic hepatocytes are formed by exposure of the liver to a "true" or complete carcinogen ("initiator"). However, these cells may remain dormant unless the liver is exposed to a second agent which, while not necessarily carcinogenic by itself, enhances development of the preneoplastic cells into malignancies ("promotion") (Pitot and Sirica, 1980; Farber, 1980).

Neurochemistry and Toxicology

The determination of the molecular and biochemical mechanisms underlying the responses of the nervous system to toxic insults remains a complex and difficult task. However, recent advances in neurochemistry have provided sufficient knowledge to suspect that compounds that interfere with such processes as energy metabolism, axoplasmic transport, protein synthesis, and chemical neurotransmission may be neurotoxic. Furthermore, the availability of new methodology permits analysis of specific, dynamic neurochemical events. These developments can provide productive, interpretable, and diverse approaches for evaluating the effects of toxic agents on nervous system functioning.

Receptors

Receptor Binding

An important approach developed in recent years is the study of membrane receptors that specifically bind neurotransmitters. These reversible, high-affinity interactions are stereospecific and nonenzymatic, have equilibria with very low dissociation constants, and involve configurational recognition (Heilbronn and Bartfai, 1978; Yamamura et al., 1981). Such binding is the first step by which the presynaptic neuron causes ionic and biochemical changes in the cell containing the receptor. Kinetic characteristics of binding of receptor density are measured by incubation of membranes with radioactively labeled compounds that bind specifically to receptor sites. Excess ligand unbound to the membranes is removed by filtrational means. Nonspecific binding of the labeled compound is estimated by repeating the incubation in the presence of a relatively high concentration of the unlabeled pharmacological agent or neurotransmitter. In general, the most potent binding ligands are the pharmacological antagonists or agonists of a given neurotransmitter, rather than the transmitter itself. Receptor binding studies may be more sensitive than assays of gross levels of a

neurotransmitter. For example, the neuroleptic drug chlorpromazine does not alter brain dopamine levels, but has major effects on dopamine binding sites and on the turnover rate of dopamine (Creese et al., 1978). This approach has led recently to several important findings in neurobiology and neuropharmacology. Among these are the discovery of endogenous brain analgesic and anxiolytic compounds, a better understanding of the mechanism of action of the major tranquilizers and anticonvulsants, and advances concerning the relation of chemistry to many neurological and psychiatric diseases (Snyder, 1979). Since the discovery that antischizophrenic drugs bind to dopamine receptors and that the degree of binding was related to the potency of the drug, receptor binding assays have been used as a quick screen for testing drugs.

Receptor density is a dynamic phenomenon subject to relatively rapid changes. There is often an inverse relation between extracellular level of a ligand and the density of its corresponding receptors on the surface of the cell (Minneman et al., 1979). Thus, receptors may regulate their own activity by increasing or decreasing their concentration or their sensitivity to the neurotransmitter. Such regulation may serve as a homeostatic mechanism which ensures appropriate physiological responses in the recipient

cell. However, in a variety of disease states, this compensatory mechanism can act against the interests of the organism. It can also frustrate pharmacological efforts to enhance or suppress a given neurotransmitter system, and may account for the development of drug tolerance and habituation (Russel et al., 1975). Malfunctioning of receptors (either by decreased sensitivity or enhanced sensitivity) have been implicated in a variety of neurological and behavioral disorders. These include such clinical manifestations as schizophrenia, myasthenia gravis, Parkinson's and Huntington's diseases, terminal shock, epilepsy, and asthma (Melnechuk, 1978). Receptor changes have also been noted during normal aging (Makman et al., 1979) and after exposure to alcohol (Michaelis et al., 1978).

Recent evidence indicates that some neurotoxic substances may affect neurotransmitter receptor modulation. In vivo and in vitro studies have shown that low concentrations of cadmium inhibit striatal muscarinic acetylcholine receptors (Hedlund et al., 1979). Other heavy metals, such as lead and mercury, have also been shown to inhibit a variety of putative neurotransmitter receptor sites by binding to the receptor site (Bondy and Agrawal, 1980). Acrylamide treatment has been shown to increase the

affinity of striatal spiroperidol binding in adult rats (Agrawal et al., 1981). Therefore, determination of the number of receptors, such as central muscarinic receptors, can provide a valuable tool in evaluating the neurochemical response to toxic agents.

Muscarinic Receptors

Muscarinic acetylcholine receptors (mAchR) are present in neurons in both the peripheral and central nervous system, in many exocrine glands, and in both cardiac and smooth muscle. Electrophysiological and pharmacological techniques have been used to study the role and action of mAChRs in mediating cholinergic synaptic transmission. These investigations have shown that mAChRs in different tissues may act in different ways. For example, activation of mAChRs causes depolarization by increasing membrane input resistance in cortical neurons, sympathetic neurons and hippocampal neurons, and causes hyperpolarization of cardiac pacemaker and atrial cells by increasing potassium permeability (Hartzell, 1981). The relatively recent development of radiolabeled ligands which bind with high affinity and specificity to the mAChR have greatly facilitated molecular studies of the mAChR. Such ligands have permitted studies on the localization of the receptor (Table III), detailed analysis of the interaction of the

Table III

REGIONAL DISTRIBUTION (IN VITRO) AND ACCUMULATION (IN VIVO) OF TRITIATED QNB IN RAT BRAIN (SNYDER ET AL., 1975)

Brain Regions	Distribution	Accumulation
Corpus striatum	478±82	426
Cerebral cortex	390±23	348
Hippocampus	243±11	278
Superior and inferior colliculi	230±83	
Midbrain and pons	150±61	
Thalamus	137±35	
Hypothalamus	131±38	59
Medulla oblongata-cervical spinal cord	51±5	15
Cerebellar cortex	34±3	0

receptor with agonists and antagonists, and investigation of mechanisms of regulation of receptor number.

The regulation by synaptic activity of the macromolecules important for neural transmission represents a mechanism for the modulation of the activity and function of nerve cells. For example, prolonged exposure of receptors to a neurotransmitter can lead to a decrease in the number of receptors remaining on the cell surface and thus decrease the sensitivity of the cell to further stimulation. Such agonist-induced decreases (`down-regulation´) in receptor number have been postulated to be a mechanism for the regulation of postsynaptic sensitivity by the overall level of synaptic activity. The mAChR is an example of a receptor whose number can be changed by exposure to receptor agonists. Thus, decreases in the number of mAChRs have been demonstrated after prolonged agonist exposure in cell cultures of neural lines (Klein et al., 1979), neurons (Siman and Klein, 1979), and cardiac muscle (Galper and Smith, 1978). These effects can be blocked by receptor antagonists; simple occupancy of the receptor by a physiologically inactive ligand is not sufficient to induce receptor loss. The kinetics of receptor loss suggest that it is due to an increase in the rate of receptor breakdown, with the half-life for turnover

of the receptor decreasing from 11 hours to 3 hours (Lanier et al., 1976). After cessation of agonist-receptor interaction, a gradual recovery of receptor number occurs which can be blocked by inhibition of protein synthesis. The loss of receptors is presumably mediated by an agonist-induced endocytosis process similar to those that are known to regulate other membrane proteins.

Studies with cultured cells have demonstrated that the loss of receptors from the cell surface is accompanied by a reduction in the physiological responsiveness to further cholinergic stimulation. In cultured neuronal cells, agonist-induced decreases in receptor number lead to a decrease in the number of mAChR-mediated inhibition of adenylate cyclase (Nathanson et al., 1978) and the mAChR-mediated turnover of membrane phospholipids, while in cultured cardiac muscle a decrease in the number of mAChRs leads to a decreased negative chronotropic response to further muscarinic stimulation (Galper and Smith, 1978). These changes in receptor number and responsiveness occur on a time scale for hours. Thus, they are distinguished from the short-term desensitization of receptor function, which both occurs and can be reversed on a time scale of minutes, and is not accompanied by a significant decrease in apparent receptor number (Taylor et al., 1979).

Muscarinic receptor number *in vivo* can be altered by a variety of pharmacological treatments. Chronic administration of acetylcholinesterase inhibitors leads to a decrease in the number of mAChRs present in the brain, presumably because the acetylcholinesterase increases the concentration of endogenous acetylcholine available for receptor binding (Smit *et al.*, 1980). Conversely, prolonged treatment of brain tissue with specific mAChR antagonists results in increased mAChR number, presumably because synaptic release of acetylcholine is prevented from interacting with the mAChR (Ben-Barak and Dudai, 1980). *In vivo* administration of cholinergic agonists can directly regulate the number and function of mAChRs in heart (Halvorsen and Nathanson, 1981) and brain (Meyer *et al.*, 1982) with characteristics strikingly similar to those of the regulation seen in cell culture. The number of mAChRs in the heart can be reduced by more than 85%; receptor number gradually returns to control levels if subsequent agonist-receptor interactions are blocked by the administration of an antagonist. The decrease in receptor number is correlated with a decrease in negative chronotropic (affecting the rate of contraction) response of isolated atria to muscarinic stimulation. These experiments also demonstrated that desensitization and down-regulation

are distinct processes; the negative chronotropic response of the atria desensitized within a minute after exposure to the agonist, while the decrease in receptor number required 6-10 hours to reach a steady state (Nathanson, 1982).

Inositol Phospholipids

Phospholipids in Developing Brain

During the first few weeks of life the brain of the rat undergoes a period of rapid growth and differentiation. Three stages of development may be distinguished: (1) The period from birth to 15 days is characterized principally by growth of unmyelinated axon processes and glial cells, resulting in a five-fold increase in brain mass. (2) At about the 10th day the deposition of myelin is initiated and achieves maximal rates in the next 10 days. Cell division and cell growth level off during this period. (3) Subsequently there is a slower continued formation of myelin. The adult levels of most components are reached by the third month (McMurray, 1964; Folch, 1955).

Studies of the lipid composition of the developing brain have shown that the lipids increasing in association with the early growth phase are the mucolipids (Folch, 1955) and many of the glycerophosphatides. Lipids typically associated with the later myelination period include

cholesterol, sphingomyelin, cerebrosides and plasmalogens (Folch, 1955; McMurray, 1964).

Distribution

Phosphatidylinositol (PI) is a minor membrane component constituting about 10% of the phospholipid content of most eucaryotic cells (Michell, 1975; Hawthorne and Kai, 1970; Becker and Lester, 1977) (Table IV). It is a precursor for the phosphoinositides, phosphatidylinositol 4-phosphate (DPI) and phosphatidylinositol 4,5-bisphosphate (TPI), which are unique phospholipids because they possess additional phosphate groups monoesterified to the inositol ring (Figure 2). They are the most polar of all the phospholipids. The polyphosphoinositide (PPI) levels, localization, and metabolism have been studied in a variety of tissues for the past 30 years. The greatest concentration of polyphosphoinositides occurs in the nervous system. A substantial body of evidence now indicates that a large proportion of the polyphosphoinositides, especially TPI, are localized in the myelin sheath that surrounds the nerve fibers. The relative concentrations of the individual polyphosphoinositides will be dependent on factors such as age. Brain homogenates from developing rats, when incubated with the appropriate precursor, display marked differences in their ability to synthesize DPI and TPI as a function of

Table IV

PHOSPHOLIPID COMPOSITION OF SYNAPTOSOMAL MEMBRANES FROM RAT BRAIN (HOFTEIG ET AL., 1985)

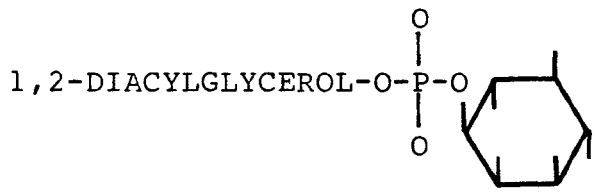
Synaptosomal Plasma Membrane	(Percent of total)
Phosphatidylcholine (PC)	42.6±4.6
Phosphatidylethanolamine (PE)	20.5±12.1
Phosphatidylinositol (PI)	10.8±0.8
Phosphatidylserine (PS)	14.2±2.8
Phosphatic acid (PA)	1.5±1.2
Phosphatidylglycerol (PG)	1.8±0.1
Lysophosphatidylcholine + Sphingomyelin (LPC + SP)	3.6±1.3

Values represent the mean ± the standard error.

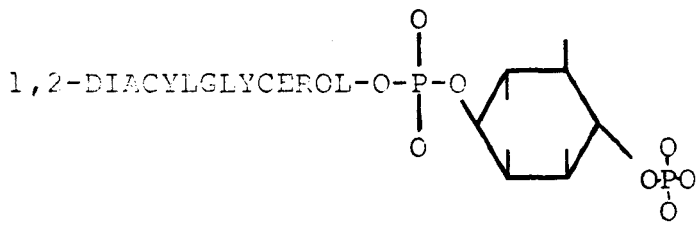
Figure 2

THE COMMON PHOSPHATIDYLINOSITOLS

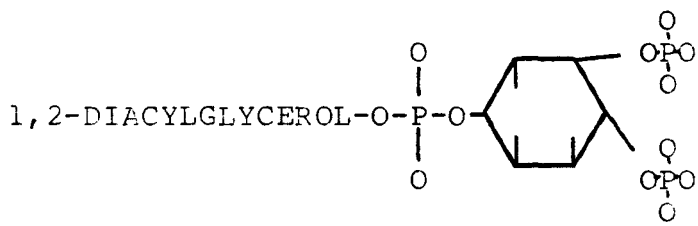
PHOSPHATIDYLINOSITOL



DIPHOSPHATIDYLINOSITOL



TRIPHOSPHATIDYLINOSITOL



age (Figure 3). The formation of DPI, expressed per milligram protein, was greatest at the youngest age examined and fell during development, so that the level in the adult was about one-third that in the newborn rat. By contrast, while substantial amounts of TPI were synthesized by homogenates from 2-day-old rats, the quantity formed was twice as high by the third week and five times as high in the mature animal (Eichberg and Hauser, 1967).

Until recently, quantitative separation of of TPI and DPI from PI presented a number of difficulties so that most published statements about their concentrations could only be regarded as minimum figures (Table V). The most reliable figures are probably derived from brains fixed by microwave irradiation (Soukup et al., 1978), where values for three brain regions are 0.1-0.13 umol DPI and 0.29-0.48 umol TPI per gram tissue. Of this, about one half is found in the myelin fractions that contain only about one quarter of the total lipid of the homogenate (Deshmukh et al., 1980). This puts the concentration of PPI in the myelin in situ at a minimum of 1 mM. The local concentration at the cytoplasmic leaflet of the membrane is almost certainly higher than this. Another estimate of local concentration has been obtained from human erythrocytes, where TPI and DPI constitute about 1% of the total phospholipids. Assuming

Figure 3

EFFECT OF AGE ON THE BIOSYNTHESIS OF BRAIN POLYPHOSPHOINOSITIDES (EICHBERG AND HAUSER, 1967)

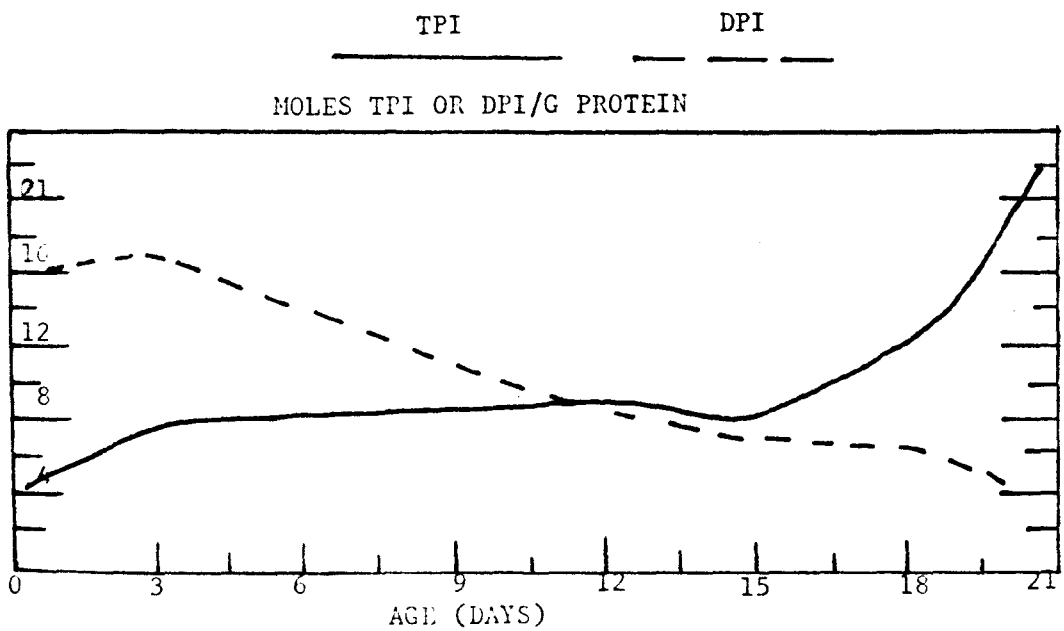


Table V

PHOSPHOINOSITIDE CONTENT OF BRAIN TISSUE
(EICHBERG AND HAUSER, 1967)

Source	Phospholipid	P as % total lipid P
Sheep brain	Phosphatidylinositol	3.2
Rabbit brain	Phosphatidylinositol	1.83
	Diphosphatidylinositol	0.87
	Triphosphatidylinositol	4.29
Ether extract of rat brain	Phosphatidylinositol	2.60
	Diphosphatidylinositol	0.42
	Triphosphatidylinositol	0.47
Ox brain diphosphoinositide fraction after ten precipitations (purified)	Phosphatidylinositol	7.0
	Diphosphatidylinositol	39.4
	Triphosphatidylinositol	37.4
	Phosphatidylserine	16.3

These figures are expressed as percentage by weight of the total solids extracted.

that phospholipids provide 25% of the mass of the hydrated erythrocyte membrane and that all of the PPIs are in the cytoplasmic leaflet of the membrane (Garrett and Redman, 1975), the total concentration of TPI and DPI at the surface is on the order of 5 mM (Sheetz et al., 1982).

Several investigators have proven that DPI and TPI are characteristic constituents of the plasma membrane (Michell, 1975; 1979; Hawthorne and White, 1975; Agranoff, 1978). This proof is largely based on the presence of PPI in myelin and erythrocyte membranes, and on the fact that PPI kinase is concentrated in plasma membranes of liver and some other tissue. There seems to be a general relationship between a tissue's PPI content and the richness of its plasma membrane complement, e.g., these lipids are present in high concentrations in myelin-rich nervous tissue and in kidney. At the other extreme would come a tissue such as liver which has large cells and hence has little of its mass contributed by plasma membrane: liver contains little PPI (Sheetz et al., 1982; Michell et al., 1970). The best, but still approximate, estimate of PPI level in rat liver comes from the measurements of acid-extractable inositol lipid and of DPI and TPI made by Michell et al. (1970). These suggest a total concentration for the two lipids of 20-40 nmol/g tissue. If PPI were to be confined to the inward-facing

half of the hepatocyte plasma membrane, which probably accounts for less than 1% of the mass of the tissue, they would have found there a local concentration of 2 mM.

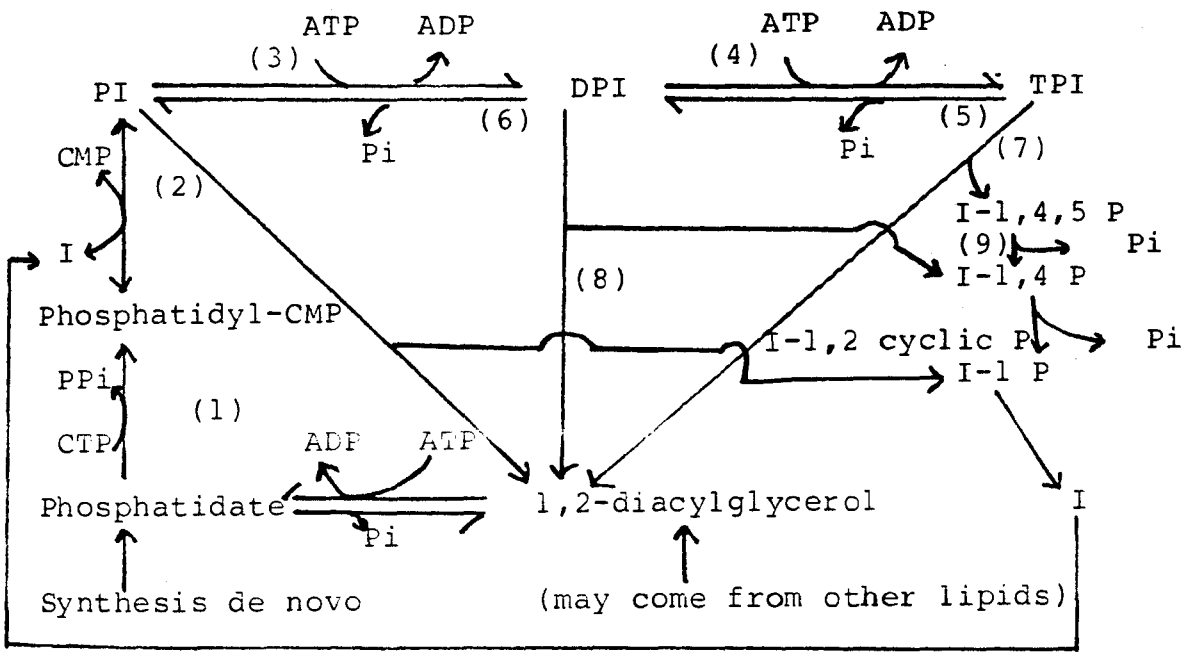
Thus, DPI and TPI are minor, indeed trace, lipid components when considered either by comparison with the major membrane phospholipids or simply as components of an entire eucaryotic cell, but this type of interpretation may conceal the fact that they are present at quite high local concentrations at the inner surface of the plasma membrane, and perhaps in related membranes such as secretory vesicles.

Biosynthesis and Degradation

There is no single cell in which all of the known steps in the metabolic pathways to and from DPI and TPI have been fully characterized, so the widely accepted set of metabolic pathways, shown in Figure 4, is based on an assemblage of information from many cells. It is generally agreed that most synthesis of PI from phosphatidate (PA) (reaction 1 and 2) occurs at the endoplasmic reticulum. Synthesis of DPI by PI kinase (reaction 3) is a widespread activity in plasma membranes (liver, erythrocytes, brain, and kidney) (Hawthorne and White, 1975) but has also been reported in a soluble fraction from brain, in adrenal medullary secretory vesicles, and in `microsomal´ fractions from liver and kidney. This `microsomal´ activity, which

Figure 4

PATHWAYS FOR THE SYNTHESIS AND DEGRADATION OF INOSITOL PHOSPHOLIPIDS (HAWTHORNE AND WHITE, 1975)



Enzymes in pathways:

(1) ER kinase
(2) ER kinase
(3) PI kinase
(4) DPI kinase
(5) PPI phosphomonoesterase
(6) TPI phosphomonoesterase
(7) TPI phosphodiesterase
(8) PPI phosphodiesterase
(9) PPI phosphodiesterase

shows characteristics different from those in plasma membrane-enriched fractions, was ascribed by Harwood and Hawthorne (1969) to a PI kinase of the endoplasmic reticulum. However, both hepatocytes and kidney tubular epithelium have plasma membranes that are differentiated into biochemically distinct domains, some of which co-sediment with endoplasmic reticulum fragments in `microsomal´ fractions. Synthesis of TPI from DPI (reaction 4) is catalyzed by a second kinase. In the erythrocyte and kidney this activity is associated with the plasma membrane (Hawthorne and Pickard, 1979), but in brain it appears principally in the soluble fraction (Hawthorne and Kai, 1970). The subcellular distribution of PI kinase and DPI kinase are particularly important because they will determine at which intracellular membrane sites PPI synthesized from PI will accumulate. Of these two enzyme activities perhaps PI kinase may be more strictly localized, since the subsequent activity of DPI kinase will be limited by the availability of its substrate.

Dephosphorylation of TPI (reaction 5) and of DPI to PI (reaction 6) were first achieved using crude extracts of brain and liver (Hawthorne, 1964; Hawthorne and White, 1975). PPI phosphomonoesterase activity initially appeared

to be largely soluble in the brain, but Sheltawy and Dawson (1969) later discovered a membrane-bound activity that required a component of the soluble phase as an activator. Comparisons with marker enzymes suggested that the membrane-bound activity was at the plasma membrane (Hawthorne and White, 1975). Studies of kidney pointed at first to a PPI phosphomonoesterase activity in the cytosol and the endoplasmic reticulum (Lee and Huggins, 1968) that could not be attributed to alkaline phosphatase. But more detailed studies using `improved' assays then revealed a substantial amount of PPI phosphomonoesterase activity that seemed to be associated with Golgi membrane (Cooper and Hawthorne, 1975). The fact that it co-purified with thiamine pyrophosphate and not galactosyltransferase may indicate that it is in the trans elements of the Golgi complex (Rothman, 1981).

From the studies just described, it appeared that one phosphomonoesterase might dephosphorylate both DPI and TPI (Cooper and Hawthorne, 1975). However, this seems intrinsically unlikely, given the different physical environments of the 5-phosphate of TPI and of the 4-phosphate of DPI; furthermore, a recent study of a soluble activity in erythrocytes by Roach and Palmer (1981) has yielded a specific TPI phosphomonoesterase that removes only the 5-phosphate and is inactive against DPI.

The other major route of breakdown of PPI is by the action of phosphodiesterase(s) (i.e. phospholipase(s) C) that remove the entire headgroup from the lipid. These were also initially reported in tissue extracts from brain and liver (Hawthorne, 1964; Hawthorne and White, 1975). TPI phosphodiesterase activity (reaction 7) in brain is found in soluble and in plasma membrane-enriched fractions, but in kidney and iris smooth muscle it appears to be soluble (Lapetina et al., 1975; Akhtar and Abdel-Latif, 1980). By contrast, the erythrocytes of man and several other species possess a PPI phosphodiesterase (reaction 7 and 8) that is tightly associated with the plasma membrane (Allan and Michell, 1978; Downes and Michell, 1981; Downes and Michell, 1982). PPI phosphodiesterases share an ability to be activated by calcium ion. However, they appear to be different enzymes.

Irving et al. (1979) have revealed a complex pattern of control of PPI rat brain phosphodiesterases, in which the expressed activity varies according to the physio-chemical state of the substrate phospholipid within the membrane.

Phosphatidylinositol Effect (PI Effect)

In 1953, Hokin and Hokin reported that acetylcholine stimulated the incorporation of radioactive phosphorus into phospholipids of the pigeon pancreas in vitro. In a

subsequent paper (Hokin and Hokin, 1955), these investigators demonstrated that the specific phospholipids responsible for the effect were phosphatidylinositol (PI) and phosphatidate (PA). This phenomenon by which stimuli enhance the radioactive labeling of phosphatidylinositol has been generally termed the 'phosphatidylinositol effect' or 'PI effect.'

By the early 1960s, interest in the phosphatidylinositols (PPIs) focused on two phenomena. The first was that a stimulation of the metabolism of PI might play a special role in receptor-mediated responses of cells to external stimuli such as hormones or neurotransmitters (Michell, 1975; 1979; Marx, 1984). These responses were originally detected in acetylcholine- or pancreozymin-treated pancreas as a rapid increase in the incorporation of radioactive phosphate or inositol into PI and PA, a precursor of PI.

Another important finding was that the phosphate groups of DPI and TPI showed rapid metabolic turnover. In many cells the metabolic turnover of the monophosphates of the whole polyphosphoinositide pool can occur within a few minutes. This rapid rate of turnover and the subcellular

localization of PPI suggest that these lipids may participate in some ubiquitous function of the plasma membrane.

The PI Response in the Function of Receptors

The ultimate aim of biochemical research into the mode of action of neurotransmitter receptors is to determine the sequence of events between receptor occupancy and the final physiological response. On their arrival at the surface of a responsive cell, most intracellular messages, including neurotransmitters, antigens, and many hormones, are read and interpreted by receptors exposed on the exterior of the cell. But the cellular responses to such chemical messages frequently occur at intracellular sites. Mechanisms must therefore exist for the translation of these signals into a language understandable within the cell. The link between receptor activation and cellular response is mediated by various effectors.

A great deal of evidence supports the notion that inositol phospholipid breakdown is an early event in the receptor-response cascade (Table VI) and that the lipid hydrolysis step may be a coupling reaction which is essential for normal receptor function (Redman and Hokin, 1964; Reddy and Sastry, 1979; Hokin-Neaverson et al., 1978).

Table VI

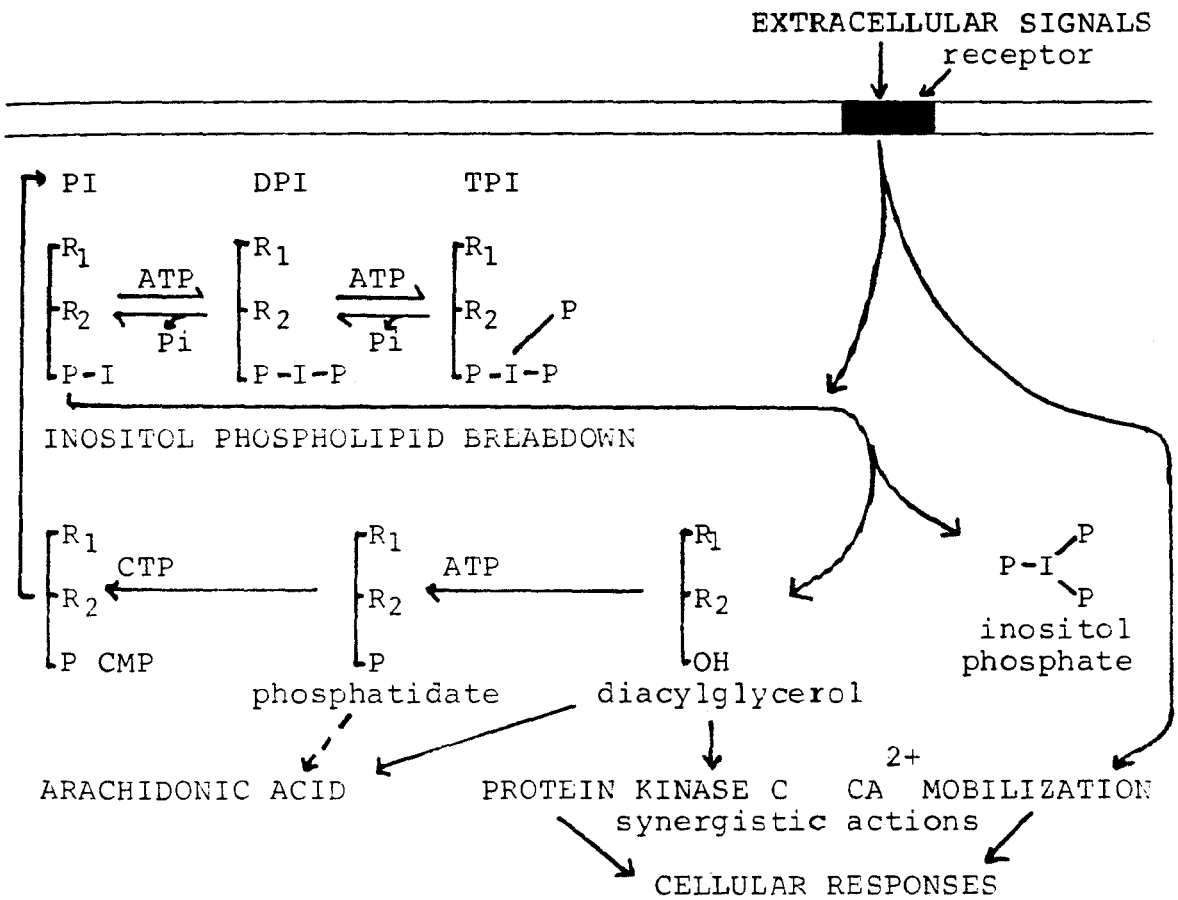
RECEPTOR COUPLED TO PHOSPHOINOSITIDE TURNOVER IN TARGET TISSUES (ABDEL-LATIF ET AL., 1974)

Tissue	Receptor
Iris smooth muscle	Muscarinic cholinergic, α_1-adrenergic
Hepatocytes	Vasopressin, angiotensin
Parotid gland	Muscarinic cholinergic, α_1-adrenergic Substance P
Platelets	Thrombin, ADP, Platelet activating factor
Brain (nerve ending preparations or slices)	Muscarinic choliergic, ACTH
Avian salt gland	Muscarinic cholinergic
Blowfly salivary gland	Serotonin
Adrenal gland	ACTH
Pancreas	Muscarinic cholinergic, caerulein

The Hokins first demonstrated that activation of certain receptors (e.g., muscarinic cholinergic or α_1-adrenergic) resulted in increased incorporation of added radioactive phosphorus into PA and PI. It was subsequently reported that there was a net loss of PI upon stimulation with the accumulation of an approximately equivalent amount of PA (Hokin-Neaverson, 1974b). This was thought to arise from an initial phosphodiesteratic breakdown of PI, liberating diacyl glycerol (DAG), which was in turn rapidly rephosphorylated in the presence of radioactive ATP to yield radiolabeled PA. The PA was then proposed to be converted to PI via (CDP-DAG), completing a `phosphatidate-phosphatidylinositol cycle´ (Figure 5). Despite this observation and the known metabolic interrelationship between the inositol lipids, a direct effect of receptor activation on DPI and TPI turnover was not proposed or examined further until 1977, when Abdel-Latif and colleagues demonstrated that exposure of the iris smooth muscle to acetylcholine resulted in increased breakdown of radiolabeled TPI. These experiments, as well as a number of other indirect indications, led to intensified efforts to identify changes in PPI associated with receptor activation. There are now numerous documented examples of receptor-ligand interactions linked to PPI turnover in brain tissue

Figure 5

PHOSPHATIDATE-PHOSPHATIDYLINOSITOL CYCLE
(HOKIN-NEAVERSON, 1974b)



(Table VII) and specifically muscarinic cholinergic receptors (Table VIII). In most instances, these have been measured by loss of PPI radioactivity from radiolabeled inositol or prelabeled cells. Such studies indicate that radiolabeled TPI, and in some cases DPI as well, is rapidly diminished following addition of a specific ligand. For example, 20% or more of label in TPI is lost within 5-30 seconds of exposure of platelets to thrombin, of hepatocytes to vasopressin, or of parotid gland slices to methacholine (Table VI). There is also evidence in the iris smooth muscle, in platelets, and in blowfly salivary gland of simultaneous release of inositol triphosphate with receptor activation. The rapidity with which the lipid breaks down following ligand addition suggests that the cleavage of TPI rather than PI constitutes the initial event following receptor activation and that the disappearance of PI is a secondary response that reflects the process of replenishment of the depleted polyphosphoinositide pool.

Table VII

RECEPTOR-COUPLED INOSITOL PHOSPHOLIPID HYDROLYSIS IN NEURONAL TISSUE (DOWNES, 1982)

RECEPTOR TYPE	LOCATION
MUSCARINIC CHOLINERGIC (SOME)	MOST BRAIN REGIONS, SUPERIOR CERVICAL GANGLIA
α_1-ADRENERGIC	CEREBRAL CORTEX
H_1-HISTAMINE	MOST BRAIN REGIONS
SEROTONIN	CEREBRAL CORTEX
SUBSTANCE P	HYPOTHALAMUS, STRIATUM, SUBSTANTIA NIGRA
NEUROTENSIN	HYPOTHALAMUS
V_1-VASOPRESSIN	HIPPOCAMPUS
CCK-OCTAPEPTIDE	CEREBRAL CORTEX
NERVE GROWTH FACTOR	PC12 CLONAL RAT CELLS

Table VIII

THE EFFECTS OF AGONISTS ON MUSCARINIC CHOLINERGIC RECEPTORS (BIRDSALL AND HULME, 1976)

CLASS OF RESPONSE	RESPONSE
	Calculated agonist-receptor interaction curve derived from dose-ratio studies (Carbamylcholine 20 uM).
Class 1: high dose-response, related to agonist-receptor interaction	Competitive inhibition of reversible binding of QNB to receptor sites (Acetylcholine 2-4 uM)
	Competitive inhibition of irreversible binding of propyl benzilylcholine mustard to receptor sites (Carbamylcholine 20 uM)
Class 2: high dose-response, Ca independent	Phosphatidylinositol breakdown, measured directly or detected through increased labeling (Acetyl- and carbamylcholine)
Class 3: high dose-response, measure of Ca permeability	Calcium ion efflux from preloaded tissue (Carbamylcholine 50 uM)
Class 4: high or moderate dose response, mediated by Ca	Potassium ion efflux from preloaded tissue (Carbamylcholine 30 uM)
	Electrical conductance of cell surface membrane (Carbamylcholine >50 mM)
Class 5: low dose-response, example of 'physiological' responses mediated by Ca	Elevation of tissue cyclic GMP concentrations (Carbamylcholine 0.1 uM)
	Protein secretion (Acetylcholine 0.1 uM)
	Contraction (Carbamylcholine 0.1 uM)

CHAPTER III

MATERIALS AND METHODS

Animals

Proven breeder males and female Sprague-Dawley albino rats were purchased from Holtzman Co. (Madison, WI). The female rats were between 45 and 60 days of age and weighed between 180 and 230 grams at the start of each study. The females were mated with proven breeder males prior to administration of the liquid diet. A male was added to each cage of the individually housed females for a five day period. During this time both animals were given access to chow.

Diet

All studies employed a dietary protocol calling for a prolonged period (3 weeks to 2 months) of consumption of a liquid diet containing chlordecone.

Chlordecone (5, 15, or 30 mg/kg/day) and control isocaloric liquid diets were prepared as described by Druse et al. (1981). Protein, fat, and carbohydrate accounted for 21.1%, 29.3%, and 49.6% of the calories, respectively. Isocaloric and chlordecone diets differed in only one respect: 5, 15, or 30 mg/kg/day of chlordecone was added to the chlordecone diet. The composition of the control diet

is described in Table IX. Daily feedings (following a pair-feeding paradigm) were given to individually housed control and chlordecone animals. Pair-feeding was accomplished by feeding the control animals the same amount of diet that the chlordecone animals consumed the previous day. The animals were maintained on a 12 hour light-12 hour dark schedule with lights on at 8:00 a.m.

Administration of Diets

Female rats were given the liquid diet ad libitum the day after the proven breeder males were removed from the cages. The pregnant females were maintained on the chlordecone (K) or control (C) diets throughout gestation and/or lactation. Females fed liquid diet only during gestation were fed laboratory chow exclusively after parturition. Laboratory chow (Wayne Lab-Blox MRH 22/5, Wayne Laboratories, Chicago, IL) contains protein (26%), carbohydrate (61%), fat (13%), and salt mixture and vitamin fortification (<1%). Rats had free access to water at all times. In all studies the litter size was adjusted to 10 pups at three days postpartum.

Animal Sacrifice and Tissue Preparation

Rats were killed by decapitation at 17 days, 24 days, 31 days, 3 months, or 9 months. Brains were quickly removed

Table IX

LIQUID DIET

Ingredient	Grams/Liter	Calories	% Calories
Protein:		225	21.1%
Casein	47.95		
L-Cysteine	0.63		
L-Methionine	0.37		
Sego	3.75		
Carbohydrates:		530	49.6%
Maltose-Dextrins	114.00		
Dextrose Vitamin Mixture	5.00		
Sego	15.00		
Fats:		313	29.3%
Corn Oil	4.35		
Olive Oil	26.90		
Safflower Oil (78% Ethyl Linoleate)	3.50		
Sego	0.75		
Salt Mixture:	10.00		
Total Calories:		1068	

by cutting the skull along the sagittal and parieto-occipital sutures and scooping from the ventral side. Brain preparations for synaptosomes and SPMs contained whole brain excluding the pons-medulla, brain stem and cerebellum. Cerebral hemispheres and striatum were used for receptor binding studies and striatum and hypothalamus for biogenic amine levels.

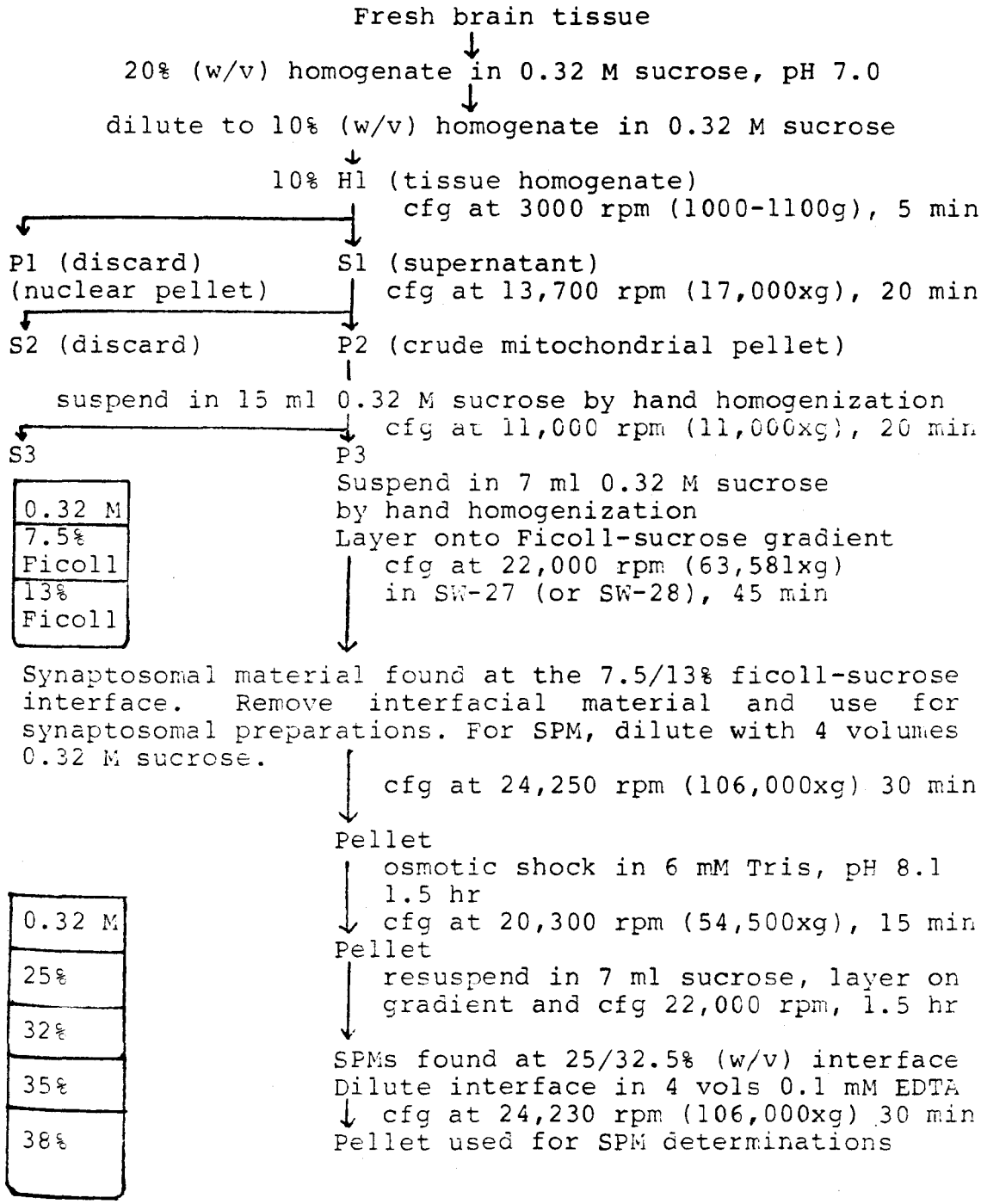
Synaptosomes and Synaptic Plasma Membrane Preparation

During mechanical homogenization of brain tissue, the nerve terminals are sheared off. In isotonic solution, the nerve terminals can seal and form "synaptosomes." Differential centrifugation techniques can be used to separate synaptosomes from cell nuclei, myelin, microsomes, mitochondria, and other subcellular components. Synaptosomes contain a characteristic self-limiting membrane, the synaptic cleft region, presynaptic mitochondria and vesicles, and other substructures. After synaptosomes are hypotonically lysed, the synaptic plasma membranes (SPM) can be separated from remnant myelin fragments and presynaptic mitochondria and vesicles by differential centrifugation techniques.

In the present study, synaptosomes and synaptic plasma membranes were prepared following a procedure of Cotman and Matthews (1971) (See Figure 6). Animals were sacrificed as

Figure 6

SYNAPTOSOME AND SYNAPTOSOMAL PLASMA MEMBRANE ISOLATION PROCEDURE (COTMAN AND MATTHEWS, 1971)



described above. Cerebral hemispheres were homogenized, 20% (w/v), in cold, 0.32 M sucrose (pH 7.0) in a glass homogenizer using a motor-driven teflon coated pestle. The homogenizer and pestle were rinsed with an equal volume of 0.32 M sucrose (pH 7.0). The rinse and homogenate were combined so that the final concentration of the homogenate was 10% (w/v). All subsequent steps were done at 0-4 ° C. An aliquot (0.5-1.0 ml) was then removed for protein determination.

The homogenate was centrifuged in a refrigerated (0-4 °C) DuPont Sorvall RC-5B at 3,000 rpm (1,000-1,100 X g) for 5 minutes using a Sorvall SS-34 rotor. This resulted in a crude nuclear pellet (P_1), containing cell bodies and nuclei, and a supernatant (S_1). The supernatant (S_1) was decanted from the nuclear pellet and centrifuged at 13,700 rpm (17,000 X g) for 10 minutes in the Sorvall. After this centrifugation, the supernatant (S_2) was decanted. The crude mitochondrial pellet (P_2) was resuspended in 15 ml of cold 0.32 M sucrose (pH 7.0) by hand homogenization using a smooth, round bottom, glass hand-held homogenizer with a teflon pestle. The suspension was centrifuged at 11,000 rpm (11,000 X g) for 20 minutes in the Sorvall. The resulting supernatant (S_3) was decanted and discarded, and the pellet (P_3) was resuspended in 7.0 ml of 0.32 M sucrose (pH 7.0) by

hand homogenization. An additional, optional rinse was omitted. The suspension was layered on top of a Ficoll gradient consisting of 13.5 ml of 13.0% (w/v) Ficoll in 0.32 M sucrose (pH 7.0) which had been previously overlayered with 13.5 ml of a 7.5% (w/v) Ficoll-sucrose solution (pH 7.0). The gradient was centrifuged at 22,000 rpm (63,500 X g) for 45 minutes. This and all subsequent centrifugations were performed in a Beckman L 5-65 ultracentrifuge and utilized either the SW-27 or SW-28 rotor. All centrifugations were stopped by an electrical braking device.

Crude myelin was found at the 0.32 M sucrose/7.5% Ficoll-sucrose interface. Synaptosomal material was found at the 7.5/13.0% Ficoll-sucrose interface. The synaptosomal material was removed, diluted with 4 volumes of 0.32 M sucrose (pH 7.0) and centrifuged at 24,250 rpm (106,000 X g) for 30 minutes. The supernatant was decanted and the pellet resuspended in a small volume of phosphate buffer for phospholipid stimulation experiments or resuspended in a small volume of 0.32 M sucrose for synaptic plasma membrane (SPM) isolation. The pellet was then osmotically shocked for 1.5 hours (0-4 ^{o}C) by resuspending it in 6 mM Tris (pH 8.1). It was shown by Cotman and Matthews (1971) that osmotic shock at alkaline pH (pH 8.1) for 1.5 hours is

necessary for the subsequent resolution of synaptosomal mitochondria from the synaptic plasma membrane. The suspension was centrifuged at 20,300 rpm (54,500 X g) for 15 minutes. The pellet was resuspended in 7 ml of 0.32 M sucrose and layered onto a sucrose gradient consisting of the following sucrose solutions in order of decreasing density (heaviest on the bottom): 5 ml of 38.0% (w/v), 7.5 ml of 35.0% (w/v), 7.5 ml of 32.5% (w/v), and 7.5 ml of 25.0% (w/v) sucrose. The gradient was centrifuged at 22,000 rpm (63,500 x g) for 1.5 hours. Synaptic plasma membrane fragments were found at the 25/32.5% (w/v) sucrose interface. The SPM bands were removed with clean Pasteur pipets and diluted with 0.1 M EDTA.

Radiolabeled Phosphorus Incorporation into Synaptosomes

Radiolabeled phosphorus incorporation into synaptosomes was assessed using a modified procedure of Abdel-Latif et al., 1968). Prepared synaptosomes were pooled in groups of 3 animals and suspended in approximately 0.5 ml of buffer (McMurray et al., 1963). The buffer contained 0.013 M sodium pyruvate, 0.002 M magnesium chloride, 0.005 M NAD, 8.3×10^{-4} M cytochrome C, 0.0025 M AMP, 0.012 M NaF, 0.005 M NaH_2PO_4, 0.0005 M CTP, 0.015 M Tris-HCl (pH 7.4), 0.75 mM calcium ion, 10 mM glucose and 10 mM sodium succinate. The synaptosomes were diluted so that

the milligrams of protein per milliliter of buffer were approximately equal. Radiolabeled orthophosphate (approximately 100 uCi/flask) was added to the buffer-synaptosome mixture and incubated in open Erlenmeyer flasks (25 ml capacity) for one hour with shaking in a water-bath at 37 °C.

After incubating for one hour, the samples were divided into two sets of test tubes for addition of agonist alone, antagonist plus agonist or antagonist alone. The agonists and their respective antagonists are listed in Table X.

The antagonist was added to the respective tubes and incubated for 5 minutes. After the incubation period, respective agonists were added to the required tubes and reincubated for 30 minutes. The reaction mixture was terminated by the addition of 2 ml chloroform, 5 ml methanol and 0.5 ml of 0.1 M EDTA-2 M KCl.

Extraction and Analysis of Phospholipids

After the incubation was terminated as described above, the synaptosomal samples were partitioned by the method of Cohn et al. (1978). The samples were left for two hours at room temperature or overnight at 5 °C. Then 2 ml of chloroform and 1 ml of EDTA-KCl was added to each sample and

Table X

AGONISTS AND THEIR RESPECTIVE ANTAGONISTS
USED FOR PHOSPHOLIPID STIMULATION STUDIES

Agonist	Concentration	Antagonist	Concentration
Carbamylcholine	1 mM	Atropine	50 uM
Dopamine	10 mM	Haloperidol	1 mM
Noradrenaline	10 mM	Regitine	1 mM
Histamine	5 mM	Bentazole	5 mM
Serotonin	10 mM	---------	----

vortexed. The samples were centrifuged at 1000 X g and the resulting chloroform layer (bottom layer) removed. The aqueous phase was washed twice with 3 ml of chloroform, centrifuged and the resulting chloroform layers combined. The phospholipids were evaporated to dryness under a gentle stream of nitrogen and stored in benzene at -20 °C until further analyzed.

The phospholipids were analyzed by thin layer chromotography (TLC) using the method of Graff et al. (1984). The phospholipid samples were thawed and dried under nitrogen. For analysis of radioactive content of individual phospholipids, a portion of each sample was spotted on a 1 cm lane in one corner of a 20 cm by 20 cm, 500 micron, silica gel G plate that had previously been activated in a 110 °C oven for one hour. The plates were developed in two dimensions for resolution of major phospholipid classes. The solvent used in the first direction was chloroform: methanol: ammonium hydroxide: water (60:30:4:1) (vol:vol). After the first dimension, the plates were thoroughly dried under nitrogen for 15-30 minutes. The second dimension solvent consisted of chloroform: acetone: methanol: glacial acetic acid: water (40:15:13:12:8) (vol:vol). Tentative identification of phospholipid classes was accomplished by comparison of the

migration of standard phospholipids to samples. The positions of the phospholipids were marked on plates that had been exposed to iodine vapors or sprayed with sulfuric acid (50% in water) and placed in a 110 °C oven for visualization. After sublimation of the iodine or sulfuric acid, positions of the silica gel corresponding to the positions of the phospholipids were scraped into vials. One ml of water was added to the vials and the samples vortexed. Ten ml of Aquasol fluor was added to the vials prior to liquid scintillation counting. The radioactivity of the orthophosphate incorporated into the individual phospholipid classes was measured in a Packard scintillation counter.

Remaining sample aliquots were used to determine total phospholipid phosphorus by the procedure of Lanzetta et al. (1979). To each sample 100 ul of 70% perchloric acid (PCA) was added and the samples digested in a 170 °C heating block for 90 minutes to hydrolize the phosphate from the phospholipid. After cooling, the samples were adjusted to 120 ul by addition of water. Then 800 ul of color reagent (90 ml 0.045% malachite green oxalate, 30 ml 4.2% ammonium molybdate in 4 N HCl and 5.0 ml sterox) was added and the mixture was vortexed. After 30 minutes of color development, the absorbance was read at 660 nm on a spectrophotometer.

Protein Determination

Protein determination was performed following the procedure of Bradford (1976). Crystalline bovine serum albumin (BSA) was used as the protein standard. A standard 1 ml BSA protein/ ml solution was prepared in deionized water. Protein standards contained 0 ug (reagent blank) to 20 ug BSA. Absorbance was measured at 700 nm in a Beckman spectrophotometer.

Receptor Binding

Offspring of control and chlordecone-exposed rats were decapitated and their brains rapidly removed. After excision of the cerebella, which were nearly devoid of receptor activity as determined by Yamamura and Snyder (1974), each brain was diluted to 0.66 mg tissue/ml of ice-cold sodium-potassium (monobasic-dibasic) phosphate buffer (pH 7.4). The brains were homogenized in a glass homogenizer fitted with a teflon pestle. The homogenate was centrifuged for 10 minutes at 1000 X g (4°C). The pellet (crude nuclear fraction) was discarded and the resultant supernatant used for receptor binding studies. For muscarinic receptor binding studies, tritiated quinuclinidyl benzilate (QNB) (30.2 Ci/mmole) was used as a ligand. Each determination was performed in quadruplicate together with

quadruplicate samples contaning atropine (10 uM) as a displacer to determine non-specific tritiated QNB binding.

The samples were incubated for 60 minutes at 37 ^{o} C; then the reaction was stopped by passing the contents through a cell culture filtration system positioned over a vacuum. The filters were washed twice under vacuum with 3 ml of ice-cold buffer. The filters were then dried and placed in vials containing 5 ml of toluene phosphor fluor (toluene:PPO:POPOP) and the radioactivity determined in a Beckman LS 7500 (microprocessor controlled) liquid scintillation counter.

For dopamine receptor binding, brain tissue was prepared as described above. Tritiated spiperone (24.5 Ci/mmole) was used as a ligand and butaclamol (1 uM) as displacer. Samples were incubated for 15 minutes and processed as described above.

Intergroup variation was analyzed using a linear regression Scatchard analysis program designed for an Apple computer (Scatchard, 1949).

Chlordecone Levels

Chlordecone levels were determined using a modified method of Moseman (1977). Whole brain excluding cerebellum was homogenized in 2.5 ml of acetonitrile with a Sorvall Omni-Mixer at high speed for approximately 30 seconds. The

macerated tissue was separated from the solvent by centrifugation at 3000 rpm for 5 minutes. The solvent was then removed by pasteur pipet and placed in a 50 ml centrifuge tube. The maceration/ extraction was repeated twice more and the extracts combined in the same centrifuge tube. The combined extract was mixed with 5 ml of 2% aqueous sodium sulfate ($Na_2 SO_4$) and partitioned with 2 ml of benzene. This extraction procedure was repeated twice more with 1 ml portions of benzene. The extracts were combined in a 10 ml test tube and evaporated to dryness with a gentle stream of nitrogen. The samples were diluted with a toluene-2% methanol mixture and injected into a Varian model 3700 gas chromatograph with an electron-capture detector. Quantitation of chlordecone levels was performed by comparisons with peak heights from standard curves with varying amounts of chlordecone and a fixed cencentration of dieldrin.

Catecholamine Extraction from Brain Tissue

Hypothalamus (~45 mg) or striatum (~55 mg) from control and chlordecone exposed offspring was homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to estimate the percent recovery. The homogenized tissue was centrifuged at 16,000 rpm for 20

minutes at 0-4 °C. The supernatant was poured into 5 ml vials and dried under a gentle stream of nitrogen. The dried sample was then diluted in 0.5 ml of mobile phase (0.1 M phosphate, 0.5 mM OSA and 8% methanol at pH 3.85) and centrifuged in a Beckman microfuge for 2 minutes. The sample was then injected directly in to a high performance liquid chromatograph (HPLC) with a 5 uM Biorad 25 cm reverse phase column with a flow rate of 0.7 ml/minute attached to an electrochemical detector (0.7 volts). The resulting catecholamine peaks were quantitated by comparison to a standard curve of known concentrations.

Statistical Determinations

For each set of data except dopaminergic receptor binding and biogenic amine levels, data represent three animals from two separate groups. Numerical calculations were generally expressed as the mean ± standard deviation of several determinations. Statistics on the difference between two related groups (control and chlordecone-exposed) were performed using the Student's t-test of probability.

CHAPTER IV

RESULTS

This study examined the offspring of female rats that were pair-fed a control or chlordecone (5, 15 or 30 mg/kg/day) diet during gestation and lactation or during gestation only. Suckling neonates were maintained with their biological mothers (noncrossfostered) or were crossfostered to control fed surrogate mothers.

Diet Consumption and Maternal Weight Gain

Diet consumption of females fed the liquid diet was 100-120 ml/day which corresponded to 210-250 kcal/kg body weight and 11-13 g protein/kg. Control and 5, 15 or 30 mg/kg chlordecone exposed mothers gained 150, 120, 100 and 90 grams, respectively, during gestation (Table XI and Figure 7). The discrepancy between the amount of weight gained during gestation could partially be accounted for by the fact that chlordecone exposed mothers gave birth to reduced litter sizes (Figure 8). However, after litter size adjustment two days postpartum, the body weights of chlordecone fed mothers did not recover to normal levels but were decreased to 85, 81 and 77% of control for 5, 15 and 30 mg/kg/day chlordecone exposed mothers, respectively.

Table XI

MATERNAL BODY WEIGHT OF RATS FED 0, 5, 15, AND 30 MG/KG/DAY CHLORDECONE THROUGHOUT PREGNANCY AND LACTATION OR DURING PREGNANCY ALONE

DAY	CONTROL (C)	5 MG/KG	15 MG/KG	30 MG/KG
1	218±3	217±4	222±6	213±8
(% of C)	(100)	(99)	(102)	(98)
8	294±4	298±2	289±3	290±7
(% of C)	(100)	(101)	(98)	(99)
15	359±10	340±6	327±8	310±5*
(% of C)	(100)	(95)	(91)	(86)
22	262±5	243±6*	232±4*	217±8*
(% of C)	(100)	(86)	(82)	(77)
29				
Noncrossfostered	280±4	246±8	229±5*	218±3*
(% of C)	(100)	(88)	(82)	(78)
Crossfostered	283±6	260±7	240±3*	226±5*
(% of C)	(100)	(92)	(86)	(80)
36				
Noncrossfostered	284±6	241±5*	230±3*	220±4*
(% of C)	(100)	(85)	(81)	(77)
Crossfostered	282±4	280±5	247±6	233±5*
(% of C)	(100)	(100)	(88)	(82)

Each value represents the mean of 14 animals ± the standard deviation. An asterisk (*) indicates that body weights of control and chlordecone exposed mothers were significantly different at $p<0.05$.

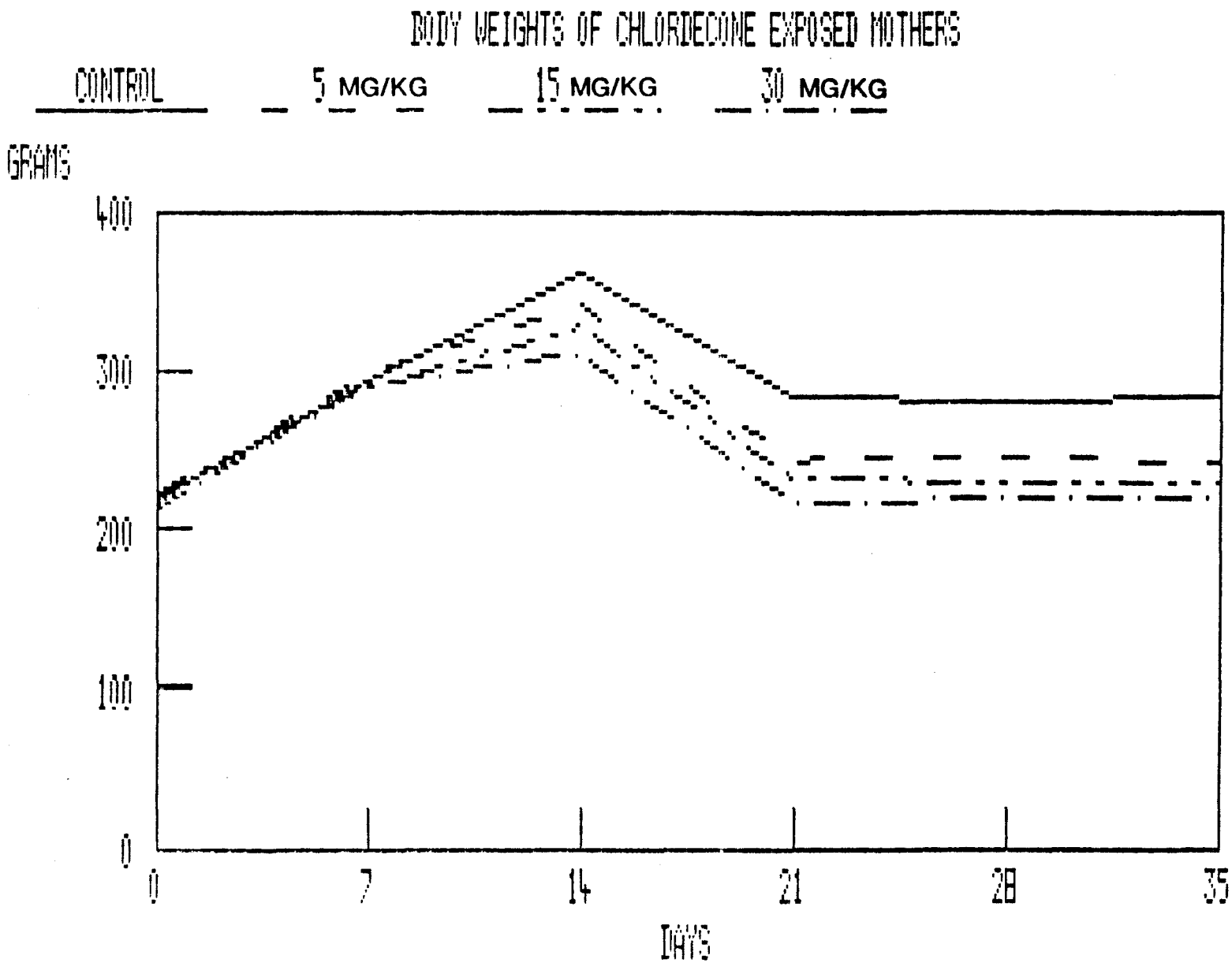
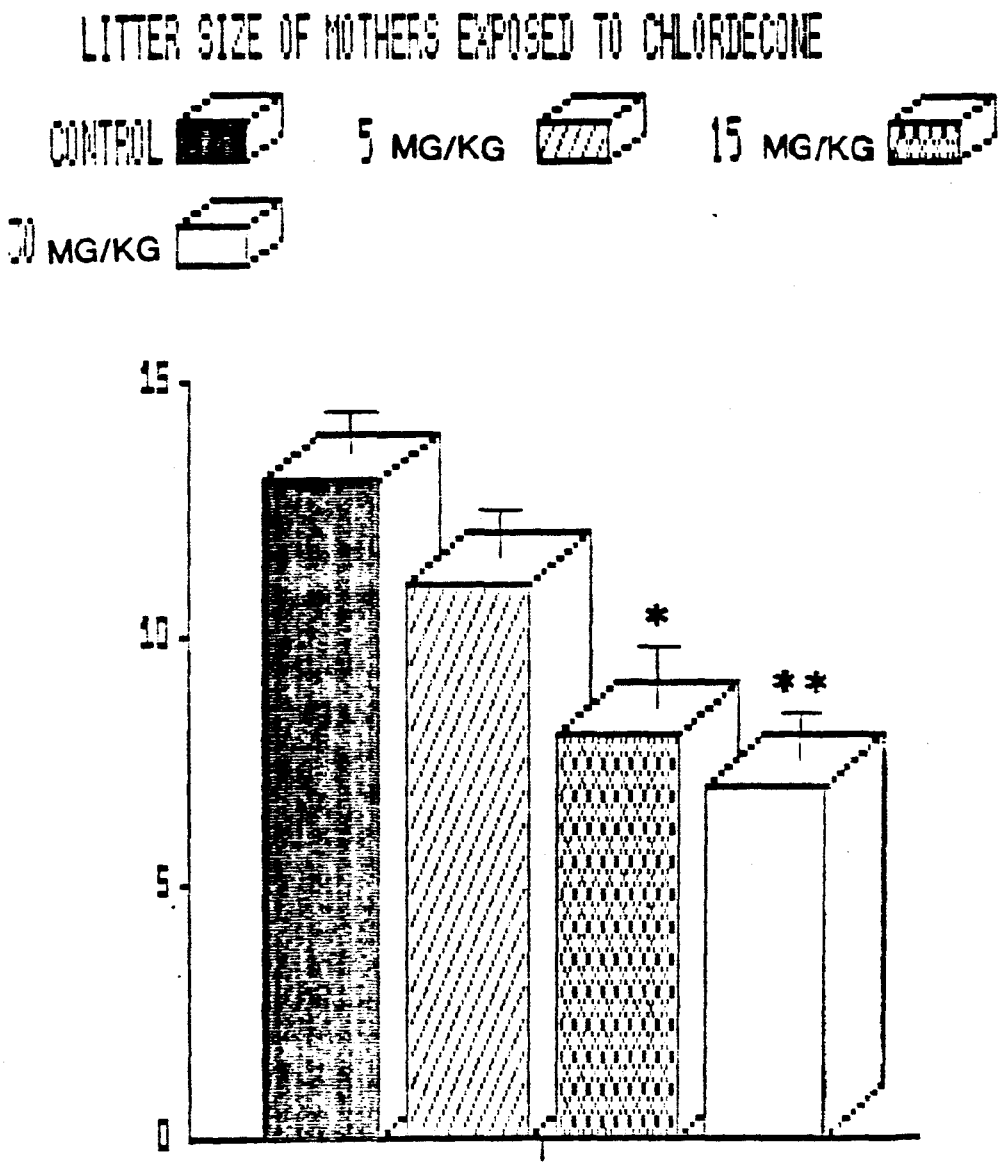

BODY WEIGHTS OF CHLORDECONE EXPOSED MOTHERS
CONTROL
5 MG/KG
15 MG/KG
30 MG/KG
GRAMS
400
300
200
100
0
0
7
14
21
28
35
DAYS

Figure 8. Litter size of mothers exposed to chlordecone. Each value represents the mean of 12 litters ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed mothers are different at $p<0.01$ and $p<0.001$, respectively.


LITTER SIZE OF MOTHERS EXPOSED TO CHLORDECONE
CONTROL
5 MG/KG
15 MG/KG
30 MG/KG
15
10
5
0
*
**

Therefore, during both gestation and lactation, chlordecone exposed mothers displayed significantly reduced body weights. When the chlordecone mothers were fed control diets during lactation, the body weights of 5 mg/kg exposed mothers were eventually rehabilitated. The 15 and 30 mg/kg exposed mothers still displayed reduced body weights but not as significantly different from normal as lactationally exposed mothers (Table XI).

Body and Brain Weights

Body weights of control and chlordecone exposed offspring were significantly different at all ages examined (Figure 9). At 17 days of age, body weights of 5, 15 and 30 mg/kg exposed offspring were 63, 46 and 34% of control, respectively (Table XII). This decline in body weight persisted at 24 and 31 days of age. Crossfostering of offspring postpartum could only partially rehabilitate the reduced body weights. Even after 31 days of a chlordecone-free diet, the body weights of gestationally exposed offspring (5, 15 or 30 mg/kg) were still significantly reduced to 68, 42 and 36% of control (See Table XII and Figure 9).

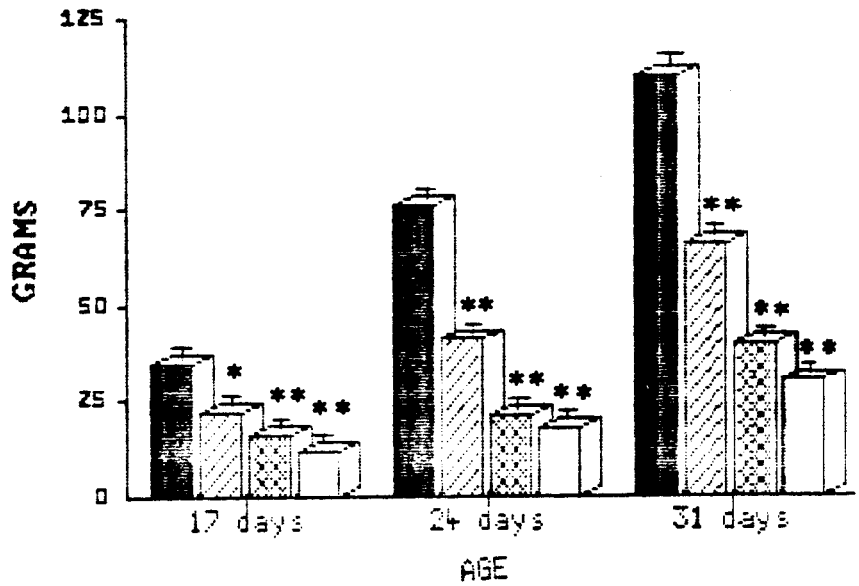
The brain weights of chlordecone exposed offspring were also significantly reduced at all ages but to a lesser degree than body weights. The brain weights of 17 day old

Figure 9. Body weights of chlordecone exposed offspring. Each value represents the mean of 20 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and 0.001, respectively.

BODY WEIGHTS OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg

NONCROSSFOSTERED OFFSPRING



CROSSFOSTERED OFFSPRING

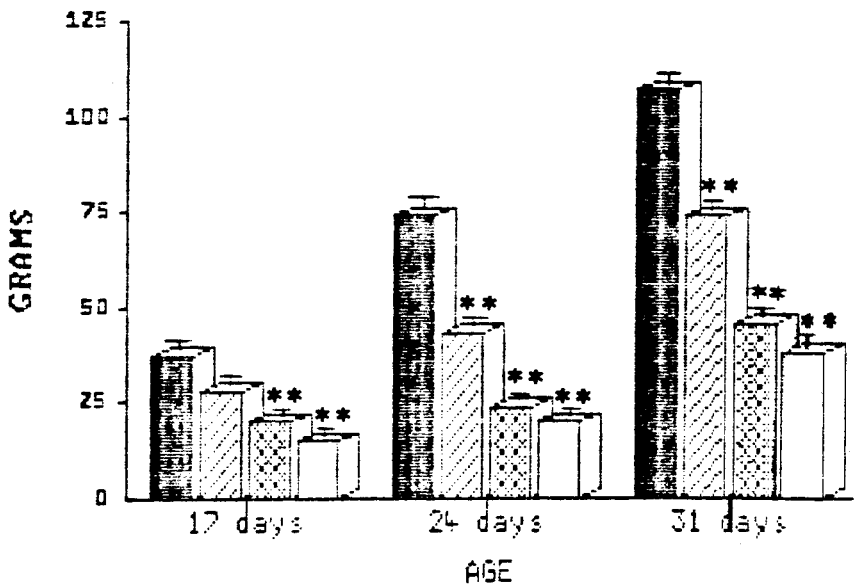


Table XII

BODY WEIGHTS OF CONTROL, 5, 15, AND 30 MG/KG/DAY CHLORDECONE EXPOSED OFFSPRING

AGE (DAYS)	BODY WEIGHT (GRAMS)			
	CONTROL (C)	5 MG/KG	15 MG/KG	30 MG/KG
17				
Noncrossfostered	35±1	22±3*	16±2**	12±1**
(% of C)	(100)	(63)	(46)	(34)
Crossfostered	37±3	28±1	20±3**	15±2**
(% of C)	(100)	(70)	(54)	(40)
24				
Noncrossfostered	76±4	41±3**	21±3**	18±2**
(% of C)	(100)	(54)	(28)	(24)
Crossfostered	74±4	43±3**	24±2**	20±1**
(% of C)	(100)	(58)	(32)	(27)
31				
Noncrossfostered	110±6	66±4**	40±3**	30±3**
(% of C)	(100)	(62)	(36)	(27)
Crossfostered	107±8	74±3**	46±5**	38±3**
(% of C)	(100)	(68)	(42)	(36)

Each value represents the mean of 20 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

offspring exposed during gestation and lactation were 63, 57 and 45% of control for 5, 15 and 30 mg/kg, respectively. By 31 days of age, the brain weights had partially recovered but were still significantly different from controls (See Table XIII and Figure 10). Crossfostering of offspring did not significantly affect the recovery of the brain weights.

In long-term studies on 3 and 9 month old control and 15 mg/kg crossfostered offspring, the body and brain weights were comparable.

In summary, body and brain weights of chlordecone exposed offspring 17 to 31 days of age were significantly different from those of controls. Crossfostering of chlordecone exposed offspring did not rehabilitate body and brain weights. By 3 months of age, brain and body weights of gestationallly exposed (15 mg/kg) offspring were comparable to those of control offspring. Therefore, chlordecone effects on body and brain weights persist at least 31 days after cessation of dosing.

Brain Chlordecone Levels

The whole brain (excluding cerebellum) chlordecone content (ug/g tissue) of all exposed offspring increased in a dose-dependent manner (Figure 11). By 17 days postpartum, all cortical chlordecone levels had reached a maximum

Table XIII

BRAIN WEIGHTS OF CONTROL, 5, 15, AND 30 MG/KG/DAY CHLORDECONE EXPOSED OFFSPRING

AGE (DAYS)	BRAIN WEIGHT (GRAMS)			
	CONTROL (C)	5 MG/KG	15 MG/KG	30 MG/KG
17				
Noncrossfostered	1.70±.08	1.07±.09*	0.97±.05*	0.76±.1*
(% of C)	(100)	(63)	(57)	(45)
Crossfostered	1.68±.06	1.10±.1*	1.03±.04*	0.82±.1*
(% of C)	(100)	(65)	(63)	(49)
24				
Noncrossfostered	1.72±.06	1.21±.01*	1.08±.04*	.96±.1*
(% of C)	(100)	(71)	(63)	(56)
Crossfostered	1.75±.07	1.32±.1*	1.12±.05*	1.00±.1*
(% of C)	(100)	(76)	(67)	(59)
31				
Noncrossfostered	1.74±.1	1.32±.1*	1.30±.04*	1.12±.1*
(% of C)	(100)	(78)	(74)	(64)
Crossfostered	1.79±.1	1.40±.1*	1.36±.1*	1.20±.04*
(% of C)	(100)	(80)	(76)	(68)

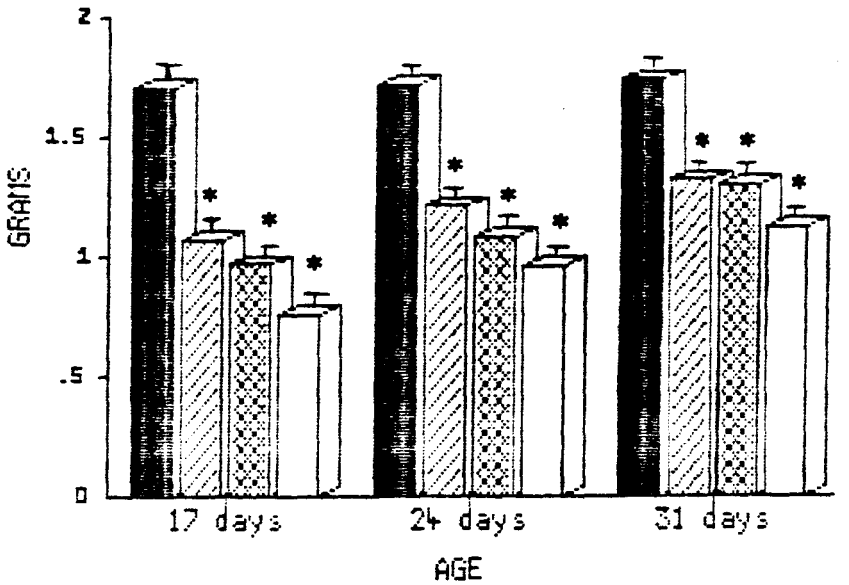
Each value represents the mean of 20 animals ± the standard deviation. An asterisk (*) indicates that values from control and chlordecone exposed offspring are different at $p<0.01$.

Figure 10. Brain weights of chlordecone exposed offspring. Each value represents the mean of 20 animals ± the standard deviation. An asterisk (*) indicates that the values from control and chlordecone exposed offspring are different at $p<0.01$.

BRAIN WEIGHTS OF CHLORDECONE EXPOSED OFFSPRING

Control 5mg/kg 15mg/kg 30 mg/kg

NONCROSSFOSTERED OFFSPRING



CROSSFOSTERED OFFSPRING

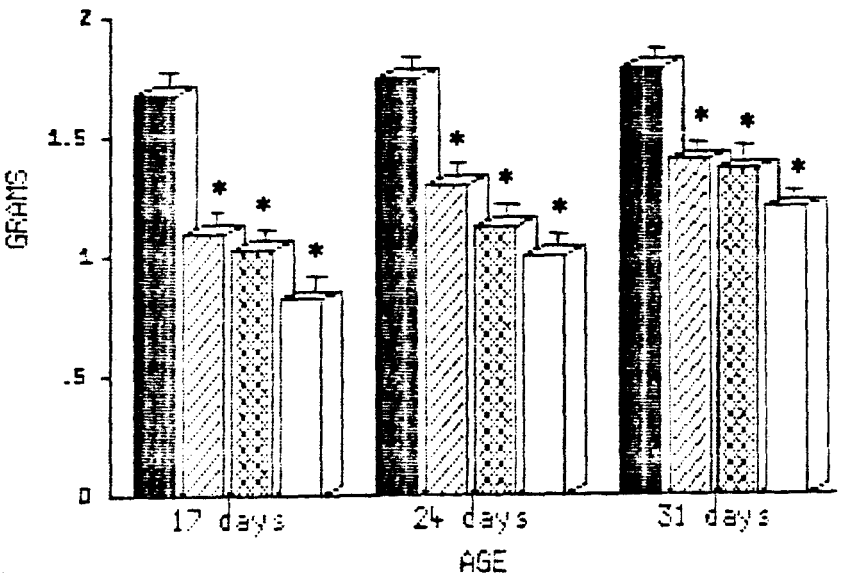
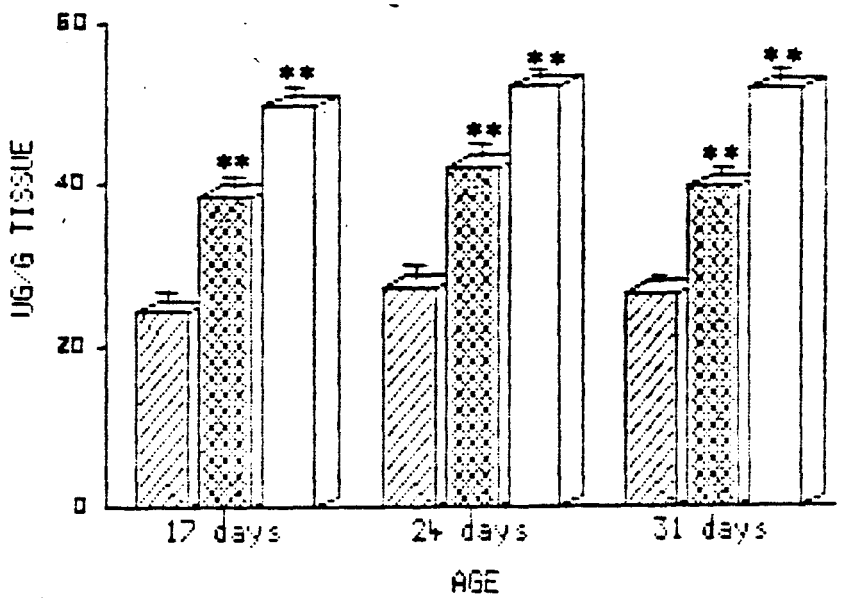


Figure 11. Chlordecone levels in cortical regions of chlordecone exposed offspring. Each value represents the mean of 8 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from 5 mg/kg are different from 15 mg/kg and 30 mg/kg exposed animals at $p<0.01$ and $p<0.001$, respectively.

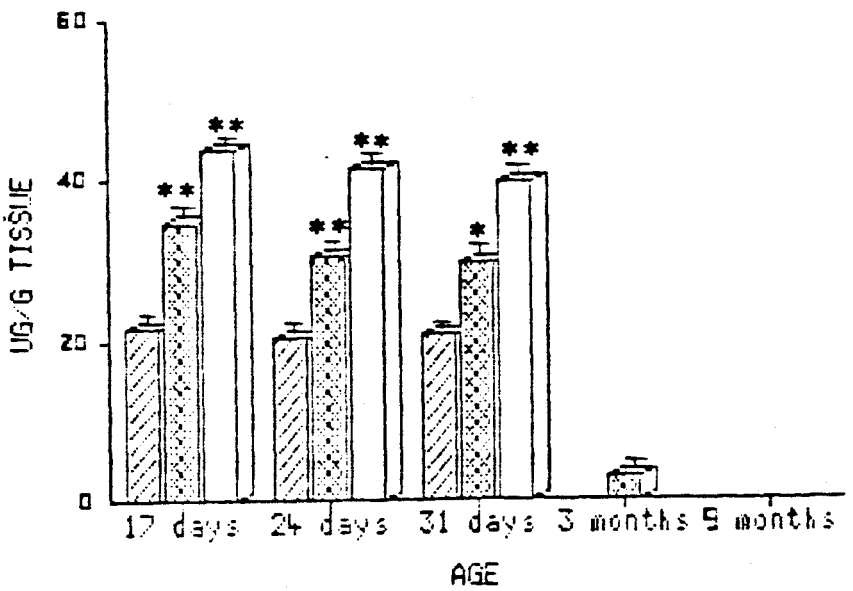
CHLORDECONE LEVELS IN CORTICAL REGIONS OF CHLORDECONE EXPOSED OFFSPRING

5mg/kg 15 mg/kg 30 mg/kg

NONCROSSFOSTERED OFFSPRING



CROSSFOSTERED OFFSPRING



concentration dependent on the daily dosage. The levels were 22, 38 and 52 ug/g tissue for noncrossfostered 5, 15 and 30 mg/kg exposed offspring, respectively. Even though the offspring were continually exposed to the respective dosages, the cortical chlordecone concentrations remained constant at 24 and 31 days of age. Crossfostering of the gestationally exposed offspring did not significantly reduce the brain chlordecone concentrations. Even at 31 days after cessation of dosing, the chlordecone levels decreased only 1-5%. Three month old 15 mg/kg gestationally exposed offspring still retained a small (2 ug/g tissue) but detectable amount of chlordecone which was cleared by 9 months. These findings are in agreement with similar studies (Fujimori et al., 1982) that chlordecone has a maximum accumulation rate and a slow clearance rate.

Synaptosomal Protein and Phospholipid Content

To assess whether chlordecone exposure affected protein composition of synaptic plasma membranes (SPM), total protein concentrations and distributions were examined by Dr. Daniel Selivonchick of Oregon State University. There were no significant differences in SPM protein concentrations and distributions between control and chlordecone exposed offspring at any of the ages or exposure

levels examined (Figure 12). Identical results were observed in crossfostered offspring.

Phospholipids from control and chlordecone exposed offspring were extracted from SPM. The samples were analyzed for total phospholipid and distribution of individual phospholipid classes. The phospholipid distributions for noncrossfostered and crossfostered chlordecone exposed offspring are shown in Table XIV. The phospholipid composition of the chlordecone exposed offspring did not differ significantly from that of the control group. Phosphatidylcholine (PC) was the major membrane constituent followed by phosphatidylethanolamine (PE), phosphatidylinositols (PI), phosphatidylserine (PS), phosphatidic acid (PA) and phosphatidylglycerol (PG). These values are in agreement with previous findings by Fontaine et al.(1980) on the phospholipid composition of synaptosomal membranes.

Phospholipid Stimulation

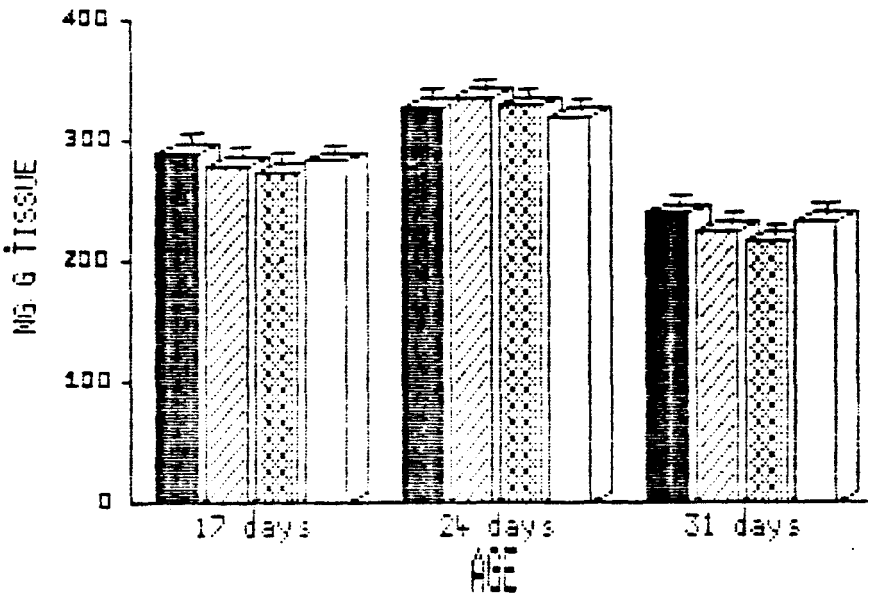
To determine whether specific receptors were coupled to enhanced phospholipid turnover, phospholipids were extracted from agonist stimulated ^{32}P-orthophosphate prelabeled synaptosomes (See Materials and Methods). Carbamylcholine (CC), dopamine (DA), noradrenaline (NA), serotonin (5HT) or histamine (HA) were used as agonists to

Figure 12. Membrane protein concentrations of chlordecone exposed offspring. Each value represents the mean of 8 animals ± the standard deviation.

MEMBRANE PROTEIN CONCENTRATIONS OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg

NONCROSSFOSTERED



CROSSFOSTERED

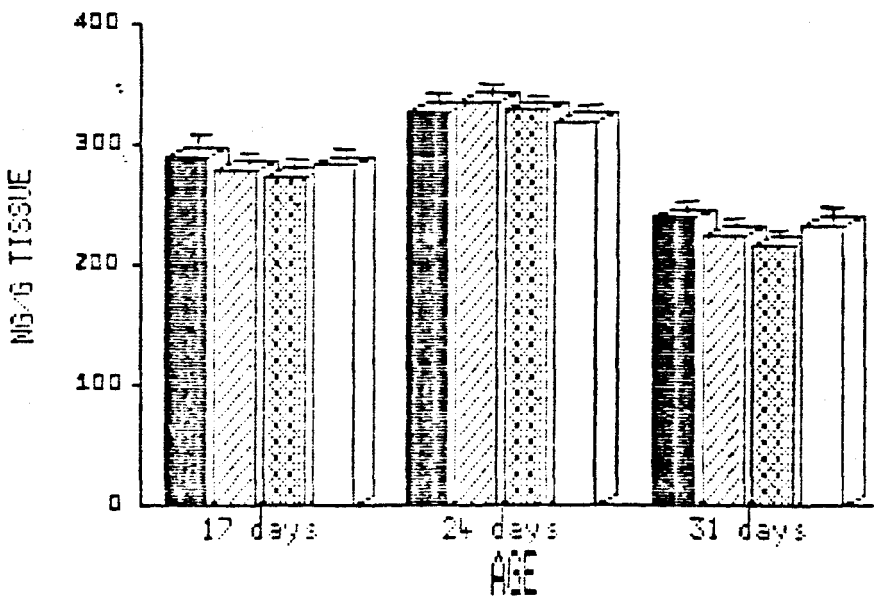


TABLE XIV

PHOSPHOLIPID DISTRIBUTION OF SYNAPTIC PLASMA MEMBRANES FROM CHLORDECONE EXPOSED OFFSPRING

GROUP	PC	PE	PI	PS	PG	PA
17 DAYS			NONCROSSFOSTERED			
CONTROL	42	30	14	6	2	6
5 MG/KG	40	29	14	8	2	7
15 MG/KG	40	29	14	8	2	6
30 MG/KG	40	30	15	7	3	5
			CROSSFOSTERED			
CONTROL	39	38	24	8	2	6
5 MG/KG	40	30	15	7	3	5
15 MG/KG	40	27	16	7	3	7
30 MG/KG	39	29	14	8	3	7
24 DAYS			NONCROSSFOSTERED			
CONTROL	44	31	12	7	2	4
5 MG/KG	42	30	14	6	2	6
15 MG/KG	43	28	15	7	2	5
30 MG/KG	41	27	15	8	3	8
			CROSSFOSTERED			
CONTROL	44	31	12	7	2	4
5 MG/KG	42	30	14	6	2	6
15 MG/KG	43	28	15	7	2	5
30 MG/KG	41	27	15	8	2	7
31 DAYS			NONCROSSFOSTERED			
CONTROL	46	27	13	7	2	5
5 MG/KG	46	30	12	6	1	5
15 MG/KG	44	31	12	7	2	4
30 MG/KG	43	28	15	7	1	5
			CROSSFOSTERED			
CONTROL	46	30	12	6	1	5
5 MG/KG	46	27	13	7	2	5
15 MG/KG	46	30	12	6	1	5
30 MG/KG	45	28	13	7	2	4

Units indicate percent of total phospholipids. Each value represents the mean of 3 animals $\pm$ the standard deviation.

stimulate radiolabeled orthophosphate incorporation into phospholipids, specifically the phosphatidylinositols and phosphatidic acid. The incorporation of ^{32}P into triphosphatidylinositol (TPI), diphosphatidylinsoitol (DPI), phosphatidylinositol (PI) and phosphatidic acid (PA) represents subsequent resynthesis of the respective phospholipid after receptor-coupled breakdown. Therefore, the resynthesis of the respective phospholipids provides a convenient way to measure the extent of initial receptor stimulated breakdown. The antagonists atropine, haloperidol, regitine or bentazole were used in conjunction with CC, DA, NA or HA, respectively, or alone to elucidate whether any observed stimulation was due to receptor-specific interactions.

The results presented below deal specifically with receptor mediated stimulation of TPI, DPI, PI and PA turnover. When the distribution of radioactivity in various phospholipid components was determined, most of the radioactivity (>70%) was found associated with the inositol phospholipids and phosphatidic acid. For all agonists, the degree of stimulation was dependent on the phospholipid measured in the order of TPI> PA> DPI> PI such that similar effects were seen without regard to exposure levels. The total amount of stimulation for each phospholipid was

dependent on the concentration of chlordecone exposure in the order of control> 5 mg/kg > 15 mg/kg > 30 mg/kg and age in the order of 17 days< 24 days< 31 days.

Antagonists added to the respective agonists or alone did not cause significant stimulation of phospholipid turnover above unstimulated control levels regardless of age or exposure levels except bentazole (discussed later). The remaining radioactivity was associated with phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) with each phospholipid containing 4-10% of the total label. For these four phospholipids, there were no significant differences between control and chlordecone exposed offspring.

Carbamylcholine

Muscarinic receptor coupling to inositol phospholipid turnover has been demonstrated by several investigators (Fisher et al., 1983; Hokin-Neaverson, 1974). Carbamylcholine was selected to determine whether the muscarinic receptor was affected by chlordecone exposure. Atropine was chosen as the antagonist to specifically link any observed stimulation to the M_1 receptor. It has been demonstrated that atropine binds preferentially to the M_1

receptor by classic receptor/antagonist interactions, thereby blocking the receptor site from agonist binding.

Carbamylcholine stimulation of ^{32}P incorporation into synaptosomes of the control group was consistent with results obtained by Reddy and Sastry (1979) for developing brain.

Appendix A and Figures 13-16 show the neurotransmitter effects of carbamylcholine. Carbamylcholine enhanced the specific activity of TPI, DPI, PI and PA in all groups. Seventeen day old offspring receiving 5 mg/kg/day chlordecone were not significantly different from the controls in any of the phospholipids measured. At 24 days of age, the effects were more pronounced in that they were significantly different from controls at the $p<0.001$ level for TPI and DPI and the $p<0.01$ level for PA. At 31 days of age, PI was also affected. The 15 and 30 mg/kg chlordecone exposed offspring displayed impaired stimulation by carbamylcholine at 24 and 31 days of age ($p<0.001$) for all phospholipids.

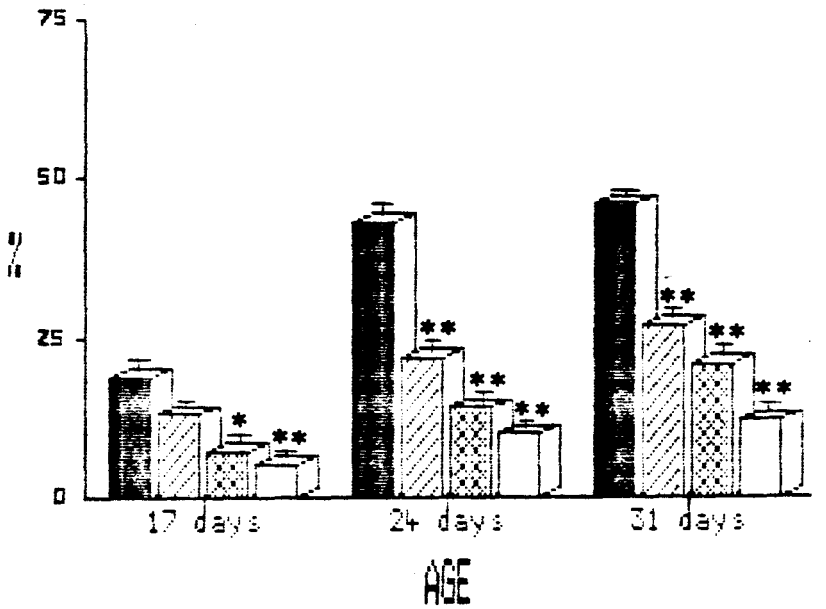
Crossfostering of chlordecone exposed offspring appeared to have no effect on the amount of stimulation for any of the phospholipids examined versus noncrossfostered offspring. Stimulation of synaptosomes from 3 and 9 month old gestationally exposed offspring (15 mg/kg) was still

Figure 13. ^{32}P incorporation into triphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by carbamylcholine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

32 P INCORPORATION INTO TRIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY CARBAMYLCHOLINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

NONCROSSFOSTERED



CROSSFOSTERED

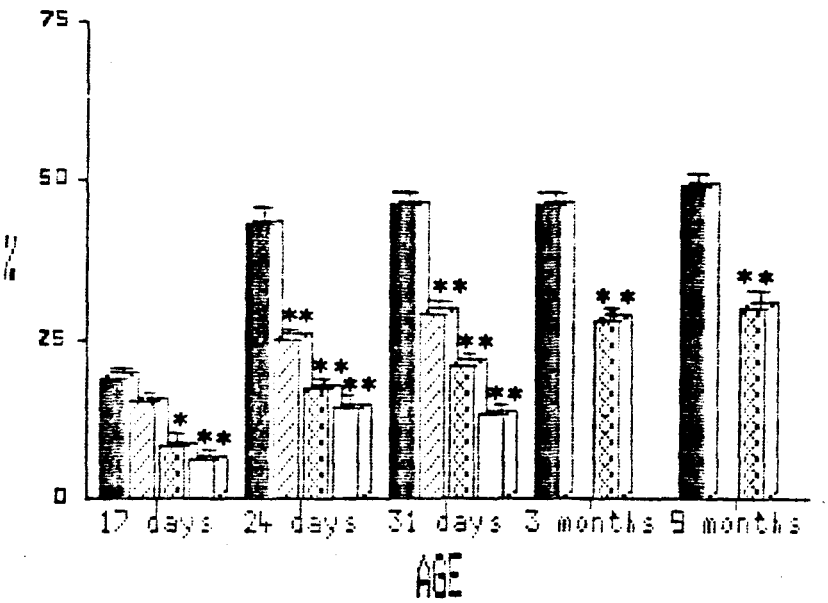


Figure 14. ^{32}P incorporation into diphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by carbamylcholine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO DIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY CARBAMYLCHOLINE

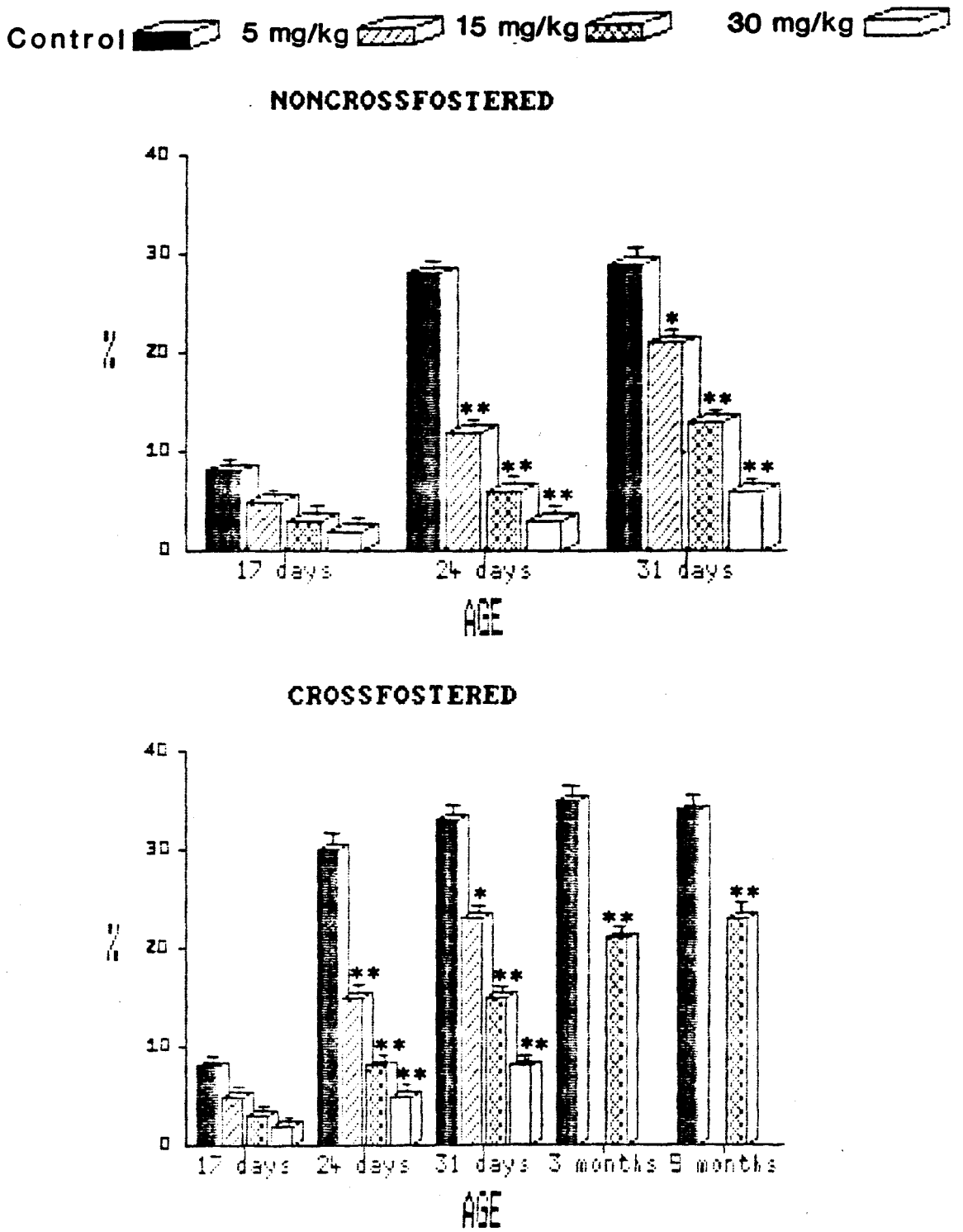
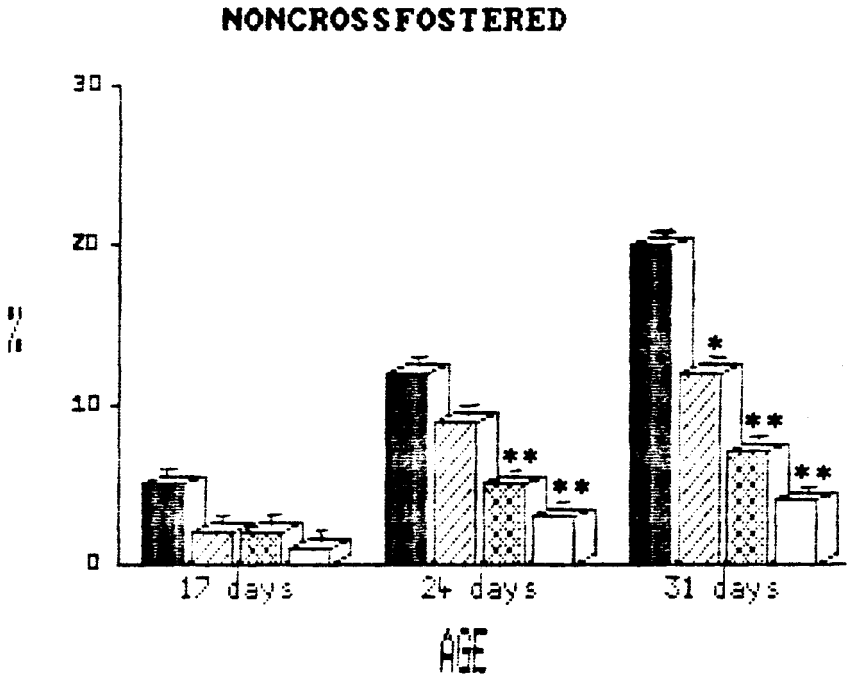


Figure 15. ^{32}P incorporation into phosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by carbamylcholine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY CARBAMYLCHOLINE

Control 5 mg/kg 15 mg/kg 30 mg/kg



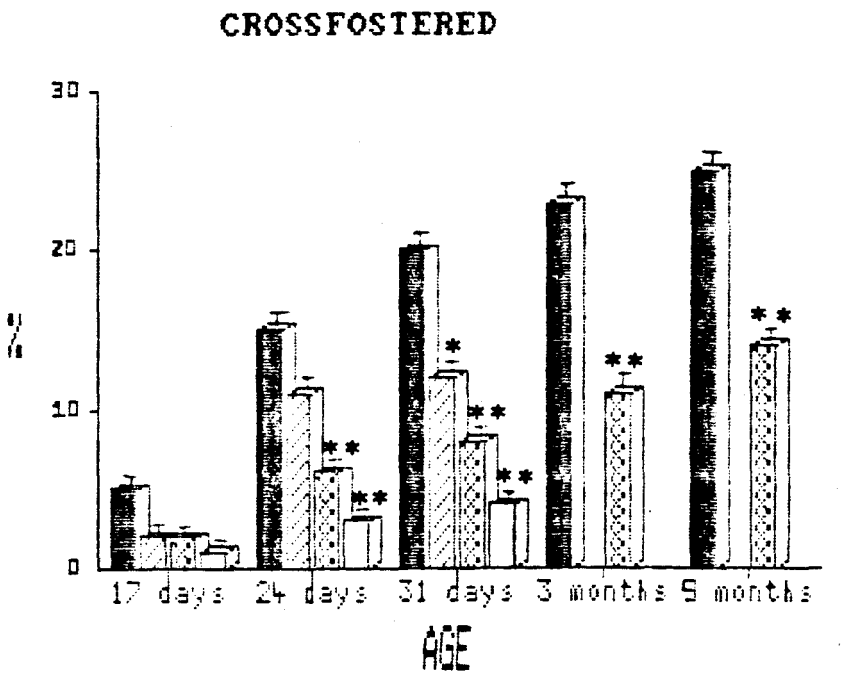
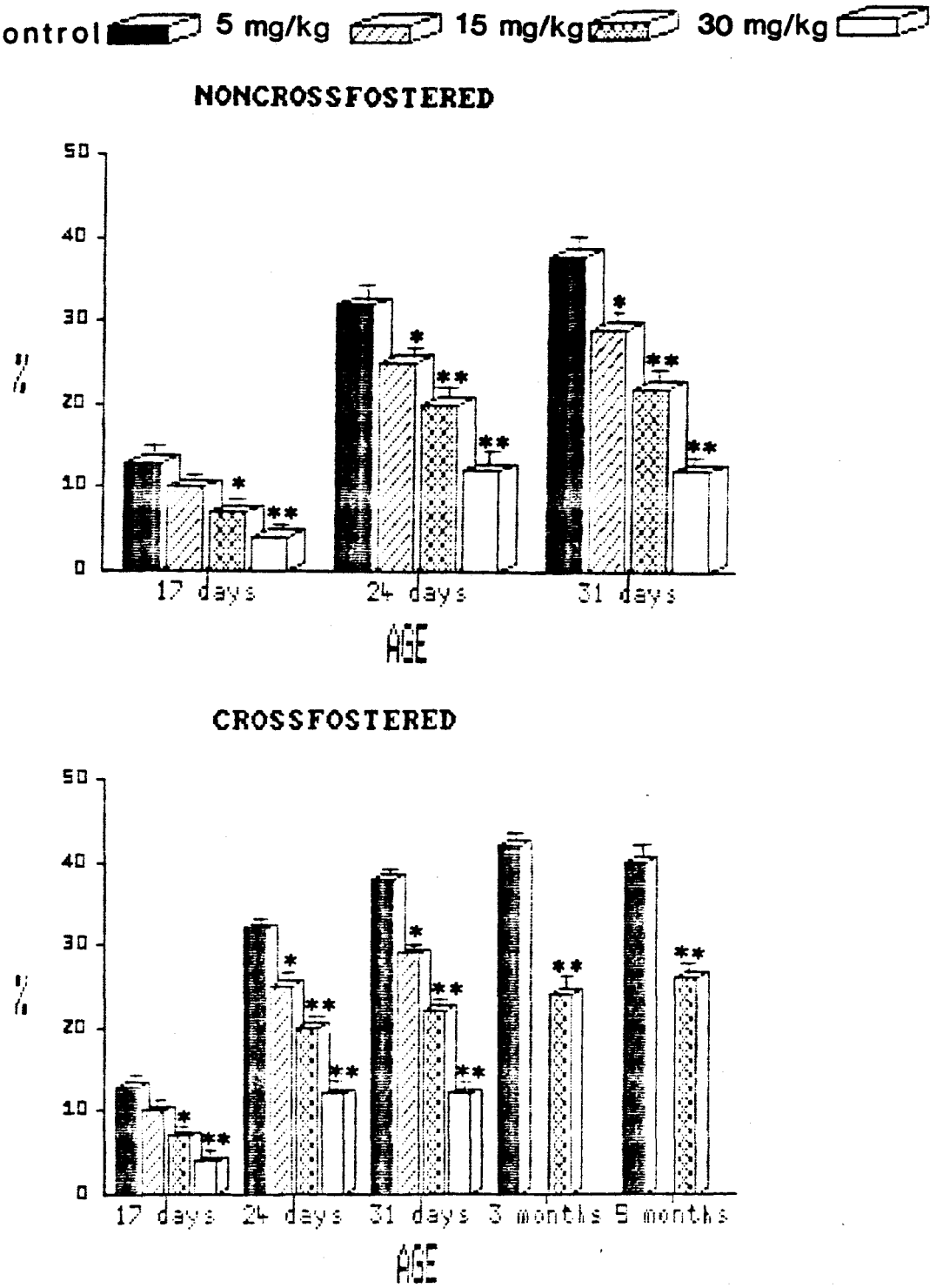


Figure 16. ^{32}P incorporation into phosphatidic acid of synaptosomes from chlordecone exposed offspring: percent stimulation by carbamylcholine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY CARBAMYLCHOLINE

Control 5 mg/kg 15 mg/kg 30 mg/kg



significantly impaired relative to control offspring but was not significantly different from 24 or 31 day old crossfostered offspring (15 mg/kg).

Addition of atropine to carbamylcholine preparations totally blocked the binding of carbamylcholine and negated any stimulation seen with carbamylcholine alone (100%±2). The incubation of synaptosomes with atropine alone did not display a significant stimulation over unstimulated controls (97%±2) of any of the phospholipids examined in all groups (Appendix B).

To distinguish chlordecone effects on inositol phospholipid labeling from in vitro effects, the following experiments were performed and subsequent results obtained. Chlordecone was added to a control synaptosome preparation from 31 day old rats and incubated for 10 or 30 minutes. The synaptosomes were then labeled with radiolabeled orthophosphate and stimulated with carbamylcholine. When the individual phospholipids were assayed there was no significant difference in control versus in vitro chlordecone exposed synaptosomes for any of the phospholipids (Table XV).

Table XV

CARBAMLYCHOLINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM 31 DAY OLD RATS EXPOSED TO CHLORDECONE IN VITRO

GROUP	TPI	DPI	PI	PA
		CONTROL		
Carbamylcholine	142±4	127±2	121±3	139±5
Carbamylcholine + Atropine	103±3	100±4	101±2	100±1
Atropine	100±2	97±1	98±3	992
		10 MINUTES		
Carbamylcholine	140±3	122±4	120±2	136±4
Carbamylcholine + Atropine	102±1	100±2	99±3	101±2
Atropine	99±1	95±3	97±2	99±2
		30 MINUTES		
Carbamylcholine	138±2	126±3	116±3	134±3
Carbamylcholine + Atropine	102±3	101±1	99±2	100±1
Atropine	99±1	100±2	97±3	98±2

Synaptosomes from 31 day old animals were preincubated with 40 ug of chlordecone for 10 or 30 minutes and then incubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 3 animals ± the standard deviation.

It was suggested that the decreased phosphatidylinositol labeling in chlordecone exposed offspring was a function of malnourishment. To test this hypothesis, protein-deficient offspring (courtesy of Dr. Manteuffel) were substituted for chlordecone exposed offspring and identical phospholipid labeling experiments were performed. Decreases in the magnitude of phosphatidylinositol and phosphatidic acid labeling were observed but not significant enough to fully account for the inhibition seen with chlordecone exposed offspring (Table XVI).

In summary, carbamylcholine stimulated incorporation of ^{32}P-orthophosphate into TPI, DPI, PI and PA was age and exposure dependent. The higher the exposure level, the more impaired the ability to cause muscarinic receptor-coupled stimulation of phospholipid turnover. Even at 3 and 9 months after cessation of dosing, stimulation was still significantly impaired. Clearance of chlordecone from the brain (9 months) did not alter the ability of carbamylcholine to stimulate phospholipid turnover. The blocking of carbamylcholine stimulated phospholipid breakdown by atropine demonstrates that the effects of chlordecone exposure were muscarinic receptor specific.

Table XVI

CARBAMLYCHOLINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM 31 DAY OLD PROTEIN DEFICIENT RATS

GROUP	TPI	DPI	PI	PA
		CONTROL		
Carbamylcholine	146±3	123±2	123±4	136±4
Carbamylcholine + Atropine	102±2	101±2	103±1	104±2
Atropine	101±3	95±2	99±2	971
		PROTEIN DEFICIENT		
Carbamylcholine	143±2	120±3	117±3	133±2
Carbamylcholine + Atropine	100±1	101±3	97±2	98±4
Atropine	97±2	96±2	98±3	97±1

Synaptosomes from 31 day old protein deficient animals were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 3 animals ± the standard deviation.

Dopamine

Dopaminergic receptor coupling to phospholipid turnover has been demonstrated only indirectly (Downes, 1982). It has been suggested that the D_2 receptor is coupled to phosphatidylinositol breakdown. Haloperidol is a potent antagonist that effectively blocks 98% of the D receptors at a concentration of 1 mM. Therefore, haloperidol in conjunction with dopamine provides a useful tool to associate phosphatidylinositol breakdown with specific D_2 dopaminergic receptor stimulation.

The effects of dopamine on radiolabeled orthophosphate incorporation into the phosphatidylinositols and phosphatidic acid of noncrossfostered chlordecone exposed offspring are represented in Figures 17-20 and Appendix C. Similar results were observed with dopamine as described earlier with carbamylcholine. The higher the exposure level, the more significant the difference between control and chlordecone exposed offspring. The differences between the percent stimulation of the individual phospholipids of the control and exposed groups were less pronounced at 5 mg/kg than at 15 or 30 mg/kg. The relative stimulation of the individual phospholipids increased with age from 17 to 31 days of age. Therefore, the incorporation of

Figure 17. ^{32}P incorporation into triphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by dopamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO TRIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY DOPAMINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

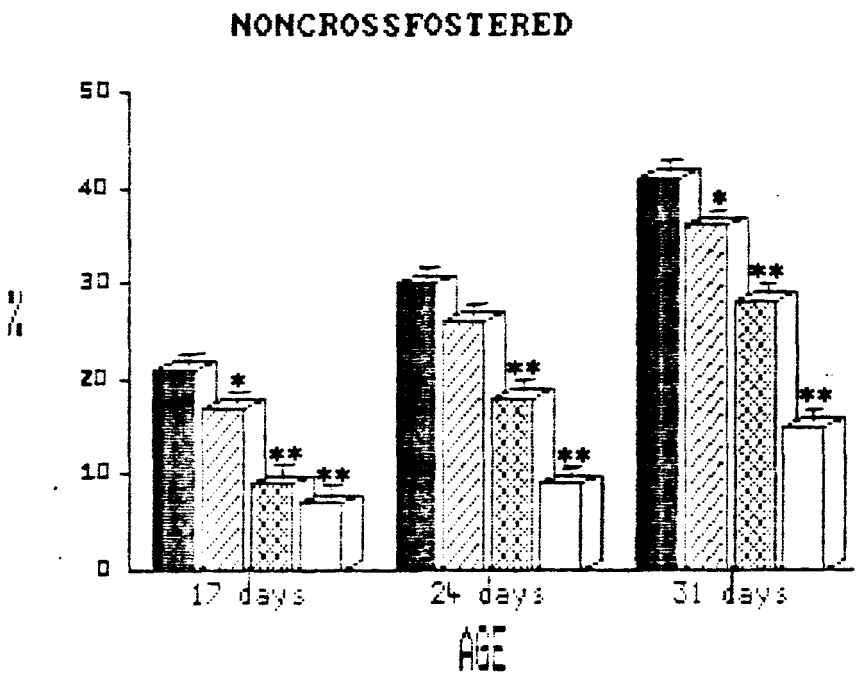


Figure 18. ^{32}P incorporation into diphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by dopamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO DIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY DOPAMINE

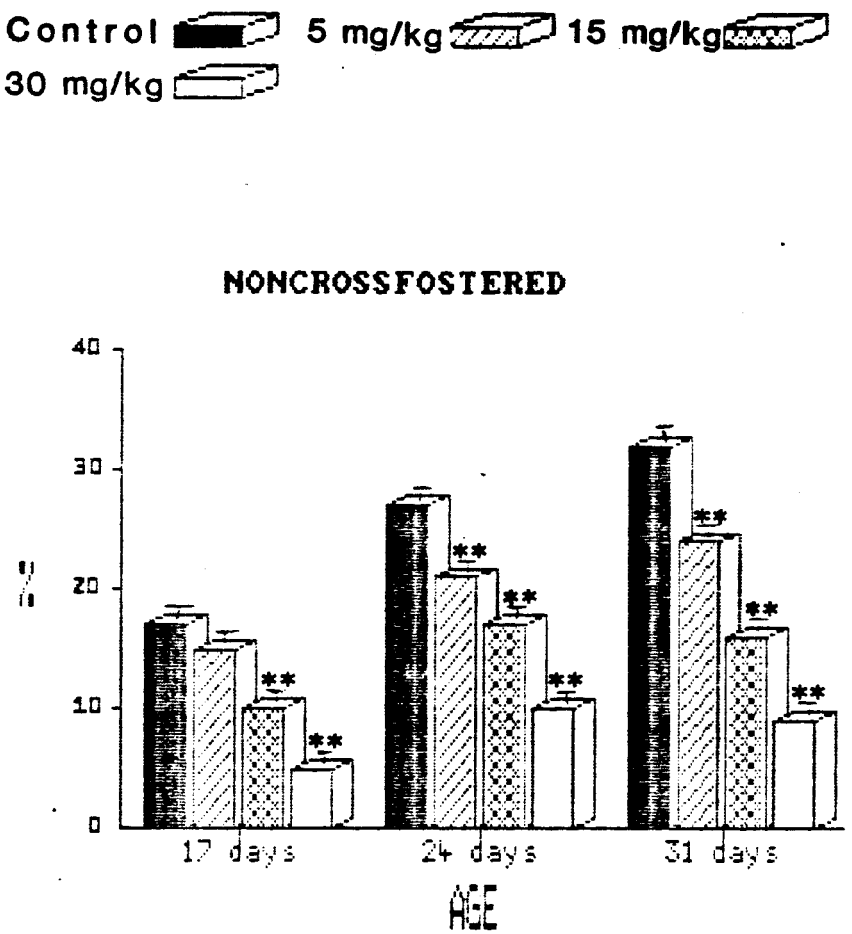


Figure 19. ^{32}P incorporation into phosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by dopamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY DOPAMINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

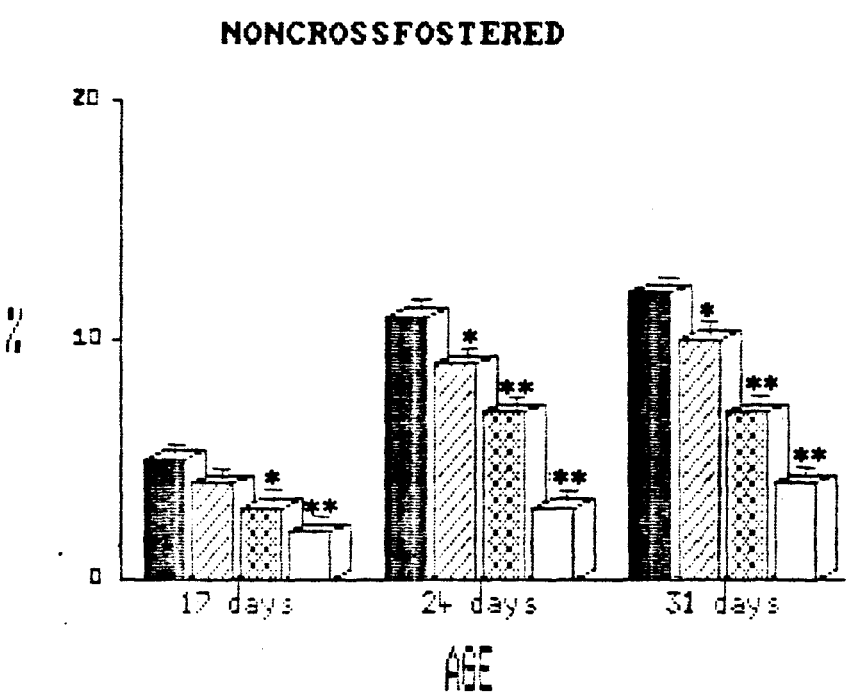
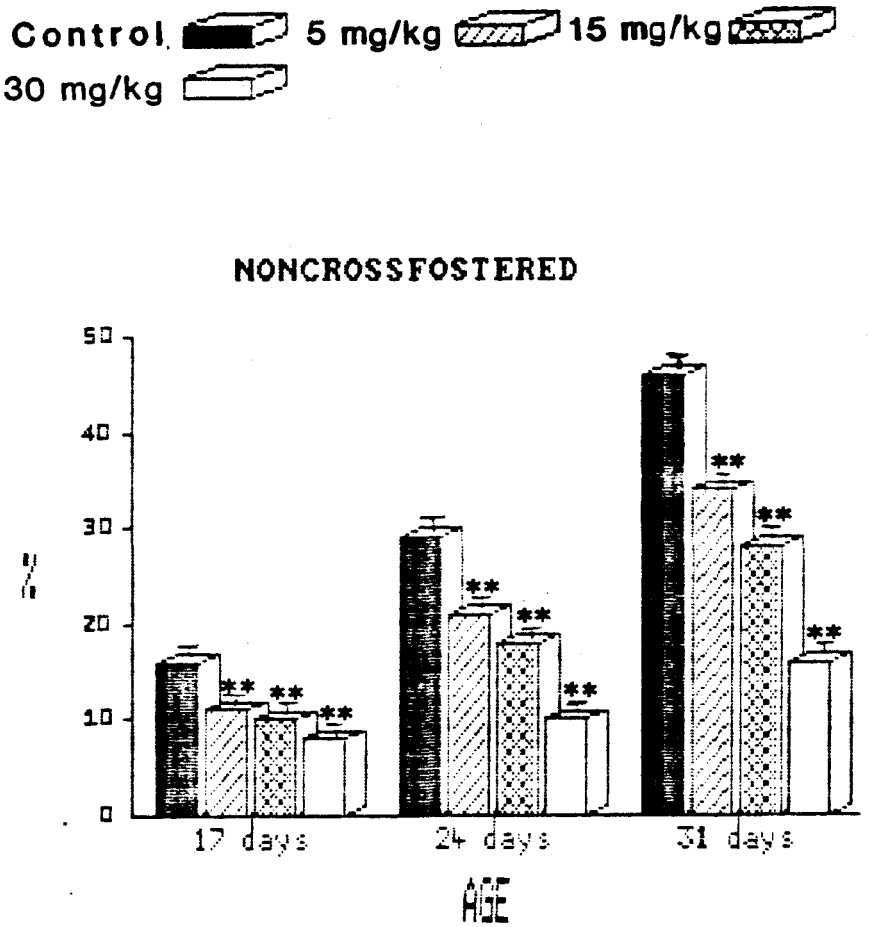


Figure 20. ^{32}P incorporation into phosphatidic acid of synaptosomes from chlordecone exposed offspring: percent stimulation by dopamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY DOPAMINE



orthophosphate into TPI, DPI, PI and PA were exposure- and age-dependent.

When synaptosomal preparations were incubated with haloperidol and dopamine or haloperidol alone, there were no significant increases over unstimulated controls for any of the phospholipids examined (Appendix D). Therefore, the phospholipid breakdown observed with dopamine was due to a D_2 receptor-coupled response.

Noradrenaline

Several investigators (Abdel-Latif and Smith, 1974) have demonstrated that α_1-adrenergic receptor activation is coupled to inositol phospholipid turnover in cerebral cortex and other target tissues. Noradrenaline is a catecholamine which binds to α_1-adrenergic receptors to elicit a stimulatory response. That action can be blocked by the addition of regitine which is a potent α_1-adrenergic receptor antagonist. Therefore, noradrenaline coupled phosphatidylinositol turnover should be effectively blocked by regitine if such breakdown is specifically an α_1-adrenergic receptor associated event.

The effects of noradrenaline associated phosphatidylinositol and phosphatidic acid breakdown are seen in Figures 21-24 and Appendix E. As was observed with carbamylcholine and dopamine, the incorporation of

Figure 21. ^{32}P incorporation into triphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by noradrenaline. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO TRIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY NORADRENALINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

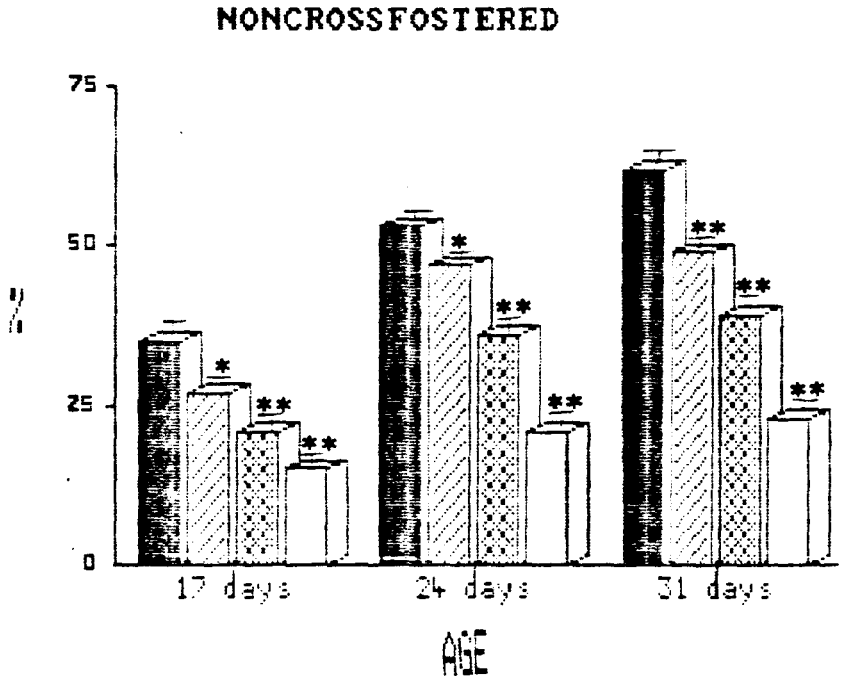


Figure 22. ^{32}P incorporation into diphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by noradrenaline. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO DIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY NORADRENALINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

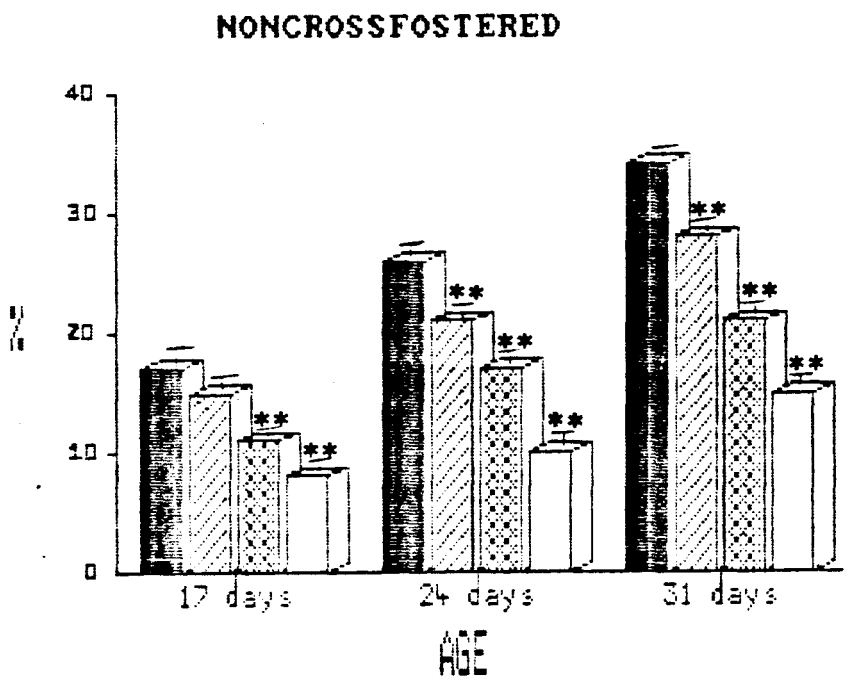


Figure 23. ^{32}P incorporation into phosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by noradrenaline. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY NORADRENALINE

Control 5 mg/kg 15 mg/kg 30 mg/kg

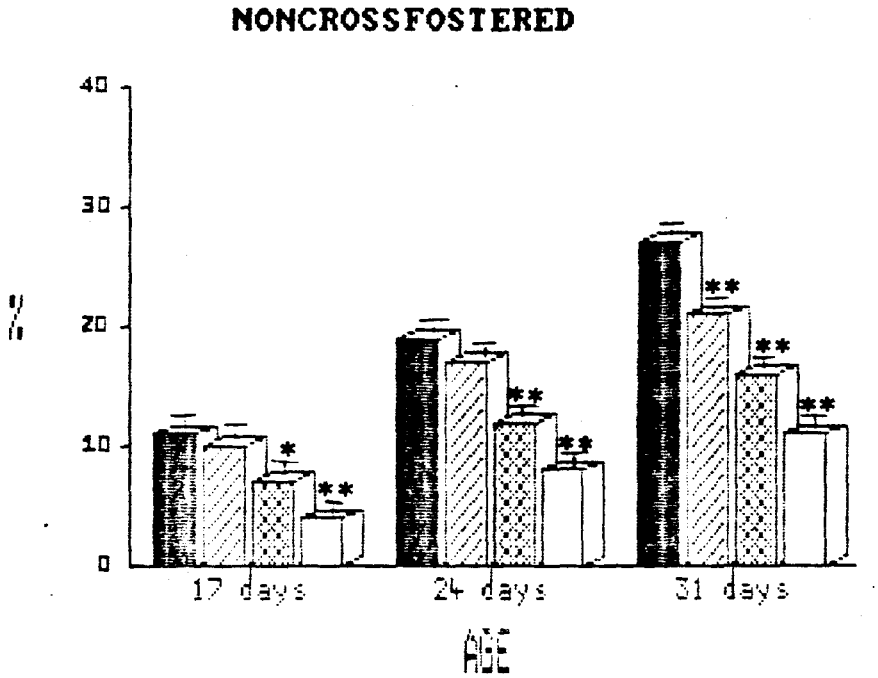
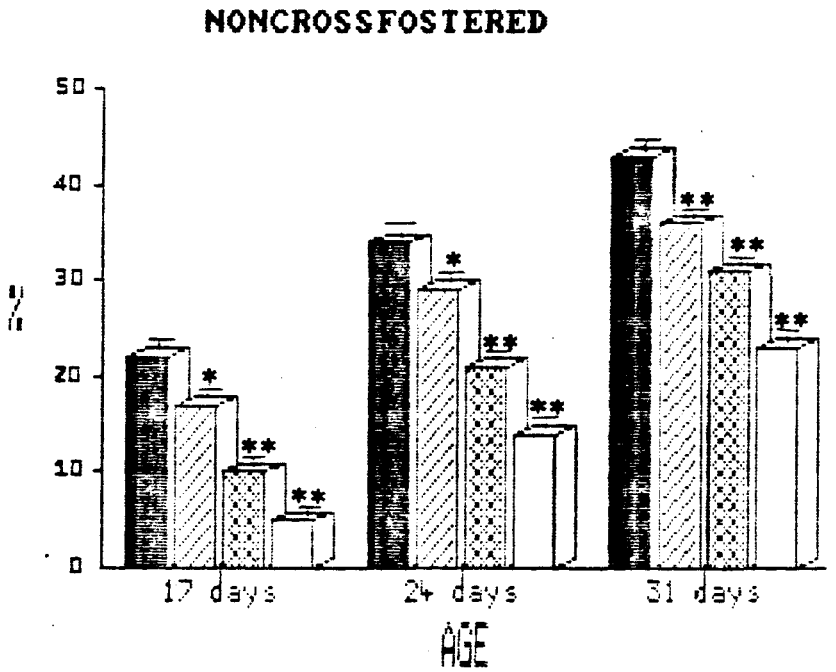


Figure 24. ^{32}P incorporation into phosphatidic acid of synaptosomes from chlordecone exposed offspring: percent stimulation by noradrenaline. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY NORADRENALINE

Control 5 mg/kg 15 mg/kg 30 mg/kg



radiolabeled orthophosphate into TPI, DPI, PI and PA was exposure- and age-dependent. The relative percent stimulation within groups and between groups paralleled the results seen for both carbamylcholine and dopamine, the only difference being that the total amount of stimulation for each individual phospholipid was greater with noradrenaline than with the two other agonists.

Addition of regitine to the incubation mixture prior to noradrenaline negated any subsequent phospholipid stimulation (Appendix F). Therefore, receptor activation and inositol phospholipid breakdown appeared to be linked specifically to the α_1-adrenergic site.

Serotonin

Serotonin receptor-coupled inositol phospholipid hydrolysis has been demonstrated in cerebral cortex (Downy, 1982) and in blowfly salivary gland (Reddy and Sastry, 1974). Doubts have been expressed as to whether this alliance between the serotonin receptor and inositol phospholipid breakdown is specific as such. This is due to the inability to demonstrate antagonist blockage of the stimulatory effects. Antagonists to serotonin receptors exist, but they are not specific enough to block greater than 60% of the receptor sites. Therefore, for this study,

serotonin stimulated phospholipid breakdown was represented without reference to receptor specific activation due to the lack of an appropriate antagonist.

Serotonin stimulated incorporation into the inositol phospholipids and phosphatidic acid (Figures 25-28 and Appendix G) paralleled the results seen previously with CC, DA and NA. There was a slightly greater overall stimulation seen with serotonin than with noradrenaline. However, the degree of stimulation seen with serotonin cannot be specifically associated with a serotonergic receptor mediated breakdown of inositol phospholipids.

Histamine

Receptor coupled inositol phospholipid breakdown by histamine had been investigated in most brain regions (Downy, 1982). This resultant turnover has been coupled to both the H_1 and H_2 receptors. This study specifically focused on the H_1 receptor activation in which bentazole can be used as an antagonist.

The relative total stimulation of TPI, DPI, PI and PA were comparable in all aspects to the effects seen with dopamine (Figures 29-32 and Appendix H). But the magnitude of these effects can not be specifically linked to the H_1 receptor. Addition of bentazole to the incubation mixture

Figure 25. ^{32}P incorporation into triphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by serotonin. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with serotonin for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO TRIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY SEROTONIN

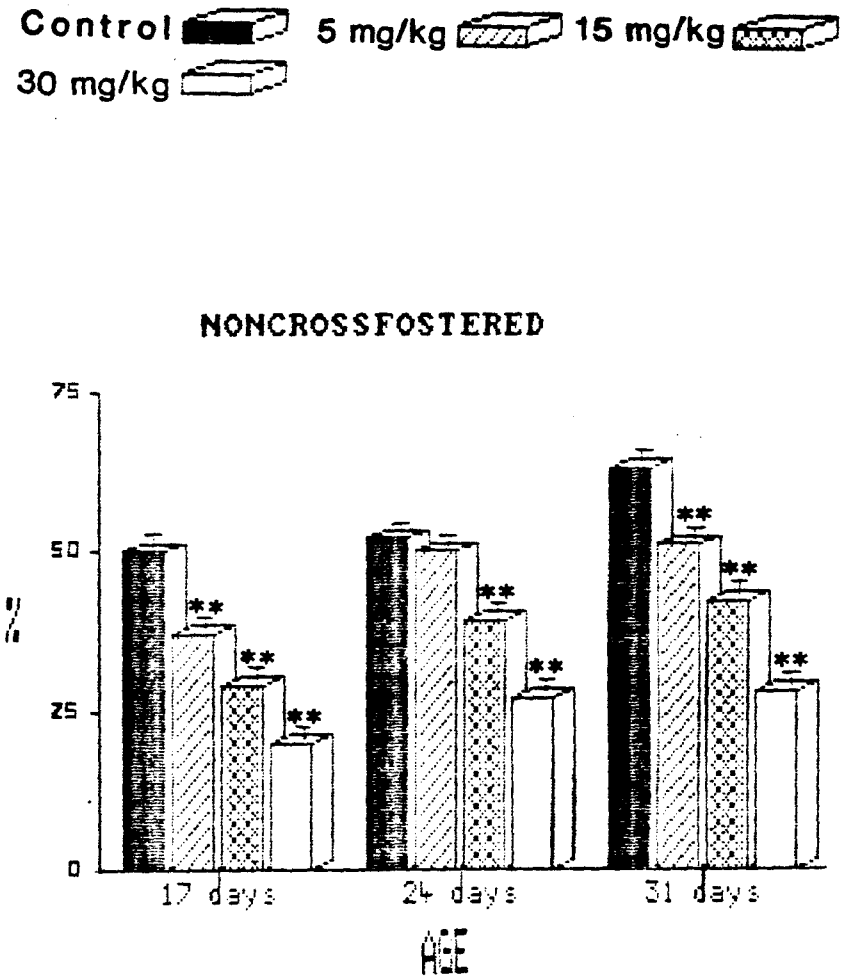


Figure 26. ^{32}P incorporation into diphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by serotonin. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with serotonin for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO DIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY SEROTONIN

Control 5 mg/kg 15 mg/kg 30 mg/kg

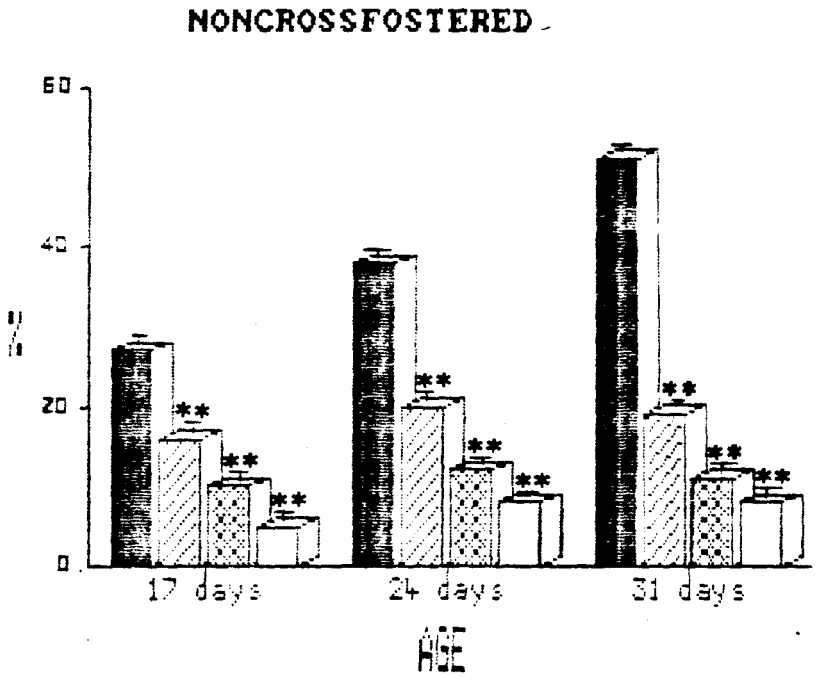


Figure 27. ^{32}P incorporation into phosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by serotonin. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with serotonin for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY SEROTONIN

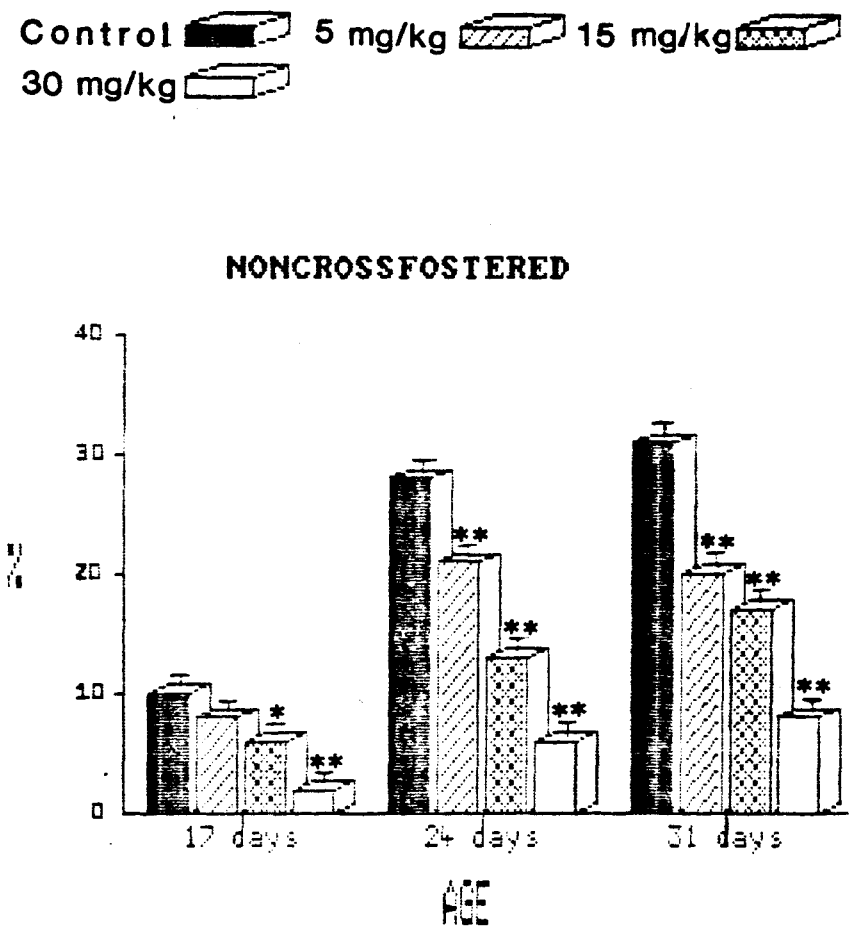


Figure 28. ^{32}P incorporation into phosphatidic acid of synaptosomes from chlordecone exposed offspring: percent stimulation by serotonin. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with serotonin for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY SEROTONIN

Control 5 mg/kg 15 mg/kg 30 mg/kg

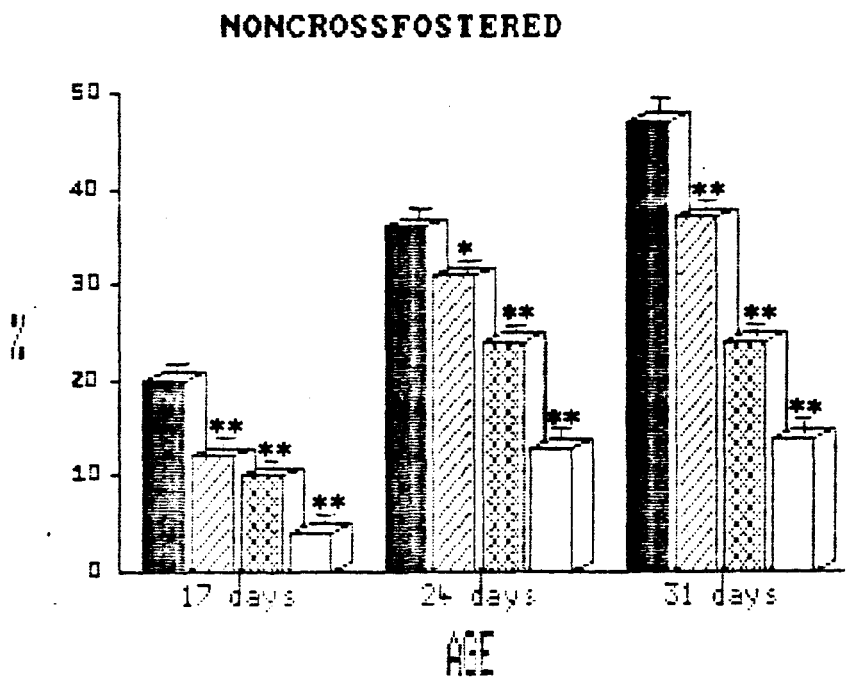


Figure 29. ^{32}P incorporation into triphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by histamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO TRIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY HISTAMINE

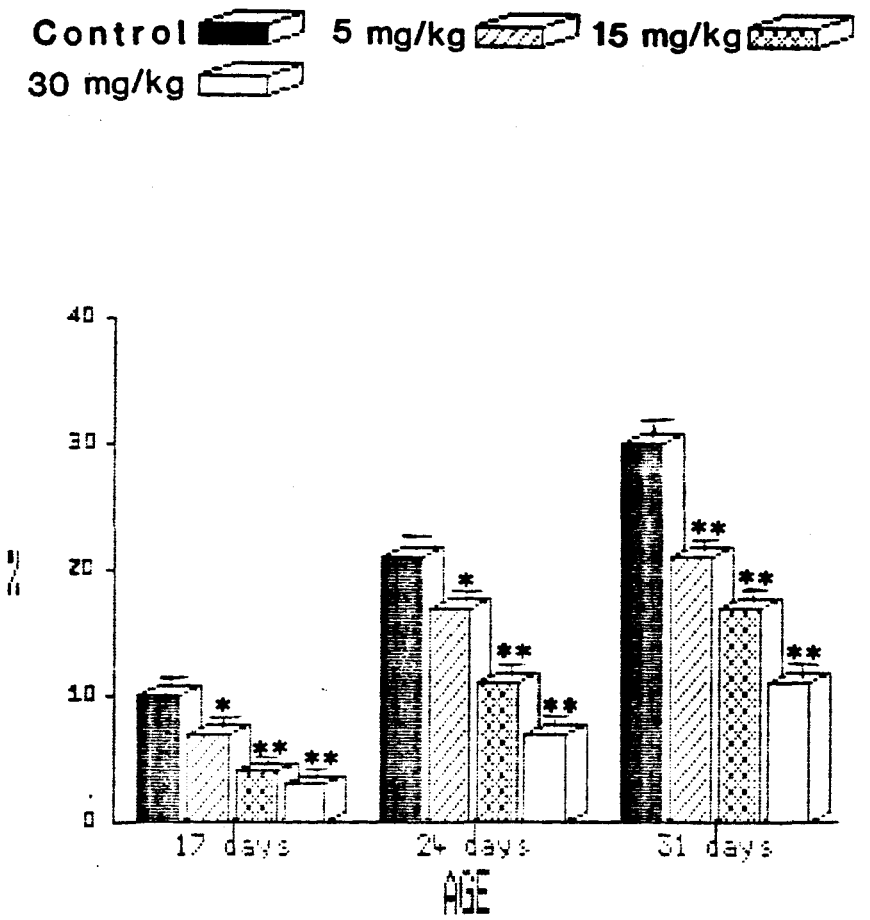


Figure 30. ^{32}P incorporation into diphosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by histamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO DIPHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY HISTAMINE

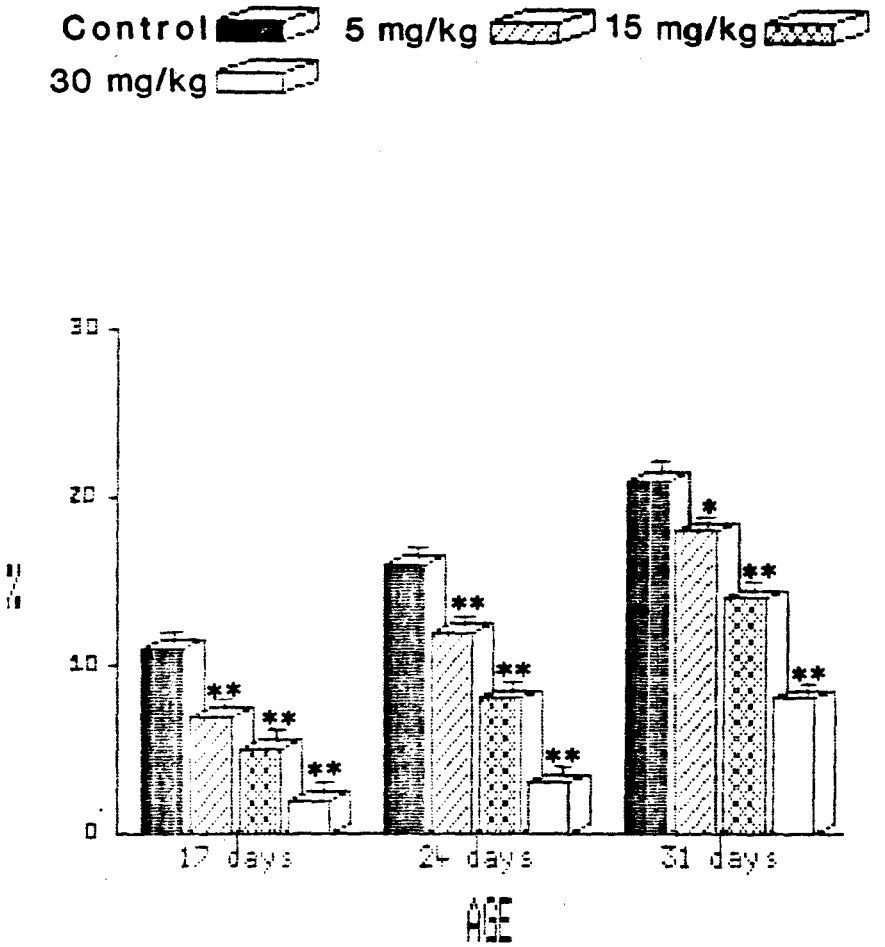


Figure 31. ^{32}P incorporation into phosphatidylinositol of synaptosomes from chlordecone exposed offspring: percent stimulation by histamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDYLINOSITOL OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY HISTAMINE

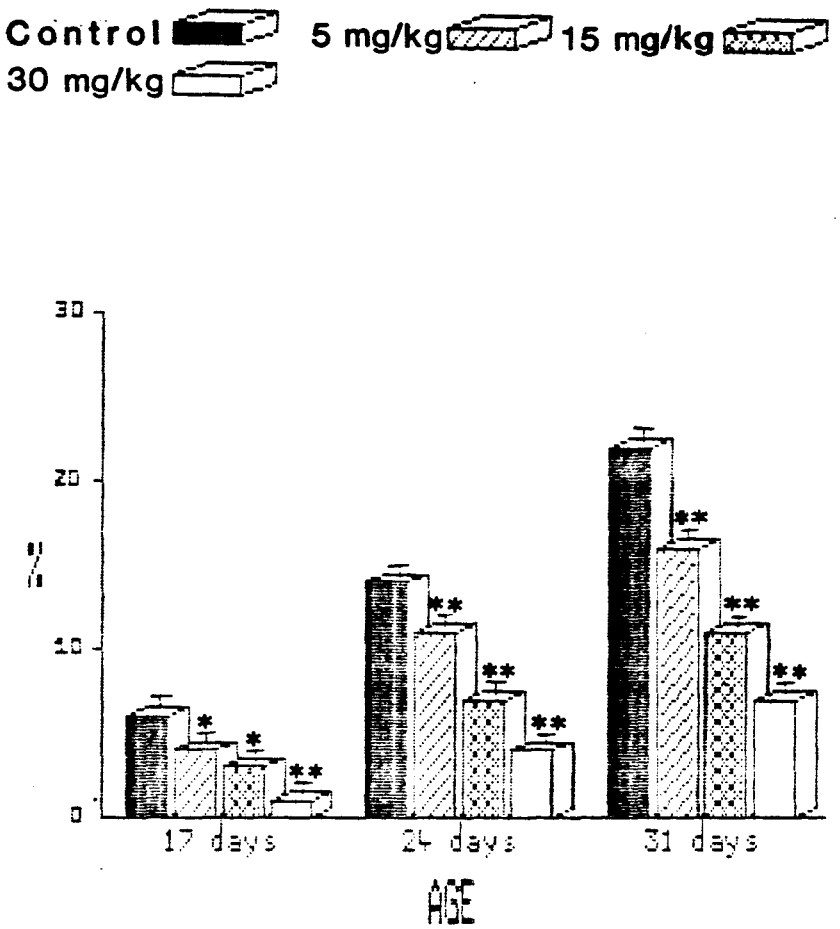
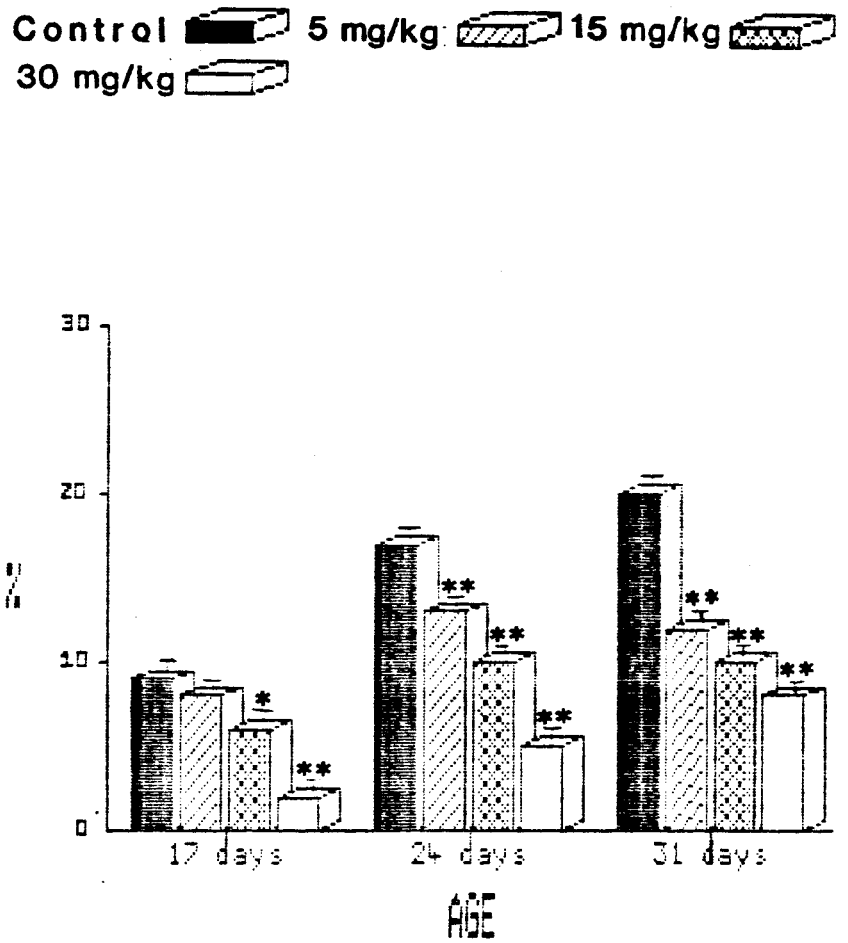


Figure 32. ^{32}P incorporation into phosphatidic acid of synaptosomes from chlordecone exposed offspring: percent stimulation by histamine. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

^{32}P INCORPORATION INTO PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING: PERCENT STIMULATION BY HISTAMINE



prior to addition of histamine only slightly decreased the overall effects (Appendix I). Therefore, the amount of stimulation of the inositol phospholipids and phosphatidic acid due to histamine H receptor coupling is very small. Subsequently, there is very little H_1 receptor-coupled phospholipid turnover in the cortical region.

Receptor-Coupled Phospholipid Turnover

In summary, neurotransmitter stimulated phospholipid labeling increased with age, being maximal at 31 days of age in the order of TPI> PA> DPI> PI, and with decreasing effectiveness from 5HT> NA> CC> DA> HA.

In chlordecone exposed offspring, neurotransmitter stimulation of phospholipid metabolism was significantly reduced in a dose- and age-dependent manner. The magnitude of the reductions was dependent on the neurotransmitter, but the overall stimulation pattern paralleled unexposed groups at all ages and exposures. The incubation of the antagonists atropine, haloperidol or regitine in the presence or absence of carbamylcholine, dopamine or noradrenaline, respectively, resulted in little or no stimulation. Bentazole in conjunction with histamine only slightly decreased the stimulation. Crossfostering of

control and chlordecone exposed offspring did not produce significant differences from offspring which were not crossfostered. The inability of carbamylcholine to stimulate receptor-coupled inositol phospholipid turnover persisted even after clearance of chlordecone from the brain.

Receptor Density and Affinity

Muscarinic Receptor Binding

Muscarinic receptor binding was examined in cortex and striatum of noncrossfostered chlordecone exposed offspring at 17, 24 and 31 days of age (Table XVII). Muscarinic receptor concentrations and affinity, measured as a function of percent tritiated QNB binding, were in agreement with the regional distributions reported by Snyder et al. (1975). This work was done in Dr. Ron Ritzmann's laboratory at the University of Illinois.

In 5, 15, and 30 mg/kg chlordecone exposed offspring, the cortical receptor concentrations were significantly reduced ($p<0.001$) at all ages and exposure levels (Figure 33). Crossfostered offspring were also analyzed for receptor concentrations at 3 and 9 months of age in addition to the ages mentioned above. When chlordecone offspring were crossfostered postpartum, the receptor concentrations

Table XVII. Muscarinic receptor binding in cortex and striatum of chlordecone exposed offspring. Cortex and striatum from control and chlordecone exposed offspring were diluted to 0.66 mg tissue/ ml of buffer. The brain was homogenized and centrifuged for 10 minutes at 1000 x g. The supernatant was used for receptor binding studies as described in Materials and Methods. Tritiated QNB was used as the ligand and atropine as the displacer. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

Table XVII

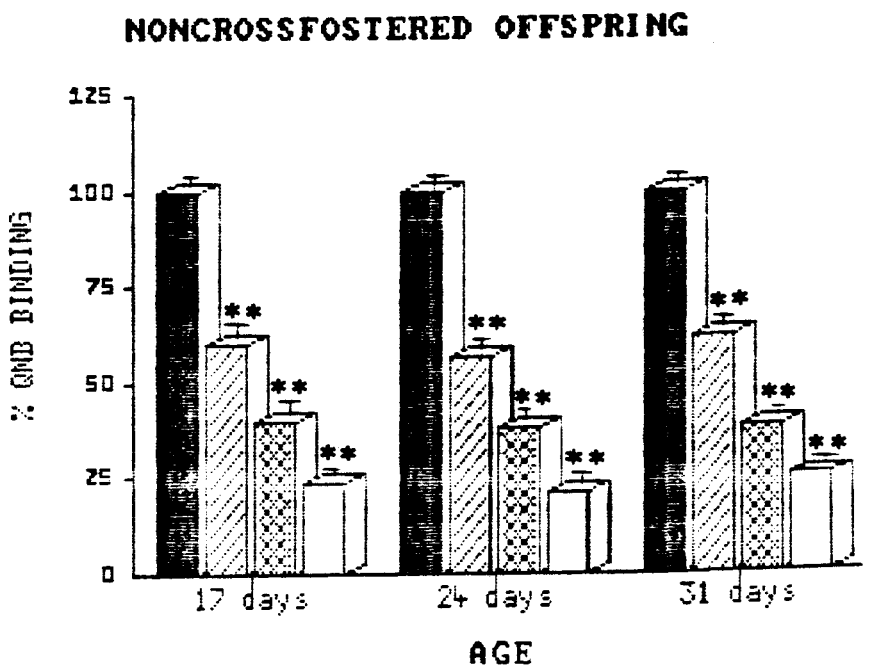
MUSCARINIC RECEPTOR BINDING IN CORTEX AND STRIATUM OF CHLORDECONE EXPOSED OFFSPRING

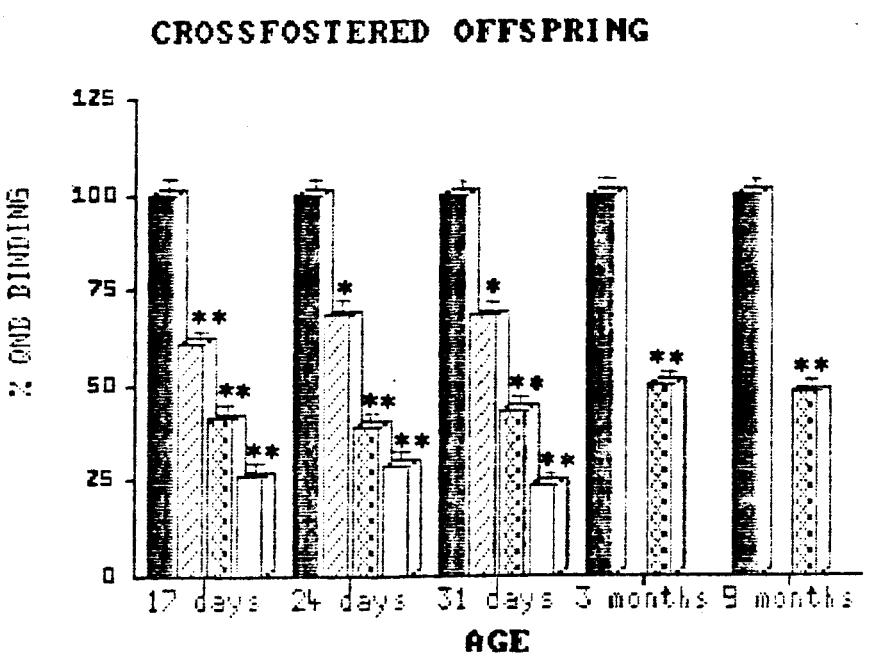
DAYS	QNB Sites (pmol/ g protein) CORTEX	STRIATUM
17 DAYS	NONCROSSFOSTERED	
Control	310±14	349±19
5 mg/kg	195±12**	220±11**
15 mg/kg	124±6**	140±5**
30 mg/kg	78±4**	94±3**
	CROSSFOSTERED	
Control	319±12	355±11
5 mg/kg	199±6**	237±9**
15 mg/kg	129±7**	149±3**
30 mg/kg	88±4**	99±4**
24 DAYS	NONCROSSFOSTERED	
Control	325±15	352±12
5 mg/kg	184±11**	229±10*
15 mg/kg	118±8**	142±6**
30 mg/kg	69±3**	97±3**
	CROSSFOSTERED	
Control	327±13	349±14
5 mg/kg	203±14**	237±10*
15 mg/kg	132±7**	148±4**
30 mg/kg	83±2**	102±6**
31 DAYS	NONCROSSFOSTERED	
Control	335±16	362±11
5 mg/kg	199±11**	239±9**
15 mg/kg	120±9**	145±6**
30 mg/kg	81±4**	101±4**
	CROSSFOSTERED	
Control	330±13	359±13
5 mg/kg	206±10*	253±11*
15 mg/kg	133±7**	157±7**
30 mg/kg	90±3**	108±5**
3 MONTH	CROSSFOSTERED	
Control	335±12	363±11
15 mg/kg	172±9**	182±7*
9 MONTH	CROSSFOSTERED	
Control	327±14	367±12
15 mg/kg	178±6**	227±10**

Figure 33. Muscarinic receptor binding in cortex of chlordecone exposed offspring. Cortex from control and chlordecone exposed offspring were diluted to 0.66 mg tissue/ ml of buffer. The brain was homogenized and centrifuged for 10 minutes at 1000 x g. The supernatant was used for receptor binding studies as described in Materials and Methods. Tritiated QNB was used as the ligand and atropine as the displacer. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks(**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

MUSCARINIC RECEPTOR BINDING IN CORTEX OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg





did not recover and were still significantly different from controls ($p<0.001$) at all ages and exposure levels except 24 and 31 day old 5 mg/kg exposed offspring. In these offspring the concentrations were significantly different at the $p<0.01$ level. Even at 3 and 9 months of age, significant rehabilitation of muscarinic receptor content had not occurred. In both noncrossfostered and crossfostered 5, 15, and 30 mg/kg exposed offspring, absolute concentrations of muscarinic receptors were inversely proportional to exposure levels.

Muscarinic receptor binding was also investigated in striatum of noncrossfostered and crossfostered offspring. Reductions in receptor concentration paralleled the results observed in cortical preparations (Figure 34). Receptor loss was dependent on concentration of chlordecone exposure. Even 3 or 9 months after cessation of dosing, significant reductions in receptor concentration were still seen.

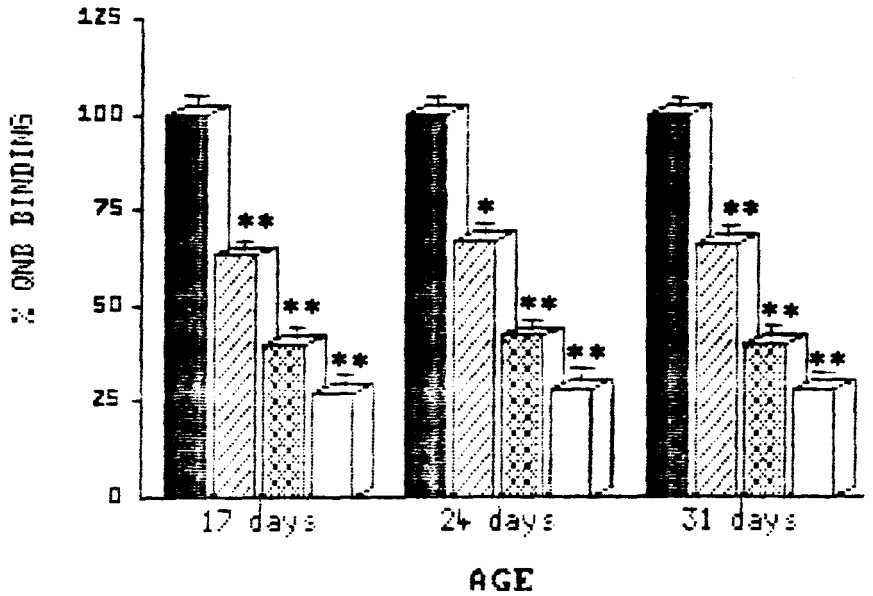
Muscarinic receptor affinity was calculated with a linear regression Scatchard analysis program. Although reference to reduction in receptor affinity utilizing this method only is not conclusive, it can be used as a basis for further investigation into possible effects on affinity of the ligand for the receptor. In both cortex and striatum, muscarinic receptor affinity was reduced (35%) at

Figure 34. Muscarinic receptor binding in striata of chlordecone exposed offspring. Striatum from control and chlordecone exposed offspring were diluted to 0.66 mg tissue/ ml of buffer. The brain was homogenized and centrifuged for 10 minutes at 1000 x g. The supernatant was used for receptor binding studies as described in Materials and Methods. Tritiated QNB was used as the ligand and atropine as the displacer. Each value represents the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks(**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

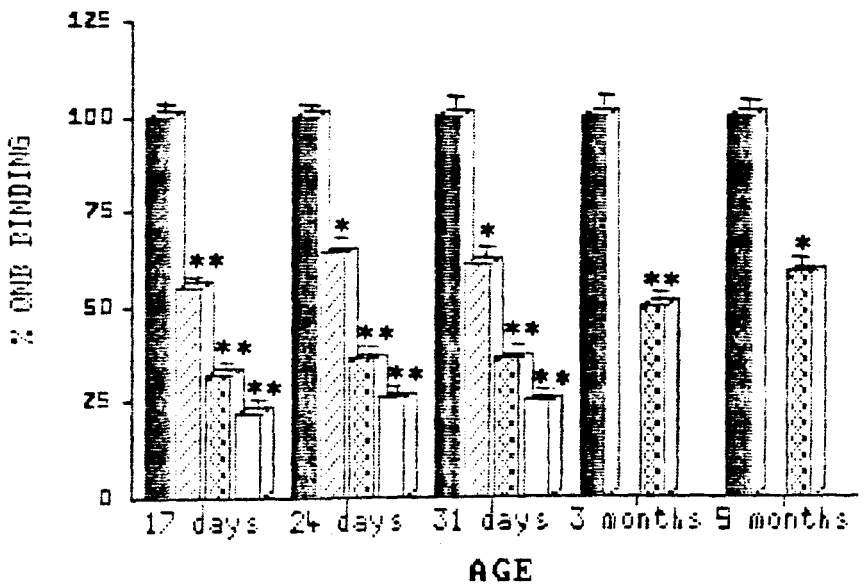
MUSCARINIC RECEPTOR BINDING IN STRIATA OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg

NONCROSSFOSTERED OFFSPRING



CROSSFOSTERED OFFSPRING



all ages and exposure levels. But the magnitude of these reductions was comparable without regard to age or concentration of chlordecone exposure.

Dopaminergic Receptor Binding

Dopaminergic (D_2) receptor binding was examined in cortex and striatum of control and 15 mg/kg noncrossfostered chlordecone exposed offspring. The results observed paralleled noncrossfostered muscarinic receptor binding respect to reduction in receptor concentration (Figures 35 and 36). The receptor concentration was decreased significantly at all ages ($p<0.001$) with respect to controls. Despite the changes seen in total receptor concentration, there was no significant difference in the receptor affinity for the ligand.

Hypothalamic and Striatal Biogenic Amine Levels

Biogenic amines concentrations from striatum and cortex were analyzed as described in Materials and Methods. The identification of individual biogenic amines was accomplished by reference between known quantities of standards (Figure 37) and tissue preparations (Figure 38).

Striatal and hypothalamic biogenic amine levels of control offspring were consistent with quantities observed by other invesigators for dopamine, DOPAC and noradrenaline

Figure 35. Dopaminergic receptor binding in cortex of chlordecone exposed offspring. Cortex from control and chlordecone exposed offspring were diluted to 0.66 mg tissue/ ml of buffer. The brain was homogenized and centrifuged for 10 minutes at 1000 x g. The supernatant was used for receptor binding studies as described in Materials and Methods. Tritiated spiperone was used as the ligand and butaclamol as the displacer. Each value represents the mean of 6 animals $\pm$ the standard deviation. Two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.001$.

DOPAMINERGIC RECEPTOR BINDING IN CORTEX OF CHLORDECONE EXPOSED OFFSPRING

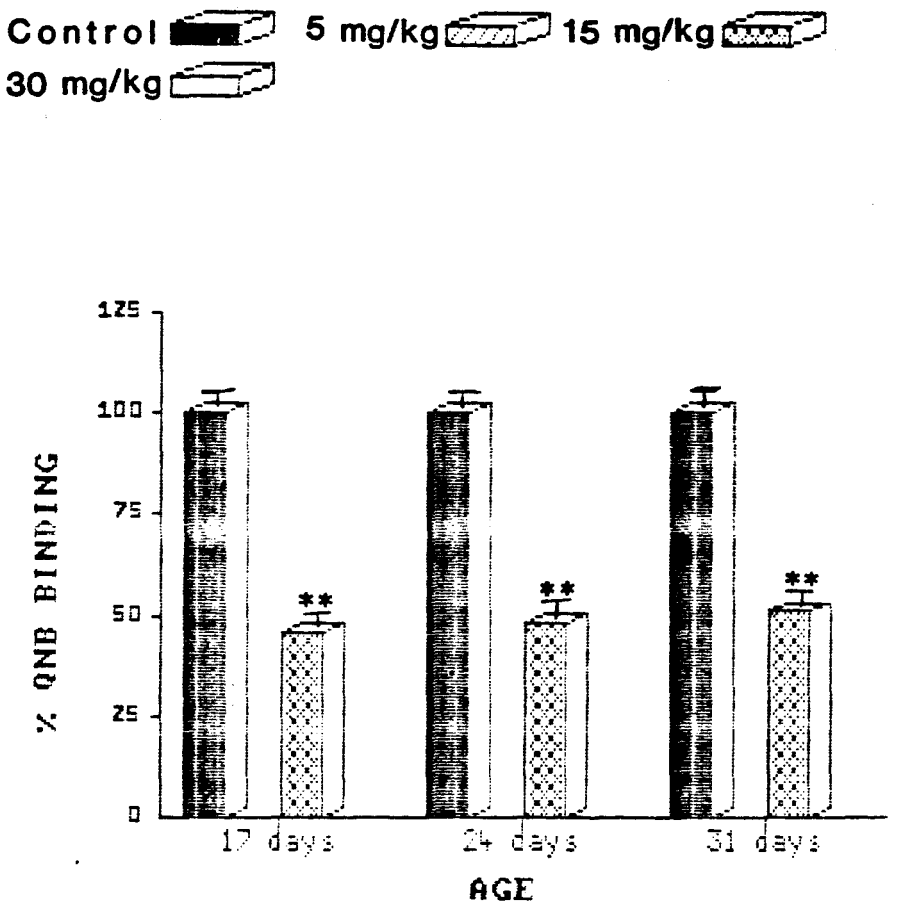


Figure 36. Dopaminergic receptor binding in striatum of chlordecone exposed offspring. Striatum from control and chlordecone exposed offspring were diluted to 0.66 mg tissue/ ml of buffer. The brain was homogenized and centrifuged for 10 minutes at 1000 x g. The supernatant was used for receptor binding studies as described in Materials and Methods. Tritiated spiperone was used as the ligand and butaclamol as the displacer. Each value represents the mean of 6 animals $\pm$ the standard deviation. Two asterisks(**) indicate that values from control and chlordecone exposed offspring were different at $p<0.001$.

DOPAMINERGIC RECEPTOR BINDING IN STRIATUM OF CHLORDECONE EXPOSED OFFSPRING

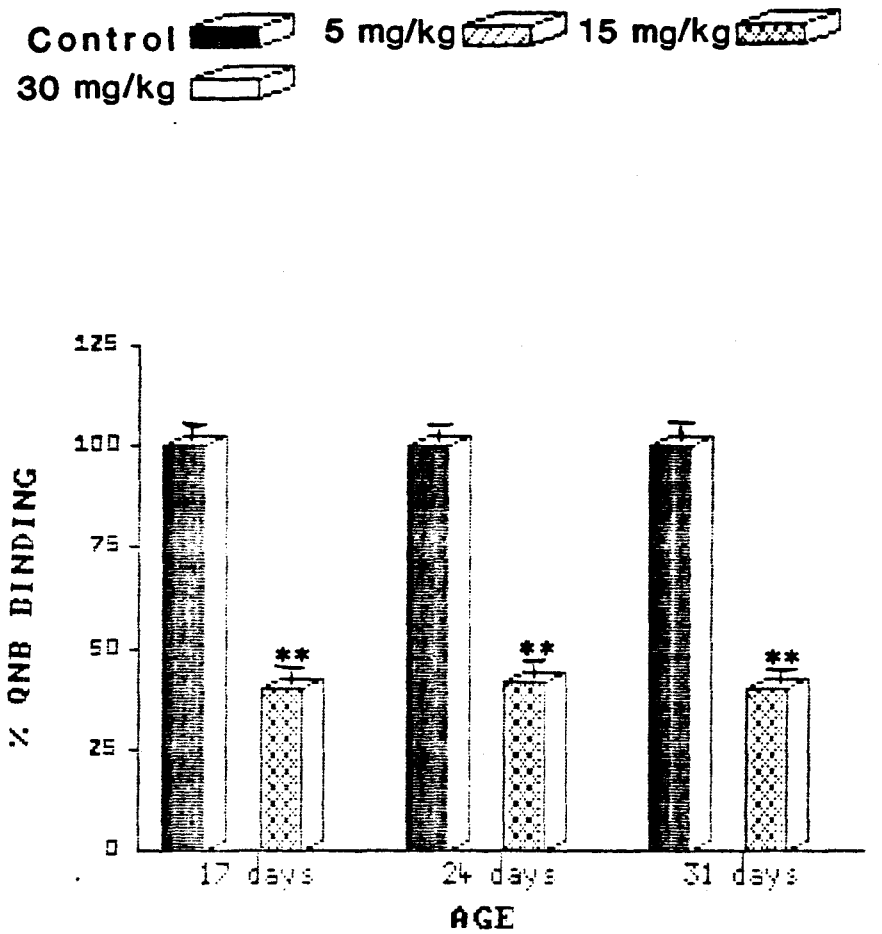


Figure 37. HPLC chromatogram of NA, DA, DOPAC, DHBA, 5HT and HIAA standards.

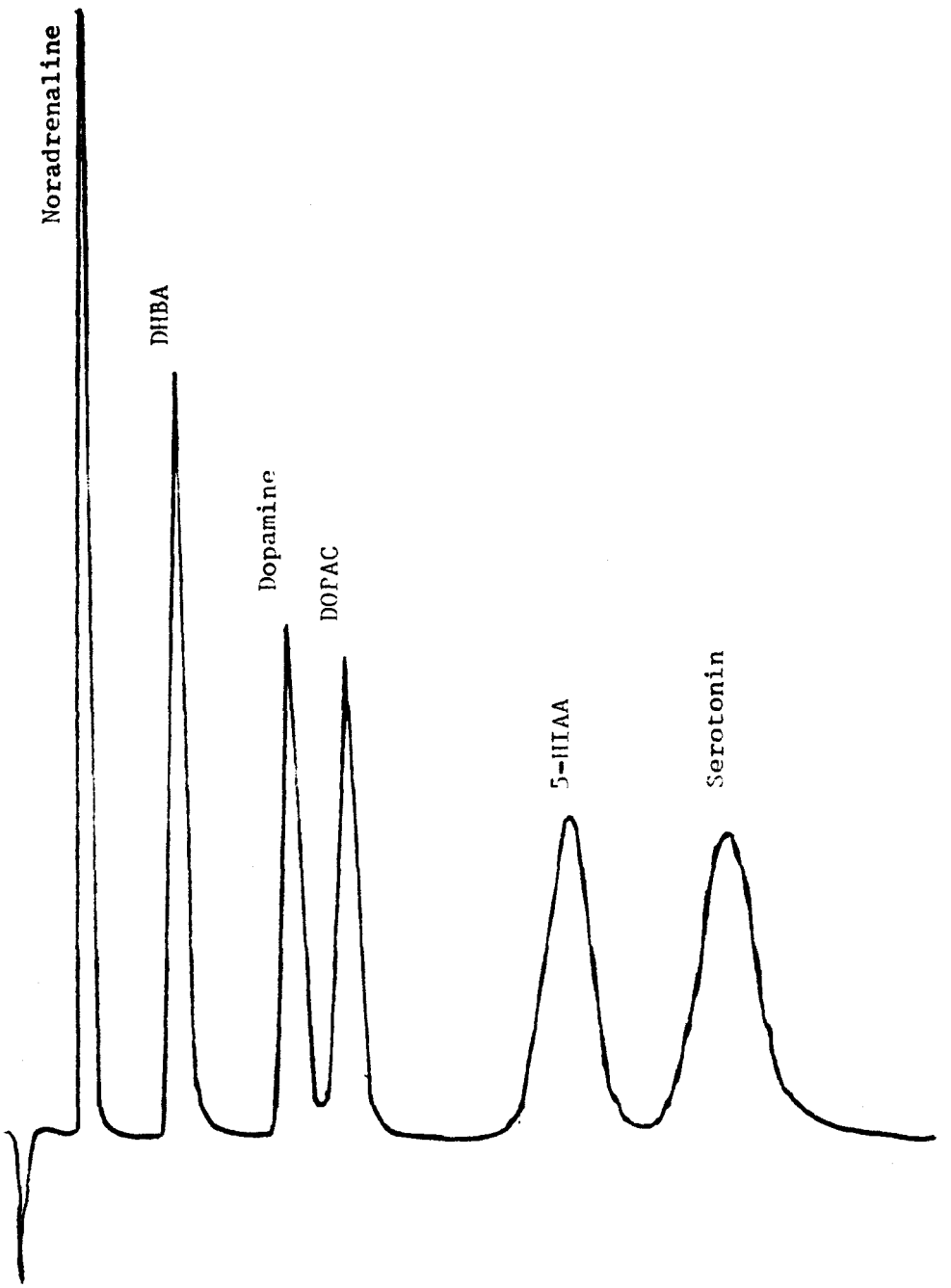
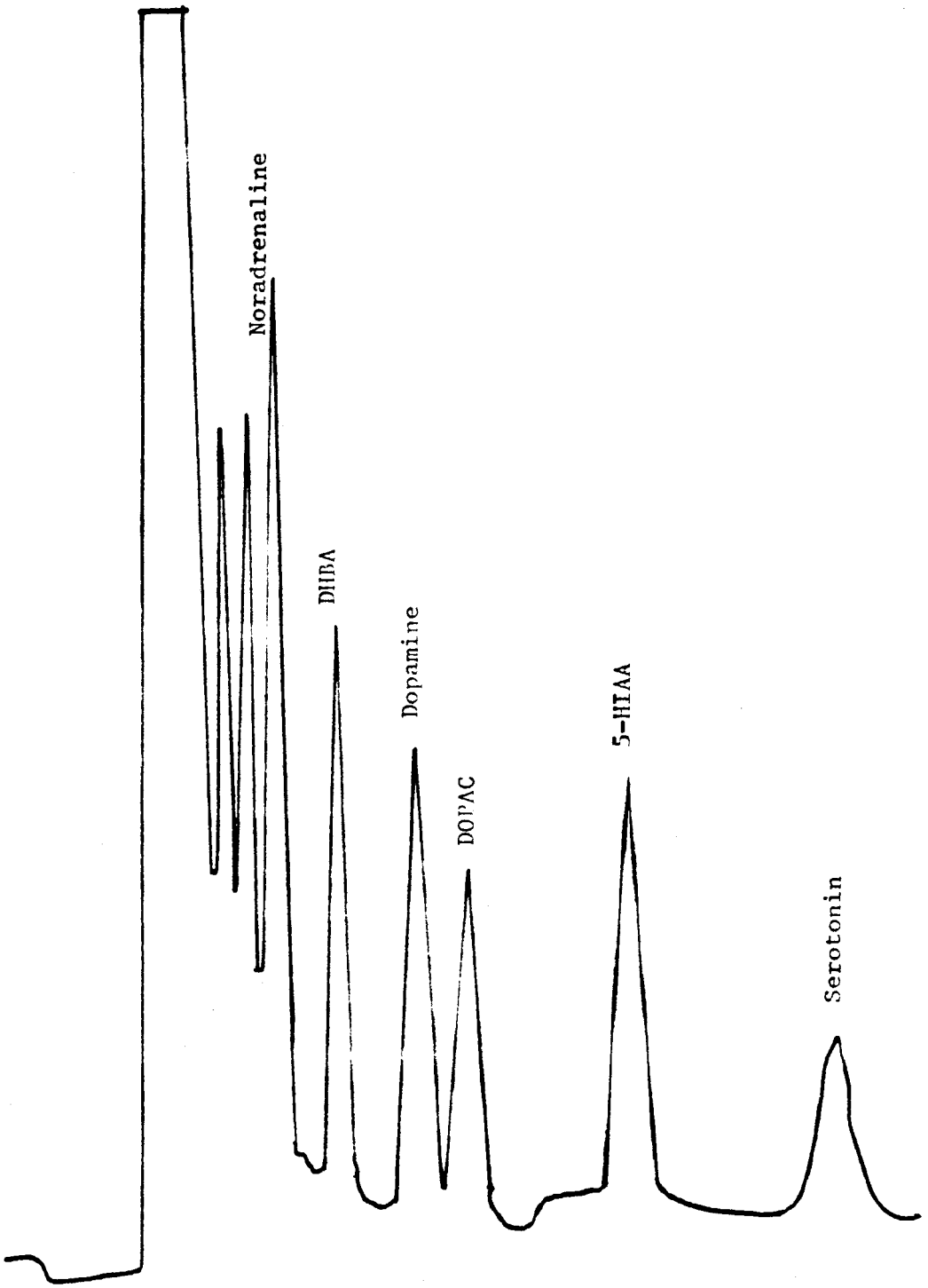

Noradrenaline
DHBA
Dopamine
DOPAC
5-HIAA
Serotonin

Figure 38. HPLC chromatogram of NA, DA, DOPAC, DHBA, 5HT and HIAA from a tissue sample.


Noradrenaline
DIBA
Dopamine
DOPAC
5-HIAA
Serotonin

(Hornykiewicz, 1966), and serotonin and HIAA (Brownstein et al., 1976). DOPAC and HIAA are the major metabolic products of dopamine and serotonin, respectively.

Striatal Biogenic Amine Levels

When offspring were exposed to 5 mg/kg chlordecone gestationally and lactationally, dopamine and DOPAC levels were significantly higher than control fed offspring (Figure 39) at all ages. At a higher dosage of 15 mg/kg/day, dopamine and DOPAC levels were only significantly different in 31 day old animals. Increasing the dosage to 30 mg/kg elicited significantly different dopamine concentrations at 24 and 31 days and DOPAC levels at all ages examined.

Serotonin levels in striatum at most ages and exposure levels were not significantly different from controls (Figure 40). Serotonin levels of 17 day old offspring were significantly increased for 5 mg/kg and significantly decreased for 30 mg/kg chlordecone exposure levels. For 24 day old animals, significant reduction in serotonin levels were observed for exposure levels of 15 and 30 mg/kg. At 31 days of age, only 30 mg/kg chlordecone exposed animals had reduced serotonin levels.

Changes in hydroxyindoleacetic acid (HIAA) levels did not parallel changes in serotonin levels (Figure 40). Significant increases were seen in 5 mg/kg exposed offspring

Figure 39. Striatal DA and DOPAC levels of chlordecone exposed offspring. Striatum from control and chlordecone exposed offspring were homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals $\pm$ the standard deviation. Two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.001$.

STRIATAL DOPAMINE AND DOPAC LEVELS OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg

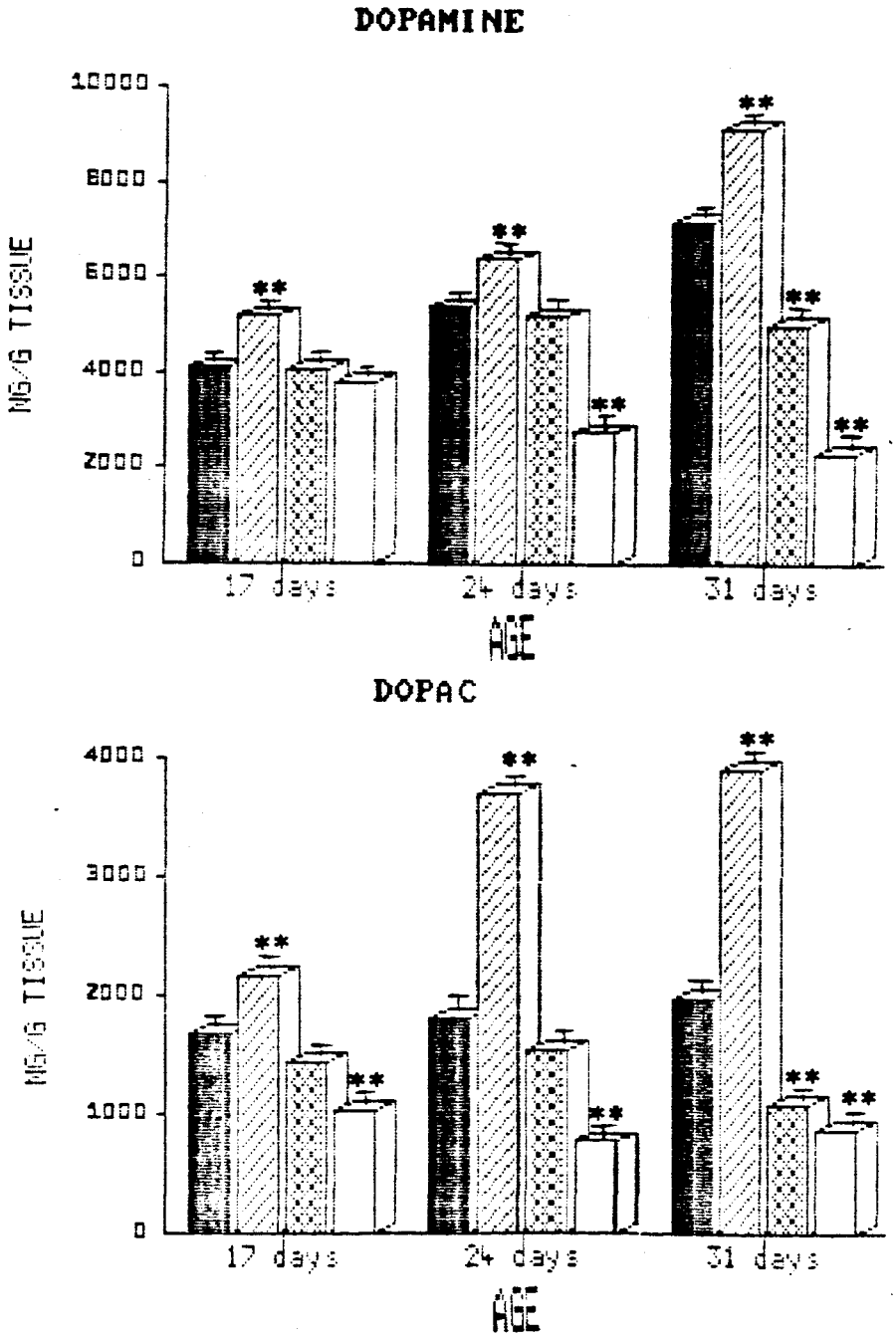
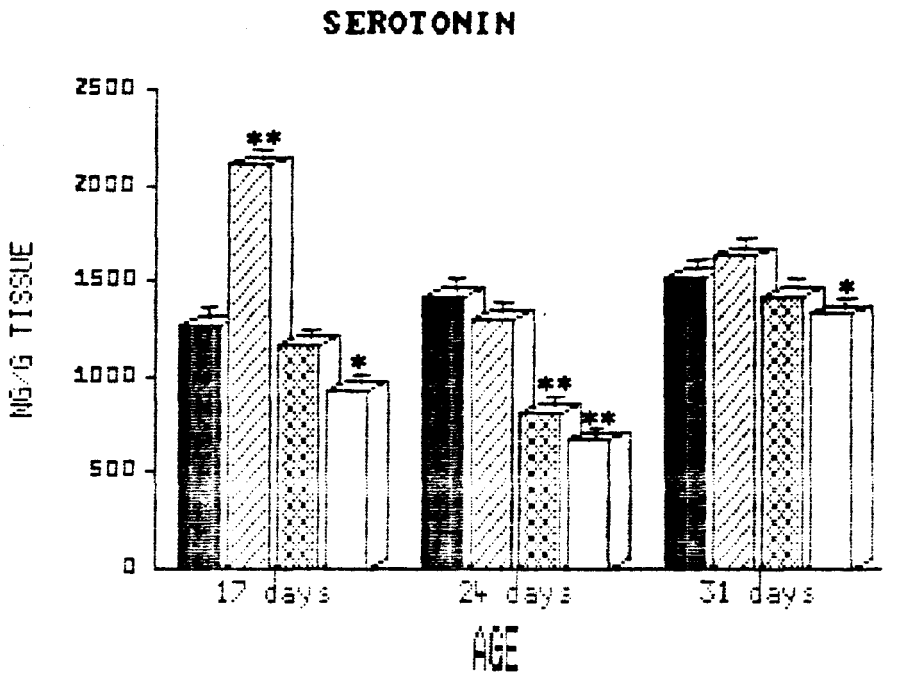
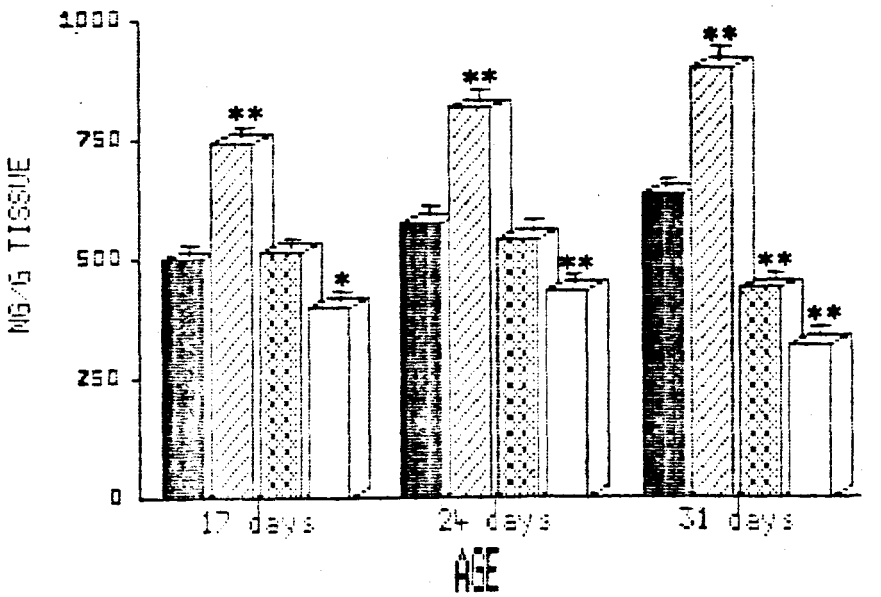


Figure 40. Striatal 5-HT and HIAA levels of chlordecone exposed offspring. Striatum from control and chlordecone exposed offspring were homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

STRIATAL SEROTONIN AND HIAA LEVELS OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg





at all ages examined. In 30 mg/kg exposed offspring, significant decreases in HIAA were observed. For 15 mg/kg chlordecone exposed offspring, significant differences were observed in 31 day old animals only.

Striatal noradrenaline displayed an exposure- and age-dependent decrease (Figure 41). Levels decreased as the age increased and these reductions were inversely proportional to the exposure levels. Noradrenaline concentrations were significantly different from controls at all ages and exposure levels. The magnitude of these differences was $p<0.01$ for 17 and 31 day old 5 mg/kg offspring and $p< 0.001$ for all other groups.

Hypothalamic Biogenic Amine Levels

Serotonin levels in the hypothalamus were significantly different from those of controls except in 24 day old 15 mg/kg exposed offspring (Figure 42). Both 5 and 30 mg/kg exposed animals were significantly different from control at the 0.001 level but the former displayed significant increases while the latter showed decreases. Serotonin levels of 15 mg/kg chlordecone exposed offspring showed a significant increase from 17 to 24 days of age but that increase was negated by 31 days of age.

Hypothalamic HIAA levels paralleled striatal levels in relative differences between exposed groups and controls

Figure 41. Striatal noradrenaline levels of chlordecone exposed offspring. Striatum from control and chlordecone exposed offspring were homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

STRIATAL NORADRENALINE LEVELS OF CHLORDECONE EXPOSED OFFSPRING

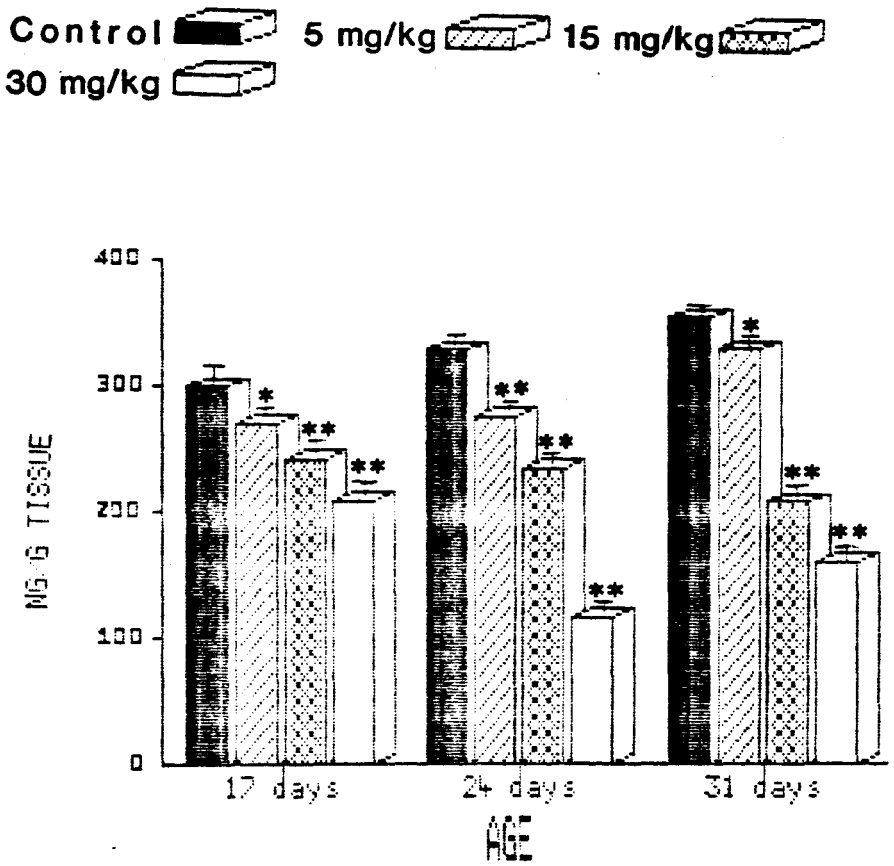
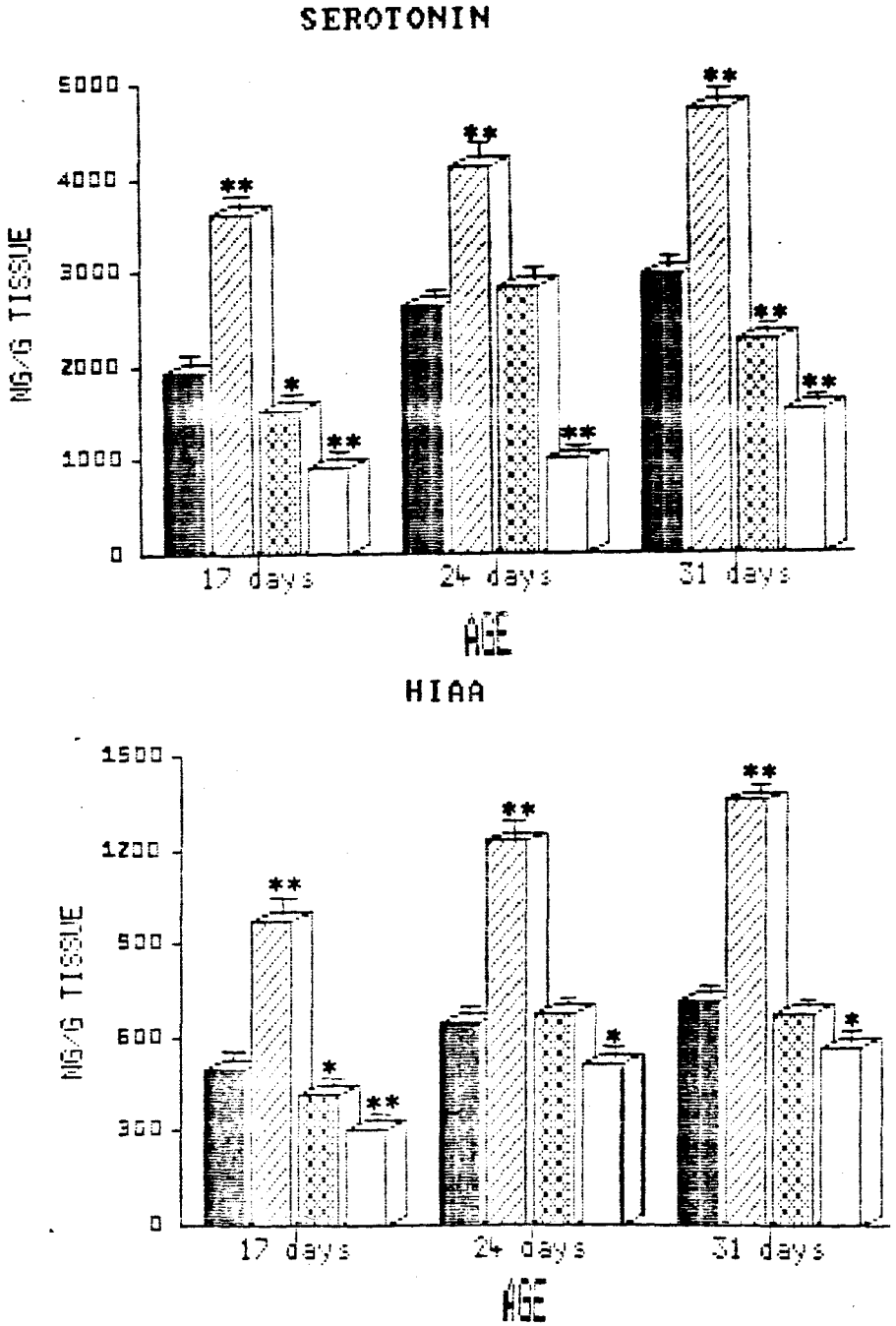


Figure 42. Hypothalamic serotonin and HIAA levels of chlordecone exposed offspring. Hypothalamic from control and chlordecone exposed offspring were homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

HYPOTHALAMIC SEROTONIN AND HIAA LEVELS OF CHLORDECONE EXPOSED OFFSPRING

Control 5 mg/kg 15 mg/kg 30 mg/kg



(Figure 42). There were significant increases in 5 mg/kg exposed offspring at all ages. Significant differences also were seen in 30 mg/kg animals but the differences were less significant at 24 and 31 days of age than at 17 days old. In offspring exposed to 15 mg/kg/day, significant differences were only observed at 17 days of age.

Noradrenaline levels in the hypothalamus were increased significantly in all 5 mg/kg and decreased in all 30 mg/kg animals at all ages examined (Figure 43). Only 31 day old 15 mg/kg chlordecone exposed offspring displayed significantly different noradrenaline levels compared to control fed offspring.

Dopamine levels in the hypothalamus significantly increased with age in 5 mg/kg exposed animals (Figure 44). For 15 and 30 mg/kg exposed offspring, significant differences were observed at 24 and 31 days of age. But the level of difference was greater for 30 mg/kg ($p<0.001$) than for 15 mg/kg animals ($p<0.01$).

Significant increases in DOPAC levels were seen in 24 day old 15 mg/kg and all ages of 5 mg/kg chlordecone exposed offspring (Figure 44). In 30 mg/kg exposed animals, significant reductions in DOPAC levels were observed at 24 and 31 days of age but not at 17 days.

Figure 43. Hypothalamic noradrenaline levels of chlordecone exposed offspring. Hypothalamus from control and chlordecone exposed offspring was homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals ± the standard deviation. Two asterisks (**) indicate that values from control and chlordecone exposed offspring were different at $p<0.001$.

HYPOTHALAMIC NORADRENALINE LEVELS OF CHLORDECONE EXPOSED OFFSPRING

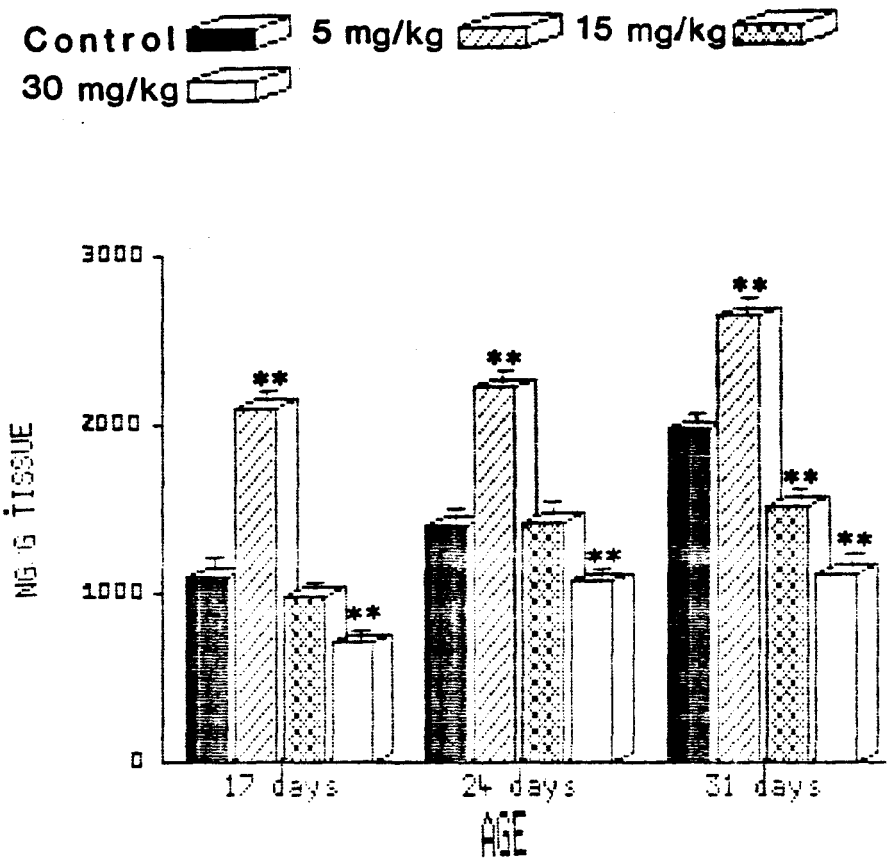
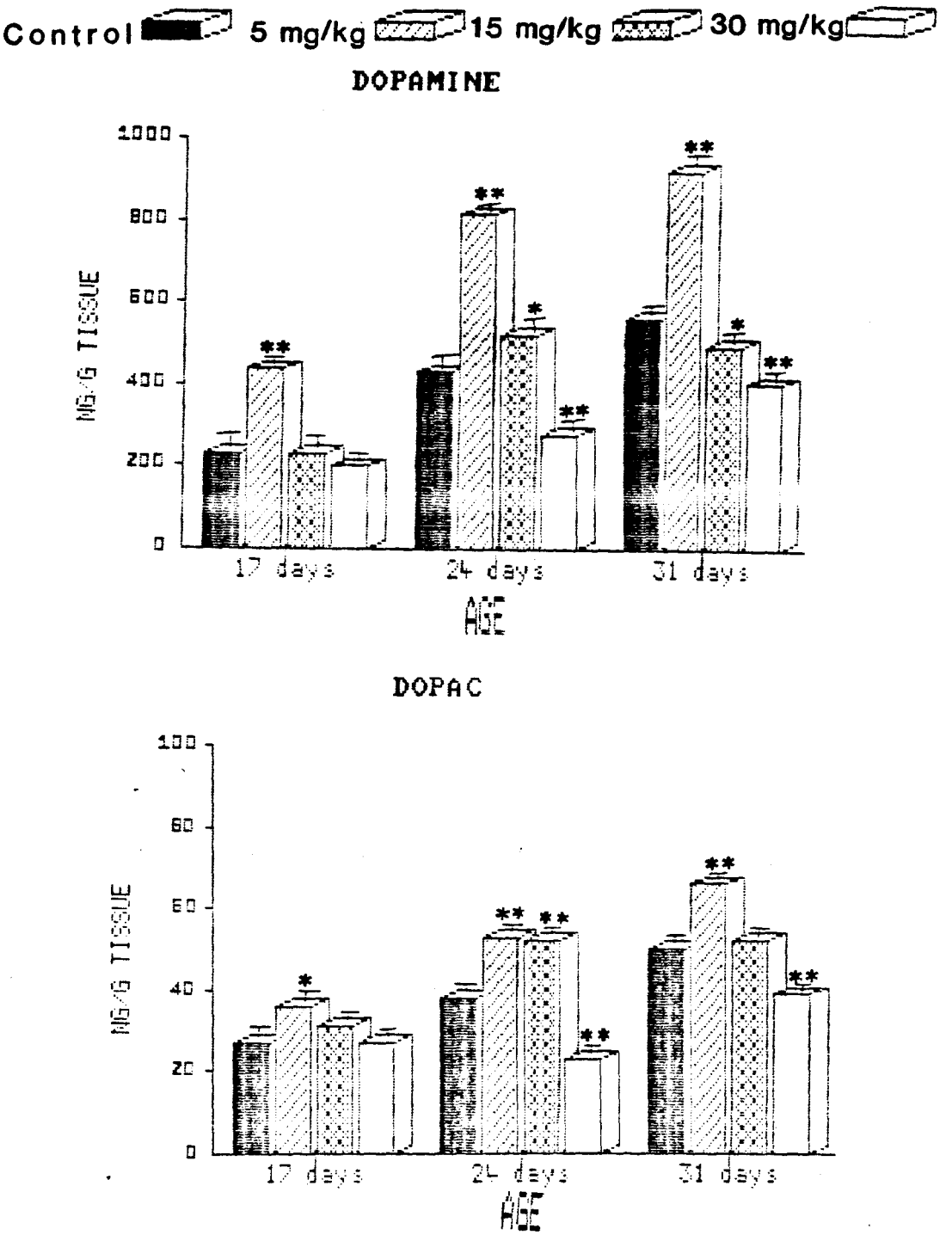


Figure 44. Hypothalamic DA and DOPAC levels of chlordecone exposed offspring. Hypothalamus from control and chlordecone exposed offspring was homogenized in 75% ethanol 20% (w/v). The internal standard DHBA was added to the homogenate to quantitate the percent recovery. The tissue was processed as described in Materials and Methods. Each value represents the mean of 3 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks(**) indicate that values from control and chlordecone exposed offspring were different at $p<0.01$ and $p<0.001$, respectively.

HYPOTHALAMIC DOPAMINE AND DOPAC LEVELS OF CHLORDECONE EXPOSED OFFSPRING



Brain Biogenic Amine Levels

In summary, striatal and hypothalamic biogenic amines increased significantly compared to controls in all ages of 5 mg/kg chlordecone exposed offspring. Conversely, 30 mg/kg exposed offspring also displayed significantly different results but those differences were manifested in absolute reductions in biogenic amine levels. The effects on brain biogenic amines from exposure to 15 mg/kg/day chlordecone were dependent on the amine or its metabolite examined.

CHAPTER V

DISCUSSION

The objective of this research was to examine the effects of maternal chlordecone exposure on receptor-mediated events in developing rat brain. It was proposed that CNS development in the presence of a lipophilic chlorinated insecticide such as chlordecone results in a significant change in the extent of neurotransmitter-stimulated synaptic membrane events.

Biochemical Changes During Development

During growth and development of the brain, there are many changes at the cellular and molecular levels. There is a rapid decline in the rate of protein synthesis soon after birth with a more gradual decline thereafter (Schain et al., 1967). The average half-life of proteins in the rat brain progressively increases with the age of the animal due to an increased synthesis of protein with longer turnover rates (Lajtha et al., 1957). Total protein concentration increases progressively in the cortex and white matter until about 30 days of age in the rat. A decrease in protein concentrations after 30 days of age is due to the accumulation of myelin lipids.

Total brain lipids increase in content during development (Sperry, 1962). Brain lipids consist of cholesterol, phospholipids, and glycolipids. Brain cholesterol and phospholipid content increases during early postnatal development, which parallels the development of membranes (Davison and Dobbing, 1968).

Gangliosides and glycoproteins are carbohydrate-containing lipids and proteins, respectively, that are located in synaptic membranes. Gangliosides and glycoproteins are known to be involved in cell-cell recognition, contact and adhesion processes, and as components of several receptors for neurotransmitters.

Gangliosides are most abundant within the grey matter where they are found in neuronal membranes, although little is known about the distribution of individual ganglioside species within these membranes. Gangliosides increase in content and concentration during brain development (Suzuki, 1965). During the first ten days of normal postnatal brain development in the rat, ganglioside content per cell greatly increases. In the normal rat cortex, ganglioside content peaks at about 20 days of age (Bass et al., 1969). This increase is correlated with the growth of dendritic processes and the formation of synaptic junctional complexes. Most of the evidence that the developmental peak

in ganglioside concentration correlates with the formation of synapses has been obtained from observations that polysialogangliosides have been found to be particularly rich within the synaptic cleft region (Wiegandt, 1967). Synaptic membranes are particularly rich in gangliosides (Tettamanti, 1971).

De Silva et al. (1979) demonstrated developmentally related alteration of rat brain synaptic membrane glycoproteins. They demonstrated a 2-3 fold increase in synaptic membrane glycoprotein sialic acid between 5 and 60 days of age. Recently, Fu et al. (1981) also reported changes in the synthesis of synaptic membrane and synaptic junctional membrane fucosyl- and sialylglycoproteins with postnatal age. In addition to age-dependent changes in glycoprotein synthesis, there appear to be environmental influences on glycoprotein synthesis. Burgoyne and Rose (1980) observed altered incorporation of tritiated fucose and lysine in the visual cortex of dark-reared rats upon first exposure to light. Studies by Sukumar et al. (1980) on learning and memory provided additional support for environmentally related alterations of glycoprotein synthesis.

Therefore, the development of the brain involves the interaction of many membrane constituents. The findings

discussed above demonstrate that synaptic plasma membranes are composed of many complex components which collectively control intra- and extramembrane processes. Therefore, these components could individually or collectively affect the cellular and molecular processes involved in the parameters examined in this study. In view of the fact that the effects of chlordecone on selected membrane components or associated events have not been previously examined except in the case of sodium-potassium ATPases, there were endless processes that could have been selected as a starting point. In an attempt to elucidate the possible effects of chlordecone on the developing membrane system, this study examined a neurotransmitter-stimulated membrane event as opposed to the parameters discussed above.

Maternal Weight Gain and Diet Consumption

Based on consumption of diet both during and after gestation, control and chlordecone fed mothers received a comparable amount of protein and calories. The average weights of the mothers were comparable during the first week of liquid diet consumption. By the third week, the relative weight gains for 5, 15 and 30 mg/kg exposed mothers were 86, 82 and 77% of control. Chernoff and Rogers (1976) observed similar reductions in maternal body weights in chlordecone exposed animals. They suggested that these differences were

due to the reduced size of the litters. But in the present studies, these weight reductions persisted three weeks after birth of the offspring in both chlordecone and control fed (chlordecone exposed during gestation) mothers. Therefore these differences were not due to variable diet consumption, litter size or continuous chlordecone consumption. Consequently, these differences could be related to the interaction of an unforeseen nutritional parameter with chlordecone such as absorption and/or utilization of nutrients.

Brain and Body Weights of Offspring

Brain and body weights of offspring reflect the different consumption of chlordecone by mothers fed the 5, 15 or 30 mg/kg diet. In general, brain and body weights of 5, 15 and 30 mg/kg exposed offspring were significantly lower than those of age-matched control pups. The brain and body weights were inversely proportional to the concentration of exposure. The normal body and brain weights of age- and diet-matched control and chlordecone exposed offspring in these studies are consistent with previous studies (Chernoff and Rogers, 1976; Seth et al., 1981; Chernoff et al., 1979; Mactutus et al., 1982; Tilson et al., 1982). However, the above studies mentioned did not utilize as carefully planned exposure schedules as

described here. This is the first study to demonstrate linear dose-dependent body and brain weight reduction in offspring following in utero chlordecone exposure. In addition, results presented in this study dispel the notion that these effects were due to maternal malnutrition alone. Crossfostered offspring still displayed reduced body and brain weights even 31 days after cessation of the chlordecone diet. Therefore, any abnormalities found in the present study are due to effects of chlordecone on the prenatal development of in utero exposed offspring.

Brain Chlordecone Levels

Fujimori et al. (1982) demonstrated that after a single dose of chlordecone, brain levels were proportional to the amount given and that these levels reached a maximum concentration at 6 hours after treatment. The chlordecone concentrations remained at that level up to 96 hours after administration. In agreement with these findings, End et al. (1978) reported that the half-life of chlordecone in the brain was in excess of 17 days.

Other studies have not attempted to investigate the effects of maternal consumption of chlordecone on brain concentrations of chlordecone in the offspring. This study has demonstrated that chlordecone levels in cerebral

hemispheres of gestationally and lactationally exposed offspring were directly proportional to the exposure levels. This level reached a maximum concentration by 17 days of age and maintained that level through at least 31 days. Therefore, the maximum brain levels of chlordecone were probably reached before 17 days of age as partially attested to in single dosed animals. A significant finding of these studies was that it appears that crossfostering of 5, 15 and 30 mg/kg exposed offspring postpartum with control mothers did not significantly reduce the brain chlordecone levels even 31 days after cessation of exposure. It was not until 3 months after cessation that the chlordecone levels were reduced to 3 ug/g tissue and 9 months until the levels were undetectable. Therefore, these studies show that after chronic dosing of chlordecone, it takes at least 3 months before chlordecone is cleared from the brain. This extends the knowledge from prior studies in which Egle et al. (1978) demonstrated that clearance of chlordecone from rat brain after a single bolus required at least 8 weeks.

Therefore, these studies have demonstrated that brain chlordecone levels (1) are proportional to exposure levels, (2) are maximum by 17 days of age and are maintained through 31 days of age, whether the exposure was throughout

gestation and lactation or during gestation alone, and (3) persist at least 3 months after cessation of dosing.

Membrane Protein and Phospholipid Concentrations

Synaptic plasma membrane protein concentrations of 17, 24 and 31 day old pups were in agreement with levels seen by Smith (1983). There are no apparent changes in total membrane protein concentrations at any of the ages or exposure levels examined.

Similar results were observed in synaptic plasma membrane phospholipid concentrations as were observed with protein concentrations. Phospholipid distribution was not affected at any of the exposure levels or ages examined. The distribution of phospholipids from 17, 24 and 31 day old pups was in agreement with levels seen by Fontaine (1980).

However, the results do not rule out the possibility that the exposure of developing membrane to varying doses of chlordecone affects the protein and phospholipid composition of those membranes. Any effects observed that involve these membranes could be due to one or more of the following factors: (1) the preparation used for isolation of synaptic plasma membranes could have selected for membranes of normal density and hence, protein yield and phospholipid composition; (2) the yield of the synaptic plasma membranes was so low that the changes in protein or phospholipid

concentration and distribution were undetectable; or (3) the effects of *in utero* chlordecone exposure were manifested in the alteration of other membrane constituents such as gangliosides and glycoproteins.

Phospholipid Stimulation

Hokin and Hokin (1958) were the first to show the neurotransmitter-stimulated phospholipid turnover with acetylcholine (ACh) in guinea pig cortex slices. Sympathetic ganglia, known to be rich in synapses, also showed a similar effect in response to ACh (Hokin *et al.*, 1960), electrical stimulation (Larrabee *et al.*, 1963) or potassium ions (Nagata *et al.*, 1973). Since then a number of investigators have demonstrated that cholinergic and adrenergic neurotransmitters stimulate the incorporation of radiolabeled orthophosphate into phospholipids of neural tissue both *in vivo* (Friedel and Schanberg, 1973; Friedel *et al.*, 1973; Margolis and Heller, 1963) and *in vitro* (Abdel-Latif *et al.*, 1974; Hokin, 1969). It is now well established that the neurotransmitter effects occur chiefly in phosphatidic acid and the inositol phospholipids and require external calcium, a condition also necessary for transmitter release (De Belleroche and Bradford, 1972). The physiological significance of the neurotransmitter effect is

not clear, but available evidence suggests that it is related to the binding of the neurotransmitter to its respective receptor and subsequent activation of a stimulus-response cascade (Gambetti et al., 1974; Hawthorne and Bleasdale, 1975).

Reddy and Sastry (1979) proposed that this phenomenon is related to an aspect of synaptic function that could serve as a measure of synaptic density or synaptogenesis in developing brain. If this hypothesis is true, a progressive increase in the magnitude of the neurotransmitter effect should be observed with increase in age in the developing brain. Furthermore, a study of the neurotransmitter effect in chlordecone-exposed offspring affords a means of assessing nutritional damage to synaptogenesis or synaptic ultrastructure and function. For this purpose, however, it was necessary to have suitable brain preparations that give reproducible neurotransmitter effects. Experiments with brain slices are highly variable (Hokin and Hokin, 1958; Hokin, 1969) and the stimulatory effect is dependent on the thickness of the slices (Abdel-Latif et al., 1974). On the other hand, the neurotransmitter effect is not usually observed with brain homogenates. Even with particulate fractions, Redman and Hokin (1964) obtained highly variable stimulation.

The experiments reported here show that purified synaptosomal preparations exhibit highly reproducible neurotransmitter effects. The results for control animals were in agreement with the magnitude of stimulation seen by Reddy and Sastry (1979) in similarly aged animals. The neurotransmitter effects with these preparations were mainly associated with phosphatidic acid and inositol phospholipids, and maximal stimulation was observed with 1 mM carbamylcholine and 10 mM noradrenaline or serotonin. These concentrations, though apparently high, are probably in a physiological range. Earlier workers (Abdel-Latif, 1974; Hokin and Hokin, 1955; Lapetina and Michell, 1972) have used 10 mM carbamylcholine, noradrenaline and serotonin in experiments with slices. In fact, Hokin (1969) estimated the levels of biogenic amines to be around 100 mM in synaptic vesicles.

The incubation of the receptor antagonists atropine, haloperidol or regitine in the presence or absence of carbamylcholine, dopamine or noradrenaline, respectively, resulted in little or no stimulation. Bentazole in conjuction with histamine only slightly decreased the stimulation. These results suggest that the stimulation of phospholipid turnover and resultant inhibition of stimulation seen in control and chlordecone exposed

offspring, respectively, are due to a receptor mediated event.

Experiments on the neurotransmitter effect on synaptosomes from cortical regions of developing rats demonstrated that there was a progressive increase in synapses during the weanling period (birth to weaning), as observed by Reddy and Sastry (1979). Therefore, the relative magnitude of stimulation for phosphatic acid and the phosphatidylinositols increased with age within an exposure group. These experiments verify the previous results by Reddy and Sastry (1979) that the neurotransmitter effect is a valid biochemical correlate of synaptogenesis in developing brain.

The neurotransmitter effect determined under comparable conditions in chlordecone-exposed offspring was significantly different from normal brains. In chlordecone exposed offspring, neurotransmitter stimulation of phospholipid metabolism was significantly reduced in a dose- and exposure time-dependent manner. The magnitude of the reduction was dependent on the neurotransmitter, but the overall stimulation pattern paralleled unexposed groups at all ages and exposures.

These results suggest that exposure to chlordecone prenatally may affect processes involved with synaptogenesis

during the weanling period. This hypothesis is supported by studies done on the effects of malnourishment (Reddy and Sastry, 1978; Balazs and Patel, 1973), DDT and related chlorinated hydrocarbon insecticides (Hrdina et al., 1975), and estrogen (Arai and Matsumoto, 1978). These investigators demonstrated that exposure to these factors during the vulnerable period of growth impairs brain maturation and might lead to an irreversible deficit in higher mental function. Morphologically these studies have shown that migration of cells is retarded, proliferation of neuronal fibres decreased and formation of synapses and myelin reduced. Reddy and Sastry (1978) also demonstrated that gangliosides but not phospholipids were significantly reduced in undernourished rats. With the knowledge that gangliosides are particularly rich in synaptic membranes and that these lipid components may be directly involved in membrane phenomena, their metabolism in chlordecone-exposed offspring could contribute either directly or indirectly to the reduction in the magnitude of the 'PI effect' observed.

Mechanism of Phospholipid Turnover

One possible mechanism of chlordecone neurotoxicity has been suggested that inhibition of mitochondrial and synaptosomal membrane bound sodium-, potassium- ATPases (Desaiah, 1981). These observations could possibly account

for the decreased turnover due to the lack of energy needed to drive the subsequent resynthesis after initial phospholipid breakdown. However, Abdel-Latif et al. (1968) demonstrated that addition of metabolic inhibitors to ATP formation did not inhibit the phosphorylation of phosphatidic acid and inositol phospholipids. Thus the small residual ATP-generating capacity was sufficient to drive the biosynthesis of phospholipids after neurotransmitter stimulated breakdown.

The results presented in this study demonstrate that the extent of phospholipid turnover was dependent on the concentration of chlordecone the offspring received and the cumulative dose to which the offspring were exposed. Therefore, a pup from a mother given 15 mg/kg/day chlordecone during gestation and lactation or gestation alone displayed significant differences in phospholipid turnover at 17 days of age compared to control, but those differences were even greater at 31 days of age. Other investigators (Cohn et al., 1978; Taylor et al., 1978) suggested that chlordecone disrupted membrane processes by being present in the membrane and that decreasing concentrations resulted in decreased effects. These investigators also speculated that when the chlordecone was removed from the brain, the "neurotoxicity" associated with

the presence of chlordecone would be reversed. The present study contradicts this assumption, in that by 3 and 9 months after cessation of dosing, the brain chlordecone concentrations are either low (3 ug/g tissue) or not detectable. Nevertheless, neurotransmitter-stimulated phospholipid labeling was suppressed to nearly the same extent in these offspring as in 31 day old offspring having cerebral chlordecone levels of 30-40 ug/g.

Chang-Tsui and Ho (1980) suggested that one of the actions of chlordecone in the central nervous system is on neurotransmitter and receptor interaction. This would seem a likely hypothesis according to the data that have been generated by other investigators. Phospholipid metabolism changes are involved in actions consequent to agonist-receptor interactions in many physiologically important systems. In particular, activation of muscarinic cholinergic receptors has been implicated in enhanced phospholipid turnover in a number of tissues (Michell, 1975; Hawthorne and Pickard, 1979; Berridge, 1981). This study has sought to utilize this phenomenon to discern whether the reduced phospholipid turnover may be due, in part, to the inability of the agonist to stimulate this receptor coupling. This loss of receptor-coupled stimulation could

be due to reduced receptor concentrations or receptor binding affinity.

Receptor Density and Affinity

Synaptic transmission provides a basis for intercellular communication in the nervous system. For many years, it has been suggested that synaptic transmission is a regulated process (Muknerjee et al., 1980). On a molecular basis, modulation of synaptic transmission could likely occur through regulation of neuroreceptor concentration (Schaeffer and Hsueh, 1980). The most widely used standard method for the identification of cell-surface receptors involves studies on the binding of specific radioactive ligands in the presence or absence of competitors in order to determine the extent of specific and nonspecific binding in preparations of the tissue being examined.

In the present study, sufficient chlordecone was administered to elicit mild to severe tremors in 5 to 30 mg/kg chlordecone exposed offspring but no lethality was observed at these dose levels. Several studies have suggested that neurons associated with central catecholamines are affected by chlordecone. Decreases in tritiated spiroperidol binding to striatal membrane preparations were observed in rats fed as little as 10 ppm chlordecone (Seth et al., 1981). This study also observed

chlordecone-dependent reductions in receptor binding but additionally demonstrated that these reductions were dependent on the exposure level of chlordecone and the age of the offspring. Seth et al. (1981) also observed decreased cerebellar tritiated QNB binding but discounted that the reductions were due to increases in non-receptor interfering proteins rather than alterations in binding sites. This study contradicts the above findings and has shown that muscarinic receptor concentrations are significantly reduced at all ages and exposure levels. The decreases in receptor concentrations were age-independent and exposure-dependent. Crossfostered offspring were examined for effects on receptor concentrations. These studies confirmed the results seen with noncrossfostered animals in that the reduction was age-independent and dependent on the level of in utero chlordecone exposure. Even 9 months after cessation of dosing and clearance of the brain chlordecone levels, significant reduction in muscarinic receptor binding was still observed. Therefore, the muscarinic receptor binding studies support the hypothesis that chlordecone may be developmentally neurotoxic.

The causes of this developmental neurotoxicity are only speculative, but the most plausible explanation for

the decreased inositol phospholipid stimulation and receptor concentration and affinity involves direct or indirect effects on the receptor complex and/or reduced numbers of synapses. One possible mechanism is that chlordecone may alter neuronal connectivity; chlordecone may induce a permanent structural modification of synaptic terminals by acting as an estrogenic agent (discussed later).

These effects could be manifested in the inhibition of synthesis of a necessary membrane or receptor component. Lapetina et al. (1967) elucidated that ganglioside-rich nerve endings contain acetylcholine, serotonin, noradrenaline, dopamine and histamine. The highest concentrations of these neurotransmitters were observed in cholinergic synaptic membranes. These investigators also demonstrated that within these ganglioside-rich nerve endings, (Na -K)-activated ATPase, adenylate cyclase, and the particulate phosphodiesterase were also concentrated. Little is known about the production or structure of the components of biogenic amine receptors.

Neurotransmitter Levels

The data collected from previous experiments regarding neurotransmitter levels in animals exposed to chlordecone are varied and contradictory. Aldous et al. (1984) observed no alterations in steady state levels of noradrenaline (NA)

and dopamine (DA), nor of their metabolites, MOPEG and DOPAC, 24 hours after a single dose of 25, 50 or 100 mg/kg. These findings were consistent with those of other investigators using moderate doses of chlordecone. End et al. (1981) found similar results in mouse brain NA or DA at 24 hours after administration of 40 mg/kg chlordecone. Negative findings by End et al. (1981) suggest that if alterations in brain catecholamine synthesis do occur, they are not of significant magnitude and are in the direction of reduced synthesis.

Earlier observation that chlordecone is a potent inhibitor of ATPases associated with uptake and storage of catecholamines (Desaiah et al., 1980; Desaiah and Koch, 1975; Chang-Tsui and Ho, 1980) led to anticipation that brain neurotransmitter levels and synthesis might be appreciably affected.

Rosecrans et al. (1982) observed that chlordecone affected indoleamine containing neurons. In each brain area evaluated, chlordecone-treated rats exhibited a higher rate of turnover of serotonin (5-HT), at least when measured by either 5-HIAA or 5-HIAA/5-HT ratio. Squibb and Tilson (1982) indicated that high doses of chlordecone increased 5-HT turnover in the adult rat, which provides additional

evidence that this neurotoxicant has effects on this biogenic amine.

Fujimori et al. (1982) reported decreases in mouse striatal DA after 9 days dosing with 25 mg/kg. They also found a decrease in mouse brain DA after 2 days administration of 50 mg/kg/day, a tremorigenic dose of chlordecone. Chang-Tsui and Ho (1980) reported the effects of chlordecone on synaptosomal uptake of DA. Both in vitro and in vivo effects of chlordecone on DA uptake into mouse brain synaptosomal fractions were shown to be inhibitory and concentration- and age-dependent.

The results from the studies here are consistent with the results discussed above but also carry the experiments a step further. All of the experiments discussed above involved acute or chronic dosing of adult animals. This study provides evidence for the neurotoxic or teratogenic effects of chlordecone on offspring exposed pre- and postnatally. In both brain areas examined, DA, 5-HT and their respective metabolites were significantly increased compared to control for 5 mg/kg exposed offspring at all ages. Conversely, all biogenic amine levels were significantly decreased at all ages in 30 mg/kg exposed offspring. Biogenic amine levels in offspring from mothers given 15 mg/kg/day chlordecone did not follow a specific

pattern but were dependent on the biogenic amine, age and brain area examined.

The data presented suggest the possible involvement of DA and 5-HT in chlordecone-induced neurotoxicity. It has been proposed that a relationship exists between DA and 5-HT in the caudate (Goldstein et al., 1971). Makseed and Baker (1973) have suggested that 5-HT plays a modulatory role in the caudate in the expression of tremor and that its action is antagonized by DA. The present study has shown that there were reduced dopamine receptor concentrations in 31 day old offspring from mothers exposed to 15 mg/kg/day and that the reduced receptor concentrations did not result in increased dopamine concentrations.

Effects of Chlordecone on Developing Rat Brain

A plausible mechanism by which the presence of chlordecone could permanently alter brain function involves chlordecone acting as an estrogenic agent during the perinatal "critical period." The term "critical period" implies a specific period during developmental differentiation when the brain can or will respond to steroid exposure with permanent changes (Tarttelin et al., 1975). Therefore, the critical period is the time during which organizational actions of the gonadal steroids are expressed. In the rat, the upper limit of this period is

generally considered to be contained within the first ten days of life. However, the sensitivity to steroids is much greater during the first half of the time period (Christensen and Gorski, 1978). Several investigators have demonstrated that high concentrations of gonadal hormones, specifically estrogen, during the perinatal critical period inhibit synaptogenesis (Gordon et al., 1977; Nomura et al., 1982; Gurney, 1981; Raisman and Field, 1973). It has been shown by several investigators that chlordecone acts as a weak estrogenic agent (Chernoff and Rogers, 1976; McFarland and Lacy, 1969). Therefore, high concentrations of chlordecone during the critical period of synaptogenesis could affect the developmental processes responsible for neurotransmitter-stimulated phospholipid turnover, receptor concentration, and synthesis and turnover of biogenic amines by exerting an "estrogenic" effect. Thereby, the results of this "estrogenic" effect are not only the loss of dendritic processes and synapses during early development but a long-term neurotoxic effect in the absence of chlordecone.

CHAPTER VI

CONCLUSIONS

It was proposed that CNS development in the presence of a lipophilic chlorinated insecticide such as chlordecone results in a significant change in the extent of neurotransmitter-stimulated membrane events. Therefore, the objective of the research described in this dissertation was to examine specific biochemical constituents and events such as receptors, biogenic amines and phospholipid labeling in central nervous system (CNS) cortical regions and synaptosomes during early development. Offspring of Sprague-Dawley rats were pair-fed a chlordecone-containing diet on a chronic basis throughout gestation and lactation or during gestation alone.

The present series of experiments provides new and exciting information concerning the effects of pre- and postnatally administered chlordecone on a neurotransmitter-stimulated membrane event throughout early adulthood. This is the first time so many interrelated biochemical parameters have been measured and correlated in chlordecone exposure ranges of this magnitude and duration. The biochemical and neurochemical evaluations of the offspring have revealed five separate findings: (1) body and brain

weights were reduced by chlordecone in a dose-dependent manner; (2) protein and phospholipid concentrations and distributions were not dependent on dose or age; (3) neurotransmitter stimulation of phospholipid turnover was significantly reduced in a dose- and age-dependent manner; (4) muscarinic and dopaminergic receptor binding were also reduced in the same manner; (5) biogenic amine levels in hypothalamic and striatal tissue were significantly increased in offspring exposed to 5 mg/kg chlordecone and significantly reduced in 30 mg/kg exposed offspring.

In all well designed experiments involving the measurement of several dependent variables, one is tempted to speculate on the relationships among all of these variables. The above results could indicate any or all of the following speculative explanations.

Brain and body weights, phospholipid turnover and receptor binding of exposed offspring could be reduced due to the interaction during the critical period between chlordecone and a developmentally necessary process such as membrane/receptor production. As the dosage of chlordecone exposure is increased, there is a severe infringement on this process or related processes that cause even greater reductions in these parameters. This may involve the inhibition of the production of membrane/receptor

constituents--for example, gangliosides or glycoproteins, as discussed.

If the membrane/receptor environment alone is disturbed by the presence of chlordecone, as in the case of noncrossfostered and crossfostered offspring up to 31 days of age, then this could serve as a possible explanation for the reduced phospholipid labeling and receptor binding seen at those ages. But this could not explain the persistent decreases in phospholipid labeling and muscarinic binding seen in 9 month old offspring in the absence of chlordecone, nor the lack of *in vitro* effects of chlordecone on these processes. Therefore, there might be a disturbance in the membrane/receptor environment by the presence of chlordecone but the effects of that disturbance are not significant enough to cause long-term neurotoxic effects.

Therefore, although significant differences have been demonstrated between pre and postnatally exposed control and chlordecone offspring, it is very difficult to pinpoint the mechanism by which these effects are manifested. One possible mechanism is that chlordecone could permanently alter brain function by acting as an estrogenic agent during the perinatal "critical period." The presence of high concentrations of chlordecone in developing brain

may alter neuronal conductivity and induce permanent structural modification of synaptic terminals.

In view of the promising directions and conclusions of these experiments as to the possible involvement of chlordecone with specific biochemical events *in utero*, further studies are necessary to elucidate the mechanisms involved in chlordecone induced neurotoxicity.

To investigate the possibility that chlordecone is acting as an estrogenic agent, the following experiments could be performed. An estrogenic agent such as diethylstilbesterol (DES) could be given to mothers, and the effects on neurotransmitter-stimulated phospholipid turnover, receptor binding and biogenic amine levels could be examined. DES has a longer biological half-life than other estrogenic compounds such as estradiol so that the concentrations *in utero* would be similar to the concentrations of chlordecone. To determine whether chlordecone exerts its effects during the "critical period," chlordecone could be administered during the first 10 days of life. In addition to examining the biochemical processes discussed in this dissertation, other parameters such as synaptic plasma membrane constituents, synaptic cell

size and number, and neuronal connectivity could be investigated.

Therefore, the work presented in this dissertation has demonstrated that chlordecone causes significant changes in membrane-associated events, and these results have opened to new avenues to explore as to the possible mechanism of chlordecone's actions.

REFERENCES

Abdel-Latif, A. A. (1983). In Handbook of Neurochemistry, Vol. 3, 2nd Ed., ed. A. Lajtha, 91-131. Plenum Press, New York.

Abdel-Latif, A. A., Akhtar, R. A., and Hawthorne, J. N. (1977). Acetylcholine increases the breakdown of triphosphoinositide of rabbit iris muscle prelabelled with 32 phosphate. Biochem. J. 162: 61-73.

Abdel-Latif, A. A., Yamaguchi, T., Yamaguchi, M., and Chang, F. (1968). Studies on radioactive phosphorus incorporation into nucleotides, phospholipids and phosphoproteins of isolated nerve endings from developing rat brain. Brain Res. 10: 307-321.

Abdel-Latif, A. A., Yau, S.-J., and Smith, J. P. (1974). Effects of neurotransmitters on phospholipid metabolism in rat cerebral-cortex slices--cellular and subcellular distribution. J. Neurochem. 22: 383-393.

Acree, F., Abramson, I., and Ceuce, J. (1963). Facts on the use of pesticides. J. Med. Pharm. 16: 425.

Agranoff, B. W. (1978). Approaches to the biochemistry of regeneration in the central nervous system. T.I.B.S. 3: 283-285.

Agranoff, B. W., Murthy, P., and Seguin, E. B. (1983). Thrombin-induced phosphodiesteratic cleavage of phosphatidylinositol biphosphate in human platelets. J. Biol. Chem. 258: 2076-2078.

Agrawal, A. K., and Squibb, R. E. (1980). Effects of acrylamide given during gestation on dopamine receptor binding in pups. Toxicol. Lett. 7: 233-238.

Agrawal, A. K., Squibb, R. S., and Bondy, S. C. (1981). The effects of acrylamide upon the dopamine receptor. Toxicol. Appl. Pharmacol. 58: 89-99.

Akhtar, R. A., and Abdel-Latif, A. A. (1980). Requirement for calcium ions in acetylcholine-stimulated phosphodiesterase cleavage of PtdIns4,5P in rabbit iris smooth muscle. Biochem. J. 192: 783-791.

Aldous, C. N., Chatty, C. S., Mehendale, H. M., and Desaiah, D. (1984). Lack of effects of chlordecone on synthesis rates, steady state levels and metabolites of catecholamines in rat brain. Neurotoxicol. 5: 59-66.

Allan, D., and Michell, R. H. (1978). A calcium-activated PPI phosphodiesterase in the plasma membrane of human erythrocytes. Biochim. Biophys. Acta 508: 277-286.

Allan, D., and Michell, R. H. (1979). The possible role of lipids in control of membrane fusion during secretion. Society for Experimental Biology Symposium No. 33: 323-336.

Anderson, J. H. Jr., Cohn, W. J., Guzelian, P., Taylor, J. R., Griffith, F. D., Blanke, R. V., dos Santos, J. G., and Blackard, W. G. (1976). Effects of kepone associated toxicity on testicular function. Presented at 24th Ann. Meet. Endocrine Soc., San Francisco.

Arai, Y., Matsumoto, A. (1978). Synapse formation of the hypothalamic arcurate nucleus during postnatal development in the female rat and its modification by neonatal estrogen treatment. Psychoneuroendocrinol. 3: 18-45.

Arias, I. M., Ohmi, N., Bhargava, M., and Listowsky, I. (1980). Ligandin: An adventure in liverland. Mol. Cell. Biochem. 29: 71-80.

Atwal, O. S. (1973). Fatty changes and hepatic cell excretion in avian liver: An electron microscopical study of kepone toxicity. J. Comp. Pathol. 83: 115-124.

Baggett, J., Thureson-Klein, A., and Klein, R. L. (1980). Effects of chlordecone on the adrenal medulla of the rat. Toxicol. Appl. Pharmacol. 52: 313-322.

Bailey, A. W., and White, C. (1970). Chlorinated hydrocarbon pesticides in major river basins. Public Health Rep. 82: 139-156.

Balazs, R., and Patel, J. (1973). Factors affecting the biochemical maturation of the brain. Effect of undernutrition during early life. Pediat. Res. 3: 407-412.

Bass, N. H., Netsky, M. G., and Young, E. (1969). Microchemical studies of postnatal development in rat cerebrum. Neurol. 19: 258-268.

Becker, G. W., and Lester, R. L. (1977). Changes in phospholipids of Saccharomyces cerevisiae associated with inositol-less death. J. Biol. Chem. 252: 8684-8691.

Ben-Barak, J., and Dudai, Y. (1980). Scopolamine induces an increase in muscarinic receptor level in rat hippocampus. Brain Res. 193: 309-313.

Berconici, B., Wasserman, M., Cucos, S., Ron, M., Wasserman, D., and Pines, A. (1983). Serum levels of polychlorinated biphenyls and some OC insecticides in women with recent and former missed pregnancies. Environ. Res. 30: 169-174.

Berlovki, C., Rosival, L., and Khan, S. U. (1983). Transplacental passage of pesticides into human embryos. Bull. Environ. Contam. Toxic. 29: 95-100.

Berridge, M. J., Dawson, R. M. C., Downes, C. P., Heslop, J. P., and Irvine, R. F. (1983). Changes in the levels of inositol phosphates after agonist-dependent hydrolysis of membrane phosphoinositides. Biochem. J. 212: 473-482.

Billah, M. M., and Lapetina, E. G. (1982). Effects of calcium blocking agents on the metabolism of low density lipoproteins in human skin fibroblasts. Biochem. Biophys. Res. Commun. 109: 217-222.

Billah, M. M., and Lapetina, E. G. (1983). Platelet-activating factor stimulates metabolism of phosphoinositides in horse platelets: Possible relationship to calcium mobilization during stimulation. Proc. Natl. Acad. Sci. 80: 965-968.

Birdsall, N. J. M., and Hulme, E. C. (1976). Biochemical studies on muscarinic acetylcholine receptors. J. Neurochem. 27: 7-16.

Birdsall, N. J. M., Burgen, A. S. V., and Hulme, E. C. (1978). The binding of agonists to brain muscarinic receptors. Mol. Pharmacol. 14: 723-736.

Bolton, T. B. (1973). In Drug Receptors, ed. H. P. Rang, 87-104. Macmillan, London.

Bondy, S. C., and Agrawal, A. K. (1980). The inhibition of cerebral high-affinity receptor sites by lead and mercury compounds. Arch. Toxicol. 46: 249-256.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Brewerton, H. U., and Slade, D. A. (1964). Kepone residues on apples. New Zealand J. Agr. Res. 7: 647.

Brownstein, M. J., Palkovits, M., and Kizer, J. S. (1976). Effect of surgical isolation of the hypothalamus on its neurotransmitter content. Brain Res. 117: 287-295.

Bulger, W. H., Muccitelli, R. M., and Kupfer, D. (1979). Studies on the estrogenic activity of chlordecone (kepone) in the rat: Effects on uterine estrogen receptor. Mol. Pharmacol. 15: 515-524.

Bungay, P. M., and Dedrick, R. L. (1979). Pharmacokinetics of halogenated hydrocarbons. N. Y. Acad. Sci. 320: 257-270.

Burgen, A. S. V., and Hiley, C. R. (1975). In Cholinergic Mechanisms, ed. P. G. Waser, 381-385. Raven Press, New York.

Burgen, A. S. V., Hiley, C. R., and Young, J. M. (1974). The binding of [34] propylbenzilycholine mustard by longitudinal muscle strips from guinea-pig small intestine. Brit. J. Pharmacol. 50: 145-151.

Burgen, A. S. V., and Spero, L. (1968). The effects of calcium and magnesium on the response of intestinal smooth muscle to drugs. Brit. J. Pharmacol. 40: 492-500.

Cannon, S. B., and Kimbrough, R. D. (1979). Short-term chlordecone toxicity in rats including effects on reproduction, pathological organ changes, and their reversibility. Toxicol. Appl. Pharmacol. 47: 469-476.

Carlson, D. A., Konyha, D. K., and Wheeler, W. B. (1976). Mirex in the environment: Its degradation to kepone and related compounds. Science 194: 939-941.

Carmines, E. L., Carchman, R. A., and Borzelleca, J. F. (1979). Kepone: Cellular sites of action. Toxicol. Appl. Pharmacol. 49: 543-550.

Chang-Tsui, Y. Y. H., and Ho, I. K. (1979). Effects of chlordecone on synaptosomal catecholamine uptake in the mouse. Neurotoxicol. 1: 357-367.

Chang-Tsui, Y. Y. H., and Ho, I. K. (1980). Effect of kepone (chlordecone) on synaptosomal catecholamine uptake in the mouse. Neurotoxicol. 1: 643-651.

Chernoff, N., Linder, R. E., Scotti, T. M., Rogers, E. H., Carver, B. D., and Kavlock, R. J. (1979). Fetotoxicity and cataractogenicity of mirex in rats and mice with notes on kepone. Environ. Res. 18: 257-269.

Chernoff, N., and Rogers, E. H. (1976). Fetal toxicity of kepone in rats and mice. Toxicol. Appl. Pharmacol. 38: 189-194.

Christensen, L. W., Gorski, R. A. (1978). Independent masculinization of neuroendocrine systems by intracerebral implants of testosterone or estradiol in the neonatal female rat. Brain Res. 136: 325-340.

Chu, I., Villeneuve, D. C., Becking, G. C., Iverson, F., and Ritter, L. (1980). Short-term study of the combined effects of mirex, photomirex, and kepone with halogenated biphenyls in rats. J. Toxicol. Environ. Health 6: 421-432.

Cohn, W. J., Boylan, J. J., Blanke, R. V., Garris, M. W., Howell, J. R., and Guzelian, P. S. (1978). Treatment of chlordecone (kepone) toxicity with cholestyramine: Results of a controlled clinical trial. N. Engl. J. Med. 298: 243-248.

Cooper, P. H., and Hawthorne, J. N. (1975). Phosphomonoesterase hydrolysis of PPI in rat kidney: Properties and subcellular localisation of the enzyme system. Biochem. J. 150: 537-551.

Cotman, C. W., and Matthews, D. A. (1971). Synaptic plasma membranes from rat brain synaptosomes: Isolation and partial characterization. Biochim. Biophys. Acta 249: 380-394.

Creba, J. A., Downes, C. P., Hawkins, P. T., Brewster, G., Michell, R. H., and Kirk, C. J. (1983). DDT and related chlorinated hydrocarbon insecticides: pharmacological basis for their toxicity in mammals. Biochem. J. 212: 733-747.

Creese, I., Burt, D. R., and Snyder, S. H. (1978). Biochemical actions of neuroleptic drugs: Focus on the dopamine receptor. In Handbook of Psychopharmacology, Vol. 10. ed. L. Iversen, S. D. Iversen, and S. H. Snyder. Plenum Press, New York.

Cueto, C. Jr., Page, N. P., and Saffioti, V. (1978). Report on carcinogenesis of technical grade chlordecone (kepone). Natl. Cancer Inst. Rep. DHEW Publ. NIH-76-1278: 27.

Curtis, L. R., and Mehendale, H. M. (1979). The effects of kepone pretreatment on biliary excretion of xenobiotics in the male rat. Toxicol. Appl. Pharmacol. 47: 295-303.

Davison, A. N., and Dobbing, J. (1968). The Developing Brain in Applied Neurochemistry, ed. A. N. Davison and J. Dobbing. Blackwell Scientific Publications, Oxford.

DeBelleroche, J. S., and Bradford, H. F. (1972). Metabolism of beds of mammalian cortical synaptosomes. Response to depolarising influences. J. Neurochem. 19: 585-602.

Desaiah, D. (1980) Comparative effects of chlordecone and mirex on rat cardiac ATPases and binding of [3H]-catecholamines. J. Environ. Pathol. Toxicol. 4: 237-248.

Desaiah, D. (1981). Interaction of chlordecone with biological membranes. J. Toxicol. Environ. Health 8: 719-730.

Desaiah, D., and Koch, R. B. (1975). Inhibition of ATPases activity in channel catfish brain by kepone and its reduction product. Bull. Environ. Contam. Toxicol. 13: 153-158.

Desaiah, D., Ho, I. K., and Mehendale, H. M. (1977). Inhibition of mitochondrial magnesium ATPase activity in isolated perfused rat liver by kepone. Biochem. Pharmacol. 26: 1155-1159.

Desaiah, D., Trottman, C. H., and Bansal, S. K. (1980a). Sensitivity of rat brain synaptosomal ATPases to several structurally related organochlorine compounds. In Mechanisms of Toxicity and Hazard Evaluation, ed. B. Holmstedt, R. Lauwerys, M. Mercier, and M. Roberfroid, 87-90. Elsevier/North-Holland, New York.

Desaiah, D., Gilliland, T., Ho, I. K., and Mehendale, H. M. (1980b). Inhibition of mouse brain synaptosomal ATPases and ouabain binding by chlordecone. Toxicol. Lett. 6: 275-285.

Deshmukh, D. S., Kuizon, S., Bear, W. D., and Brockerhoff, H. (1980). Distribution of phosphoinositides among subfractions of rat brain myelin. Lipids 15: 14-21.

DeSilva, N. S., Gurd, J. W., and Schwartz, C. (1979). Developmental alteration of rat brain synaptic membranes. Brain Res. 165: 283-293.

DeWitt, J. B., Crabtree, D. G., Finley, R. B., and George, J. L. (1962). Effects of pesticides on fish and wildlife: A review of investigations during 1960. U.S. Dept. Inter. Fish Wildl. Serv. Circ. 167. 36 pp.

Dietz, D. D., McMillan, D. E., and Dewey, W. L. (1979). Comparative effects of mirex and kepone on schedule-controlled behavior in the rat. I. Multiple fixed-ratio 12 fixed-interval 2-minute schedule. Neurotoxicol. 1: 369-385.

Downes, C. P., and Michell, R. H. (1981). The PPI phosphodiesterase of erythrocyte membranes. Biochem. J. 198: 133-140.

Downes, C. P., and Michell, R. H. (1982). The control by calcium of the PPI phosphodiesterase and the calcium-pump ATPase in human erythrocytes. Biochem. J. 202: 53-58.

Druse, M. J., Waddell, C., and Haas, G. (1981). Maternal ethanol consumption during the third trimester of pregnancy, synaptic plasma membrane glycoproteins and gangliosides in offspring. Sub. Alc. Actions/Misuse 2: 359-368.

Eaton, P., Carlson, L., Lombardo, P., and Yates, P. (1960). Pyrolysis of the cage kepone. J. Org. Chem. 25: 1225.

Ebel, R. E. (1980). In vitro effects of chlordecone (kepone) on hepatic microsomal cytochrome P-450. Pestic. Biochem. Physiol. 14: 221-226.

Edwards, F., Baggett, M, and Soine, P.J. (1970). Environmental insectides and their impact. Bull. Environ. Contam. Toxicol. 12: 332-340.

Egle, J. L. Jr, Fernandez, S. B., Guzelian, P. S., and Borzelleca, J. F. (1978). Distribution and excretion of chlordecone (kepone) in the rat. Drug Metab. Dispos. 6: 91-95.

Egle, J. L. Jr., Guzelian, P. S., and Borzelleca, J. F. (1979). Time course of the acute toxic effects of sublethal doses of chlordecone (kepone). Toxicol. Appl. Pharmacol. 48: 533-536.

End, D. W., Carchman, R. A., Ameen, R., and Dewey, W. L. (1979). Inhibition of rat brain mitochondrial calcium transport by chlordecone. Toxicol. Appl. Pharmacol. 51: 189-196.

End, D. W., Carchman, R. A., and Dewey, W. L. (1981). Neurochemical correlates of chlordecone neurotoxicity. J. Toxicol. Environ. Health 8: 707-718.

Eichberg, J., and Hauser, G. (1967). Polyphosphoinositol biosynthesis in developing rat brain homogenates. Fed. Proc. 26: 675-684.

Eroschenko, V. P. (1979). Changes in the reproductive performance of Japanese quail fed kepone in different calcium diets. Bull. Environ. Contam. Toxicol. 21: 631-638.

Eroschenko, V. P., and Palmiter, R. D. (1980). Estrogenicity of kepone in birds and mammals. In Estrogens in the Environment, ed. J. A. McLachan, 305-324. Elsevier/North Holland, New York.

Eroschenko, V. P. and Place, T. A. (1977). Prolonged effects of kepone on strength and thickness of eggshells from Japanese quail fed different calcium level diets. Environ. Pollut. 13: 255-264.

Eroschenko, V. P., and Place, T. A. (1978). Variations in dimensions and shell weights of eggs collected from Japanese quail fed kepone with different calcium diets. Environ. Pollut. 16: 123-127.

Eroschenko, V. P., and Wilson, W. O. (1974). Photoperiods and age as factors modifying the effects of kepone in Japanese quail. Toxicol. Appl. Pharmacol. 29: 329-339.

Eroschenko, V. P. and Wilson, W. O. (1975). Cellular changes in the gonads, livers, and adrenal glands of Japanese quail as affected by the insecticide kepone. Toxicol. Appl. Pharmacol. 31: 491-504.

Farber, E. (1980). The sequential analysis of liver cancer induction. Biochim. Biophys. Acta 605: 149-166.

Fariss, M. W. (1980). Comparative metabolism of chlordecone (kepone) in mammals. Ph. D. dissertation, Med. Coll. Va. 131 pp.

Farese, R. V., Sabir, A. M., and Larson, R. E. (1980). On the mechanism whereby ACTH and cAMP increase adrenal PPI: Rapid stimulation of the synthesis of phosphatidic acid and derivatives of CDP-diacylglycerol. J. Biol. Chem. 255: 7232-7237.

Fisher, S. K., and Agranoff, B. W. (1981). Enhancement of the muscarinic synaptosomal phospholipid labeling effect by the ionophore A23187. J. Neurochem. 37: 968-977.

Fisher, S. K., Hootman, S. R., Heacock, A. M., Ernst, S. A., and Agranoff, B. W. (1983). Muscarinic stimulation of phospholipid turnover in dissociated avian salt glands. FEBS Lett. 155: 43-46.

Fisher, S. K., Klinger, P. D., and Agranoff, B. W. (1983). Muscarinic agonist binding and phospholipid turnover in brain. J. Biochem. 258: 7358-7363.

Folch, J. (1955). In Biochemistry of the Developing Nervous System, ed. H. Wallsch, 268. Academic Press, New York.

Fontaine, R. N., Harris, R. A., and Schroeder, F. (1980). Amino phospholipid asymmetry in murine synaptic plasma membranes. J. Neurochem. 34: 269-277.

Friedel, R. O., Johnson, J. R., and Schanberg, S. M. (1973). The effects of sympathomimetic drugs on incorporation in vivo of intracisternally injected 32Pi into phospholipids of rat brain. J. Pharmacol. Exp. Ther. 184: 583-589.

Friedel, R. O., and Schanberg, S. M. (1973). Effects of carbamylcholine and atropine on incorporation in vivo of intracisternally injected 32Pi into phospholipids of rat brain. J. Pharmacol. Exp. Ther. 183: 326-332.

Fu, S. C., Cruz, T. F., and Gurd, J. W. (1981). Development of synaptic glycoproteins. Effect of postnatal age on the synthesis and concentration of synaptic membranes and synaptic junctional fucosyl and sialyl glycoproteins. J. Neurochem. 36: 1338-1351.

Fujimori, K., Benet, H., Mehendale, H. M., and Ho, I. K. (1982). Comparison of discrete area distributions of chlordecone and mirex in mouse. Neurotoxicol. 3: 125-130.

Fujimori, K., Nabeshima, T., Ho, I. K., and Mehendale, H. M. (1982). Effects of oral administration of chlordecone and mirex on brain biogenic amines in mice. Neurotoxicol. 3: 149-158.

Furchgott, R. F., and Burtsztyn, P. (1967). Comparison of dissociation constants and of relative efficacies of selected agonists on parasympathetic receptors. Ann. N.Y. Acad. Sci. 144: 882-899.

Gaines, T. B. (1969). Acute toxicity of pesticides. Toxicol. Appl. Pharmacol. 14: 515-534.

Galper, J. B., and Smith, T. W. (1978). Properties of muscarinic acetylcholine receptors in heart cell cultures. Proc. Natl. Acad. Sci. U.S.A. 75: 5831-5835.

Gambetti, P., Autilio-Gambetti, L., Ruzzuto, N., Shafer, B., and Pfaff, L. (1974). Synapses and malnutrition: Quantitative ultrastructure study of rat cerebral cortex. Exp. Neurol. 43: 464-473.

Gardner, J., Conlon, T. P., Klaeveman, H. L., Adams, T. D., and Ondetti, M. A. (1975). Action of cholecystokinin and cholinergic agents on calcium transport in isolated pancreatic acinar cells. J. Clin. Invest. 56: 366-375.

Garrett, R. J. B., and Redman, C. M. (1975). Localisation of enzymes involved in polyphosphoinositide metabolism on the cytoplasmic surface of the human erythrocyte membrane. Biochim. Biophys. Acta 382: 58-64.

Gellert, R. J. (1978). Kepone, mirex, dieldrin, and aldrin: Estrogenic activity and the induction of persistent vaginal estrus and anovulation in rats following neonatal treatment. Environ. Res. 16: 131-138.

Gerhart, J. M., Hong, J. S., Uphouse, L. L., and Tilson, H.A. (1982). Chlordecone-induced tremor: Quantitative and pharmacological analysis. Toxicol. Appl. Pharmacol. 66: 234-243.

Gilbert, E. E., Lombardo, P., and Walker, G. L. (1966). Preparation and insecticide evaluation of alcohol and amine adducts of kepone. J. Agr. Food Chem. 14: 115.

Goldstein, S., Himwich, W. A., Leiner, K., and Stout, M. (1971). Psychoactive agents in dogs with bilateral lesions in subcortical structure. Neurol. 21: 847-852.

Good, E. E., Ware, G. W., and Miller, D. F. (1965). Effects of insecticides on reproduction in the laboratory mouse. I. Kepone. J. Econ. Entomol. 58: 754-757.

Gordon, J. H., Nance, D. M., Wallis, C. J., Gorski, R. A. (1977). Effects of estrogen on dopamine turnover, glutamic acid decarboxylase activity and lordosis behavior in septal lesioned rats. Brain Res. Bull. 2: 341-346.

Goring, D., Crosby, D. G., and Benson, W. R. (1967). General aspects of pesticide use. The chemistry of pesticides. Ann. N.Y. Acad. Sci. 160: 7-29.

Graff, G., Nahas, N., Nikolopoulou, M., Natarajan, V., and Schmid, H. H. O. (1984). Possible regulation of phospholipase C activity in human platelets by phosphatidylinositol 4,5-biphosphate. Arch. Biochem. Biophys. 228: 299-308.

Griffen, G. W., and Price, A. K. (1964). Perichloro cage compounds. I. Structural studies. J. Org. Chem. 29: 3192.

Gullis, R. J., Traber, J., Fischer, K., Buchen, C., and Hamprecht, B. (1975). Effects of cholinergic agents and sodium ions on the levels of guanosine and adenosine 3′:5′ cyclic monophosphates in neuroblastoma and neuroblastoma X glioma hybrid cells. FEBS Lett. 59: 74-79.

Gurney, M. E. (1981). Hormonal control of cell form and number in the zebra finch song system. J. Neurosci. 1: 658-673.

Guzelian, P. S., Vranian, G. Boylan, J. J., Cohn, W. J., and Blanke, R. V. (1980). Liver structure and function in patients poisoned with chlordecone (kepone). Gastroenterol. 78: 206-213.

Hall, C. D., Weiss, E. A., Morris, C. E., and Prange, A. J. (1972). Rapid deterioration in patients with Parkinsonism following tryptophanpyridoxine administration. Neurol. 22: 231-237.

Halvorsen, S. W., and Nathanson, N. M. (1981). In vivo regulation of muscarinic acetylcholine receptor number and function in embryonic chick heart. J. Biol. Chem. 256: 7941-7948.

Hammond, B., Katznellenbogen, B. S., Krauthammer, N., and McConnell, J. (1979). Estrogenic activity of the insecticide chlordecone (kepone) and interaction with uterine estrogen receptors. Proc. Natl. Acad. Sci. U.S.A. 76: 6641-6645.

Harris, W. M., Babad, H., and Headley, J. C. (1969). Epidemiological considerations of human exposure to pesticides. Ann. N.Y. Acad. Sci. 160: 299-313.

Hartzell, H. C. (1981). Mechanisms of slow postsynaptic potentials. Nature (London) 291: 539-544.

Harwood, J. L., and Hawthorne, J. N. (1969). The properties and subcellular distribution of PtdIns kinase in mammalian tissues. Biochim. Biophys. Acta 1: 75-88.

Hawthorne, J. N. (1964). The biochemistry of the inositol lipids. Vitamins and Hormones 22: 57-79.

Hawthorne, J. N., and Bleasdale, J. E. (1975). Phosphatidic acid metabolism, calcium ions and transmitter release from electrically stimulated synaptosomes. Mol. Cell. Biochem. 8: 83-87.

Hawthorne, J. N., and Kai, M. (1970). Metabolism of phosphoinositides. In Handbook of Neurochemistry, ed. A. Lajtha, 491-508. Plenum Press, New York.

Hawthorne, J. N., and Pickard, M. R. (1979). Phospholipids in synaptic function. J. Neurochem. 32: 5-14.

Hawthorne, J. N., and White, D. A. (1975). Myo-inositol lipids. Vitamins and Hormones 33: 529-573.

Haymaker, E., and Ginzler, A. M. (1946). The toxic effects of prolonged ingestion of DDT on dogs with special reference to lesions in the brain. Am. J. Med. Sci. 212: 423-431.

Hedlund, B., Gamara, M., and Bartfai, I. (1979). Inhibition of striatal muscarinic receptors in vivo by cadmium. Brain Res. 168: 216-218.

Heilbronn, E., and Bartfai, I. (1978). Muscarinic acetylcholine receptor. Prog. Neurobiol. 11: 171-188.

Hendrickson, C. M., and Bowden, J. A. (1975). The in vitro inhibition of rabbit muscle lactate dehydrogenase by mirex and kepone. J. Agric. Food Chem. 23: 407-409.

Hewitt, W. R., Miyajima, H., Cote, M. G., and Plaa, G. L. (1979). Acute alteration of chloroform-induced hepato- and nephrotoxicity by mirex and kepone. Toxicol. Appl. Pharmacol. 48: 509-527.

Ho, I. K., Fujimori, K., Huang, T.-P., and Chang-Tsui, H. (1981). Neurochemical evaluation of chlordecone toxicity in the mouse. J. Toxicol. Environ. Health 9: 181-190.

Hodgson, P. W., Kanton, E. J., and Mann, J. B. (1978). Analytical methodology for the detection of kepone in fish, shellfish, and high volume air filter. Arch. Environ. Contam. Toxicol. 7: 99-112.

Hokin, L. E., and Hokin, M. R. (1955). Effect of acetylcholine on the turnover of phospholipids of pancreas and brain cortex slices. Biochim. Biophys. Acta 18: 102-110.

Hokin, L. E., and Hokin, M. R. (1958). Acetylcholine and exchange of inositol and phosphate in brain phosphoinositide. J. Biol. Chem. 233: 818-821.

Hokin, M. R. (1968). Studies on chemical mechanisms of the action of neurotransmitters and hormones. Arch. Biochem. Biophys. 124: 280-284.

Hokin, M. R. (1969). Effect of norepinephrine on 32Pi incorporation into individual phosphatides from different areas of the guinea pig brain. J. Neurochem. 16: 127-134.

Hokin, M. R., and Hokin, L. E. (1953). Enzyme secretion and the incorporation of radioactive phosphorus into phospholipids of pancreas slices. J. Biol. Chem. 203: 967-977.

Hokin, M. R., Hokin, L. E., and Shelp, W. D. (1960). The effects of acetylcholine on the turnover of phosphatidic acid and phosphoinositide in sympathetic ganglia and in various parts of the central nervous system in vitro. J. Gen. Physiol. 44: 217-226.

Hokin-Neaverson, M. R. (1974a). Acetylcholine causes a net decrease in phosphoinositides and a net increase in phosphatidic acid in mouse pancreas. Biochem. Biophys. Res. Commun. 58: 763-768.

Hokin-Neaverson, M. R. (1974b). In Secretory Mechanisms of Exocrine Cells, ed. N. A. Thorn and O. H. Pederson, 701-712. Munksgaard, Copenhagen.

Hokin-Neaverson, M. R., Sadeghian, K., Harris, P. W., and Merrin, J. S. (1978). The mechanism of stimulated phosphatidylinositol breakdown. In Cyclitols and Phosphoinositides, ed. F. Eisenberg and W. W. Wells. Academic Press, New York.

Hong, J. S., Tilson, H. A., Uphouse, L. L., Gerhart, J., and Wilson, W. E. (1983). Effects of chlordecone exposure on brain neurotransmitters: Possible involvement of serotonin system in chlordecone-elicited tremor. Toxicol. Appl. Pharmacol. 73: 339-344.

Hornykiewicz, O. (1966). Dopamine (3 hydroxytyramine) and brain function. Pharmacol. Rev. 18: 925-964.

Hrdina, P. D., Singhal, R. I., and Ling, G. M. (1975). DDT and related chlorinated hydrocarbon insecticides. Pharmacological basis of their toxicity in mammals. Adv. Pharmacol. Chemother. 12: 31-60.

Huang, T.-P., Ho, I. K., and Mehendale, H. M. (1980). Assessment of neurotoxicity induced by oral administration of chlordecone (kepone) in the mouse. Neurotoxicol. 2: 113-124.

Huber, J. J. (1965). Some physiological effects of the insecticide kepone in the laboratory mouse. Toxicol. Appl. Pharmacol. 7: 516-524.

Hulme, E. C. (1976). Interactions of agonists and antagonists with muscarinic receptors. In Smooth Muscle Pharmacology and Physiology, ed. M. Worcel and G. Vassant, 49-67. Plenum Press, New York.

Hulme, E. C., Burgen, A. S. V., and Birdsall, N. J. M. (1976). Mol. Pharmacol. 14: 737-750.

Hunter, J., Maxwell, J. D., and Stewart, D. A. (1972). Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides. Nature 237: 399-400.

Irving, R. F., Hemington, N., and Dawson, R. M. C. (1979). The calcium-dependent PtdIns phosphodiesterase of rat brain, mechanisms of suppression and stimulation. Eur. J. Biochem. 99: 525-530.

Ishikawa, T. T., McNeely, S., Steiner, P. M., Glueck, C. J., Mellies, M., Gartside, P. S., and McMillan, C. (1978). Effects of chlorinated hydrocarbon on plasma beta-lipoprotein cholesterol in rats. Metabolism 27: 89-96.

Jafferji, S. S., and Michell, R. H. (1976). Effects of calcium-antagonistic drugs on the stimulation by carbamylcholine and histamine of phosphatidylinositol turnover in longitudinal smooth muscle of guinea-pig ileum. Biochem. J. 160: 163-169.

Jolles, J., Zwiers, H., Dekker, A., Wirtz, K. W. A., and Gispen, W. H. (1981). ACTH affects protein phosphorylation and PPI metabolism in rat brain. Biochem. J. 194: 283-291.

Jones, L., and Michell, R. H. (1974). Breakdown of phopshatidylinositol provoked by muscarinic cholinergic stimulation of rat parotid-gland fragments. Biochem. J. 142: 583-590.

Kavlock, R. J., Chernoff, N., Rogers, E., and Whitehouse, D. (1980). Comparative tissue distribution of mirex and chlordecone in fetal and neonatal rats. Pestic. Biochem. Physiol. 14: 227-235.

Klein, W. L., Nathanson, N., and Nirenberg, M. (1979). Muscarinic acetylcholine receptor regulation by accelerated rate of receptor loss. Biochem. Biophys. Res. Commun. 90: 506-512.

Koch, R. B., Desaiah, D., Glick, B., Subba Rao, D. S. V., and Stinson, R. (1977). Antibody reactivation of kepone inhibited brain ATPase activities. Gen. Pharmacol. 8: 231-234.

Koch, R. B., Patil, T. N., Glick, B., Stinson, R. S., and Lewis, E. A. (1979). Properties of an antibody to kelavan isolated by affinity chromatography: Antibody reactivation of ATPase activities inhibited by pesticides. Pest. Biochem. Physiol. 12: 130-140.

Lajtha, A., Furst, S., Gerstein, A., and Wallsch, H. (1957). Amino acid and protein metabolism of the brain. I. Turnover of free and protein bound lysine in brain and other organs. J. Neurochem. 1: 289-300.

Landrigan, P. J., Wilcox, K. R., Silva, J. Jr., Humphrey, H. E. B., Kauffman, C., and Heath, C. W. Jr. (1977). Cohort study of Michigan residents exposed to polybrominated biphenyls: Epidemiologic and immunologic findings. Ann. N.Y. Acad. Sci. 320: 284-294.

Lanier, L. P., Dunn, A. J., and van Hartesveldt, C. (1976). Development of neurotransmitters and their function in brain. Rev. Neurosci. 2: 195-256.

Lanzetta, P. A., Alvarez, L. J., Reinach, P. S., and Candia, O. A. (1979). An improved assay for nanomole amounts of inorganic phosphate. Anal. Biochem. 100: 95-97.

Lapetina, E. G., and Michell, R. H. (1972). Stimulation by acetylcholine of phosphatidyl inositol labeling: Subcellular distribution in rat cerebral cortex slices. Biochem. J. 126: 1141-1147.

Lapetina, E. G., Seguin, E. B., and Agranoff, B. W. (1975). Preparation of 32P-labelled inositides and their degradation by soluble kidney enzymes. Biochim. Biophys. Acta 398: 118-124.

Lapetina, E. G., Soto, E. F., and DeRobertis, E. (1967). Gangliosides in brain membranes. Biochim. Biophys. Acta 135: 33-43.

Larrabee, M. G., Klingman, J. D., and Leicht, W. S. (1963). Effects of temperature, calcium and activity of phospholipid metabolism in a sympathetic ganglion. J. Neurochem. 10: 549-570.

Larson, P. S., Egle, J. L. Jr., Hennigar, G. R., Lane, R. W., and Borzelleca, J. F. (1979). Acute, subchronic, and chronic toxicity of chlordecone. Toxicol. Appl. Pharmacol. 48: 29-41.

Lee, T. C., and Huggins, C. G. (1968). PdtIns4,5P phosphomonoesterase in rat kidney cortex I and II. Arch. Biochem. Biophys. 126: 206-213, 214-220.

Lee, T.-P., Kuo, J. F., and Greengard, P. (1972). Role of muscarinic cholinergic receptors in regulation of quanisine 3′-5′ monophosphate content in mammalian brain, heart muscle, and intestinal smooth muscle. Proc. Natl. Acad. Sci. U.S.A. 69: 3287-3291.

Lichtenstein, B. W., and Schultze, P. R. (1960). Early clinical manifestations of neurological disorders. Med. Clin. N. Amer. 44: 225-236.

Lindstrom, F. T., Gillett, J. W., and Rodecap, S. E. (1974). Distribution of HEOD (dieldrin) in mammals. I. Preliminary model. Arch. Environ. Contam. Toxicol. 2: 9-42.

Mactutus, C. F., Unger, K. L., and Tilson, H. A. (1982). Neonatal chlordecone exposure impairs early learning and memory in the rat on a multiple passive avoidance task. Neurotoxicol. 3: 27-44.

Makman, M. M., Ahn, H. S., Thai, L. J., Sharpless, N. S., Dvorkin, B., Horowitz, S. G., and Rosenfeld, M. (1979). Aging and monoamine receptors in brain. Fed. Proc. 38: 1922-1926.

Malseed, R. T., and Baker, W. W. (1973). Analysis of tremorogenic effect of intracaudate serotonin. Proc. Soc. Exper. Biol. Med. 143: 1088-1093.

Margolis, R. U., and Heller, A. J. (1966). The effect of cholinergic and other pharmacological agents on brain monophosphoinositide turnover in vivo. J. Pharmacol. Exp. Ther. 151: 307-321.

Marx, J. L. (1984). The polyphosphoinositides revisited. Science 224: 271.

Matsuzawa, H., and Nirenberg, M. (1975). Receptor-mediated shifts in cGMP and cAMP levels in neuroblastoma cells. Proc. Natl. Acad. Sci. U.S.A. 72: 3472-3476.

McBee, E. T., Roberts, C. W., Idol, J. D., and Earle, R. H. (1956). An investigation of the chlorocarbon, MP485 and the kepone MP349. J. Amer. Chem. Soc. 78: 1511.

McFarland, L. Z., and Lacy, P. B. (1969). Physiologic and endocrinologic effects of the insecticide kepone in the Japanese quail. Toxicol. Appl. Pharmacol. 15: 441-450.

McMurray, W. C. (1963). Metabolism of phosphatides in rat brain. I. J. Neurochem. 11: 287-299.

Mehendale, H. M. (1977). Effect of preexposure to kepone on the biliary excretion of imipramine and sulfobromophthalein. Toxicol. Appl. Pharmacol. 40: 247-259.

Mehendale, H. M., Takanaka, A., Desaiah, D., and Ho, I. K. (1977). Kepone induction of hepatic mixed function oxidases in the male rat. Life Sci. 20: 991-998.

Mehendale, H. M., Takanaka, A., Desaiah, D., and Ho, I. K. (1978). Effect of preexposure to kepone on hepatic mixed-function oxidases in the female rat. Toxicol. Appl. Pharmacol. 44: 171-180.

Melnechuk, T. (1978). Cell Receptor Disorders. Western Behavioral Sciences Institute, La Jolla, California.

Meyer, M. R., Gainer, M. W., and Nathanson, N. M. (1982). In vivo regulation of muscarinic cholinergic receptors in embryonic chick brain. Mol. Pharmacol. 21: 280-286.

Meyer, U. A., and Schmid, R. (1978). The porphrias. In The Metabolic Basis of Inherited Disease, ed. J. B. Stanbury, J. B. Wyngaarden, and D. S. Frederickson, 1166-1220. McGraw-Hill, New York.

Michaelis, E. K., Mulvanez, M. J., and Freed, W. J. (1978). Effects of acute and chronic ethanol intake on synaptosomal glutamate ginding activity. Biochem. Pharmacol. 27: 1685-1691.

Michell, R. H. (1975). Inositol phospholipids and cell surface receptor function. Biochim. Biophys. Acta 415: 81-87.

Michell, R. H. (1979). Inositol phospholipids in membrane function. Trends Bioch. Sci. 4: 128-131.

Michell, R. H., Hawthorne, J. N., Coleman, R., and Karnovsky, M. L. (1970). Extraction of PPI with neutral and acidified solvents: A comparison of brain and liver, and measurements of rat liver inositol compounds that are resistant to extraction. Biochim. Biophys. Acta 210: 86-91.

Michell, R. H., Jafferji, S. S., and Jones, L. M. (1977). The possible involvement of phosphatidylinositol breakdown in the mechanism of stimulus-response coupling at receptors which control cell-surface calcium gates. Adv. Exp. Med. Biol. 83: 447-464.

Minneman, K. P., Dibner, M. D., Wolfe, B. B., and Molinoff, P. B. (1979). B1 and B2 adrenergic receptors in rat adrenal cortex are independently regulated. Science 204: 866-868.

Mishra, S. K., Joury, M. and Desaiah, D.(1980). Inhibition of calcium ATPase activity in rat brain and muscle by chlordecone. Bull. Environ. Contam. Toxicol. 25: 262-268.

Morgan, D. P., and Roan, C. C. (1974). The metabolism of DDT in man. Essays Toxicol. 12: 39-97.

Moseman, R. F., Crist, H. L., Edgerston, T. R., and Ward, M. K. (1977). EC-GC detection of kepone in environmental samples. Arch. Environ. Contam. Toxicol. 6: 221-231.

Muknerjee, A., Snyder, G., and McCann, S. M. (1980). Characterization of muscarinic cholinergic receptors on intact rat anterior pituitary cells. Life Sci. 27: 475-482.

Naber, E. C., and Ware, G. W. (1964). Effect of kepone and mirex on reproductive performance in the laying hen. Ohio Agric. Exp. Stn. J. 80-64: 875-880.

Nagata, Y., Mikoshiba, K., and Tsukada, Y. (1973). Effect of potassium ions on glucose and phospholipid metabolism in rat's cervical sympathetic ganglia with and without axotomy. Brain Res. 56: 259-269.

Nathanson, N. M. (1982). Regulation and development of muscarinic acetylcholine receptors. T.I.N.S Nov.:401-404.

Nathanson, N. M., Klein, W. L., and Nirenberg, M. (1978). Regulation of adenylate cyclase activity mediated by muscarinic acetylcholine receptors. Proc. Natl. Acad. Sci. U.S.A. 75: 1788-1791.

National Cancer Institute, Carcinogenesis Program, Division of Cancer Cause and Prevention (1976). Report on Carcinogenesis Bioassay of Technical Grade Chlordecone (Kepone). 38 pp.

Nebert, D. W., Levitt, R. C., Orlando, M. M., and Felton, J. S. (1977). Effects of environmental chemicals on the genetic regulation of microsomal enzyme systems. Clin. Pharmacol. Ther. 22: 640-658.

Nomura, Y., Oki, K., Segawa, T. (1982). Ontogenetic development of the striatal (3H) spiperone binding: regulation by sodium and guanine nucleotide in rats. J. Neurochem. 38: 902-908.

Oron, Y., Sharoni, Y., Lefkowitz, H., and Selinger, Z. (1978). PdtIns kinase and PdtIns4P kinase in the rat parotid gland. In Cyclitols and Phosphoinositides, ed. W. W. Wells and F. Eisenberg, 383-398. Academic Press, New York.

Palmiter, R. D., and Mulvihill, E. R. (1978). Estrogenic activity of the insecticide kepone on the chicken oviduct. Science 201: 356-358.

Phillips, J. H. (1973). PdtIns kinase, a component of the chromaffin granule membrane. Biochem. J. 136: 579-587.

Pitot, H. C., amd Sirica, A. E. (1980). The stages of initiation and promotion in hepatocarcinogenesis. Biochim. Biophys. Acta 605: 191-215.

Probst, G. S., McMahon, R. E., Hill, L. E., Thompson, C. Z., Epp, J. K., and Neal, S. B. (1981). Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: A comparison with bacterial mutagenicity using 218 compounds. Environ. Mutat. 3: 11-32.

Putney, J. W., Burgess, G. M., Halenda, S. P., McKinney, J. S., and Rubin, R. P. (1983). Effects of secretagogues on 32P phosphatidylinositol 4,5-bisphosphate metabolism in the exocrine pancreas. Biochem. J. 212: 483-488.

Rabie, A., and Legrand, J. (1973). Effects of thyroid hormone and undernourishment on the amount of synaptosomal fraction in the cerebellum of the young rat. Brain Res. 61: 267-278.

Raisman, G., Field, P. M. (1973). Sexual dimorphism in the neutrophil of the preoptic area of the rat and its dependence on neonatal androgen. Brain Res. 94: 1-29.

Reddy, P. V., and Sastry, P. S. (1978). Effects of undernutrition on the metabolism of phospholipids and gangliosides in developing brain. Br. J. Nutr. 40: 403-410.

Reddy, P. V., and Sastry, P. S. (1979). Studies on neurotransmitter-stimulated phospholipid metabolism with cerebral tissue suspension: A possible biochemical correlate of synaptogenesis in normal and undernourished rats. Brain Res. 168: 287-298.

Redman, C. M., and Hokin, L. E. (1964). Stimulation of the metabolism of phosphatidylinositol and phosphatidic acid in brain cytoplasmic functions by low concentrations of cholinergic agents. J. Neurochem. 32: 5-14.

Reiter, L., Kidd, K., Ledbetter, G., Gray, L. E. Jr., and Chernoff, N. (1977). Comparative behavioral toxicology of mirex and kepone in the rat. Toxicol. Appl. Pharmacol. 41: 143 (Abstr.).

Reuber, M. D. (1978). Carcinogenicity of kepone. J. Toxicol. Environ. Health 4: 895-911.

Reuber, M. D. (1979). Carcinomas of the liver in rats ingesting kepone. Neoplasms 26: 231-235.

Rhodes, D. Prpic, V., Exton, J. H., and Blackmore, P. F. (1983). Stimulation of phosphatidylinositol 4,5-biphosphate hydrolysis in hepatocytes by vasopressin. J. Biol. Chem. 258: 2770-2773.

Roach, P. D., and Palmer, F. B. St. C. (1981). Human erythrocyte cytosol PdtIns4,5P phosphatase. Biochim. Biophys. Acta 661: 323-333.

Robinson, J., and Hunter, C. G. (1966). Organochlorine insecticides: Concentrations in human blood and adipose tissue. Arch. Environ. Health 13: 558-563.

Rosalki, S. B., Tarlow, D., and Rau, D. (1971). Plasma gamma-glutamyl transpeptidase elevation in patients receiving enzyme-inducing drugs. Lancet 1: 376-377.

Rosecrans, J. A., Hong, J. S., Squibb, R. E., Johnson, J. H., Wilson, W. E., and Tilson, H. A. (1982). Effects of perinatal exposure to chlordecone (kepone) on neuroendocrine and neurochemical responsiveness of rats to environmental challenges. Neurotoxicol. 3: 131-142.

Rothman, J. (1981). The Golgi apparatus: Two organelles in tandem. Science 213: 1212-1219.

Russel, R. W., Overstreet, D. H., Cotman, C. W., Carson, V. G., Churchill, L. Dalglish, F. W., and Vasquez, J. (1975). Experimental tests of hypotheses about neurochemical mechanisms underlying behavioral tolerance to the anticholinesterase diisopropyl fluorophosphate. J. Pharmacol. Exp. Ther. 192: 73-85.

Sanborn, G. E., Selhorst, J. B., Calabrese, V. P., and Taylor, J. R. (1979). Pseudomotor cerebri and insecticide intoxication. Neurol. 29: 1222-1227.

Scatchard, G. (1949). The attraction of proteins for small molecules and ions. Ann. N.Y. Acad. Sci. 51: 660-672.

Schaeffer, J. M., and Hsueh, A. J. W. (1980). Acetylcholine receptors in the rat anterior pituitary gland. Endocrinol. 106: 1377-1381.

Schain, R. J., Carver, M. J., and Copenhaver, J. H. (1967). Protein metabolism in the developing brain: Influence of birth and gestational age. Science 156: 984-986.

Schoeny, R. S., Smith, C. C., and Loper, J. C. (1979). Non-mutagenicity for salmonella of the chlorinated hydrocarbons 1,2,4-trichlorobenzene, mirex and kepone. Mutat. Res. 68: 125-132.

Selivonchick, D. P., Schmid, P., Natarajan, V., and Schmid, H. (1980). Structure and metabolism of phospholipids in bovine epidydimal spermatozoa. Biochim. Biophys. Acta 618: 242-254.

Seth, P. K., Agrawal, A. K., and Bondy, S. C. (1981). Biochemical changes in the brain consequent to dietary exposure of developing and mature rats to chlordecone (kepone). Toxicol. Appl. Pharmacol. 39: 262-267.

Sheetz, M. P., Febbroriello, P., and Koppel, D. (1982). PtdIns4,5P increases glycoprotein lateral mobility in erythrocyte membranes. Nature 296: 91-93.

Sheltawy, A., and Dawson, R. M. C. (1969). The metabolism of polyphosphoinositides in the brain and sciatic nerve. Biochem. J. 111: 147-153.

Sherman, M., and Ross, E. (1961). Acute and subacute toxicity of insecticides to chicks. Toxicol. Appl. Pharmacol. 3: 521-533.

Siman, R. G., and Klein, W. L. (1979). Cholinergic activity regulates muscarinic receptors in central nervous system cultures. Proc. Natl. Acad. Sci. U.S.A. 76: 4141-4145.

Sjolund, B., Bjorklund, A., and Wiklund, L. (1977). The indoleminergic innervation of the inferior olive. 2. Relation to harmaline induced tremor. Brain Res. 131: 23-37.

Skalsky, H. L., Fariss, M. W., Blanke, R. V., and Guzelian, P. S. (1979). The role of plasma proteins in the transport and distribution of chlordecone (kepone) and other polyhalogenated hydrocarbons. Ann. N. Y. Acad. Sci 320: 231-237.

Smart, J. L., Adlard, B. P., and Dobbing, J. (1974). Further studies of body growth and brain development in "small-for-dates" rats. Biol. Neonate 25: 135-150.

Smit, M. H., Ehlert, F. J., Yamamura, S., Roeske, W. R., and Yamamura, H. I. (1980). Differential regulation of muscarinic agonist binding sites following chronic cholinesterase inhibition. Eur. J. Pharmacol. 66: 379-380.

Smith, D. (1983). The Effects of Maternal Protein Deficiency on the Development of Synaptic Plasma Membranes in Rat Offspring. Ph. D. dissertation, Loyola University of Chicago: 75.

Smith, J. C., and Avant, F. S. (1967). Residues of kepone in milk from cows receiving treated feed. J. Econ. Entomol. 60: 925-927.

Snyder, S. H. (1979). Receptors, neurotransmitters, and drug responses. N. Engl. J. Med. 300: 465-472.

Snyder, S. H., Chang, K. J., Kuhar, M. J., and Yamamura, H. (1975). Biochemical identification of the mammalian muscarinic cholinergic receptor. Fed. Proc. 34: 1915-1921.

Soine, P. J., Blanke, R. V., Guzelian, P. S., and Schwartz, C. C. (1981). Preferential binding of chlordecone to the protein and high density lipoprotein fractions of plasma from man and other species. J. Toxicol. Environ. Health 9: 107-118.

Soukup, J. F., Friedel, R. O., and Schanberg, S. M. (1978). Cholinergic stimulation of polyphosphoinositide metabolism in brain in vivo. Biochem. Pharmacol. 27: 1239-1243.

Sperry, W. M. (1962). The biochemistry of brain during early development. Science 178: 708-713.

Squibb, R. E., and Tilson, H. A. (1982). Neurobehavioral changes in adult Fischer 344 rats exposed to dietary levels of chlordecone (kepone). Neurotoxicol. 3: 50-57.

Sukumar, R., Rose, S. P., and Burgoyne, R. D. (1980). Increased incorporation of [3H] fucose into chick brain glycoproteins following training on a passive avoidance task. J. Neurochem. 34: 1000-1006.

Suzuki, K. (1965). The pattern of mammalian brain gangliosides. J. Neurochem. 12: 629-638.

Tarttelin, M. F., Shryne, J. E., Gorski, R. A. (1975). Patterns of body weight change in rats following neonatal hormone manipulation. Acta Endocrinol. 79: 177-191.

Taylor, J. R., Selhorst, J. B., Houff, S. A., and Martinez, A. J. (1978). Chlordecone intoxication in man. I. Clinical observations. Neurol. 28: 626-630.

Taylor, J. E., El-Fakahany, E., and Richelson, E. (1979). Long-term regulation of muscarinic acetylcholine receptors on cultured nerve cells. Life Sci. 25: 2181-2187.

Tettamanti, G. (1971). Persistent pesticides. Science 164: 633-635.

Tilson, H. A., Byrd, N., and Riley, M. (1980). Neurobehavioral effects of exposing rats to kepone via the diet. Environ. Health Perspect. 33: 321-329.

Tilson, H. A., Squibb, R. E., and Burne, T. A. (1982). Neurobehavioral effects following a single dose of chlordecone (kepone) administered to rats. Neurotoxicol. 3: 45-58.

Trifaro, J., and Dworkind, J. (1975). Phosphorylation of the membrane components of chromaffin granules: Synthesis of PtdIns4P and presence of PtdIns kinase in granule membranes. Can. J. Physiol. Pharmacol. 53: 479-492.

Vernadakis, A. (1982). Neurotoxic effect of phenytoin: The developing organism. In Mechanisms of Actions of Neurotoxic Substances, ed. K. N. Prasid and A. Vernadakis, 155-179. Raven Press, N. Y.

Vettorazzi, G. (1975). The role of the WHO in pesticide research. Toxicol. 4: 31-40.

Vickers, J. D., Kinlough-Rathbone, R. L., and Mustard, J. F. (1982). Changes in phosphatidylinositol 4,5-biphosphate 10 seconds after stimulation of washed rabbit platelets with ADP. Blood 60: 1247-1249.

Weber, K., Klemmer, H. W., and Stenersen, J. (1972). Human health and pesticides: Community pesticide studies. Residue Rev. 42: 4158-4163.

Weiss, S. J., McKinney, J. S., and Putney, J. W. Jr. (1982). Receptor-mediated net breakdown of phosphatidylinositol 4,5-biphosphate in parotid acinar cells. Biochem. J. 206: 555-560.

Westlake, A., Westlake, W. E., and Gunther, F. A. (1970). Detection of residues of GC-9160 in cabbage, lettuce and citrus fruit. J. Agr. Food Chem. 18: 159.

Whitfield, J. B., Moss, D. W., and Neale, G. (1973). Changes in plasma gamma-glutamyl transpeptidase activity associated with alterations in drug metabolism in man. Br. Med. J. 1: 316-318.

Wiegandt, N. (1967). The subcellular localization of gangliosides in the brain. J. Neurochem. 14: 671-674.

Williams, G. M. (1980). Classification of genotoxic and epigenetic hepatocarcinogens using liver culture assays. Ann. N.Y. Acad. Sci. 349: 273-282.

Wilson, N. K., and Zehr, R. D. (1979). Structures of some kepone photoproducts and related chlorinated pentacyclodecanes by carbon-13 and proton nuclear magnetic resonance. J. Org. Chem. 44: 1278-1282.

Yamamura, H. I., Enna, S. J., and Kuhar, M. J. (1981). Neurotransmitter Binding. Raven Press, N. Y.

Yamamura, H. I., and Snyder, S. H. (1974). Muscarinic cholinergic receptor binding in rat brain. Proc. Nat. Acad. Sci. U.S.A. 71: 1725-1729.

Young, J. M. (1975). Desensitization and agonist binding to cholinergic receptors in intestinal smooth muscle. FEBS Lett. 46: 354-356.

APPENDIX A

Appendix A. Carbamylcholine stimulated radiolabeled orthophosphate incorporation into inositol phospholipids and phosphatidic acid of synaptosomes from chlordecone exposed offspring. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents cpms for the mean of 6 animals $\pm$ the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

Appendix A

CARBAMLYCHOLINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS		NONCROSSFOSTERED		
Control	3701±14	2052±21	924±27	5085±208
5 MG/KG	3457±28	1995±42	900±20	4950±96
15 MG/KG	3383±28*	1957±60	902±28	4815±37*
30 MG/KG	3201±14**	1940±21	890±27	4680±64**
		CROSSFOSTERED		
Control	3840±30	2090±42	918±10	5130±127
5 MG/KG	3712±31	2033±60	900±30	4890±115
15 MG/KG	3488±48*	1977±20	906±17	4770±54*
30 MG/KG	3424±30**	1920±39	894±9	4725±36**
24 DAYS		NONCROSSFOSTERED		
Control	4576±65	2432±60	1012±28	5940±132
5 MG/KG	4320±64**	2128±63**	980±16	5620±66*
15 MG/KG	4064±14**	2014±41**	932±26**	5400±34**
30 MG/KG	3648±42**	1920±80**	910±35**	5030±60**
		CROSSFOSTERED		
Control	4704±28	2470±61	1038±16	5985±54
5 MG/KG	4352±40**	2242±21**	985±25	5805±63*
15 MG/KG	3980±26**	2055±63**	924±15**	5490±60**
30 MG/KG	3744±41**	1980±41*	906±17**	5040±32**
31 DAYS		NONCROSSFOSTERED		
Control	4672±63	2460±40	1056±28	6210±54
5 MG/KG	4128±32**	2300±41*	980±10*	5808±63*
15 MG/KG	3872±15**	2160±63**	940±15**	5490±60**
30 MG/KG	3616±45**	2030±45**	920±9**	5040±32**
		CROSSFOSTERED		
Control	4768±54	2530±84	1082±15	6345±122
5 MG/KG	4192±26**	2337±20*	995±16**	5762±133*
15 MG/KG	4061±27**	2185±41**	950±10**	5614±85**
30 MG/KG	3712±12**	2050±21**	925±16**	5120±87**
3 MONTH		CROSSFOSTERED		
Control	4736±51	2570±65	1074±15	6390±203
15 MG/KG	4064±48**	2250±63**	983±23**	5535±66**
9 MONTH		CROSSFOSTERED		
Control	4672±26	2500±88	1064±16	6170±124
15 MG/KG	4128±30**	2280±44**	1003±14**	5563±130**

APPENDIX B

Appendix B. Effects of carbamylcholine and atropine on ^{32}P orthophosphate incorporation into inositol phospholipids and phosphatidic acid of synaptosomes from chlordecone exposed offspring. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were incubated with atropine for 5 minutes. Half of these were then incubated with carbamylcholine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the percent stimulation over control of 6 animals $\pm$ the standard deviation.

Appendix B

EFFECTS OF CARBAMYLCHOLINE AND ATROPINE ON ^{32}P-ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS		CARBAMYLCHOLINE + ATROPINE		
Control	103±3	103±1	102±2	100±1
5 MG/KG	102±2	101±3	100±1	101±2
15 MG/KG	102±2	104±2	103±1	100±2
30 MG/KG	102±3	99±2	100±3	99±2
		ATROPINE		
Control	99±3	98±2	97±2	99±2
5 MG/KG	97±2	95±3	99±1	97±1
15 MG/KG	95±1	99±1	98±3	97±1
30 MG/KG	96±3	99±2	96±3	99±2
24 DAYS		CARBAMYLCHOLINE + ATROPINE		
Control	103±2	99±2	103±3	102±3
5 MG/KG	102±2	99±3	100±2	102±2
15 MG/KG	100±3	97±3	99±2	103±1
30 MG/KG	99±3	99±1	98±2	100±3
		ATROPINE		
Control	101±2	97±2	98±3	97±1
5 MG/KG	100±2	97±2	96±2	97±2
15 MG/KG	100±3	99±2	99±3	97±1
30 MG/KG	101±2	96±1	98±1	98±2
31 DAYS		CARBAMYLCHOLINE + ATROPINE		
Control	102±2	99±1	99±2	100±3
5 MG/KG	103±3	96±2	97±2	98±3
15 MG/KG	100±2	98±2	97±3	99±2
30 MG/KG	101±2	96±3	98±2	98±1
		ATROPINE		
Control	100±2	98±3	97±1	97±3
5 MG/KG	101±3	99±2	98±1	98±2
15 MG/KG	99±1	97±3	97±1	96±3
30 MG/KG	100±2	99±2	95±3	98±1

APPENDIX C

APPENDIX C

DOPAMINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS				
Control	3860±46	2250±63	982±27	5490±94
5 MG/KG	3750±29	2155±42	920±16	4980±120
15 MG/KG	3470±15**	2070±21**	930±9*	4870±45**
30 MG/KG	3425±43**	1995±43**	910±20**	4850±97**
24 DAYS				
Control	4170±36	2430±43	1030±28	5810±145
5 MG/KG	4063±21	2240±21**	960±10*	5570±90**
15 MG/KG	3760±34**	2215±40**	930±18**	5250±87**
30 MG/KG	3490±30**	2090±62**	920±16**	4960±85**
31 DAYS				
Control	4550±46	2502±41	1034±8	6550±145
5 MG/KG	4350±30*	2380±39**	987±17*	5700±44**
15 MG/KG	4030±19**	2120±63**	940±9**	5680±40**
30 MG/KG	3670±28**	2040±42**	920±18**	5220±86**

Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents cpms for the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

APPENDIX D

Appendix D. Effects of dopamine and haloperidol on ^{32}P orthophosphate incorporation into inositol phospholipids and phosphatidic acid of synaptosomes from chlordecone exposed offspring. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were incubated with haloperidol for 5 minutes. Half of these were then incubated with dopamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation.

APPENDIX D

DOPAMINE AND HALOPERIDOL STIMULATED ^{32}P-ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS		DOPAMINE + HALOPERIDOL		
Control	105±3	103±2	104±1	105±3
5 MG/KG	104±1	101±1	105±1	102±1
15 MG/KG	102±3	100±2	106±3	103±2
30 MG/KG	100±2	97±2	102±2	98±1
		HALOPERIDOL		
Control	97±2	97±1	99±2	99±2
5 MG/KG	98±1	94±2	98±1	97±1
15 MG/KG	94±2	96±2	98±2	97±1
30 MG/KG	96±2	98±1	97±1	99±2
24 DAYS		DOPAMINE + HALOPERIDOL		
Control	103±2	99±2	103±3	102±3
5 MG/KG	102±2	99±3	100±2	102±2
15 MG/KG	100±3	97±3	99±2	103±1
30 MG/KG	99±3	99±1	98±2	100±3
		HALOPERIDOL		
Control	101±2	97±2	98±3	97±1
5 MG/KG	100±2	97±2	96±2	97±2
15 MG/KG	100±3	99±2	99±3	97±1
30 MG/KG	101±2	96±1	98±1	98±2
31 DAYS		DOPAMINE + HALOPERIDOL		
Control	104±1	99±1	99±2	100±3
5 MG/KG	101±3	96±2	97±2	98±3
15 MG/KG	102±1	98±2	97±3	99±2
30 MG/KG	103±2	96±3	98±2	98±1
		HALOPERIDOL		
Control	100±2	98±3	97±1	97±3
5 MG/KG	101±3	99±2	98±1	98±2
15 MG/KG	99±1	97±3	97±1	96±3
30 MG/KG	100±2	99±2	95±3	98±1

APPENDIX E

APPENDIX E

NORADRENALINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS				
Control	4320±46	2223±16	999±18	5625±42
5 MG/KG	4064±21*	2185±10	968±9	5265±85*
15 MG/KG	3872±20**	2052±9**	941±10*	4950±88**
30 MG/KG	3680±44**	2014±18**	914±11**	4725±40**
24 DAYS				
Control	4960±63	2394±10	1047±28	6075±123
5 MG/KG	4704±42*	2204±11**	1020±9	5670±87*
15 MG/KG	4352±21**	2166±36**	950±18**	5445±46**
30 MG/KG	3872±40**	2109±23**	924±17**	5130±92**
31 DAYS				
Control	5185±62	2546±34	1117±8	6435±132
5 MG/KG	4768±40**	2375±36**	1038±16**	6040±79**
15 MG/KG	4448±60**	2299±21**	1003±26**	5895±83**
30 MG/KG	3936±41**	2185±11**	977±9**	5535±132**

Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents cpms for the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

APPENDIX F

Appendix F. Effects of noradrenaline and regitine on ^{32}P orthophosphate incorporation into inositol phospholipids and phosphatidic acid of synaptosomes from chlordecone exposed offspring. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were incubated with regitine for 5 minutes. Half of these were then incubated with noradrenaline for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation.

APPENDIX F

EFFECTS OF NORADRENALINE AND REGITINE ON ^{32}P-ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS		NORADRENALINE + REGITINE		
Control	106±1	102±2	104±2	102±3
5 MG/KG	103±1	100±2	103±1	103±1
15 MG/KG	101±2	101±2	103±1	103±2
30 MG/KG	102±1	99±1	102±2	99±1
		REGITINE		
Control	99±1	98±1	97±2	99±2
5 MG/KG	99±2	95±2	99±1	96±1
15 MG/KG	97±3	96±2	98±2	97±1
30 MG/KG	96±2	98±1	96±1	98±2
24 DAYS		NORADRENALINE + REGITINE		
Control	105±1	96±2	103±3	104±3
5 MG/KG	104±2	99±2	102±2	101±3
15 MG/KG	103±2	101±3	99±2	102±2
30 MG/KG	99±3	100±1	98±2	100±1
		REGITINE		
Control	101±2	97±2	98±3	97±1
5 MG/KG	100±2	97±2	96±2	97±2
15 MG/KG	100±3	99±2	99±3	97±1
30 MG/KG	101±2	96±1	98±1	98±2
31 DAYS		NORADRENALINE + REGITINE		
Control	104±1	99±1	99±2	100±3
5 MG/KG	101±3	96±2	97±2	96±3
15 MG/KG	102±1	98±2	97±3	99±2
30 MG/KG	103±2	96±3	98±2	98±1
		REGITINE		
Control	100±2	98±3	97±1	97±3
5 MG/KG	101±3	99±2	98±1	98±2
15 MG/KG	99±1	97±3	97±1	96±3
30 MG/KG	100±2	99±2	95±3	98±1

APPENDIX G

APPENDIX G

SEROTONIN STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS				
Control	4800±62	2850±41	1117±18	5625±122
5 MG/KG	4384±30**	2603±21**	968±17	5265±88*
15 MG/KG	4128±32**	2451±20**	941±8*	4950±130**
30 MG/KG	3840±60**	2280±42**	915±9**	4725±46**
24 DAYS				
Control	4864±93	2888±63	1126±28	5400±126
5 MG/KG	4768±66	2831±41**	1047±17**	5040±89**
15 MG/KG	4448±97**	2641±66**	976±19**	4950±120**
30 MG/KG	4064±88**	2413±65**	950±15**	4680±92**
31 DAYS				
Control	5216±88	3097±60	1151±8	6615±132
5 MG/KG	4832±63**	2869±41**	1056±16**	6165±82**
15 MG/KG	4544±91**	2698±62**	1030±25**	5580±87**
30 MG/KG	4065±65**	2432±39**	950±7**	5130±136**

Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with serotonin for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents cpms for the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

APPENDIX H

APPENDIX H

HISTAMINE STIMULATED RADIOLABELED ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS				
Control	3872±62	2128±20	959±18	4905±132
5 MG/KG	3360±30*	2033±42**	915±17*	4900±84
15 MG/KG	3328±66**	1995±21**	906±8*	4680±46*
30 MG/KG	3296±68**	1938±18**	888±7**	4590±92**
24 DAYS				
Control	3872±88	2204±21	1038±9	5355±92
5 MG/KG	3744±62*	2090±20**	976±19**	5085±47**
15 MG/KG	3552±30**	2052±43**	941±10**	4950±44**
30 MG/KG	3424±31**	1950±20**	915±10**	4725±88**
31 DAYS				
Control	4160±84	2338±65	1110±10	5435±46
5 MG/KG	3872±34**	2240±21*	1020±22**	5040±90**
15 MG/KG	3744±30**	2166±43**	986±9**	4950±40**
30 MG/KG	3552±66**	2050±44**	941±8**	4860±42**

Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were then stimulated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents cpms for the mean of 6 animals ± the standard deviation. An asterisk (*) and two asterisks (**) indicate that values from control and chlordecone exposed offspring are different at $p<0.01$ and $p<0.001$, respectively.

APPENDIX I

Appendix I . Effects of histamine and bentazole on ^{32}P orthophosphate incorporation into inositol phospholipids and phosphatidic acid of synaptosomes from chlordecone exposed offspring. Synaptosomes from control and chlordecone exposed offspring were preincubated with radiolabeled orthophosphate for 1 hour. The prelabeled synaptosomes were incubated with bentazole for 5 minutes. Half of these were then incubated with histamine for 30 minutes. The reaction was terminated by adding 2 ml chloroform, 5 ml methanol, and 0.5 ml EDTA-KCl. The phospholipids were extracted as described in Materials and Methods. Each value represents the mean of 6 animals $\pm$ the standard deviation.

APPENDIX I

EFFECTS OF HISTAMINE AND BENTAZOLE ON ^{32}P-ORTHOPHOSPHATE INCORPORATION INTO INOSITOL PHOSPHOLIPIDS AND PHOSPHATIDIC ACID OF SYNAPTOSOMES FROM CHLORDECONE EXPOSED OFFSPRING

DAYS	TPI	DPI	PI	PA
17 DAYS		HISTAMINE + BENTAZOLE		
Control	109±1	110±2	104±2	109±3
5 MG/KG	106±1	107±2	104±1	107±1
15 MG/KG	104±2	104±2	103±1	105±2
30 MG/KG	102±1	102±1	100±2	101±1
		BENTAZOLE		
Control	100±1	101±1	98±2	102±2
5 MG/KG	101±2	99±2	100±1	101±1
15 MG/KG	101±3	100±2	96±2	97±1
30 MG/KG	97±2	99±1	94±1	96±2
24 DAYS		HISTAMINE + BENTAZOLE		
Control	120±1	113±2	115±3	115±3
5 MG/KG	118±2	111±2	111±2	112±3
15 MG/KG	111±2	107±3	108±2	109±2
30 MG/KG	108±3	103±1	103±2	104±1
		BENTAZOLE		
Control	102±2	100±2	97±3	100±1
5 MG/KG	98±2	100±2	99±2	99±2
15 MG/KG	101±3	98±2	101±3	101±1
30 MG/KG	100±2	101±1	98±1	99±2
31 DAYS		HISTAMINE + BENTAZOLE		
Control	128±1	119±1	120±2	119±3
5 MG/KG	120±3	116±2	118±2	116±3
15 MG/KG	114±1	111±2	114±3	112±2
30 MG/KG	108±2	105±3	106±2	105±1
		BENTAZOLE		
Control	96±2	99±3	100±1	97±3
5 PPM	97±3	99±2	101±1	96±2
15 PPM	96±1	97±3	99±1	98±3
30 PPM	97±2	99±2	100±3	97±1

APPROVAL SHEET

The dissertation submitted by Kristine Diane Dahl has been read and approved by the following committee:

Dr. Michael Collins, Ph.D., Director
Professor, Biochemistry and Biophysics
Loyola Stritch School of Medicine

Dr. Mary Manteuffel, Ph.D.
Associate Professor, Biochemistry and Biophysics
Loyola Stritch School of Medicine

Dr. William Simmons, Ph.D.
Assistant Professor, Biochemistry and Biophysics
Loyola Stritch School of Medicine

Dr. Daniel Selivonchick, Ph.D.
Associate Professor, Food Science and Nutrition
Oregon State University
Corvallis, Oregon

Dr. Samuel Epstein, M.D., Ph.D.
Professor, Environmental and Occupational Health
School of Public Health
University of Illinois at Chicago
Chicago, Illinois

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Aug 5, 1985
Date

Michael A. Collins
Director's Signature