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PHYSIOLOGICAL AND BIOCHEMICAL SEQUELAE TO PERINATAL EXPOSURE TO POLYBROMINATED BIPHENYLS

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PHYSIOLOGICAL AND BIOCHEMICAL SEQUELAE TO PERINATAL EXPOSURE TO POLYBROMINATED BIPHENYLS

By

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ABSTRACT

Physiological and Biochemical Sequelae to Perinatal Exposure to Polybrominated Biphenyls

by

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Polybrominated biphenyls (PBBs) were directly added to the food supply in Michigan. Of particular concern are possible effects of PBBs on mothers and progeny since high concentrations of PBBs have been detected in human milk and PBBs have been reported to undergo transplacental movement. The purpose of this investigation was to determine if physiological and biochemical alterations are produced by prenatal and/or postnatal exposure to PBBs.

Dietary exposure of Sprague-Dawley rats to 100 ppm PBBs (Firemaster BP-6) from day 8 through day 20 of gestation did not alter litter size, resorption rate, fetal body weight or length or incidence of gross, soft tissue or skeletal anomalies. Food deprivation in combination with this treatment increased fetal resorption rate and decreased fetal body weight. Treatment of rat pups with 150 or 500 mg/kg PBBs on day 1 postpartum had no effect on growth, development or mortality. However, after 1 wk postpartum growth and development were retarded and mortality increased in pups born to and suckled by dams fed 100 ppm PBBs from the eighth day of pregnancy. Consistent with these effects, concentrations of PBBs in tissues from animals exposed

to PBBs transplacentally and via mother's milk were higher than concentrations in tissues from rats neonatally treated with a single injection of PBBs.

Kidneys from rats exposed to PBBs did not have prominent, if any, macroscopic or microscopic morphological changes. The paucity of renal structural alterations was correlated with a lack of effect of PBBs on renal function. Similarly, perinatal exposure to PBBs did not affect lung or heart weights and had little effect on pulmonary or cardiac function.

The liver weight-to-body weight ratio was increased following perinatal exposure to 10 or 100 ppm PBBs and after neonatal treatment with 150 or 500 mg/kg PBBs. Liver enlargement was dose dependent and directly related to hepatic concentrations of PBBs. Increased liver weight may have been due, at least in part, to elevated protein content as hepatic microsomal protein was increased by PBBs. Microscopic morphologic alterations were also produced by PBBs. Liver from rats perinatally exposed to 100 ppm PBBs had vacuolation, hepatocyte swelling, necrosis, absent or pycnotic nuclei and myelin bodies. Liver from rats treated with PBBs also had a decreased concentration of vitamin A and an increased concentration of coproporphyrin and uroporphyrin.

Activity of microsomal enzymes in liver and extrahepatic organs was altered by PBBs in a manner that was dependent on dose, age and time following administration. These enzymatic changes produced by PBBs were correlated with modifications in the toxicity and/or duration of action of certain subsequently administered therapeutic agents and environmental chemicals. Thus, even animals that exhibited no visible

expression of PBBs toxicity were more susceptible to drug interactions after exposure to PBBs.

Activity of progesterone hydroxylases was increased in hepatic microsomes prepared from rats perinatally exposed to 100 ppm PBBs. Accelerated metabolism of progesterone in vitro was reflected in vivo by a reduced duration of anesthesia following a pharmacologic dose of progesterone. Similarly, responses to exogenously administered estradiol-17β and testosterone were diminished by perinatal exposure to PBBs. Radioactivity in serum and target tissues following administration of labeled steroid hormones was also reduced by PBBs. Reproductive capacity may be diminished by PBBs as a consequence of enhanced steroid metabolism.

Effects of PBBs not only persisted for long periods of time in directly exposed rats but were produced in their descendants. PBBs were transferred from one generation to the next via transplacental movement and excretion into milk. Transferred PBBs produced alterations in liver morphology and ability to metabolize xenobiotic and endogenous compounds. Therefore, the health hazard associated with exposure to PBBs may not be limited to a single generation.

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TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS---------- ii

LIST OF TABLES---------- vi

LIST OF FIGURES----------viii

INTRODUCTION---------- 1

A. Characteristics of Polybrominated Biphenyls---------- 2

B. Environmental Contamination by Polybrominated Biphenyls 4

C. Biological Effects of Polybrominated Biphenyls--------- 8

D. Biological Effects of Agents that Increase Microsomal Enzyme Activity---------- 13

E. Objectives---------- 28

MATERIALS AND METHODS---------- 29

A. Animals---------- 29

B. Teratology---------- 29

C. Postnatal Development---------- 30

D. Vaginal Cycle Length---------- 31

E. Histopathology---------- 31

F. Milk Collection---------- 31

G. Quantitation of PBBs---------- 31

H. Liver---------- 33

1. Coproporphyrin and uroporphyrin concentrations in liver and urine---------- 33
2. Vitamin A concentration in liver and serum-------- 34

I. Preparation of Postmitochondrial Supernatants and Microsomes---------- 34

J. Enzyme Assays---------- 35

K. Kidney---------- 39

1. Function in vitro---------- 39
2. Function in vivo---------- 41

TABLE OF CONTENTS (continued)

Page

L. Heart---------- 42

1. Function in vitro---------- 42
2. Arterial pressure---------- 43

M. Lung---------- 44

1. Function in vitro---------- 44
2. Isolated perfused lung---------- 45

N. Response to Xenobiotics---------- 47

O. Response to Pharmacological Doses of Steroid Hormones-- 48

P. Persistence of Effects---------- 49

1. Single injection---------- 49
2. Perinatal exposure---------- 49
3. Multiple generations---------- 50

Q. Statistics---------- 50

RESULTS---------- 51

A. Survival, Growth and Development---------- 51

B. Liver---------- 56

C. Kidney---------- 83

D. Heart---------- 99

E. Lung---------- 107

F. Testis and Ovary---------- 107

G. Response to Xenobiotics---------- 119

H. Response to Exogenously Administered Steroid Hormones-- 128

I. Persistence of Effects---------- 143

DISCUSSION---------- 178

A. Survival, Growth and Development---------- 178

B. Liver---------- 182

C. Kidney---------- 192

D. Heart---------- 194

E. Lung---------- 194

F. Extrahepatic Microsomal Enzyme Stimulation---------- 195

G. Response to Xenobiotics---------- 198

TABLE OF CONTENTS (continued)

Page

H. Response to Exogenously Administered Steroid Hormones-- 200

I. Persistence of Effects---------- 204

SUMMARY AND CONCLUSIONS---------- 209

BIBLIOGRAPHY---------- 215

LIST OF TABLES

Table		Page
1	Resorption rate, fetal size and whole carcass concentration of PBBs among offspring of pregnant rats treated with PBBs and food deprived	52
2	Postnatal development of rats following neonatal or perinatal treatment with PBBs	55
3	Tissue concentrations of PBBs following neonatal or perinatal and continuous treatment with PBBs	59
4	Effect of perinatal exposure to PBBs on the concentration of vitamin A in serum and liver of rats	72
5	Concentration of coproporphyrin and uroporphyrin in liver from female rats exposed to PBBs	73
6	Concentration of coproporphyrin and uroporphyrin in urine from female rats exposed to PBBs	74
7	Packed cell volume (hematocrit) in female rats exposed to PBBs	75
8	Effect of PBBs on blood urea nitrogen (BUN)	91
9	Effect of PBBs on glomerular filtration rate (GFR) and effective renal plasma flow (ERPF)	92
10	Serum calcium concentration and bone mineral mass in female rats exposed to PBBs	95
11	Effect of perinatal exposure to PBBs on the concentration of vitamin A in kidney of rats	96
12	Effect of PBBs on the accumulation of PAH and NMN	97
13	Effect of PBBs on ammoniagenesis and gluconeogenesis	98
14	Effect of PBBs on the accumulation of (^{3}H)-d,l-metaraminol	106

LIST OF TABLES (continued)

Table		Page
15	Effect of PBBs on left atrial susceptibility to ouabain-induced arrhythmia, twitch tension and inotropic response to ouabain or calcium	108
16	Effect of PBBs on mean systolic blood pressure	109
17	Effect of perinatal treatment with polybrominated biphenyls on the clearance of angiotensin I (AI) and 5-hydroxytryptamine (5-HT) by isolated perfused rat lungs	112
18	Concentration of testosterone and progesterone in serum from rats exposed to PBBs	120
19	Vaginal (estrus) cycle length and age of vaginal opening and testicular descent in rats exposed to PBBs	121
20	Median time to death after administration of bromobenzene (2820 mg/kg) in 49 day old male rats neonatally treated with polybrominated biphenyls	126
21	Median time to death after administration of digitoxin in female rats pretreated with PBBs	127
22	Tissue concentrations of PBBs following a single injection	148
23	Residual effect of PBBs-exposure on blood urea nitrogen (BUN) and activity of serum glutamic pyruvic transaminase (SGPT)	159
24	Tissue concentrations of PBBs following perinatal exposure to PBBs	162
25	Concentration of PBBs in liver following perinatal exposure to PBBs	176

LIST OF FIGURES

Figure		Page
1	Effect of neonatal or perinatal treatment with PBBs on body wt gain	53
2	Effect of neonatal or perinatal treatment with PBBs on survival rate-to-weaning	57
3	Concentration of PBBs in milk from lactating rats fed diet containing 50 ppm PBBs from the 8th day of pregnancy	60
4	Effect of neonatal or perinatal treatment with PBBs on liver wt to body wt and kidney wt to body wt ratios	62
5	Hepatic tissue from control rat	65
6	Hepatic tissue from rat 28 days of age that had been exposed to 100 ppm PBBs from 8th day of gestation	67
7	Hepatic tissue from rat fed diet containing 100 ppm PBBs for 90 days	69
8	Hepatic microsomal protein and enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum	77
9	Hepatic enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum	79
10	Effect of neonatal or perinatal treatment with PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver	81
11	Hepatic progesterone hydroxylase activities in rats treated with 0 or 100 ppm PBBs from day 8 of gestation until they were killed at day 28 postpartum	84
12	Hepatic monoamine oxidase activity in rats treated with 0 or 100 ppm PBBs from day 8 of gestation until they were killed at day 28 postpartum	86

LIST OF FIGURES (continued)

Figure		Page
13	Renal tissue from rat fed diet containing 100 ppm PBBs for 90 days	88
14	Effect of PBBs on fractional sodium excretion	93
15	Renal postmitochondrial supernatant protein and enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum	100
16	Effect of neonatal or perinatal treatment with PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney	102
17	Effect of PBBs on the heart weight-to-body weight ratio	104
18	Effect of PBBs on the lung weight-to-body weight ratio-	110
19	Effect of perinatal treatment with PBBs on the activity of angiotensin-converting enzyme and monoamine oxidase in lung	113
20	Effect of PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in lung	115
21	Effect of PBBs on the testis weight- or ovary weight-to-body weight ratios	117
22	Effect of neonatal or perinatal treatment with PBBs on the duration of anesthesia produced by pentobarbital---	122
23	Effect of PBBs on the duration of anesthesia produced by diethyl ether	124
24	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the duration of anesthesia produced by progesterone-	129
25	Effect of perinatal exposure to 0 or 100 ppm PBBs on the concentration of equivalents of progesterone-^{14}C in brains of rats treated with progesterone-^{14}C	131
26	Effect of perinatal exposure to 0 or 100 ppm PBBs on the concentration of equivalents of progesterone-^{14}C in serum of rats treated with progesterone-^{14}C	133
27	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the increase in seminal vesicle wt-to-body wt ratio produced by testosterone	135

LIST OF FIGURES

Figure		Page
28	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of testosterone-^{3}H in testis of rats treated with testosterone-^{3}H	137
29	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of testosterone-^{3}H in serum of rats treated with testosterone-^{3}H	139
30	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the increase in uterus wt-to-body wt ratio produced by estradiol-17β	141
31	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of estradiol-17β-6,7-^{3}H in uterus of rats treated with estradiol-17β-6,7-^{3}H	144
32	Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of estradiol-17β-6,7-^{3}H in serum of rats treated with estradiol-17β-6,7-^{3}H	146
33	Disappearance of PBBs from the peritoneal cavity of rats after a single intraperitoneal injection	150
34	Residual effect of perinatal exposure to PBBs on liver wt-to-body wt and kidney wt-to-body wt ratios	152
35	Residual effect of perinatal exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver	155
36	Residual effect of perinatal exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney	157
37	Residual effect of perinatal exposure to PBBs on duration of anesthesia produced by pentobarbital	160
38	Effect of exposure to PBBs on body weight and the liver wt-to-body wt ratio in subsequent generations	164
39	Effect of exposure to PBBs on percent survival-to-weaning in subsequent generations	166
40	Effect of exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver in subsequent generations	168

LIST OF FIGURES (continued)

Figure		Page
41	Effect of exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney in subsequent generations----------	170
42	Effect of exposure to PBBs on duration of anesthesia produced by progesterone in subsequent generations-----	172
43	Effect of exposure to PBBs on duration of anesthesia produced by pentobarbital in subsequent generations----	174

INTRODUCTION

Accidental addition of polybrominated biphenyls (PBBs) to dairy cow feed in Michigan has resulted in contamination of the food supply (Dunckel, 1975). As PBBs are quite lipid soluble, they accumulate in biological tissues, especially those with a high fat content. Most PBBs are biologically stable, efficiently absorbed from the gastrointestinal tract, and very slowly eliminated from the body. Extrapolation of data obtained in animals suggests that PBBs will persist in tissues throughout a human lifetime (Matthews et al., 1977).

Biological effects of PBBs in man and animals have not been fully characterized. Hepatic and renal histopathological changes have been produced by PBBs; however, data presently available are inadequate to assess changes in organ function. Although PBBs have been shown to increase activity of microsomal mixed function oxidases (MFO) in liver, the effect of PBBs on MFO activity in extrahepatic tissues has not been characterized (Dent et al., 1976a,b). These microsomal enzymes catalyze metabolism of a variety of xenobiotic and endogenous compounds including certain carcinogens, therapeutic agents, vitamins and hormones (Conney, 1967). Thus, some effects of PBBs may be mediated through alterations in MFO activity. The potential hazard to man of such metabolic alterations following PBBs has not been conclusively established.

Of particular concern are possible effects of PBBs on mothers and progeny since high concentrations of PBBs have been detected in human milk and PBBs have been reported to undergo transplacental movement (Dunckel, 1975; Rickert et al., 1978). Major organ systems are morphologically and functionally immature in very young animals and deficient in their capacity to maintain homeostasis when challenged with stressful stimuli. Therefore, mammals including man, may be particularly vulnerable to damage produced by PBBs during organogenesis and early postnatal life.

A. Characteristics of Polybrominated Biphenyls

Polybrominated biphenyls (PBBs) are composed of two six-membered aromatic carbon rings, connected by a carbon-carbon bond, which may be brominated at any position, except the bridge carbons. Firemaster FF-1 is the commercial name for the product that contained PBBs (Firemaster BP-6) and calcium polysilicate and was added to the food supply. Firemaster BP-6 has been shown by electron capture gas chromatography to consist of a mixture of PBBs. Analysis of the Firemaster mixture using gas chromatography-mass spectrometry demonstrated 12 peaks whose mass spectrum corresponded to compounds with molecular weights indicative of 2 penta-, 4 hexa-, 4-hepta-, and 2 octabromobiphenyl congeners (Anderson et al., 1974; Sundstrom et al., 1976). Nuclear magnetic resonance was used to identify the structures of congeners in Firemaster BP-6. The two main congeners, 2,4,5,2',4',5'-hexabromobiphenyl and 2,3,4,5,2',4',5'-heptabromobiphenyl, account for 54-68 and 27 percent of the Firemaster BP-6 mixture by weight, respectively (Sundstrom et al., 1976; Jacobs et al., 1976; Moore et al.,

1978a). Other congeners identified include: 2,4,5,4',5'-penta-, 2,4,5,3',4'-penta-, 2,3,4,2',4',5'-hexa-, 2,4,5,3',4',5'-hexa-, 2,3,4,5,2',3',4',5'-octa-, and 2,3,4,5,6,2',3',4',5'-nonabromobiphenyl (Moore and Aust, 1978). Firemaster may contain 30 or more components. Trace quantities of hexa-, penta-, and tetrabromonapthalene have been found in this mixture as well as several polar contaminants which have been partially characterized (O'Keefe, 1976; Hass et al., 1978).

Firemaster is a gray powder that is nearly insoluble in water (11 ppb) but soluble in most organic solvents. Firemaster has a comparatively low vapor pressure, begins to melt at 72°C and decomposes in the range 300-400°C (Sundstrom et al., 1976). The major congener, 2,4,5,2',4',5'-hexabromobiphenyl, melts at 159-160°C and 2,3,4,5,2',4',5'-heptabromobiphenyl melts at 165-166°C (Moore et al., 1978a, 1979). The stability of PBBs to oxidation and hydrolysis in the environment has not been determined. However, part of the Firemaster mixture is unstable to alkaline hydrolysis as reflected by the fact that treatment with 2% potassium hydroxide in ethanol resulted in degradation of the major hexa-isomer (Pomerantz et al., 1978). PBBs are more photoreactive than polychlorinated biphenyls (PCBs) and should be protected from light in the laboratory. When dissolved in methanol and exposed to ultraviolet light (3000 Å), 2,4,5,2',4',5'-hexabromobiphenyl was 7 times more photolytically reactive than 2,4,5,2',4',5'-hexachlorobiphenyl (Ruzo and Zabik, 1975). 2,4,5,2',4',5'-Hexabromobiphenyl was found to undergo photolytic reductive debromination in methanol to penta- and tetrabromobiphenyl as well as (approximately 1%) dimethoxytetrabromobiphenyl (Ruzo and Zabik, 1975). PBBs may be more photoreactive than PCBs because of enhanced intersystem crossing to a

triplet state due to virbonic coupling with the bromines and low carbon-bromine bond energy (71 Kcal/mole) (Kerst, 1974; Ruzo and Zabik, 1975; Matthews et al., 1978). The rates and extent of photolytic reactivity of PBBs in the environment have not yet been determined.

B. Environmental Contamination by Polybrominated Biphenyls

Contamination of the environment by PBBs was discovered in the spring of 1974, at least 8 months after 500-1000 pounds of Firemaster had been inadvertently substituted for magnesium oxide in dairy cattle feed (Dunckel, 1975). According to formula, animals were being fed diet with a concentrate containing 4,000 to 5,200 ppm PBBs but some concentrate contained as much as 13,500 ppm PBBs while other livestock and poultry feed mixed in contaminated mills had smaller amounts. Two other probable routes of indirect contamination are recycling of contaminated products and feed swapping between individual farms and feed mills.

Milk from Michigan farms had concentrations of PBBs in excess of 600 ppm which if consumed at 500 to 1000 ml per day during the period between contamination and identification of PBBs would have resulted in human intakes of approximately 150 mg PBBs per kg body weight (Fries and Marrow, 1975). Additional human exposures could have come from consumption of poultry, beef or eggs which had concentrations as high as 4,600 (fat), 2,700 (fat) and 4,000 ppm PBBs, respectively. PBBs are usually quantified by the major hexabromobiphenyl peak (2,4,5,2',4',5'-hexa) despite the possibility that other congeners are more toxic. Guidelines for permissible concentrations of PBBs were

first established in May, 1974 by the U.S. Food and Drug Administration and included milk and meat, 1.0 ppm; eggs, 0.1 ppm; and feed, 0.3 ppm. These guidelines were revised in November, 1974 to milk and meat, 0.3 ppm; and eggs and furnished feeds, 0.05 ppm, and have been reduced several times since then. All products containing PBBs at or exceeding those guideline concentrations were confiscated. To date more than 30,000 cattle, 5,000 swine and sheep, 1.5 million chickens, 2,600 pounds of butter, 34,000 pounds of dry milk products, 1500 cases of canned evaporated milk, 18,000 pounds of cheese, 5 million eggs, and 865 tons of feed have been destroyed. It has been estimated that between the onset of contamination in the fall of 1973 and the establishment of quarantine of contaminated livestock in the spring of 1974, over 10,000 Michigan residents were exposed to PBBs through consumption of contaminated milk and meat (Dunckel, 1975; Kay, 1977). Extrapolation of data obtained in 1976 suggested that approximately 90% of the residents in Michigan had detectable body burdens of PBBs (Brilliant et al., 1978).

Although once considered to be solely a Michigan problem, PBBs have recently been found in New York, New Jersey, Pennsylvania, Iowa, Indiana, Wisconsin, Alabama, Mississippi and Texas (Report, 1976; Carter, 1976; Michigan Dept. Agriculture, 1977; Anonymous, 1977). Products from Michigan contaminated with PBBs may have been transported to other states for consumption. It has been estimated that over 600,000 pounds of PBBs were directly added to the environment at sites of PBBs manufacture (Neufeld et al., 1977). Industrial pollution included emission to the air from vents of the hydrogen bromide recovery system, losses in waste waters resulting from quenching and

washing PBBs as they were recovered from the reaction mixture, and losses occurring with landfills resulting from drying, handling and transportation (Neufeld et al., 1977). The general population may also be exposed to PBBs from the use of Firemaster as a flame retardant. Between 1971 and 1974 almost 12 million pounds of PBBs were marketed as fire retardants used in thermal plastics such as typewriter, television and business machine casings (Kerst, 1974). Most of the products containing PBBs are or will be eventually buried in refuse dumps. PBBs have little tendency to migrate from the thermal plastics into which they are incorporated, however, small quantities of PBBs may leak into streams or the water table, be consumed by scavengers, or enter the atmosphere upon flameless combustion of products containing PBBs (e.g., in refuse dumps or office fires). PBBs entering the atmosphere may then be deposited within a few days onto land or into water and eventually enter food chains.

Polybrominated biphenyls are quite lipid soluble and stable and thus, would be anticipated to accumulate in biological tissues especially those with a high fat content. Fish accumulate PBBs as do a variety of mammals including rodents, farm animals and man (Zitko, 1977; Fries and Marrow, 1975; Matthews et al., 1977; Willet and Irving, 1975; Kimbrough et al., 1978). Investigations with Firemaster in rats and farm animals indicate that PBBs are efficiently absorbed from the gastrointestinal tract and very slowly eliminated from the body (Gutenmann and Lisk, 1975; Willett and Irving, 1976; Matthews et al., 1976). Studies with 2,4,5,2',4',5'-hexabromobiphenyl suggest that under conditions of adequate feed and good health, the

concentration of this congener in rat adipose tissue would not be expected to decline appreciably during the animal's lifetime (Matthews et al., 1977). Lactating females may eliminate PBBs more rapidly than males or nonlactating females, since lipophilic compounds such as PBBs are eliminated in the lipid portion of milk. Cows consuming PBBs had milk concentrations of PBBs that plateaued at approximately 4 times the dietary concentration (Fries, 1978; Fries et al., 1978). Transfer of PBB congeners from diet to milk is dependent on degree of bromination (Fries and Marrow, 1975). Hexabromobiphenyls have been transferred 5 to 6 times more efficiently than 2,3,4,5,2',4',5'-heptabromobiphenyl, in cows, suggesting that there is a greater resistance to movement of more brominated congeners across membranes (Fries and Marrow, 1975; Fries, 1978). Concentrations of PBBs (2,4,5,2',4',5'-hexabromobiphenyl) were lower in body fat than milk fat while cows were being fed PBBs (Fries, 1978; Fries et al., 1978). Following termination of PBB exposure in cows, the relationship between concentration of PBBs in milk and body fat approached a 0.4 to 1.0 ratio (Fries, 1978; Fries et al., 1978).

Of women selected at random from Michigan's lower peninsula, 96% had PBBs in their milk (Eyster, 1976). Human breast milk contains relatively high concentrations of PBBs, which ranged from 0.21 to 92.66 ppm in milk from mothers on quarantined farms in 1974 and 1975 (Cordle et al., 19778 Brilliant et al., 1978). The concentration of PBBs in human milk ranged from 70 to 131 times the concentration detected in paired blood (plasma) with an average ratio of 100 to 1 (Cordle et al., 1978). The concentration of PBBs in blood (plasma) of

individuals on quarantined farms ranged from 0.002 to 2.26 ppm in 1974 (Cordle et al., 1978). Firemaster handlers employed at the Michigan Chemical Corporation had plasma concentrations of PBBs that ranged from 0.006 to 0.085 ppm (Michigan Chemical Corporation, 1975). The concentration of PBBs in human fat ranged from 61 to 370 times the concentration detected in plasma with ratios of fat to plasma around 175 (Cordle et al., 1978). These findings suggest that the lipo-soluble PBBs are biomagnified within individuals and are probably biomagnified from species to species while ascending in the food chain.

C. Biological Effects of Polybrominated Biphenyls

Although at present no human health effects have been unequivocally attributed to PBBs, dietary exposure to PBBs has resulted in a variety of toxic manifestations in animals (Kimbrough et al., 1978). Cows fed high concentrations of PBBs lost body weight as a result of aversion to food containing PBBs as well as developing diarrhea (Jackson and Halbert, 1974; Mercer et al., 1976). Body weight gain was reduced in rats and monkeys fed lower doses of PBBs (Sleight and Sanger, 1976; Garthoff et al., 1977; Allen et al., 1978). However, this effect resulted, at least in part, from decreased food efficiency since weight differences were not attributable to differences in food consumption alone. General weakness, slow wound healing, thymus atrophy, abnormal hoof growth, hematomas and abscesses in peritoneal and thoracic cavities and dermal lesions including subcutaneous hemorrhage, hyperkeratosis with accumulation of keratin in hair follicles of the epidermis and squamous metaplasia with keratin cysts

in eyelid tarsal glands have been observed in cows following PBBs (Jackson and Halbert, 1974; Mercer et al., 1976; Moorhead et al., 1978). Dermal lesions similar to those in cattle have been reported in rhesus monkeys fed PBBs (Lambrecht et al., 1978; Allen et al., 1978). Hyperkeratosis, keratotic hair follicles and atrophy and squamous metaplasia of the sebaceous glands were observed on microscopic examination of monkey skin (Allen et al., 1978).

Modifications in erythroid and lymphoid tissues have been produced by dietary exposure to PBBs. Rhesus monkeys fed PBBs had reduced packed cell volume which was associated with bone marrow hypoactivity (Lambrecht et al., 1978; Allen et al., 1978). Leukopenia and immunosuppression have been produced in monkeys, rats, mice and guinea pigs by PBBs (Kimbrough et al., 1978; Allen et al., 1978; Lambrecht et al., 1978; Fraker and Aust, 1979). Rhesus monkeys fed PBBs had decreased immunoglobulins and altered T-cell function (Lambrecht et al., 1978; Allen et al., 1978). Exposure to PBBs reduced the in vivo immunoglobulin response and had an adverse effect on B-cells and helper T-cells in mice (Fraker and Aust, 1979).

Endocrine alterations also may be caused by PBBs. Rhesus monkeys had lengthened menstrual cycles after PBBs and this effect was correlated with flattened and lengthened serum progesterone peaks (Lambrecht et al., 1978; Allen et al., 1978). In addition to decreased concentrations of serum progesterone, rhesus monkeys fed PBBs had excessive postconceptional bleeding (Allen et al., 1978). Rhesus monkeys fed PBBs had hypoactive seminiferous tubules (Allen et al., 1978). Bulls fed PBBs had testicular atrophy and reduced spermatogenesis (Jackson and Halbert, 1974; Kimbrough et al., 1978). Prenatal

administration of PBBs to rats caused a delay in vaginal opening (Harris et al., 1978). Milk production was decreased in lactating cows which also may have been more susceptible to stress following PBBs (Jackson and Halbert, 1974). Pregnant cows, that did not abort, went overdue 2-4 weeks and their udders did not develop (Jackson and Halbert, 1974; Mercer et al., 1976). Although PBBs undergo transplacental movement and may be lethal to embryos and/or fetuses, their teratogenic potential is low, at least in rodents (Corbett et al., 1975; Preache et al., 1976; Kimbrough et al., 1978; Rickert et al., 1978).

Kidneys from cows fed diet containing PBBs have been reported to be pale tan-to-gray and almost twice normal size (Jackson and Halbert, 1974; Willett and Irving, 1976). Microscopically, bovine kidneys had collecting ducts that were extremely dilated, convoluted tubules with epithelial degenerative changes such as cloudy swelling, hydropic degeneration and separation from the basement membrane (Moorhead et al., 1977,1978). Kidney enlargement and renal lesions including petechial hemorrhage and hyaline degenerative cytoplasmic changes have been observed in rats following treatment with octabromobiphenyl (Aftmosis et al., 1972; Norris et al., 1974). Suggestive of an alteration in renal function were findings of polyuria, low urine specific gravity and moderately elevated urinary protein concentration in both cows and rats following PBBs (Sleight and Sanger, 1976; Mercer et al., 1976). Total protein, albumin and cholesterol were reduced in serum from monkeys after consumption of diet containing PBBs (Allen et al., 1978). Cows with low tissue concentrations of PBBs had significantly decreased concentrations of serum calcium, glucose and cholesterol and

urinary potassium and increased excretion of calcium and protein (Willett and Irving, 1976; Mercer et al., 1976,1978). Changes in serum and urinary calcium concentrations may result from renal lesions and/or effects on several hormones including parathyroid hormone and adrenal glucocorticoids. Alterations in renal function and/or calcium homeostasis may be responsible, at least in part, for depressed heart and respiratory rates produced in cows fed PBBs (Jackson and Halbert, 1974; Mercer et al., 1976).

Heart enlargement has been produced in rhesus monkeys fed PBBs (Allen et al., 1978). Dietary treatment of white leghorn cockerals has resulted in a variety of cardiovascular effects including hydropericardium and alterations in cardiac output, heart rate and arterial pressure (diastolic, systolic and mean) (Heineman and Ringer, 1976).

Ingestion of PBBs by cows also resulted in hepatic degenerative changes. Abscesses, foci of fatty degeneration and glycogen depletion were seen in liver and serum glutamic oxaloacetic transaminase (SGOT) was increased following PBBs (Jackson and Halbert, 1974; Moorhead et al., 1978; Durst et al., 1978). Livers from rhesus monkeys fed PBBs had hyperplasia of bile duct epithelium and enlarged hepatocytes, the cytoplasm of which contained markedly proliferated smooth endoplasmic reticulum (Allen et al., 1978). Exposure of rhesus monkeys to PBBs also resulted in increased serum glutamic pyruvic transaminase (SGPT) activity (Allen et al., 1978). Livers from rats consuming PBBs were enlarged and had lesions consisting of swelling, centrilobular cytoplasmic myelin bodies and vacuolation (Norris et al., 1974; Sleight and Sanger, 1976; Dent et al., 1976b). Correlated with hepatocyte hypertrophy were increased liver lipid (total lipid, cholesterol,

phospholipid and neutral lipid) and massive proliferation of the smooth endoplasmic reticulum (Sleight and Sanger, 1976; Garthoff et al., 1977). Elimination of certain drugs via liver was stimulated in rats by PBBs in an age-dependent manner (Cagen and Gibson, 1977; Cagen and Gibson, 1978).

Microsomal mixed function oxidase (MFO) activity has been increased in liver by PBBs (Farber and Baker, 1974; Dent et al., 1976a,b). PBBs belong to a class of compounds termed "mixed inducers"; that is, PBBs exhibited stimulating characteristics of both phenobarbital and 3-methylcholanthrene (3MC), two agents which induce distinct types of hepatic microsomal enzymes (Sladek and Mannering, 1969a,b). Although mixed stimulation following PBBs may result from combined effects of individual mixture congeners, which are either phenobarbital or 3MC-like stimulators, one or more congeners, such as 2,4,5,3', 4',5'-hexabromobiphenyl could produce both types of stimulation (Dannan et al., 1978). Following a single i.p. injection of Firemaster BP-6, the pattern of hepatic microsomal enzyme stimulation in adult rats initially resembled phenobarbital, however, later the effect more closely resembled that of 3MC (Dent et al., 1976a). Consequently, animals pretreated with a single i.p. injection of PBBs displayed a time-dependent change in susceptibility to bromobenzene (which is metabolized by hepatic MFOs to toxic epoxides) toxicity that coincided with the pattern and time course of stimulation of hepatic MFO activities (Dent et al., 1977; Roes et al., 1977; Zampaglione et al., 1973). These findings suggest that interactions with endogenous and xenobiotic compounds may be dependent upon time following exposure to PBBs.

D. Biological Effects of Agents that Increase Microsomal Enzyme Activity

Numerous drugs and environmental contaminants stimulate or inhibit microsomal enzyme function in animals, and this is reflected *in vivo* by modified metabolism and action of therapeutic agents, carcinogens, and a variety of endogenous compounds, such as steroid hormones, vitamins, fatty acids, thyroxin, and bilirubin (Conney, 1967). Since PBBs are environmental contaminants that increase microsomal enzyme activity, they may have pharmacological and toxicological actions resembling those of other agents that stimulate mixed function oxidases.

Phenobarbital, administered to rats for several days, increased the activity of hepatic microsomal enzymes that metabolize certain drugs. The anticoagulants bishydroxycoumarin and warfarin are examples of therapeutic agents which are metabolized by MFOs (Cucinell *et al.*, 1965; Ikeda *et al.*, 1966). Administration of phenobarbital to man reduced the concentration of bishydroxycoumarin and warfarin in plasma and reduced their pharmacological action (Cucinell *et al.*, 1965; MacDonald *et al.*, 1969).

Treatment of rats with phenobarbital for several days increased the activity of hepatic microsomal enzymes that hydroxylate androgens, estrogens, progestational steroids, and adrenocortical steroids (Kuntzman *et al.*, 1964; Conney and Klutch, 1963; Conney *et al.*, 1976; Levin *et al.*, 1968). Accelerated hydroxylation of steroid hormones by hepatic microsomal enzymes of rats treated with phenobarbital was reflected *in vivo* by an altered metabolism and modified physiological action of steroids. Increased progesterone hydroxylase activity

induced by phenobarbital was associated with a decrease in the anesthetic action of large doses of progesterone and a decreased concentration of progesterone and its metabolites in the brain and total body of rats (Conney et al., 1966). Prolonged administration of phenobarbital also decreased the anesthetic action of deoxycorticosterone, androsterone, and Δ^4-androstene-3,17-dione and accelerated their metabolism by hepatic microsomes. Pretreatment of immature rats with phenobarbital for several days prior to administration of testosterone or testosterone propionate inhibited the growth-stimulating effect of these androgens on the seminal vesicles (Levin et al., 1969). Estrone and 17β-estradiol metabolism was also increased by phenobarbital (Kuntzman et al., 1964; Welch et al., 1971). Stimulated catabolism of these estrogens as well as commonly used oral contraceptives was associated with a reduced ability of these compounds to increase uterine wet weight (Levin et al., 1968).

Patients treated chronically with phenobarbital for anticonvulsant therapy have an increased incidence of rickets, osteomalacic bone changes, hypocalcemia and elevated serum concentrations of alkaline phosphatase (Kruse, 1968; Dent et al., 1970). These symptoms are similar to those resulting from vitamin D deficiency and respond rapidly to vitamin D supplementation (Dent et al., 1970). Further suggesting that increased MFO activity is correlated with accelerated catabolism of vitamin D are findings that rats pretreated with phenobarbital were protected from hypercalcemia and renal calcinosis produced by calciferol and that patients chronically administered phenobarbital rapidly converted injected vitamin D_3-^3H to more polar

metabolites, some of which are biologically inactive (Richens and Rowe, 1970; Hahn et al., 1972).

Calcium homeostasis may also be disrupted by other mechanisms following phenobarbital. In vivo, phenobarbital had an inhibitory effect on vitamin D-25-hydroxylase kinetics and stimulated bile excretion resulting in reduced serum concentrations of 25-OH-vitamin D (Delvin et al., 1977). Inhibition of the energy-dependent calcium transport system in the intestinal mucosa and inhibition of calcium-binding protein synthesis are two additional postulated effects of phenobarbital on calcium homeostasis that may contribute to osteomalacia (Harrison and Harrison, 1976).

Metabolism of vitamin K may also be altered by treatment with phenobarbital. Hemorrhagic episodes occur in some babies born to mothers taking phenobarbital for epilepsy (Mountain et al., 1970). The coagulation defect mimics that found in vitamin K deficiency and has been prevented by administration of vitamin K, suggesting that phenobarbital stimulates metabolism of vitamin K.

Administration of phenobarbital to animals enhanced the enzymatic glucuronidation of bilirubin by liver microsomes, stimulated bile flow and accelerated metabolism of bilirubin in vivo (Roberts et al., 1967). Investigations in man indicate that chronic treatment with phenobarbital results in a decreased serum bilirubin concentration in patients with intrahepatic cholestasis and in jaundiced infants (Thompson and Williams, 1967; Crigler and Gold, 1969; Arias et al., 1969).

The mechanism by which phenobarbital and a host of other chemicals stimulate the synthesis of MFOs is different from the mechanism whereby polycyclic aromatic hydrocarbons produce their inductive effects (Sladek and Mannering, 1969a,b). Early evidence for this difference was the observation that agents such as phenobarbital induced the increased metabolism of endogenous compounds as well as a much larger number of drugs and other xenobiotics than did polycyclic aromatic hydrocarbons such as 3-methylcholanthrene (3MC) or benzo(a)-pyrene (BP). Administration of phenobarbital resulted in parallel increases in rates of metabolism of certain drugs and hepatic cytochrome P450 concentrations and withdrawal of phenobarbital resulted in parallel decreases to basal values suggesting that cytochrome P450 is the rate-limiting component of the microsomal drug metabolizing system (LaDu et al., 1971). The observation that polycyclic aromatic hydrocarbons increased hepatic cytochrome P450 concentrations with a differential effect on two hepatic microsomal enzymes suggested that P450 was either not rate-limiting or polycyclic aromatic hydrocarbons cause the synthesis of a unique P450 hemoprotein (Sladek and Mannering, 1969a,b). A number of observations led to the latter conclusion and the 3MC- or BP-sensitive hemoprotein was termed cytochrome P_1-450 or cytochrome P448 (Sladek and Mannering, 1969a,b).

Induction of cytochrome P_1-450 by polycyclic aromatic hydrocarbons such as BP, which is found in tobacco smoke and polluted atmosphere, parallels the induction of an enzyme system in the microsomal fraction of liver and several extrahepatic tissues including kidney, lung, intestine, skin, placenta and mammary gland (Wattenberg et al.,

1962; Welch et al., 1968; Nebert and Gelboin, 1969). Various chemicals including nicotine, zoxazolamine, phenacetin and polycyclic aromatic hydrocarbons are metabolized into alkene and arene oxides in reactions catalyzed by this NADPH-dependent enzyme complex, activity of which is reflected by activity of the enzyme BP hydroxylase (aryl-hydrocarbon hydroxylase - AHH)(Beckett and Triggs, 1967; Welch et al., 1969; Pantuck et al., 1972). This process, and particularly subsequent metabolism by the microsomal enzyme epoxide hydratase (EH) and/or conjugation with glutathione by a family of cytosolic transferases can result in detoxification (Jerina and Daly, 1974). However, certain intermediary metabolites (epoxides) produced by AHH are more electrophilic than the parent compound and react readily with critical cellular nucleophiles including DNA, RNA and protein to produce toxic responses (Miller, 1970; Daly et al., 1972; Oesch, 1973; Jerina and Daly, 1974). Although EH often results in detoxification of epoxides such as BP 4,5-oxide, it is also capable of catalyzing the transformation of certain arene oxides, such as BP 7,8-oxide, to precursors, such as BP-7,8-dihydrodiol, of the ultimate carcinogenic and mutagenic forms of parent compounds, such as the isomeric BP 7,8-dihydrodiol-9,10-epoxides of BP (Sims et al., 1974; Wood et al., 1976). Thus, EH plays a dual role in the metabolic activation and inactivation of certain polycyclic aromatic hydrocarbons into mutagenic and carcinogenic metabolites. The toxicity and/or carcinogenicity of a compound depends on the region in which the molecule is oxidized, which is dependent on the positional specificities of different forms of cytochrome P-450 and on MFO activity (Wiebel et al., 1975).

Since metabolites which are ultimate carcinogens or toxicants are highly reactive and unstable, they may not be transported from liver to extrahepatic tissues. Thus, if metabolic activation to an ultimate carcinogen or toxicant is an essential intermediate step in tissue specific toxicity it may occur in target tissues. Tissue differences in metabolism, whether quantitative or qualitative, may be responsible, at least in part, for site specific deleterious effects. The steady-state concentration of an epoxide metabolite within cells of an organ depends, at least in part, on its rates of synthesis and further metabolism. Consequently, the rates of epoxide forming and detoxifying enzyme activities in various tissues or cells and the sensitivity of cells or tissues to such toxic metabolite(s) may be important determinants of tissue-specific toxicity, including the initial step in chemical mutagenesis or carcinogenesis. Different metabolites of the potential carcinogens 7,12-dimethylbenz(a)anthracene (7,12-DMBA) and N2-fluorenylacetamide (2-FAA) were produced by microsomes from mammary than from liver and treatment with 3MC produced a shift in 7,12-DMBA metabolism from side chain to ring hydroxylation in liver (Tamulski et al., 1973; Malejka-Giganti et al., 1977). However, no such alteration occured in mammary tissue. These findings are suggestive of tissue specific qualitative differences in metabolism. Tissue differences in metabolism may also vary with age. Several studies have revealed that immature animals lack, or possess low activities of many hepatic microsomal enzymes (Fouts and Devereux, 1972). Perinatal development of enzymes involved in epoxide metabolism in extrahepatic tissues has not yet been fully characterized despite the

potential importance of interactions between chemical carcinogens, (e.g., BP) and extrahepatic tissues.

Polychlorinated biphenyls (PCBs) are widespread commercial pollutants that persist in the food chain and have been detected in tissues and milk from many species including man (Risebrough et al., 1968; Price and Welch, 1972; Hamano et al., 1974). Since PBBs are structurally analogous to PCBs and similar in chemical and biological stability they may share many biological and toxicological properties (Jacobs et al., 1976; Lee et al., 1977; Rickert et al., 1978). Like PBBs, PCBs have increased the liver weight-to-body weight ratio, produced histological changes in liver including fatty infiltration, formation of myelin bodies and proliferation of smooth endoplasmic reticulum and stimulated hepatic microsomal enzymes sensitive to both phenobarbital and BP (Nishizumi, 1970; Kimbrough et al., 1972; Norback and Allen, 1972; Alvares et al., 1973). On a weight basis, PBBs (Firemaster BP-6 or hexabromobiphenyl) may be three times more potent than PCBs (Aroclor 1254 - 54% chlorine) at increasing hepatic MFO activity in male rats (Farber and Baker, 1974; Garthoff et al., 1977). Hepatic microsomal enzyme activity has been increased for at least a month following a single i.p. injection of PCBs (Parkki et al., 1977). Induction patterns of MFOs in liver exhibit time-dependent profiles after PCBs (Bickers et al., 1974; Parkki et al., 1977). Microsomal enzymes have also been stimulated in extrahepatic tissues such as kidney, lung, skin and placenta following exposure to PCBs (Vainio, 1974; Bickers et al., 1974). Administration of PCBs to pregnant rats has also resulted in detectable fetal concentrations of PCBs and

stimulation of fetal hepatic microsomal enzymes (Hamano et al., 1974; Takagi et al., 1976; Alvares and Kappas, 1975).

Rats treated with PCBs had a marked reduction in pentobarbital sleeping time, an indirect measure of pentobarbital metabolism in vivo (Villeneuve et al., 1972). The insecticides dieldrin and DDT were more toxic when administered with PCBs (Lichenstein et al., 1969). Pretreatment with PCBs also potentiated the acute toxicity of carbon tetrachloride (Grant et al., 1971; Carlson, 1975). In addition to modifying biological responses to certain xenobiotics metabolized by the MFO system, PCBs may alter the metabolism of endogenous compounds such as fat-soluble vitamins, fatty acids and steroid hormones.

Vitamin A functions in maintaining reproduction, growth and development (Mason, 1939; Thompson, 1969). Decreased growth rate is one of the earliest and most sensitive indices of vitamin A deficiency (Corey and Hayes, 1972). Insufficient vitamin A can also result in xerophthalmia, night blindness, disturbances in the central nervous system (CNS), respiratory difficulties, skin lesions including hyperpigmentosis and hyperkeratosis and renal lesions including epithelial vacuolization, hyperkeratization and distention of tubules as well as albuminuria, polyuria and decreased urine osmolality which is potentiated in rats by cold stress (Herrin and Nicholes, 1930; Herrin, 1939; Wolbach, 1954; Odagiri and Koyamagi, 1961; Arvy, 1968; Webb et al., 1968,1970).

A variety of clinical symptoms exhibited by patients with Yusho disease were similar to those symptoms noted in vitamin A deficiency. Yusho disease was diagnosed in approximately 1400 Japanese in 1968 and caused by consumption of rice oil contaminated with Kanechlor 400, a

commercial mixture of PCBs mainly composed of tetrachlorobiphenyls but also containing numerous chlorinated trace impurities including dibenzofurans (Kuratsune et al., 1972; Nagayama et al., 1975). These individuals had an increased incidence of CNS disturbances, such as short-term memory loss, behavioral changes, and numbness, and respiratory problems such as bronchitis, dyspnea and cough (Kuratsune et al., 1972; Umeda, 1972). More prominent symptoms suggestive of vitamin A deficiency were hyperpigmentation of the skin, mucus membranes and nails, acne-like skin eruptions with follicular accentuation and transient visual disturbances (Kuratsune et al., 1972; Umeda, 1972; Kimbrough, 1974). Dermal application of PCBs to rabbit ear resulted in hyperplasia and hyperkeratosis of the epidermal and follicular epithelium (Vos and Beems, 1971). Dietary treatment with PCBs reduced the concentration of vitamin A in liver of rats and pregnant rabbits (Cecil et al., 1973; Innami et al., 1976; Villeneuve et al., 1971). Body weight gain was retarded in these animals and PCBs were reported to cause renal lesions in rats including hydropic degeneration of the convoluted tubules and tubular dilation which are similar to microscopic changes observed in kidneys of vitamin A deficient rats (Vos and Beems, 1971; Bruckner et al., 1973, 1974).

Calcium homeostasis may be altered following PCBs secondary to modification of vitamin D metabolism. Vitamin D mediated calcium metabolism has been reported to be altered in chickens after treatment with PCBs (Wong et al., 1974). Yusho patients also had symptoms suggestive of an alteration in calcium homeostasis such as an increased incidence of dental disorders including exfoliation and

fracture of teeth, abnormal eruption of decidious and permanent teeth and abnormal formation and growth of erupted teeth and facial bones (Kuratsune et al., 1972; Umeda, 1972).

Vitamin E acts as an antioxidant possibly to prevent oxidation of essential cellular constituents (Wasserman and Taylor, 1972). Chickens fed PCBs had an increased incidence of exudative diathesis which could be reduced by dietary supplementation with vitamin E or selenium (Combs et al., 1975). Thus, the metabolism of this fat soluble vitamin may also be altered following exposure to PCBs.

The porphyrin nucleus is an integral component of hemoglobin and cytochromes. Cytochromes P450 and P448 were increased in liver and hemoglobin, hematocrit and spleen size were reduced following PCBs suggesting that porphyrin metabolism is altered by PCBs (Alvares et al., 1972; Vos and Beems, 1971; Bruckner et al., 1974). Consistent with these findings, PCBs have been reported to increase fluorescence of tissues including liver, kidney and bone marrow (Vos et al., 1970, 1971). Excretion of a porphyrin fraction in feces and hepatic iron content were also increased and PCBs produced hepatic porphyria (Vos et al., 1970, 1971; Vos and Notenboom-Ram, 1972; Kimbrough et al., 1972). Hepatic porphyria in rats following PCBs resembles human porphyria cutanea tarda, a condition resulting from an acquired defect in hepatic porphyrin metabolism, symptoms of which include uroporphyrinuria, photosensitivity and mechanical fragility of skin (Kimbrough, 1974). Porphyria produced by PCBs is characterized by slow onset, increased excretion of uroporphyrins (URO), coproporphyrin (COPRO), delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) in urine, accumulation of 8- and 7-carboxyporphyrins in liver and

induction of hepatic drug metabolizing enzymes and ALA synthetase, the rate-limiting enzyme in heme synthesis (Goldstein et al., 1974; Granick, 1966). Porphyria produced by many hepatoporphyrinogenic chemicals is due primarily to increased activity of ALA synthetase followed by overproduction of porphyrins. However, PCBs may act differently because induction of ALA synthetase occurs rapidly whereas porphyria has a delayed onset (Goldstein et al., 1974).

Many chemicals that produce hepatic porphyria also induce AHH and other microsomal monoxoygenases as well as cytochrome P448 and the cytosolic enzyme glutathione S-epoxide transferase. Coordinate induction of AHH and cytochrome P448 and possibly these other enzymes by xenobiotics may be a result of binding to a receptor (Poland and Glover, 1974). An induction receptor theory was initially based on observations regarding effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most potent inducer of AHH and cytochrome P448 known, and 3MC on induction of AHH (Poland and Glover, 1974). TCDD and 3MC produced parallel log dose-response curves for induction of hepatic AHH in the rat, both agents produced the same maximal response and concomitant administration of maximally inducing doses of both compounds resulted in no additive effect. Induction of AHH by TCDD has been correlated with the binding of ^{3}H-TCDD to a high-affinity low capacity stereospecific cytosol receptor protein (i.e., TCDD-receptor) (Poland et al., 1976). Structurally related polycyclic aromatic hydrocarbons such as 3MC and halogenated aromatic compounds such as PCB congeners chlorinated symmetrically in both the meta and para positions, that also induce AHH, compete for the binding of ^{3}H-TCDD to

the receptor (Poland and Glover, 1974, 1977; Poland et al., 1976; Goldstein et al., 1977). In addition to being porphyrinogenic, many compounds that bind to the TCDD receptor are acnogenic and immunosuppressant (Poland et al., 1976).

Phenobarbital, pregnenolone-16α-carbonitrile and other PCB congeners, which induce cytochrome P450 and are less efficient inducers of AHH, do not compete with TCDD for binding to a receptor (Poland et al., 1976; Poland and Glover, 1977). By analogy, there may be a similar recognition site for phenobarbital and phenobarbital-like compounds which controls the induction of cytochrome P-450 and its associated enzymes (Poland et al., 1976). As previously noted, phenobarbital has produced a variety of biological effects including accelerated steroid metabolism.

Metabolism of steroid hormones by hepatic microsomal enzymes was increased in vitro by pretreatment with PCBs (Nowicki and Norman, 1972). Pretreatment of rodents with PCBs accelerated the catabolism of exogenously administered testosterone, estradiol and progesterone (Orberg and Lundberg, 1974; Orberg and Ingvast, 1977; Orberg and Kihlstrom, 1973; Derr, 1978). These findings suggest that increased microsomal enzyme activity following PCBs is correlated with enhanced metabolism of steroid hormones. Physiological functions controlled by steroid hormones such as reproduction would be expected to be affected least by lowly chlorinated PCBs because of their relative low efficiency at increasing mixed function oxidase activity (Bickers et al., 1972; Orberg, 1976). However, certain PCBs with low chlorine content have estrogenic activity which is reflected as an increase in uterine

glycogen content and inhibition of binding of tritiated estradiol to rat uterus cytosol *in vitro* (Nelson, 1974; Bitman and Cecil, 1970; Ecobichon and MacKenzie, 1974). Following administration to rat neonates, an estrogenic mixture of PCBs produced precocious puberty as well as persistent vaginal estrus and anovulation by six months of age (Gellert, 1978). When estrogenic PCB congeners are components of complex PCB mixtures which contain other PCBs and in some cases toxic contaminants such as dioxins and dibenzofurans, direct effects may be obscured by factors including indirect actions resulting from enhanced steroid metabolism.

A variety of effects observed after administration of mixtures of PCBs suggest that these compounds may modify endogenous steroid metabolism to the extent that physiological sequelae are noted. Adrenal weights and plasma corticosterone concentrations have been increased in mice, rats and rhesus monkeys by PCBs (Wasserman *et al.*, 1973; Sanders *et al.*, 1974; Barsotti and Allen, 1975). Urinary androgen in boars and seminal vesicle weight and spermatogenesis in mice have been reduced by pretreatment with PCBs (Platonow *et al.*, 1972; Sanders *et al.*, 1974; Sanders and Kirkpatrick, 1975). Rhesus monkeys consuming diet containing PCBs had lengthened menstrual cycles (Allen and Barsotti, 1976). Women with Yusho disease also had menstrual cycle irregularities, dysmenorrhea and altered serum concentrations of ketosteroids (Kuratsune *et al.*, 1972). Various mixtures of PCBs caused uterine atrophy, reductions of plasma progesterone concentration, ovarian stromal changes, and lengthened estrus cycle in rodents

(Vos and Beems, 1971; Johnsson et al., 1976; Orberg and Kihlstrom, 1973; Kimbrough et al., 1978).

Endocrine changes in mammalian females, especially primates, exposed to PCBs suggest that the uterine hormonal environment may be suboptimum for blastocyst implantation and maintenance of pregnancy (Smith and Biggers, 1968; McCormack and Greenwald, 1974; Orberg, 1978). Ova implantation frequency has been reduced in mice treated with PCBs (Orberg and Kihlstrom, 1973; Kihlstrom et al., 1975; Orberg, 1978). Dose and mixture-dependent reductions in conception rate occurred in monkeys maintained on diet containing PCBs (Allen, 1975; Allen and Barsotti, 1976). Exposure to PCBs reduced the number of rats producing litters after mating (Linder et al., 1974; Johnsson et al., 1976; Keplinger et al., 1972). Implantation rate reduction in mice may not occur if only one mate is treated with PCBs (Kihlstrom et al., 1975).

Fetal tissues from numerous species including monkey, rat and human contain PCBs, indicating that PCBs readily cross the placenta and accumulate in the fetus (Allen and Barsotti, 1976; Takagi et al., 1976; Kuratsune et al., 1972; Umeda et al., 1978). Although the teratogenic potential of PCBs is low, resorption and abortion frequencies are increased in rabbits, mink and rhesus monkeys fed PCBs (Villeneuve et al., 1971; Ringer et al., 1972; Allen and Barsotti, 1976). Of those women with Yusho who gave birth, 2 of 13 had stillborn infants (Kuratsune et al., 1972). Infants born to Yusho patients exhibited skin, liver and growth disorders including low birth weights (Kuratsune et al., 1972; Umeda et al., 1978). Infant monkeys also had decreased birth weights and focal areas of hyperpigmentation of the

skin (Allen and Barsotti, 1976; Allen et al., 1978). Rabbits transplacentally exposed to PCBs had reduced thymus size and white cell count, which are associated with a reduced capacity to produce antibodies and general immunosuppression (Vos and Bemms, 1971; Miller, 1963). A factor in addition to immunosuppression and vitamin A deficiency which could contribute to reduced birth weights and body weight gain following PCBs may be altered energy metabolism. Inhibition of oxidative phosphorylation and respiration have been associated with addition of PCBs in vitro to either rat liver mitochondria or heavy beef heart mitochondria (Chesney and Allen, 1974; Pardini, 1971).

Elevated concentrations of PCBs in tissues from stillborn babies born to Yusho mothers have been detected even 10 years after the rice-oil incident (Umeda et al., 1978). Extrapolation of data obtained in rats indicated that less than 20% of administered hexachlorobiphenyl would ever be excreted (Matthews and Anderson, 1975). Persistence of PCBs in biological tissue is further exemplified by a report that one year after initial diagnosis in 159 patients, 40% showed no remission of symptoms while 10% appeared more severely affected (Kuratsune et al., 1972). Even 10 years later, symptoms of Yusho were observed and PCBs, especially highly chlorinated congeners, detected in tissues of patients diagnosed as having Yusho disease (Umeda et al., 1978).

Body burdens of PCBs may be reduced in lactating females as PCBs are mobilized with body fat during lactation and excreted with milk lipids (Hamano et al., 1974; Takagi et al., 1976). Signs of PCBs intoxication quickly became more severe in monkeys and rats suckled by mothers maintained on diet containing PCBs (Allen and Barsotti, 1976; Takagi et al., 1976). Survival-to-weaning has been reduced and

liver weight-to-body weight ratio increased in F_2 rat pups following dietary exposure of both F_0 and F_1 generations to PCBs (Linder et al., 1974). Thus, PCBs, like PBBs, may be passed from one generation to the next via transplacental movement and especially through milk and may produce effects in each generation.

E. Objectives

The purpose of this investigation was to determine if functional and metabolic alterations are produced by prenatal and/or postnatal treatment with PBBs. Identification of physiological and biochemical sequelae to perinatal exposure to PBBs will assist assessment of the potential health hazard posed by PBBs to developing mammals. Three specific objectives of this research were identified: 1) to determine effects of perinatal exposure to PBBs on survival, growth and development, organ function, morphology and microsomal enzyme activity; 2) to determine if enzymatic alterations produced by PBBs result in modifications in metabolism of xenobiotic and endogenous compounds; 3) to determine the persistence of effects of PBBs and whether or not effects of PBBs are correlated with tissue concentrations of PBBs.

MATERIALS AND METHODS

A. Animals

Sprague-Dawley rats were either purchased directly or descendants of rats from Spartan Research Animals, Inc., Haslett, Michigan. Adults were female unless otherwise specified. Timed-pregnant rats were obtained between days 1 and 5 of pregnancy or impregnated by males of the same age and treatment from our breeding stock. At birth all litters were normalized to 10 pups; 5 males and 5 females. Pups were weaned at 28 days of age.

Rats were not used until after at least 2 days of acclimatization. Animals were maintained in clear polypropylene cages at 22°C with a 12 hr light cycle (0700-1900 hr) and were allowed free access to food (Wayne Lab Blox, Anderson Mills, Maumee, Ohio) and water. Rats in dietary studies received ground Lab Blox; all others received pelleted chow. Ground diet was prepared by dissolving appropriate quantities of PBBs (Firemaster BP-6, Velsicol Chemical Co., St. Louis, Michigan) in acetone and mixing this solution (20 ml) with 2 kg of ground Lab Blox for approximately 10 min. Controls were fed ground diet with which only acetone had been mixed and allowed to evaporate.

B. Teratology

Pregnant rats were placed on diet containing 0 or 100 ppm PBBs (approximately 7.0 mg/kg/day) on day 8 of gestation and deprived of

food for 0 or 48 hr beginning the 10th day of gestation. On the 20th day of gestation, fetuses were removed by caesarean section and the number and position of live, dead and resorbed fetuses was recorded. Fetuses were dried on absorbant paper, weighed, measured for crown-rump length with a vernier caliper, sex determined and examined for external anomalies. Each litter was divided into 3 subgroups for further examination and 1 subgroup for determination of whole carcass concentration of PBBs. One was fixed in Bouin's solution for 2 weeks, after which fetuses were sectioned by hand into 2-3 mm sections and examined for soft-tissue anomalies by the method of Wilson (1965). A second subgroup was fixed in 95% ethanol, skinned and eviscerated. Fetal cartilage and ossified skeletons were subsequently stained with alcian blue 8GS and alizarin red S, respectively, by the method of Inouye (1976). Stained skeletons were examined for cartilaginous and skeletal anomalies. Organ weight-to-body weight ratios were determined in the third-subgroup following decapitation.

C. Postnatal Development

Pups were randomly cross-fostered within treatment groups and litters were normalized to 5 males and 5 females on day 1 postpartum. Control values were obtained by pooling data from animals treated with peanut oil alone or suckled by dams maintained on diet containing 0 ppm PBBs. Rats were weighed at 7, 14, 28 (whole litter) 56 and 84 (individual female) days of age. Daily observations were made to determine the age of pinna detachment, incisor eruption, fur development, opening of external auditory duct, eye opening, testes descent and vaginal opening.

D. Vaginal Cycle Lenth

Vaginal cycle length was monitored in 10-14 week old rats that had been exposed to 0 or 100 ppm PBBs from day 8 of gestation. Daily vaginal smears were obtained for at least 14 consecutive days. The interval in days between 2 successive peaks in the frequency of cornified cells was taken as the length of each individual vaginal (estrus) cycle.

E. Histopathology

Animals used for histologic examination were killed by cervical dislocation and pieces of tissue were immediately cut and fixed in 10% buffered formalin. After fixation, tissues were embedded in paraffin, sectioned at 5 μM and stained with hematoxylin and eosin.

F. Milk Collection

To obtain milk, animals were anesthetized with ether and treated i.m. with 0.05 USP units (approximately 9 ng/kg) synthetic oxytocin (Haven-Lockhart Laboratories, Shawnee, Kansas). Mammary areas were washed and milk collected using an in line pulsating vacuum pump.

G. Quantitation of PBBs

Concentrations of PBBs in peritoneal cavity and tissues following treatment of 7 day old rats with 150 mg/kg PBBs and in milk and tissues from rats dietarily exposed to 50 ppm PBBs were determined by the method of Fehringer (1975). Petroleum (pet) ether (5 ml) was added to tubes containing milk (1 ml), peritoneal swabs (one 2 inch square gauze sponge) shaved fat (1 g) or other tissues (200 mg homogenized in 0.8 ml water). Samples were mixed with 20 ml acetonitrile

saturated with pet ether. Phases were separated and 100 mg NaCl was added to the acetonitrile phase. The acetonitrile phase was extracted 3 times with 10 ml portions of pet ether. Pet ether fractions were pooled and reduced in volume to 2 ml which was placed on a Florisil (60-100 mesh) (Fisher, Pittsburgh, Pa.) column (100 mm x 5 mm). PBBs were eluted with 6% diethyl ether in pet ether. After evaporation of pet ether and diethyl ether residues were reconstituted in pet ether (20-100 µl) for injection onto a gas chromatograph. Quantitation was by gas-liquid chromatography with electron capture detection on a Varian model 2100 gas chromatograph (Varian, Palo Alto, Calif.) using a 1.7 M column packed with 1% OV-1. Carrier gas (N_2) flow was 30 ml/min. Column temperature was held at 230° and only area of the major peak (2,4,5, 2',4',5'-hexabromobiphenyl) was calculated.

Concentrations of PBBs in tissues from all other rats used in this investigation were determined using the general AOAC procedure for chlorinated hydrocarbon pesticides (1970). Samples were weighed and ground with sufficient anhydrous sodium sulfate to render them completely dry and pulverized. Powdered tissues were extracted 5 times with 15 ml portions of hexane. Pooled extracts were reduced in volume to 1-2 mls which was placed on a Florisil (60-100 mesh) (Fisher, Pittsburg, Pa) column (500 mm x 22 mm). PBBs were eluted with 200 ml hexane which was evaporated to dryness and then brought up to desired volume with hexane. Quantitation was by gas-liquid chromatography as described except that column temperature was held at 245°C and heights of both major peaks (2,4,5,2',4',5'-hexa- and 2,3,4,5, 2',4',5'-heptabromobiphenyl) were measured. Concentration of PBBs was

expressed as μg PBBs (usually as 2,4,5,2',4',5'-hexabromobiphenyl) per g (wet wt tissue) or ml (milk).

H. Liver

1. Coproporphyrin and uroporphyrin concentrations in liver and urine

Coproporphyrin (COPRO) and uroporphyrin (URO) were extracted from liver and urine by the method of Schwartz et al. (1951) and quantified fluorimetrically. Urine was collected for 24 hr in aluminum foil-covered flasks containing 300 mg sodium carbonate and 1 ml toluene. After centrifugation to remove sediment, urine was transferred to separatory funnels containing 75 ml ethyl acetate (EA), 10 ml water and 5 ml acetate buffer (glacial acetic acid, saturated sodium acetate, water; 1:4:3, v/v, pH 4.8) (EA-A buffer). Liver was homogenized (Potter-Elvehjem homogenizer with a Teflon pestle) in 9 ml EA-A buffer and treated as urine. Samples were extracted for 2 min and phases allowed to separate for 30 min. Aqueous phase was drained into a centrifuge tube containing 0.5 g aluminum oxide. The organic phase was washed twice with 10 ml 1% sodium acetate. Sodium acetate washings and aqueous phase were pooled for URO quantitation; COPRO was extracted from organic phase with four 5 ml 1.5 N HCl washings. Fluorescence was measured using an excitation wavelength of 405 nm and emission wavelength of 603 nm. URO was extracted from aluminum oxide with 20 ml 50% saturated sodium acetate and 40 ml water. URO was subsequently extracted from washings and quantified fluorimetrically as described for COPRO. URO values were multiplied by 0.75. URO and COPRO concentrations were expressed as μg URO or COPRO per 24 hr (urine) or per g wet liver wt.

2. Vitamin A concentration in liver and serum

Concentration of vitamin A in liver and serum was quantified fluorimetrically by the method of Hansen and Warwick (1968, 1978). Serum (100 µl), water (1 ml) and absolute ethanol (3 ml) were added to a screwcap test tube and shaken for 1 min. Liver (100 mg) was homogenized (Potter-Elvehjem homogenizer with a Teflon pestle) in water (1 ml) and absolute ethanol (3 ml) and treated as serum. Petroleum ether (5 ml) was added to each sample. Following extraction for 5 min and centrifugation at 600 g for 2 min, vitamin A was quantified in organic phase using an excitation wavelength of 369 nm and emission wavelength of 483 nm. Concentration was expressed as µg vitamin A per ml (serum) or g wet liver wt.

Serum glutamic pyruvic transaminase (SGPT) was assayed spectrophotometrically by the method of Reitman and Frankel (1957). Serum (200 µl), NADH (200 µl of 1 mg/ml stock in 0.01 N NaOH), analine (500 µl of stock 0.2 M, pH 7.5), lactic dehydrogenase (800 units) and distilled water (1.8 ml) were added to a cuvette. α-Ketoglutarate (200 µl of stock 0.1 M, pH 7.5) was mixed into the cuvette to initiate the reaction. Decrease in O.D. at 340 nm was measured. Activity was expressed as units per ml serum.

I. Preparation of Postmitochondrial Supernatants and Microsomes

Animals were killed and organs were excised, weighed and then coarsely chopped into ice-cold 1.15% KCl, pH 7.4, (liver) or 66 mM Tris, pH 7.4 (extrahepatic tissues). Postmitochondrial supernatants (PMS) were prepared by homogenization (Potter-Elvehjem homogenizer with a Teflon pestle) in 3 volumes of medium into which they were

excised followed by centrifugation at 10,000 g for 20 min. Microsomes were prepared by centrifuging PMS at 105,000 g for 60 min. The microsomal pellet was resuspended in 0.25 M sucrose containing 5.4 nm EDTA and 20 mM Tris-HCl, pH 7.4, to a final concentration of 1-3 mg wet weight tissue per ml as described by Dent et al. (1976). All assays were performed on the day of supernatant or microsomal preparation. Protein was measured by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

J. Enzyme Assays

In all assays, each incubation mixture contained 0.5 to 2.0 mg protein per milliliter. Reaction mixtures were incubated for 10 (hepatic) or 30 (extrahepatic) minutes at 37°C. Radioactivity was determined as described in Renal Function. Spectrophotometric measurements were made using a Beckman dual beam spectrophotometer (Beckman Instruments, Fullerton, Calif.). Fluorescence was quantified after spectrophotofluorimeter (American Instrument Corp., Model SPF 12S, Silver Springs, Maryland) standardization with 0.1 µg/ml quinine sulphate (QS) in 0.05 M H_2SO_4 using an excitation wavelength of 365 nm and emission wavelength of 460 nm.

Arylhydrocarbon hydroxylase (AHH) was assayed fluorimetrically by the method of Nebert and Gelboin (1968) as modified by Oesch (1976). Benzo(a)pyrene (BP) (Sigma Chemical Co., St. Louis, Mo.) and cofactors were added in 66 mM Tris, pH 7.4, to beakers such that the final volume was 0.95 ml. The incubation mixture contained approximately 0.1 µmole BP, 5.8 µmol glucose-6-phosphate (G-6-P), 0.95 units G-6-P dehydrogenase (G-6-PD), 3.0 µmol $MgCl_2$, 0.25 µmol β-nicotinamide

adenine dinucleotide, reduced form (NADH) (from yeast), 0.4 µmol NAD phosphate (NADP) (from yeast) and 0.27 µmol NADPH, reduced form (NADPH) (Type I). The reaction was stopped by addition of 1 ml ice-cold acetone. Acetone was added to blanks prior to incubation. After addition of 6 ml petroleum (pet) ether, samples were extracted with 3 ml 1.0 M NaOH and the pet ether aspirated. Fluorescence was measured in the NaOH layer using an excitation wavelength of 396 nm and an emission wavelength of 522 nm. Activitiy was expressed as fluorescent units (relative to QS standard) per mg protein per min.

Biphenyl-2-hydroxylase (BP-2-OH) and biphenyl-4-hydroxylase (BP-4-OH) were assayed fluorimetrically by the method of Creaven et al. (1965). Biphenyl (Mallinckrodt Chemical, St. Louis, Mo.) and co-factors were added in 50 mM Tris, pH 8.5, to beakers such that the final volume was 2.0 ml. The incubation mixture contained approximately 20.0 µmol biphenyl, 7.5 µmol G-6-P, 0.5 units G-6-PD, 5.0 µmol $MgCl_2$, 0.6 µmol NADP and 0.27 µmol NADPH. Reactions were stopped by addition of 0.5 ml ice-cold 4.0 M HCl. Blanks were treated with HCl prior to incubation. After addition of 5 ml n-heptane containing 1% v/v isoamyl alcohol, samples were extracted with 3 ml 0.1 M NaOH and the heptane layer aspirated. To the aqueous layer, 0.5 ml 0.25 M succinic acid was added. Fluorescence was measured 20 min later using excitation wavelength of 302 nm and emission wavelength of 410 nm (BP-4-OH) and again using excitation wavelength of 312 nm and emission wavelength of 422 nm (BP-2-OH). Sample fluorescence was compared to fluorescence of 2-OH BP and 4-OH BP standards. Activity was expressed as nmol of 2- or 4-hydroxybiphenyl produced per mg protein per min.

Ethoxyresorufin-O-deethylase (EROD) was assayed fluorimetrically by the method of Burke and Mayer (1974). Ethoxyresorufin (ER) (gift from R.T. Mayer, College Station, Texas) and cofactors were added in 66 mM Tris, pH 7.4, to beakers to give a final volume of 1.0 ml. The incubation mixture contained approximately 2.5 nmol ER, 0.8 units G-6-PD, 10.0 µmol G-6-P, 0.4 µmol NADP and 0.4 µmol NADPH. The reaction was stopped by addition of 1 ml ice-cold acetone. Acetone was added to blanks prior to incubation. The reaction mixture was diluted with 5 ml distilled water and fluorescence was measured at excitation and emission wavelengths of 560 nm and 580 nm, respectively. Sample fluorescence was compared to fluorescence of resorufin standards. Activity was expressed as nmol resorufin formed per mg protein per min.

Hexobarbital hydroxylase (Hex-OH) was assayed by the method of Kupfer and Rosenfeld (1973). (2-^{14}C)Hexobarbital (New England Nuclear, Boston, Mass.) and cofactors were added in 66 mM Tris, pH 7.4, to beakers to give a final volume of 2 ml. The incubation mixture contained approximately 1.2 µmol (2-^{14}C)hexobarbital, 0.8 units G-6-PD, 10.0 µmol G-6-P, 10.0 µmol $MgCl_2$, 0.4 µmol NADP and 0.25 µmol NADPH. Reactions were stopped by addition of 3 ml ice-cold 1 M citrate buffer, pH 5.5. Blanks were treated with citrate buffer prior to incubation. After addition of 10 ml 1-chlorobutane, samples were extracted with 10 ml ethyl acetate and 1-chlorobutane layer aspirated. Radioactivity was determined in 200 µl of ethyl acetate layer and in 10 ml substrate. Activity was expressed as nmol 3-hydroxyhexobarbital per mg protein per min.

Epoxide hydratase (EH) was assayed by the method of Oesch et al. (1971). To screwcap test tubes containing 50 µl 0.5 M Tris, pH 9.0, and sufficient distilled water to give a final volume of 200 µl was added 10 µl of 43.6 mM (7-^{3}H)styrene oxide (Amersham Corp., Arlington Heights, Ill.) in acetonitrile. The reaction was stopped by addition of 3 ml pet ether. Pet ether was added to blanks prior to incubation. Samples were shaken for 5 min, centrifuged at 600 g for 2 min and placed at -20°C. When the aqueous layer was frozen, pet ether was aspirated. An additional 3 ml pet ether was placed in test tubes and this process was repeated. To the aqueous layer 1 ml ethylacetate was added and samples were extracted for 5 min, centrifuged at 600 g for 2 min. Radioactivity was determined in 200 µl of ethylacetate layer and in 10 µl substrate. Activity was expressed as nmol styrene glycol formed per mg protein per minute.

Glutathione-S-transferase (GSH-transferase) was assayed spectrophotometrically by the method of Goldstein and Combes (1966). Reduced GSH (Sigma Chemical Co., St. Louis, Mo.) and 227 µmol sulfobromophthalein (BSP) were added in 0.1 M Na pyrophosphate, pH 8.2, to beakers such that the final volume was 4.4 ml. Conjugating activity was measured by recording 5 min change in optical density (O.D.) at 330 mµ from the time BSP was added. Activity was expressed as nmol BSP conjugate (BSP-GSH) produced per mg protein per min.

Progesterone hydroxylases (16α and 6βPH) were assayed using high pressure liquid chromatography (HPLC). Progesterone (Sigma) and cofactors were added in 66 mM Tris, pH 7.4 to beakers to give a final volume of 1.05 ml. The incubation mixture contained approximately 1.1

μmol progesterone, 5.8 μmol G-6-P, 0.95 units G-6-PD, 3.0 μmol $MgCl_2$, 0.25 μmol NADH, 0.4 μmol NADP and 0.3 μmol NADPH. Following 60 min incubation, reactions were stopped by addition of 20 ml chloroform/methanol (2:1 v/v). Chloroform/methanol was added to the blanks prior to incubation.

Progesterone and metabolites in chloroform were partitioned from methanol with 5 ml saline. Following filtration through 0.45 μM Teflon Millipore filters and evaporation of chloroform phase to dryness, progesterone and metabolites were redissolved in acetonitrile/water (1:1, v/v). Quantitation was by HPLC on a Waters 6000A HPLC with a Waters 450 variable wavelength detector (Waters Inst. Co., Milford, Mass.) using a wavelength of 240 nm and a 300 nm x 3.9 nm column packed with reverse phase Bondapak C_{18}. Solvent (acetonitrile/water - 49:51, v/v) flow was 1.3 ml/min. Column pressure was held at 750 psi. Standard curves were prepared using progesterone, 6β- and 16α-hydroxyprogesterone (Steraloids, Inc., Wilton, N.H.). Activity was expressed as pmol 16α and 6β HP per mg protein per min.

K. Kidney

1. Function in vitro

Organic ion transport capacity was estimated in vitro 3 days following an i.p. injection of either peanut oil (10 ml/kg) or 150 mg/kg PBBs in peanut oil to 11 day old pups and after 30 or 90 days exposure of adults to diet containing 0 or 100 ppm PBBs. Following cervical dislocation, kidneys were quickly removed, weighed and placed in ice-cold normal saline. Thin (approximately 0.5 mm) renal cortical slices were prepared freehand. Transport capacity was quantified as

the ability of renal cortical slices to accumulate a representative anion, p-aminohippuric acid (PAH), and cation, N-methylnicotinamide (NMN) by the method of Cross and Taggart (1950). Slices were placed in 3.0 ml Ringer's solution, pH 7.4, containing 10 mM sodium acetate and 7.4×10^{-5}M PAH and 6.0×10^{-6}M NMN (New England Nuclear, Boston, Mass.). Following incubation for 90 min at 25°C under 100% oxygen, slices were removed from the medium, blotted on gauze and weighed. Concentrations of PAH and NMN were determined using ^{14}C-labeled compounds (Ecker et al., 1975). Tissue and medium (0.1 ml) were solubilized in 1.0 ml Soluene-100 (Packard, Downers Grove, Ill.). After 24 hr 10 ml Dimilume-30 (Packard) scintillation cocktail was added to each sample. Following 48 hr storage in dark at room temperature, radioactivity was determined using a Packard model 3380 liquid scintillation spectrometer. Results were expressed as slice-to-medium ratio, calculated by dividing disintegrations per minute per ml of tissue by disintegrations per minute per ml of medium (Ecker et al., 1975).

Ability of renal slices to produce ammonia and glucose was determined by the method of Roobol and Alleyne (1974). Slices were incubated in 5 ml Krebs-bicarbonate medium, pH 7.4, containing 2 mM glutamine as substrate. Slices and medium were placed in 15 ml screwcap test tubes, flushed with O_2-CO_2 (95:5 v/v), capped and incubated for 60 min at 37°C. Following incubation, slices were removed from the medium, blotted on gauze and weighed. Immediately after removal of slices, 0.5 ml 10% perchloric acid was added to the incubation medium, the suspension centrifuged at approximately 600 g, and the supernatant was assayed for ammonium by the method of Kaplan (1965) and glucose using Glucostat reagents (Worthington Biochemical

Corp.). Net production of ammonia and glucose was expressed as micromoles per milligram of wet tissue weight per hour.

Vitamin A was quantified in kidney as described for liver.

2. Function in vivo

To determine renal function in vivo, animals exposed to 100 ppm PBBs for 30 or 90 days were anesthetized with 50 mg/kg sodium pentobarbital, i.p., and body temperature was maintained at 37±1°C using heat lamps. A PE50 cannula was inserted into the bladder and urine was collected under mineral oil in preweighed vials. The left femoral vein was cannulated for infusion. Both femoral arteries were cannulated to monitor blood pressure using a Statham transducer and a Beckman type RS dynograph (Beckman, Schuler Park, Ill.) and to obtain blood samples. The infusion solution contained 1.0% inulin and 0.6% PAH in normal saline. (^{3}H)Inulin (0.5 μCi/ml) and (^{14}C)PAH (0.5 μCi/ml) were added to the solution (saline), which was infused at 0.019 ml/min using a Harvard infusion pump (Harvard Apparatus, Millis, Mass.). A minimum of 90 min elapsed from the beginning of infusion to initiation of urine collection. Four 30 min urine samples were taken. Blood (0.4 ml) was sampled at the middle of each urine collection. Following the initial clearance period, animals were infused with 1:4 rat plasma/saline solution (4% body weight) and three additional 30 min collections were made. Radioactivity (^{14}C-PAH and ^{3}H-inulin) in urine and plasma was determined as previously described. Sodium concentrations were determined by flame photometry (Instrumentation Labs, Model 143, Boston, Mass.). Blood urea nitrogen (BUN) was determined by the method of Kaplan (1965) and expressed as mg urea nitrogen per 100 ml of whole blood.

L. Heart

1. Function in vitro

Sympathetic neuronal transport capacity was estimated in vitro in hearts from animals 14, 28, 56, 84 or 98 days of age that had been continuously exposed to 0 or 100 ppm PBBs from day 8 of gestation. Following cervical dislocation, hearts were quickly excised, weighed and placed in ice-cold preoxygenated Krebs-Henseleit solution. Thin ventricular slices were prepared with a Stadie-Riggs microtome (A.H. Thomas Co., Philadephia, Pa.). Transport capacity was quantified as the ability of ventricular slices to accumulate a nonmetabolized catacholamine, d,l-metaraminol (MET), by the method of Stickney (1976). Slices were placed in 10 ml modified Krebs-Henseleit solution, pH 7.4, of the following composition (mM): $NaHCO_3$, 27.2; NaCl, 118.0; KCl, 4.8; KH_2PO_4, 1.0; $MgSO_4$, 1.2; $CaCl_2$, 2.5; anhydrous dextrose, 11.1. The solution also contained 0.75 mg/ml ascorbic acid and $1x10^{-7}$M MET (approximately 20,000 DPM/ml) (New England Nuclear, Boston, Mass.). Following incubation for 60 min at 37°C under 95% O_2-5% CO_2, slices were removed from the medium, washed with ice-cold saline, blotted on gauze and weighed. Concentration of MET was determined using ^{3}H-MET (Stickney, 1976). Slices were solubilized in 0.5 ml tetraethylammonium hydroxide. Double-distilled water (1.5 ml) was added to each sample and mixed thoroughly. Of this resultant suspension, 0.1 ml was mixed with 10.0 ml Bray's solution (88% p-dioxane, 10% methanol and 2% ethylene glycol). Sample radioactivity was determined using a Packard model 3380 liquid scintillation spectrometer. Results were expressed as slice-to-medium ratio, calculated

by dividing disintegrations per min per g heart tissue by disintegrations per min per ml medium (Stickney, 1976).

Inotropic response to calcium and ouabain was quantified in hearts from animals 70 days of age that had been exposed to 0 or 100 ppm PBBs from day 8 of gestation. Following cervical dislocation, hearts were quickly excised, weighed and placed in ice-cold preoxygenated Krebs-Henseleit solution modified as previously described. Left atria were separated from ventricles, trimmed, placed in an atria holder and lowered into a 90 ml bath of modified Krebs-Henseleit solution which was maintained at 30°C and bubbled with 95% O_2-5% CO_2 (Stickney, 1978). Atria were attached to a Grass FT-03C force transducer (Grass Med. Instruments, Quincy, Mass.). A resting tension of approximately 1 g was applied to each atrium. Following determination of the threshold voltage for electrical stimulation, a voltage of 1.1 times threshold was employed to drive the atrium at a frequency of 1 Hz. Atria were allowed to equilibrate for 60 min during which the bathing solution was changed 4 times (15, 30, 45 and 55 min). Following equilibration, twitch tension was recorded and Ca^{2+} ($1.0x10^{-2}M$) or ouabain ($1.5x10^{-6}M$) was added to the bath. Contractile height was recorded and results were expressed as percentage increase over control (contractile height prior to addition of Ca^{2+} or ouabain).

2. Arterial pressure

After cannulation of the left femoral artery in pentobarbital (50 mg/kg, i.p.) anesthetized female rats exposed to 100 ppm PBBs for 90 days, systolic arterial blood pressure was monitored via a Statham transducer (Statham, Hato Rey, Puerto Rico) attached to a

Beckman-Type RS Dynograph (Beckman Inst., Schiller Park, Ill.). Systolic arterial pressure was also measured indirectly in unanesthetized female rats using tail plethysmography (Narco-Bio Systems, Inc., Houston, Texas).

M. Lung

1. Function in vitro

Activity of angiotensin converting enzyme (ACE) was quantified by the method of Cushman and Cheung (1971) as modified by Wallace et al. (1978). Lungs were excised, weighed and homogenized using a polytron homogenizer (Brinkman Instruments, Westbury, N.Y.) in 4 volumes of ice-cold 100 mM potassium phosphate-300 mM sodium chloride buffer, pH 7.0. Homogenate was centrifuged at 600 g for 10 min and supernatant assayed for ACE activity. Hippuryl-L-histidyl-L-leucine (HHL) was added in 100 mM potassium phosphate-300 mM sodium chloride buffer, pH 8.3, to give a final concentration of HHL of 5.0 mM and final volume of 0.25 ml. Following 30 min incubation at 37°C, the reaction was stopped by addition of 0.25 ml 1.0 N HCl. After addition of 1.5 ml ethyl acetate and centrifugation for 10 min at 2,000 g, 1.0 ml acetate phase was evaporated at 40°C in 3.0 ml 1.0 M NaCl and the optical density determined at 228 nm using a Beckman dual beam spectrophotometer (Beckman Instruments, Fullerton, Calif.). A molar extinction coefficient of 9.8 mM^{-1} cm^{-1} was employed to convert optical density to molar units. Activity was expressed as nmol hippuric acid produced per mg protein per min.

Activity of monoamine oxidase (MAO) was quantified by the method of Roth et al. (1979). MAO activity was determined in

supernatants prepared as described for ACE activity. (^{14}C)-5-HT was added in 50 mM potassium phosphate-150 mM sodium chloride buffer, pH 7.0, to give a final volume of 2.2 ml. The reaction mixture contained 0.2 μmole ^{14}C-5-HT. Following 15 min incubation at 37°C, the reaction was stopped by addition of 0.2 ml $Ba(OH)_2$ followed by 0.2 ml 0.2 M $ZnSO_4$. After centrifugation for 10 min at 2,000 g the resulting supernatant was analyzed for 5-HT and 5-HIAA by the method of Roth et al. (1977). Samples were added to columns (0.5 x 1.0 cm) of Bio-Rex 70 (sodium form; pH 6.0) cation exchange resin (Bio-Rad Labs, Richmond, Calif.) to separate parent (unreacted) 5-HT from its metabolite 5-hydroxyindoleacetic acid (5-HIAA). 5-HIAA was eluted from the column with 2.5 ml distilled water, 5-HT was eluted from the column with 3.0 ml 0.2 N HCl. Radioactivity in each fraction was quantified after addition of 10 ml ACS (Amersham/Searle Corp., Arlington Heights, Ill.) using a Packard model 3380 liquid scintillation spectrometer. Activity was expressed as nmol 5-HIAA produced per mg protein per min.

2. Isolated perfused lung

Lungs were perfused by the method of Wallace et al. (1979). Rats were anesthetized with sodium pentobarbital, 50 mg/kg, i.p. and administered sodium heparin, 2,000 U/kg, i.v. A PE90 cannula was inserted into the pulmonary artery via the right ventricle. A PE160 cannula was inserted into the trachea and the lungs excised and suspended in a perfusion apparatus maintained at 37±1°C with a heat lamp. Lungs were ventilated by negative pressure ventilation at 30 strokes per min with a 95% O_2-5% CO_2 gas mixture and perfused with Krebs-bicarbonate medium, pH 7.4, containing 4% bovine serum albumin in a single pass (nonrecirculating) system. Inflow pressure was continuously

monitored using a Grass model 7 polygraph (Grass Med. Instruments, Quincy, Mass.) and a P23AC Statham pressure transducer (Statham, Hato Rey, Puerto Rico).

Following 5-10 min equilibration, angiotensin I (AI) was added to the perfusate at a concentration of 1 ng AI/ml. Samples (0.5 ml) of effluent medium were taken at the middle of a 4 min collection period (approximately 2 min following onset of perfusion with AI). After a brief equilibration with drug-free perfusion medium, lungs were perfused with medium containing ^{14}C-5-hydroxytryptamine (5-HT) (Amersham/Searle Corp., Arlington Heights, Ill.) at a concentration of 0.1 μM. Samples (0.5 ml) of effluent medium were taken at the middle of a 4 min collection period. When the experiment was terminated lungs were removed from perfusion chamber, blotted on gauze and weighed.

Concentration of AI was quantified by radioimmunoassay using a specific antibody to pure Asp^1-Ile^5-AI (CIBA Pharmaceuticl Co., Summit, N.J.) according to the method of Haber et al. (1969). Following incubation of sample at 4°C for 18 hr with antibody and Asp^1-[^{125}I]-Ile^5-AI, free and antibody-bound AI were separated by activated charcoal (10% suspension) binding of free AI. After centrifugation, radioactivity in resultant fractions was quantified using a Searle model 1185 gamma counter. Concentrations of 5-HT and 5-HIAA were quantified as described for MAO.

N. Response to Xenobiotics

Duration of anesthesia following 40 mg/kg sodium pentobarbital in water, i.p., was determined in female rats 84 days of age that had been neonatally treated with 150 or 500 mg/kg PBBs or exposed to 100 ppm PBBs from day 8 of gestation. Sleeping time was recorded as the interval from time of injection until the righting reflex was regained.

Duration of anesthesia following 90 sec in dessicator saturated with diethyl ether was determined in female rats 90 days of age that had been exposed to 100 ppm PBBs from day 8 of gestation. Sleeping time was recorded as the interval from the time animals (pair control and PBBs treated) were removed from the dessicator until the righting reflex was regained.

Median time to death following bromobenzene administration was determined in male rats that had been neonatally treated with 150 or 500 mg/kg PBBs. At 49 days of age, 2820 mg/kg bromobenzene in DMSO (approximately a 24 hr LD_{85} dose which was established in preliminary experiments) was administered to the animals, i.p., and the time to death was measured.

Median time to death following digitoxin was determined in female rats that had been exposed to 100 ppm PBBs from day 8 of gestation. At 90 days of age, 10 mg/kg digitoxin in DMSO (approximately a 24 hr LD_{85} dose which was established in preliminary experiments) was administered to the animals, i.p., and the time to death was measured.

O. Response to Pharmacological Doses of Steroid Hormones

Rats were exposed to 0, 10 or 100 ppm PBBs from day 8 of gestation until 28 days postpartum when experiments were conducted in offspring, unless otherwise specified. Methods employed in these experiments were similar to those used by others to determine effects of phenobarbital on steroid hormone metabolism (Conney et al., 1966; Levin et al., 1968, 1969). Radioactively labeled steroids were purchased from New England Nuclear (Boston, Mass.) and unlabeled steroids from Sigma Chemical Co., (St. Louis, Mo.). Sample radioactivity was determined as described in Renal Function.

Male rats were treated with an i.p. injection of 150 mg/kg (^{14}C)progesterone ([4-^{14}C], 0.4 μCi/mg) (approximately a LD_{30} dose in controls) in dimethylsulfoxide (DMSO). Duration of anesthesia was recorded as time of injection until righting reflex was regained. Following decapitation and blood collection, brains were excised, weighed and homogenzed (Potter-Elvehjem homogenizer with a Teflon pestle) in 3 volumes 66 mM Tris, pH 7.4, at waking or 3 hr after progesterone administration. Aliquots (2.5 ml) of whole brain homogenate were extracted initially with n-hexane (Hex) and subsequently with water saturated ethyl acetate (EA). Homogenates were extracted twice, 4 volumes and then 2 volumes which were pooled, with each solvent. Following evapoaration to dryness, radioactivity was determined in extracts (1.5 ml). Radioactivity was also quantified in whole brain homogenates (500 μl) and serum (50 μl).

Male rats were treated with a s.c. injection of 0 (corn oil) or 20 mg/kg (^{3}H)testosterone ([7-^{3}H(N)], 5 μCi/mg) on day 25 postpartum. At least 6 animals in each greatment group were decapitated 24 hr later, blood was collected and testes excised and weighed. Radioactivity was determined in serum (50 μl) and whole testis. Remaining animals were weighed 72 hr later, sacrificed by cervical dislocation, and seminal vesicles were excised and weighed.

Female rats were treated with an i.p. injection of 0 (DMSO), 1 or 3 μg/kg (^{3}H)estradiol-17β ([6,7-^{3}H], 156 μCi/μg). Body weights were recorded, animals decapitated, blood collected and uteri excised and weighed 4 hr later. Radioactivity was quantified in serum (50 μl) and whole uterus.

P. Persistance of Effects

1. Single injection

Nursing dams and pups (10 per litter) were purchased 3 days postpartum and were acclimatized for 4 days prior to experimental treatment. Following normalization of the litter to 5 males and 5 females on day 7, pups of each litter were treated with a single i.p. injection of 0 (peanut oil, 10 ml/kg) or 150 mg/kg PBBs in peanut oil. Pups were sacrificed by decapitation 1, 2, 3, 7, 14, 28 or 63 days following treatment at which times enzyme assays and quantification of tissue PBBs were performed.

2. Perinatal exposure

Time pregnant rats were obtained on day 2 of gestation and were acclimatized for 6 days prior to experimental treatment. On day 8 of gestation, the experimental diet containing 0 or 100 ppm PBBs was

substituted for the Lab Blox. Litters were normalized to 10 pups (5 male, 5 female) each at birth. At 28 days postpartum all animals were weaned onto Lab Blox containing no PBBs. Enzyme assays, pentobarbital sleeping time, tissue PBBs quantification and histologic examinations were performed at 28 days of age and during the residual phase; 150 and/or 328 days of age.

3. Multiple generations

Timed pregnant rats (F_0) were obtained on day 2 of gestation and were acclimatized for 6 days prior to experimental treatment. On day 8 of gestation, the experimental diet containing 0, 10 or 100 ppm PBBs was substituted for the Lab Blox. Litters were normalized to 10 pups (5 male, 5 female) at each generation. At 28 days postpartum all progeny (F_1) of dams (F_0) dietarily exposed to PBBs were weaned onto Lab Blox containing no PBBs. After an additional 10-12 wk for maturation, littermates (F_1) were bred to produce the next generation (F_2). Similarly, F_2 littermates were bred at 14-16 wk of age to produce F_3. Enzyme assays, pentobarbital sleeping times, progesterone sleeping times and tissue PBBs quantification were performed and survival determined in F_1, F_2 and/or F_3 at weaning.

Q. Statistics

Data were analyzed statistically by analysis of variance, either randomized complete block or completely random design. Treatment differences were detected by the least significant difference test (Steel and Torrie, 1960). The 0.05 level of probability was used as the criterion of significance.

RESULTS

A. Survivial, Growth and Development

Dietary exposure of pregnant rats to 100 ppm PBBs with maternal food deprivation for 48 hr produced a decrease in number of live fetuses and fetal body weight and an increase in resorption rate and whole fetal carcass concentration of PBBs (Table 1). Dietary treatment with 100 ppm PBBs alone increased whole fetal carcass concentration of PBBs when compared to controls but not 100 ppm PBBs with food deprivation. Separate treatment with 100 ppm PBBs or food deprivation had no effect on number of live fetuses, resorption rate or fetal size. No statistically significant treatment differences in fetal liver or kidney weight-to-body weight ratios or incidence of gross, soft-tissue or skeletal anomalies were detected.

Body weight gain of pups suckled by dams fed 100 ppm PBBs and weaned onto 100 ppm PBBs was decreased by 14 days of age (83% of control value) and remained decreased through 84 days of age (82% of control value) (Figure 1). Growth rate was not affected by neonatal treatment with a single i.p. injection of 150 or 500 mg/kg PBBs. Fur development, external auditory direct opening and eye opening were delayed in pups suckled by dams fed 100 ppm PBBs (Table 2). Postnatal physical development, assessed using these development markers as well as earlier occurring pinna detachment and incisor eruption, was not affected by neonatal treatment with 150 or 500 mg/kg PBBs (Table 2). Survival rate was reduced in pups exposed to 100 ppm PBBs by 14 days

TABLE 1

Resorption Rate, Fetal Size and Whole Carcass Concentration of PBBs Among Offspring of Pregnant Rats Treated with PBBs and Food Deprived[a]

PBBs (ppm)	Deprivation (hr)	No. of Live Fetuses	No. of Resorbed or Dead Fetuses (%)	Fetal Crown Rump Length (cm)	Fetal Body Weight (g)	Whole Carcass PBBs (μg/g)
0	0	13±1	6.9±2.0	4.0±0.1	4.39±0.21	0.0
0	48	12±1	8.7±5.1	3.9±0.1	4.04±0.25	0.0
100	0	14±1	4.5±1.8	3.9±0.1	4.05±0.18	4.0±0.3[b]
100	48	10±1[b]	19.2±4.2[b]	3.8±0.1	3.88±0.15[b]	3.7±0.4[b]

[a]Values are means ± S.E.M. for 8 litters. Rats were treated with 0 or 100 ppm PBBs from day 8 of gestation until day 20 of gestation unless food deprived (gestational days 10 and 11).

[b]Significantly different from the nondeprived control value, $p<0.05$.

Figure 1. Effect of neonatal or perinatal treatment with PBBs on body wt gain. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

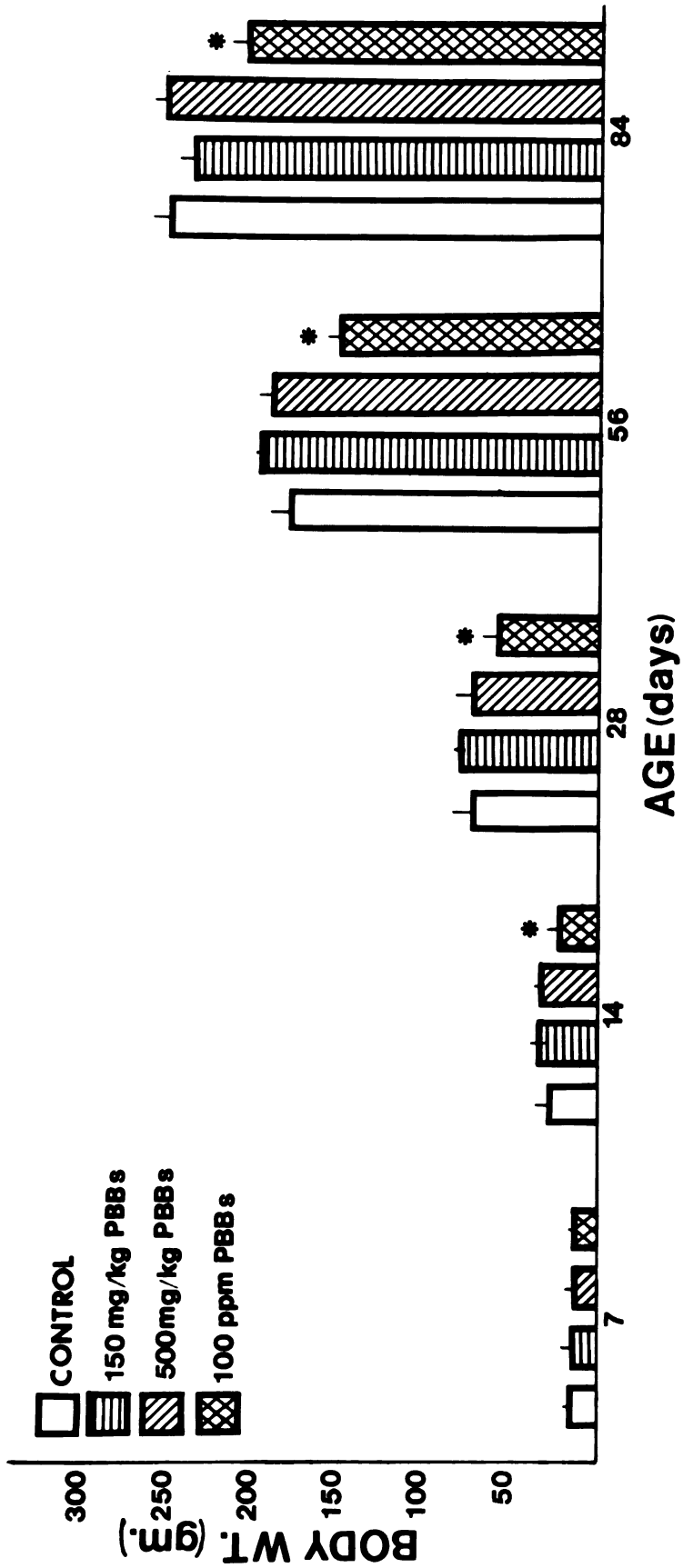
CONTROL
150 mg/kg PBBs
500mg/kg PBBs
100 ppm PBBs
BODY WT. (gm.)
300
250
200
150
100
50
7
14
28
56
84
AGE (days)
*
*
*
*

Figure 1

TABLE 2

Postnatal Development of Rats Following Neonatal or Perinatal Treatment with PBBs[a]

Parameter	Treatment			
	Control	150 mg/kg PBBs	500 mg/kg PBBs	100 ppm PBBs
Pinna detachment	2.7±0.1	2.7±0.2	3.0±0.2	2.9±0.2
Incisor eruption	8.1±0.2	8.1±0.2	8.6±0.3	8.6±0.3
Fur development	9.2±0.1	9.1±0.1	9.6±0.2	10.5±0.2[b]
Ext. and duct opening	11.6±0.2	11.6±0.2	11.8±0.2	12.4±0.3[b]
Eye opening	14.7±0.2	14.8±0.2	14.4±0.2	15.3±0.2[b]

[a]Values are means in days ± S.E.M. for at least 4 litters. Animals were treated with peanut oil (control), 150 mg/kg PBBs or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed.

[b]Significantly different from the control value, $p<0.05$.

of age (88% of control value) and remained lower than control through 28 days of age (87% of control value) (Figure 2). Pup mortality was not affected by neonatal treatment with 150 or 500 mg/kg PBBs.

Liver, kidney, ovary and fat all contained PBBs following neonatal or perinatal treatment with PBBs (Table 3). Tissue concentrations of PBBs were dose-dependent as neonatal treatment with 150 mg/kg PBBs resulted in lower concentrations of PBBs than 500 mg/kg PBBs. Perinatal and continuous exposure to 100 ppm PBBs resulted in higher tissue concentrations of PBBs than neonatal treatment with a single injection of 500 mg/kg PBBs. Of the tissues examined, kidney had the lowest and fat the highest concentration of PBBs. More PBBs were found in ovary than in either kidney or liver.

Concentration of PBBs in milk was highest at parturition and decreased with time for 14 days (Figure 3). At parturition, the concentration of PBBs in milk was approximately 3-4 times higher than the concentration of PBBs in the maternal diet. By 14 days postpartum, concentration of PBBs in milk and maternal diet were similar.

B. Liver

The liver weight-to-body weight ratio was increased in a dose-dependent manner by neonatal treatment with 150 or 500 mg/kg PBBs when determined at 28 days of age (120% and 145% of control value, respectively) (Figure 4). At 56 days of age, liver was enlarged after 500 mg/kg PBBs, however, not after 150 mg/kg PBBs (115% and 105% of control value, respectively). The liver weight-to-body weight ratio was increased to a greater extent by 100 ppm PBBs than 150 or 500 mg/kg PBBs (approximately 165% of control value by 100 ppm PBBs at

Figure 2. Effect of neonatal or perinatal treatment with PBBs on survival rate-to-weaning. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

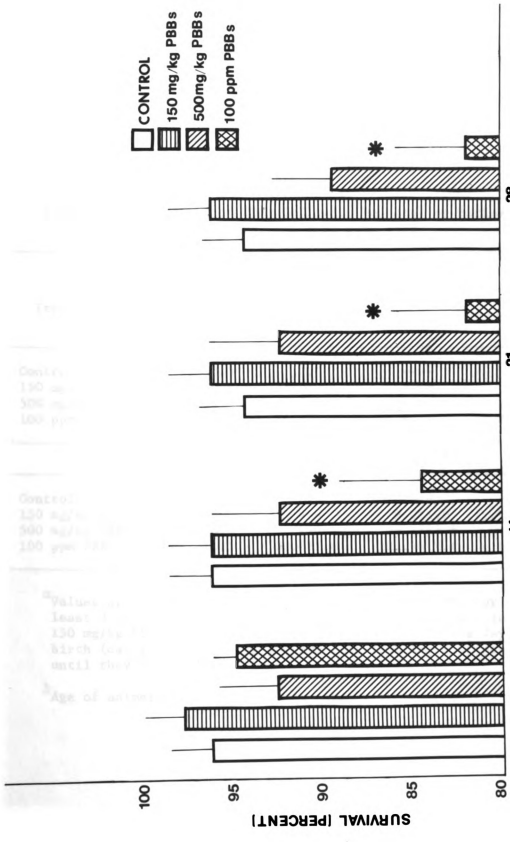
CONTROL
150 mg/kg PBBs
500mg/kg PBBs
100 ppm PBBs
SURVIVAL (PERCENT)
100
95
90
85
80
7
14
21
28
AGE (days)
*
*
*

Figure 2

TABLE 3

Tissue Concentrations of PBBs Following Neonatal or Perinatal and Continuous Treatment with PBBs[a]

Treatment	TISSUE			
	Liver	Kidney	Ovary	Fat
		28[b]		
Control	0.3±0.3	0.0	----	0.6± 0.6
150 mg/kg PBBs	2.6±0.4	1.2±0.1	----	45.0±13.4
500 mg/kg PBBs	12.5±4.0	4.2±1.1	----	101.7±41.1
100 ppm PBBs	27.2±6.9	16.5±4.2	----	282.2±17.3
		56[b]		
Control	0.0	0.0	0.0	0.0
150 mg/kg PBBs	0.9±0.3	---	9.8±1.9	19.9± 4.1
500 mg/kg PBBs	5.5±0.3	---	26.1±11.6	52.3± 6.2
100 ppm PBBs	44.7±5.5	20.6±1.8	122.3± 4.4	449.6±25.8

[a]Values are means in µg PBBs/g wet wt tissue ± S.E.M. for at least 3 animals. Animals were treated with peanut oil (control), 150 mg/kg PBBs or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed.

[b]Age of animals in days.

Figure 3. Concentration of PBBs in milk from lactating rats fed diet containing 50 ppm PBBs from the 8th day of pregnancy. Values are μg PBBs/ml milk ± S.E.M. for at least 4 animals.

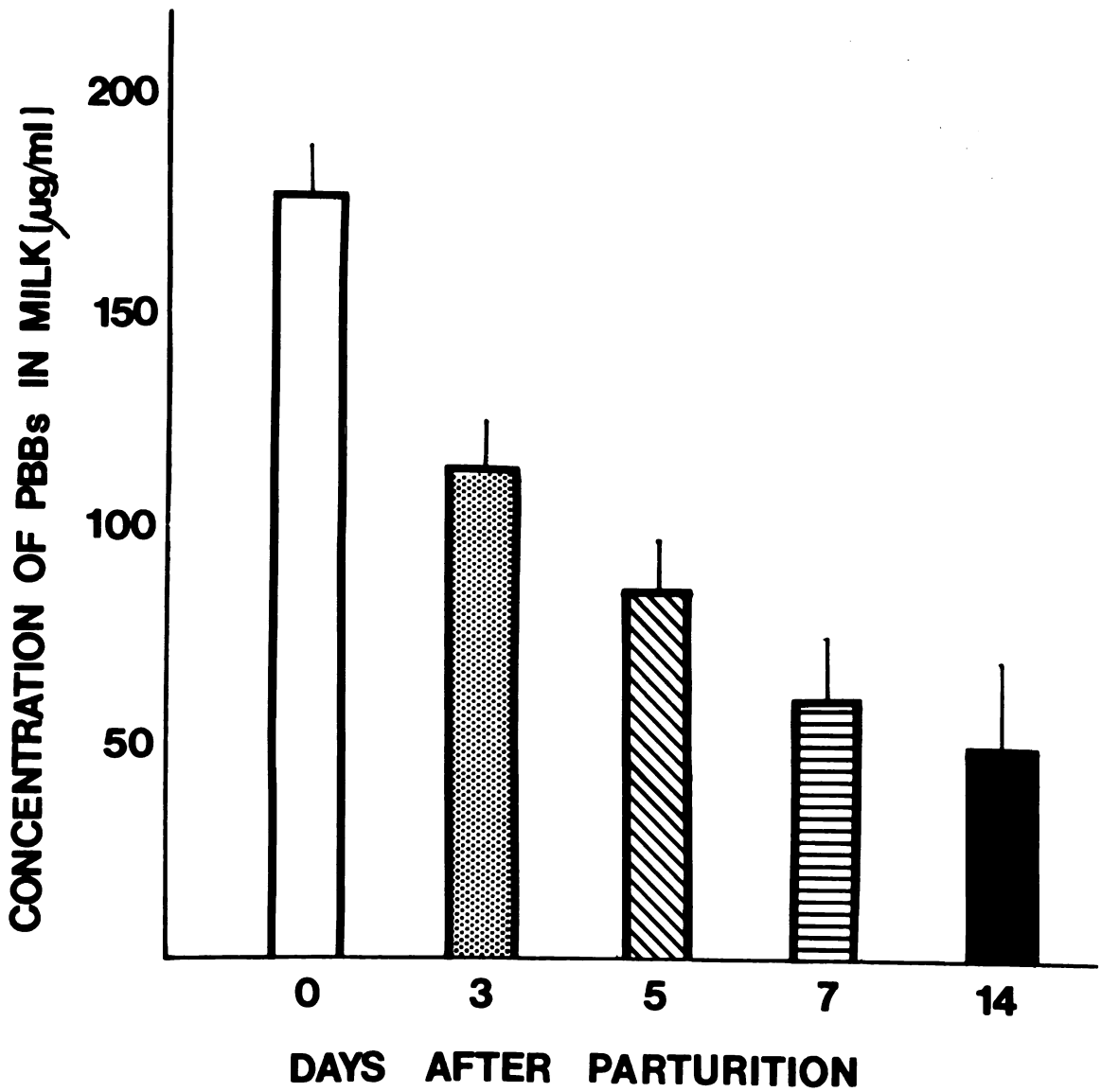
CONCENTRATION OF PBBs IN MILK (µg/ml)
200
150
100
50
0
3
5
7
14
DAYS AFTER PARTURITION

Figure 3

Figure 4. Effect of neonatal or perinatal treatment with PBBs on liver wt to body wt and kidney wt to body wt ratios. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

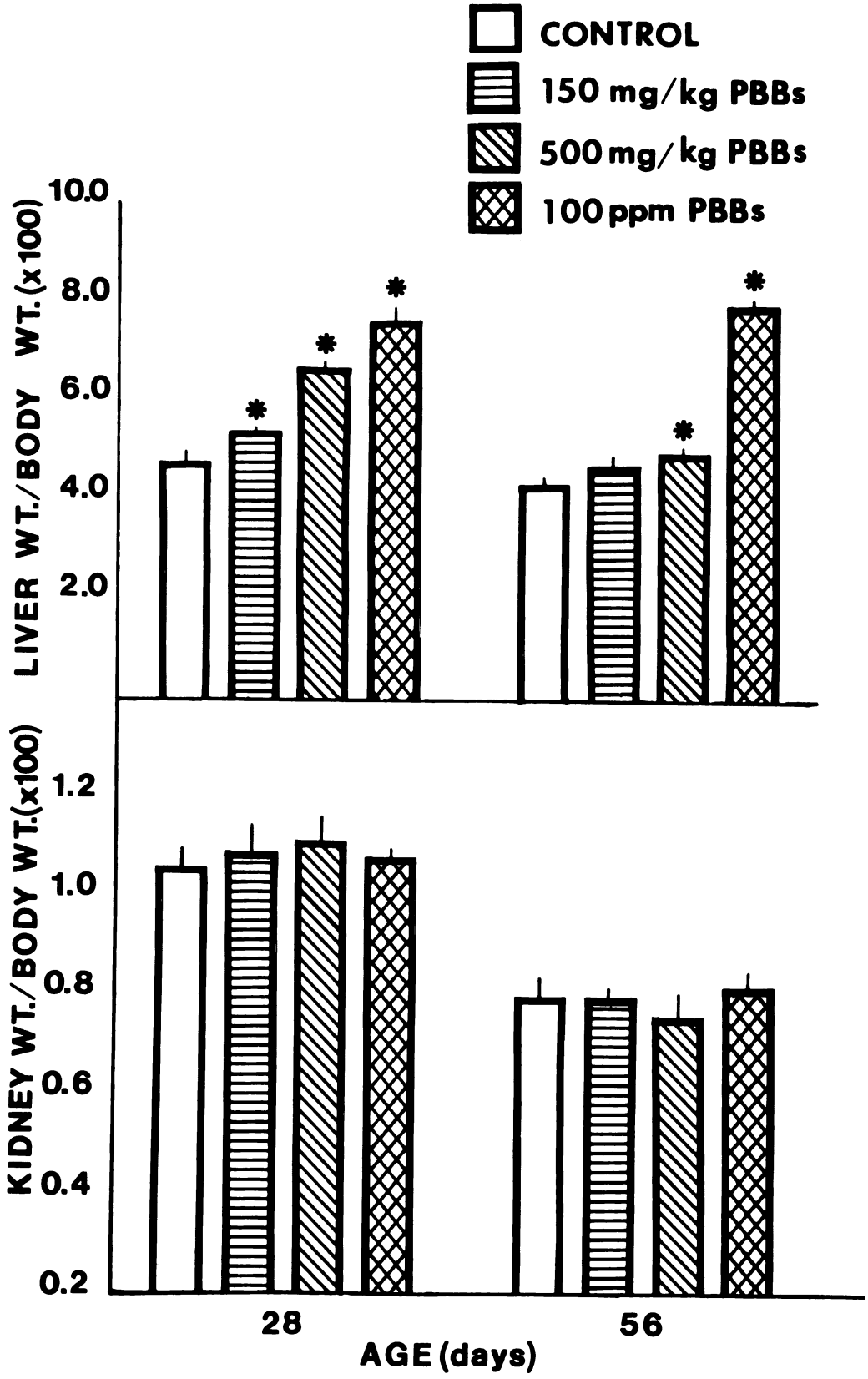
CONTROL
150 mg/kg PBBs
500 mg/kg PBBs
100 ppm PBBs
LIVER WT./BODY WT.(x100)
10.0
8.0
6.0
4.0
2.0
KIDNEY WT./BODY WT.(x100)
1.2
1.0
0.8
0.6
0.4
0.2
28
56
AGE (days)

Figure 4

both 28 and 56 days of age). Liver enlargement produced by PBBs was accompanied by an increase in hepatic microsomal protein. Treatment of 7-day old pups with 150 mg/kg PBBs increased microsomal protein in liver after 7, 14 and 28 days (approximately 130% of control values) (Figure 8).

Compared to control liver (Figure 5), liver from animals perinatally exposed to 100 ppm PBBs had histopathological alterations (Figure 6). At 28 days the most prominent structural change was vacuolation which gave the cytoplasm a foamy appearance. Some cells had large intracytoplasmic vacuoles that displaced most of the cytoplasm and pressed the nucleus to the periphery. Occasionally vacuolation and necrosis progressed centrolobularly but most was midzonal. Cellular swelling caused the sinusoids to become less apparent. Similar hepatic degenerative changes were observed in adult rats following 90 days exposure to 100 ppm PBBs (Figure 7). Hepatic cells were uniformly enlarged so that sinusoids appeared only as small, clear spaces with an occasional Kupfer cell visible. Many cells were vacuolated. Focal necrosis was found occasionally, however, as in liver following perinatal exposure to PBBs midzonal necrosis was most apparent. Hepatocytes were disrupted, nuclei were absent in some cells and pycnotic in others. Many hepatocytes contained large myelin bodies. Some of these bodies were uniform in appearance, while others appeared to have a lighter central mass surrounded by a wide, darker band. In contrast to effects produced by dietary exposure to PBBs, hepatic degenerative changes were not observed following neonatal treatment with a single injection of 150 or 500 mg/kg PBBs.

Figure 5. Hepatic tissue from control rat. Hematoxlyin and eosin stain; x100.

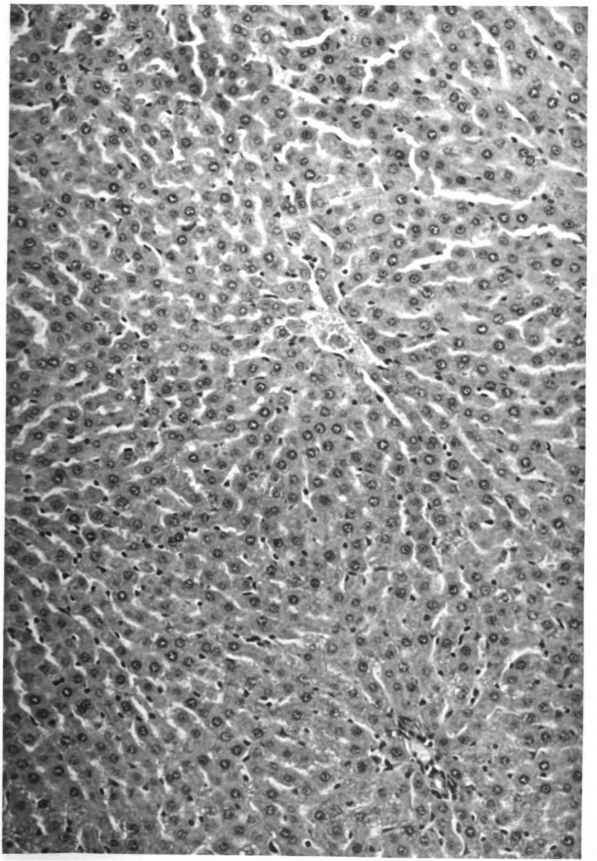

Figure 5

Figure 6. Hepatic tissue from rat 28 days of age that had been exposed to 100 ppm PBBs from 8th day of gestation. There is vacuolation. Hematoxylin and eosin stain; x40.

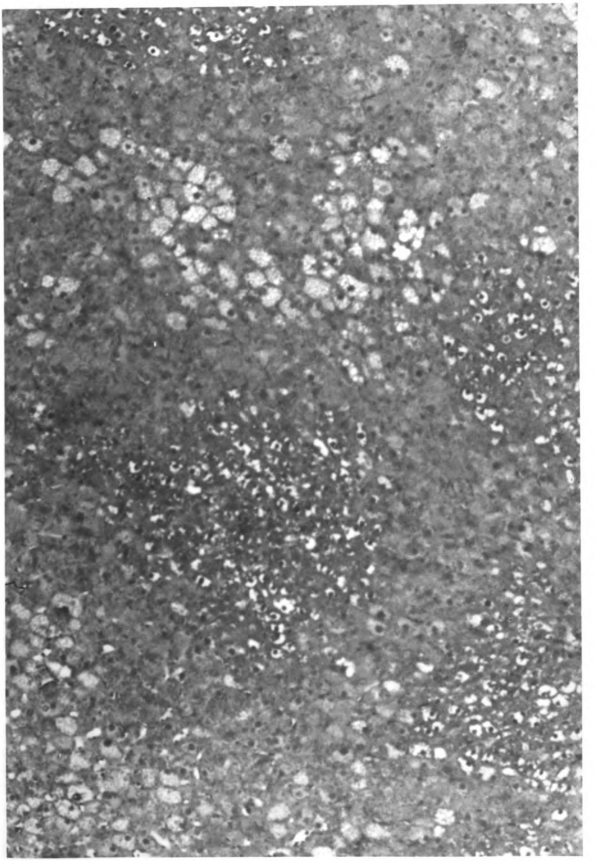

Figure 6

Figure 7. Hepatic tissue from rat fed diet containing 100 ppm PBBs for 90 days. There are inclusion bodies. Hematoxylin and eosin stain; x400.

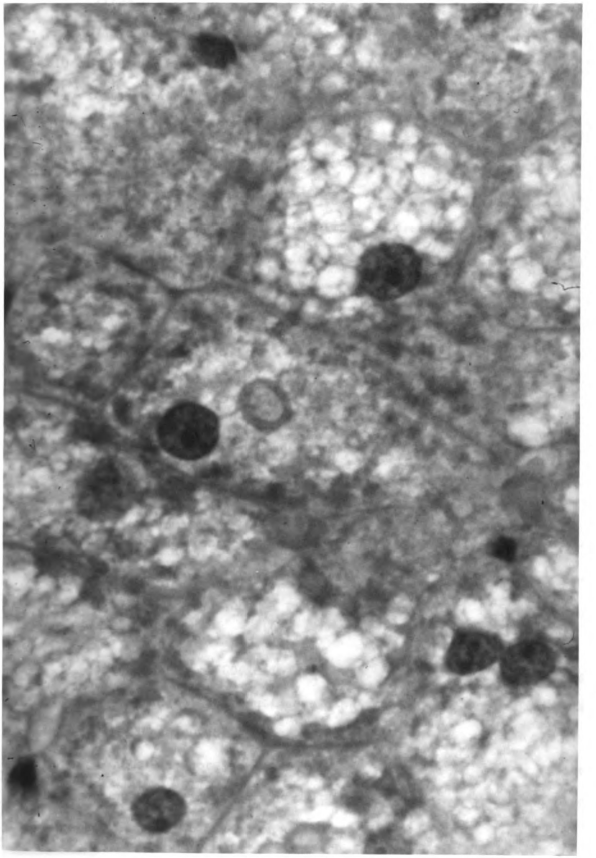

Figure 7

Vitamin A concentration (μg/g wet wt tissue) in liver was reduced at 28 days of age in a dose-dependent manner by perinatal exposure to 10 or 100 ppm PBBs (60% and 43% of control value, respectively) (Table 4). Vitamin A per liver was also reduced at 28 days of age by 10 or 100 ppm PBBs (76% and 71% of control value, respectively). Similarly, vitamin A concentration in liver was reduced at 56 and 100 days of age by perinatal and continuous exposure to 100 ppm PBBs (50% and 60% of control values, respectively) (Table 4). Treatment with PBBs had no effect on vitamin A concentration in serum when determined at 28, 56 or 100 days of age (Table 4).

Concentration of COPRO and URO in liver decreased with age in control and PBBs treated rats (Table 5). At 28 days of age, hepatic COPRO concentration was increased by perinatal exposure to 100 ppm PBBs (approximately 350% of control value). Hepatic URO concentration was increased in a dose dependent manner at 28 days of age by perinatal exposure to 10 or 100 ppm PBBs (approximately 150% and 350% of control value, respectively). At 112 days of age, hepatic COPRO concentration was increased by 10 or 100 ppm PBBs (approximately 150% and 350% of control value, respectively). Hepatic URO concentration was only increased by 100 ppm PBBs at 112 days of age (approximately 150% of control value).

Concentration of COPRO in urine was increased at 28 and 112 days of age by perinatal and continuous exposure to 100 ppm PBBs (approximately 150% and 200% of control values, respectively) (Table 6). Urinary COPRO was not increased by 10 ppm PBBs at 112 days of age and URO concentration in urine was not affected by PBBs. Perinatal exposure to PBBs had no effect on packed cell volume (Table 7).

TABLE 4

Effect of Perinatal Exposure to PBBs on the Concentration of Vitamin A in Serum and Liver of Rats[a]

PBBs (ppm)	Age (days)	Vitamin A Concentration	
		Serum (µg/ml)	Liver (µg/g)
0	28	0.32±0.04	66.1± 5.3
10	28	0.34±0.03	40.0± 4.7[b]
100	28	0.31±0.04	28.4±10.1[b]
0	56	0.34±0.05	94.5±12.6
100	56	0.36±0.03	47.2± 9.7[b]
0	100	0.37±0.04	187.0±22.4
100	100	0.34±0.06	112.6±18.2[b]

[a]Values are means ± S.E.M. for at least 4 animals. Rats were treated with 0, 10 or 100 ppm PBBs from day 8 of gestation until 28 days postpartum at which time vitamin A was quantified.

[b]Significantly different from the control value, $p<0.05$.

TABLE 5

Concentration of Coproporphyrin and Uroporphyrin in Liver from Female Rats Exposed to PBBs[a]

Treatment	Coproporphyrin	Uroporphyrin
	Age: 28 Days	
Control	0.084±0.012	0.056±0.028[b]
10 ppm PBBs	0.109±0.024[b]	0.094±0.017[b]
100 ppm PBBs	0.282±0.009[b]	0.206±0.039[b]
	Age: 112 Days	
Control	0.039±0.005[b]	0.017±0.004
10 ppm PBBs	0.066±0.015[b]	0.015±0.005[b]
100 ppm PBBs	0.147±0.029[b]	0.027±0.004[b]

[a]Values represent μg/gm wet liver, expressed as means ± S.E.M. for at least 3 animals. Animals were exposed to 0, 10 or 100 ppm PBBs from day 8 of gestation until measurements were made.

[b]Significantly different from control value, $p<0.05$.

TABLE 6

Concentration of Coproporphyrin and Uroporphyrin in Urine from Female Rats Exposed to PBBs[a]

Treatment	Coproporphyrin	Uroporphyrin
	Age: 28 Days	
Control	3.15±0.46	0.12±0.01
100 ppm PBBs	4.67±0.24[b]	0.11±0.01
	Age: 112 Days	
Control	1.73±0.41	0.35±0.08
10 ppm PBBs	2.29±0.63	0.41±0.07
100 ppm PBBs	3.62±0.55[b]	0.28±0.05

[a]Values represent µg/gm wet liver, expressed as means ± S.E.M. for at least 3 animals. Animals were exposed to 0, 10 or 100 ppm PBBs from day 8 of gestation until measurements were made.

[b]Significantly different from control value, $p<0.05$.

TABLE 7

Packed Cell Volume (Hematocrit) in Female Rats Exposed to PBBs[a]

Treatment	Packed Cell Volume (%)
Control	43.0±0.7
100 ppm PBBs	42.3±0.4

[a]Values are means ± S.E.M. for 10 animals. Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation until 16 wks postpartum, when measurements were made.

Activity of hepatic microsomal and cytosolic enzymes increased with age in control and PBBs treated rats (Figures 8 and 9). Administration of 150 mg/kg PBBs to 7-day old rats increased the relative activity of all hepatic enzymes investigated. EROD and AHH activities were increased above controls by 2 days following administration of PBBs with maximal activities occurring after 7 to 14 days (approximately 2000% and 900% of control values, respectively) (Figure 8). Hepatic EH activity was higher in PBBs treated pups 7 days following PBBs and was maximally stimulated by 14 days (approximately 650% of control value) (Figure 8). BP-4-OH, Hex-OH and GSH-transferase activities were all increased above control values by 7 days after administration of PBBs and reached maximal activity 28 days following treatment (approximately 450%, 400% and 140% of control values, respectively) (Figure 9). BP-2-OH was stimulated 1 day following treatment of 7 day old rats with PBBs and reached a maximum by 14 days (approximately 500% of control value) (Figure 9).

Hepatic AHH and EH were stimulated at both 28 and 56 days of age by perinatal and continuous exposure to 100 ppm PBBs and by neonatal treatment with 150 or 500 mg/kg PBBs (Figure 10). Of these treatments, hepatic AHH was increased to the greatest extent in animals exposed to 100 ppm PBBs (approximately 1500% of control value at both 28 and 56 days of age). Stimulation of AHH in liver following 500 mg/kg PBBs was greater than after 150 mg/kg PBBs approximately 550% and 200% of control value, respectively, at 28 days of age). Activity of hepatic AHH following 500 or 150 mg/kg PBBs was greater at 56 than 28 days of age (approximately 1250% and 350% of control value,

Figure 8. Hepatic microsomal protein and enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum. Each point represents the mean ± S.E.M. for at least 3 rats. Asterisk indicates values significantly different from corresponding control, $p<0.05$.

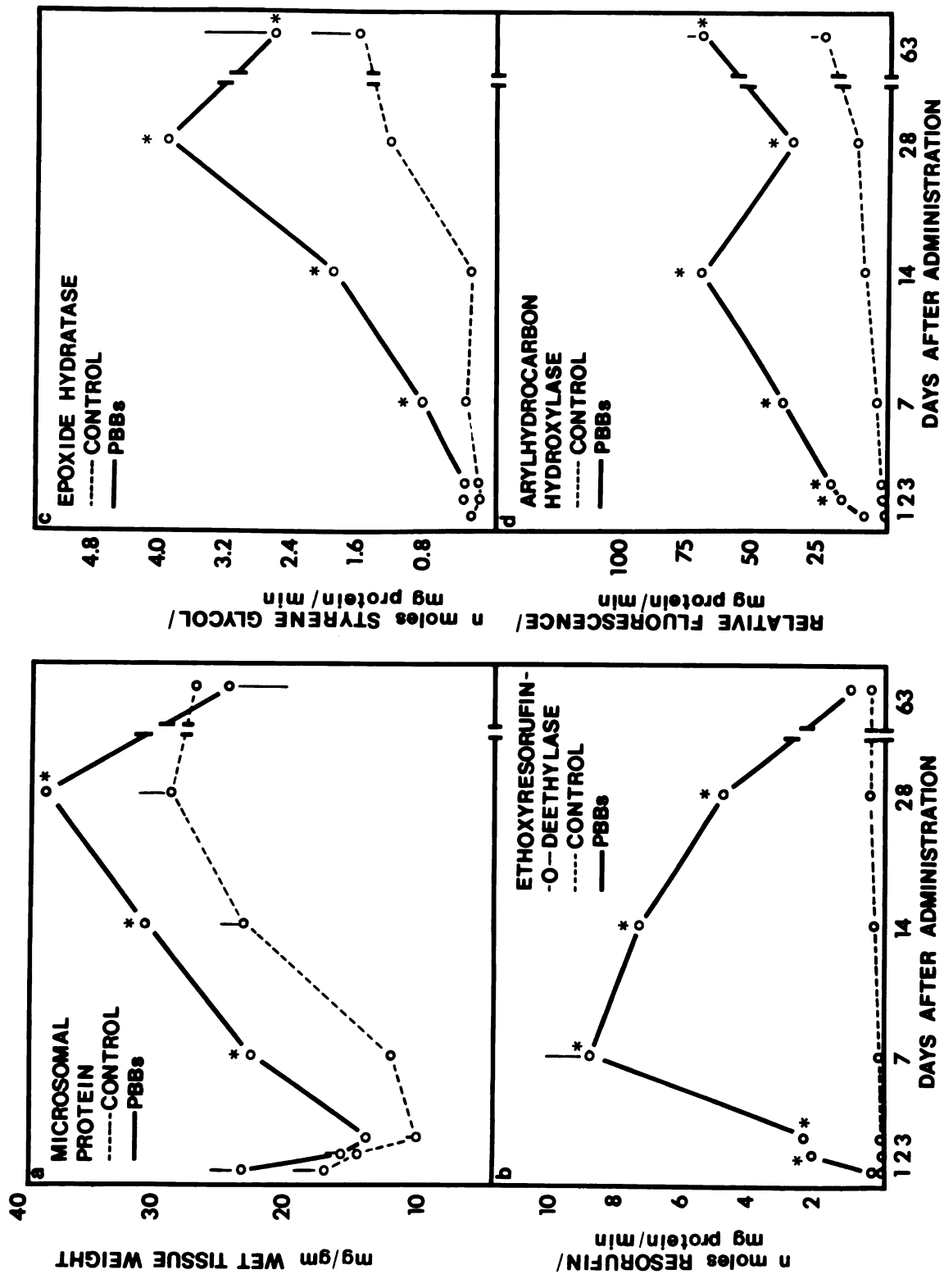
a
MICROSOMAL PROTEIN
CONTROL
PBBs
mg/gm WET TISSUE WEIGHT
40
30
20
10
b
ETHOXYRESORUFIN-O-DEETHYLASE
CONTROL
PBBs
n moles RESORUFIN/ mg protein/min
10
8
6
4
2
1 2 3 7 14 28 63
DAYS AFTER ADMINISTRATION
c
EPOXIDE HYDRATASE
CONTROL
PBBs
n moles STYRENE GLYCOL/ mg protein/ min
4.8
4.0
3.2
2.4
1.6
0.8
d
ARYLHYDROCARBON HYDROXYLASE
CONTROL
PBBs
RELATIVE FLUORESCENCE/ mg protein/ min
100
75
50
25
1 2 3 7 14 28 63
DAYS AFTER ADMINISTRATION

Figure 8

Figure 9. Hepatic enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum. Each point represents the mean ± S.E.M. for at least 3 rats. Asterisk indicates values significantly different from corresponding control, $p<0.05$.

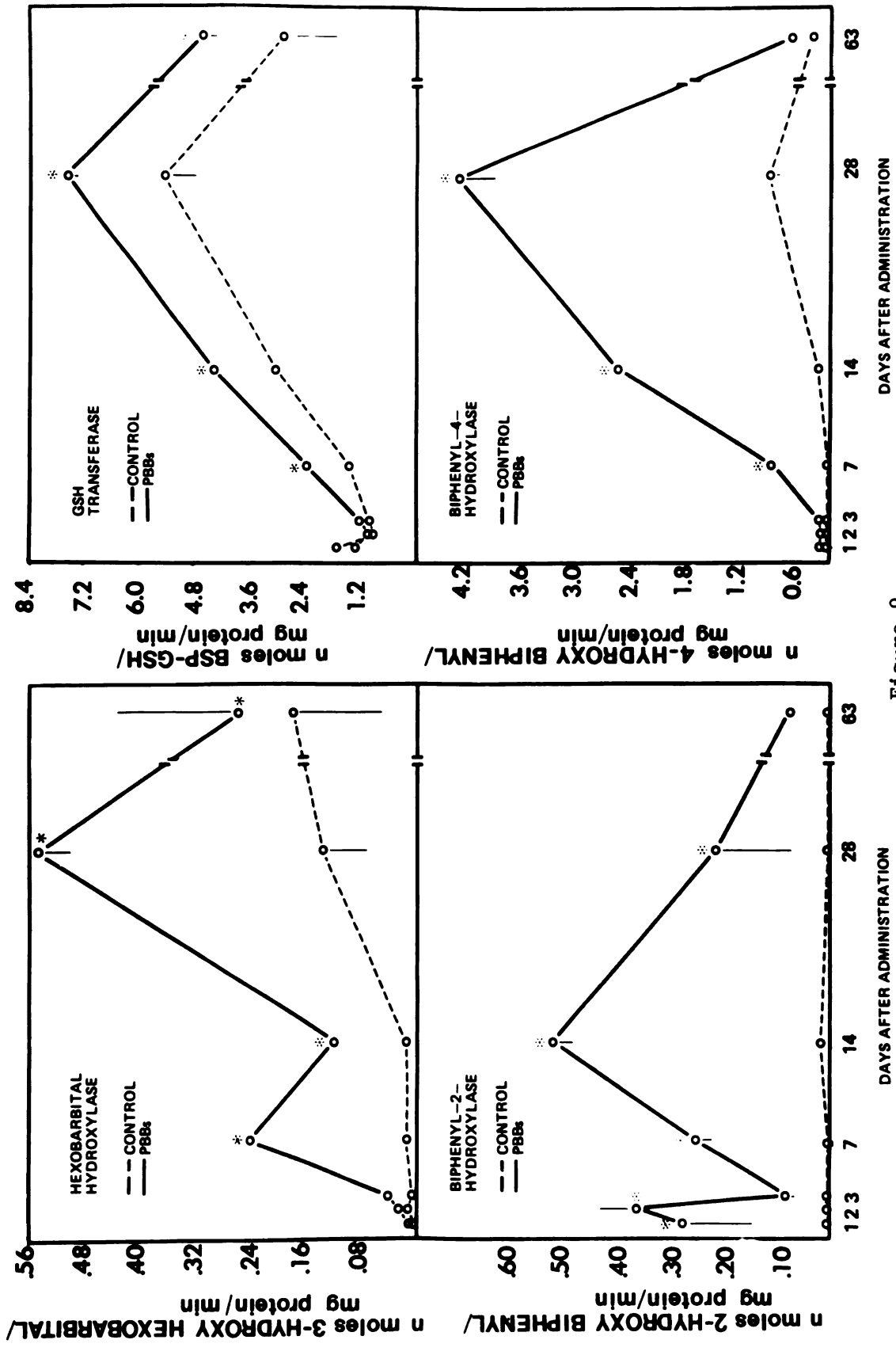
n moles 3-HYDROXY HEXOBARBITAL/ mg protein/min
HEXOBARBITAL HYDROXYLASE
CONTROL
PBBs
n moles 2-HYDROXY BIPHENYL/ mg protein/min
BIPHENYL-2-HYDROXYLASE
CONTROL
PBBs
DAYS AFTER ADMINISTRATION
n moles BSP-GSH/ mg protein/min
GSH TRANSFERASE
CONTROL
PBBs
n moles 4-HYDROXY BIPHENYL/ mg protein/min
BIPHENYL-4-HYDROXYLASE
CONTROL
PBBs
DAYS AFTER ADMINISTRATION

Figure 9

Figure 10. Effect of neonatal or perinatal treatment with PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

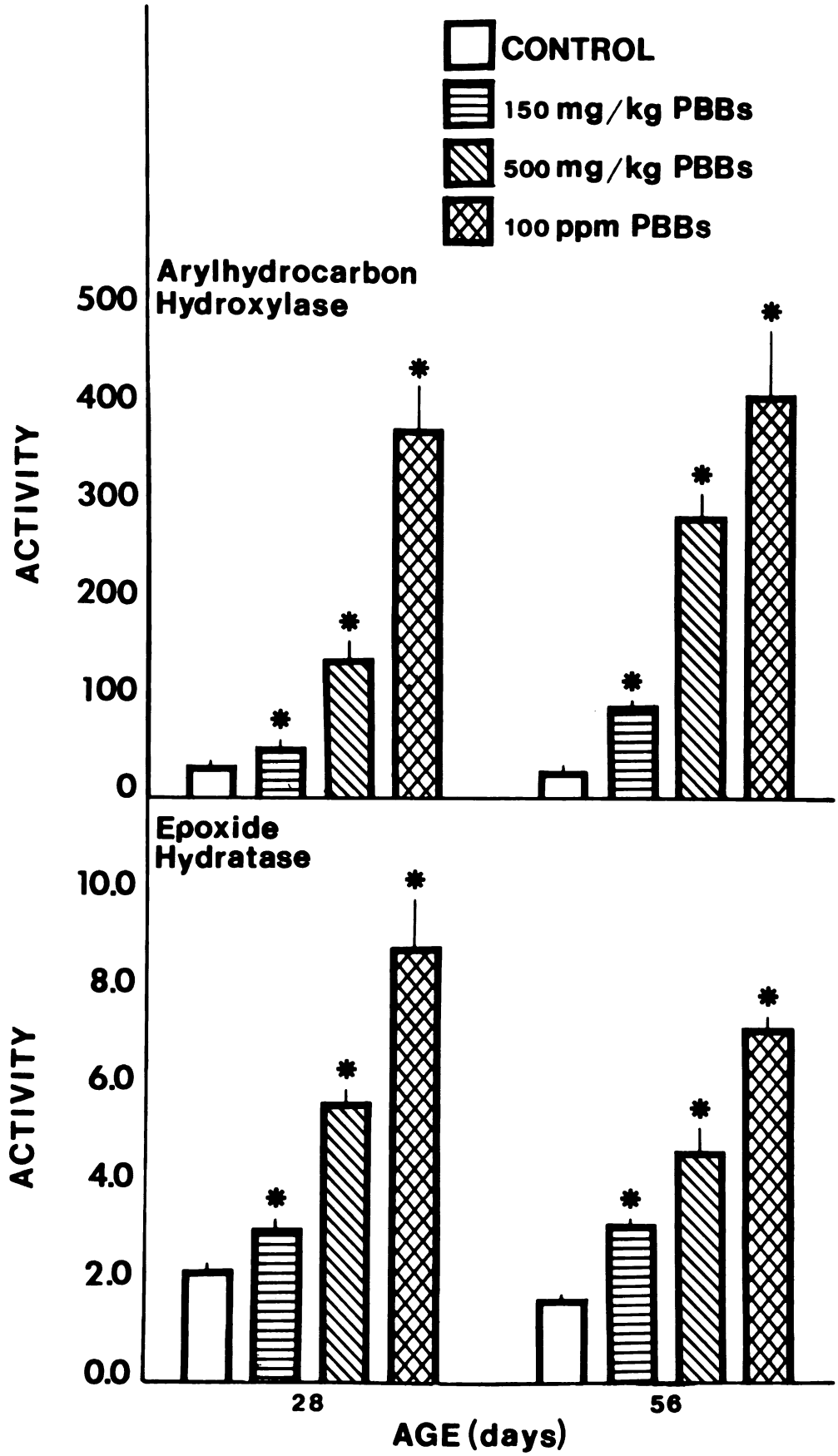
CONTROL
150 mg/kg PBBs
500 mg/kg PBBs
100 ppm PBBs
Arylhydrocarbon Hydroxylase
ACTIVITY
500
400
300
200
100
0
Epoxide Hydratase
ACTIVITY
10.0
8.0
6.0
4.0
2.0
0.0
28
56
AGE (days)

Figure 10

respectively, at 56 days of age). Of these treatments hepatic EH activity was also highest following 100 ppm PBBs (approximately 400% of control value at both 28 and 56 days of age). Stimulation of EH in liver following 500 mg/kg PBBs was greater than after 150 mg/kg PBBs (approximately 250% and 150% of control values, respectively, at both 28 and 56 days of age).

Metabolism of progesterone, in reactions catalyzed by microsomal enzymes was accelerated in vitro following PBBs. At 28 days of age, 16αPH and 6βPH activities were increased in liver from animals perinatally exposed to 100 ppm PBBs (approximately 450% and 700% of control value, respectively) (Figure 11). Although microsomal enzyme activities were increased following perinatal exposure to 100 ppm PBBs, activity of the mitochondrial enzyme MAO was decreased in liver by PBBs (approximately 80% of control value)(Figure 12).

C. Kidney

The kidney weight-to-body weight ratio was not affected by neonatal or perinatal treatment with PBBs (Figure 4). PBBs also had no consistent effect on renal PMS protein although protein was increased 28 days after treatment of 7-day old pups with 150 mg/kg (135% of control value) (Figure 15).

Histopathological changes were not observed in kidney after neonatal treatment with 150 or 500 mg/kg PBBs or perinatal treatment with 100 ppm PBBs when examined at 28 or 56 days of age. However, degenerative histological alterations were noted in adult rats treated with 100 ppm PBBs for 90 days (Figure 13). Renal changes included progressive absolescence of glomeruli. Glomerular tufts were shrunken.

Figure 11. Hepatic progesterone hydroxylase activities in rats treated with 0 or 100 ppm PBBs from day 8 of gestation until they were killed at day 28 postpartum. Values are means ± S.E.M. for at least 3 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

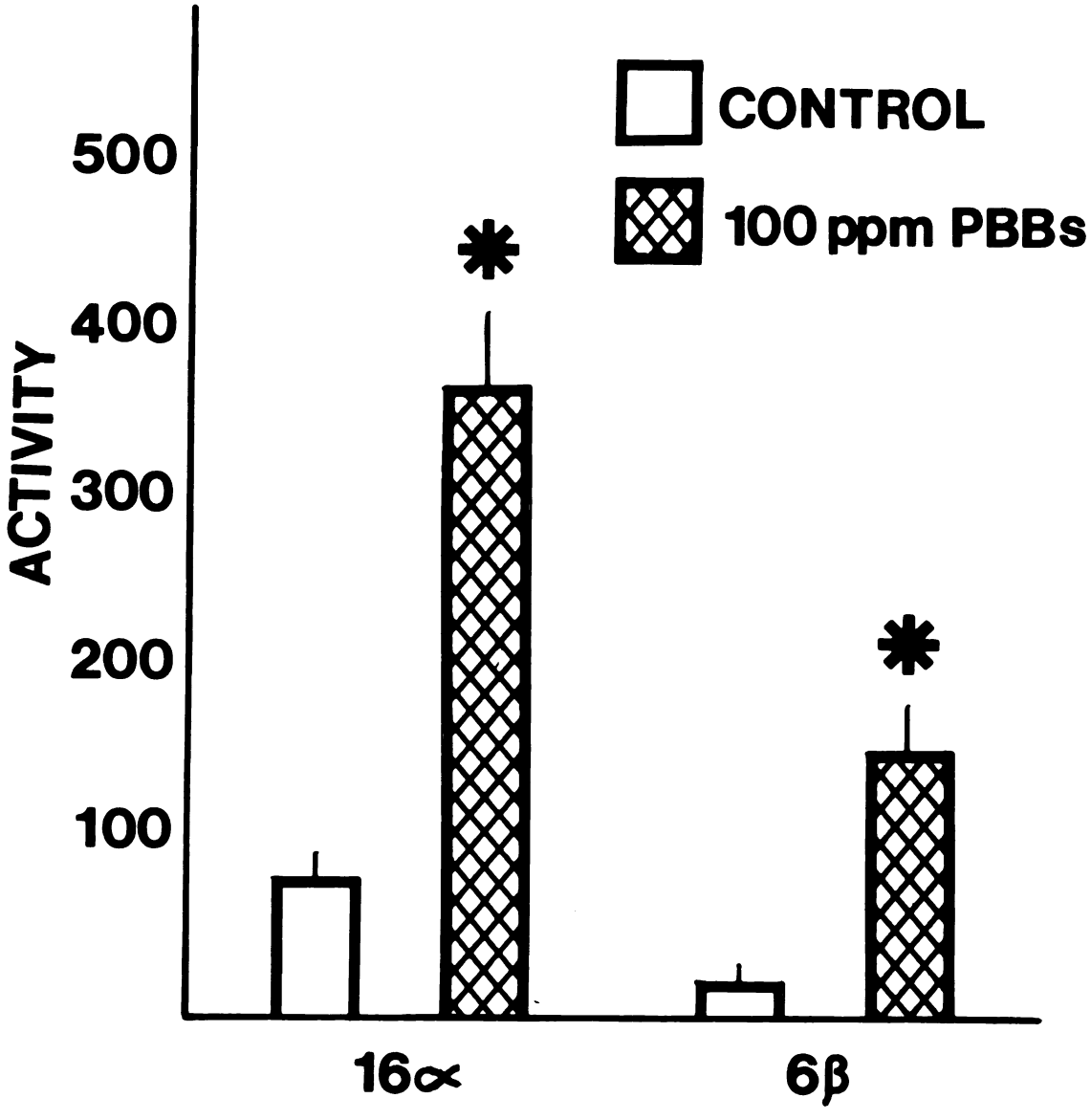

Figure 11

Figure 12. Hepatic monoamine oxidase activity in rats treated with 0 or 100 ppm PBBs from day 8 of gestation until they were killed at day 28 postpartum. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

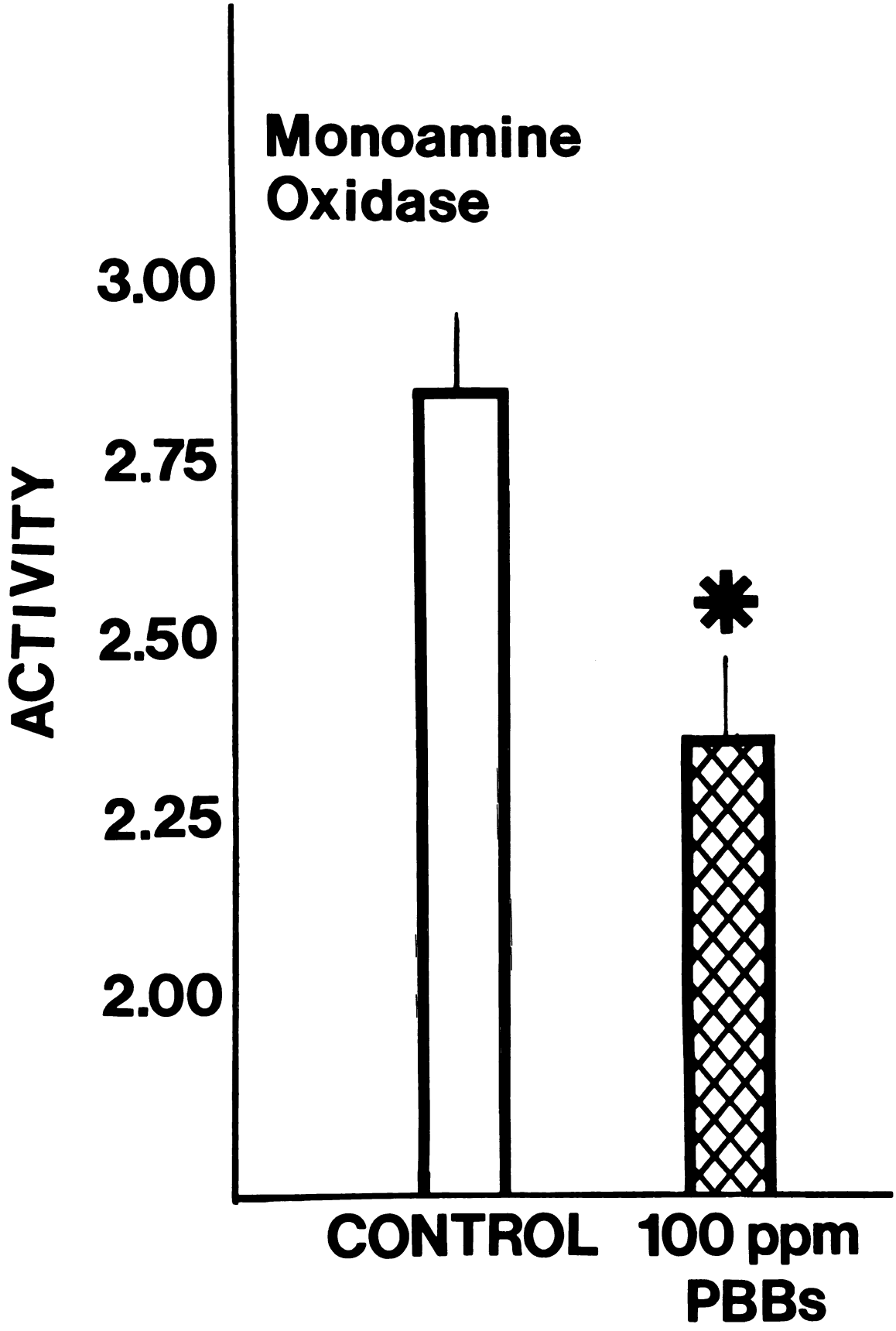
Monoamine
Oxidase
ACTIVITY
3.00
2.75
2.50
2.25
2.00
*
CONTROL
100 ppm
PBBs

Figure 12

Figure 13. Renal tissue from rat fed diet containing 100 ppm PBBs for 90 days. There are shrunken and fibrotic glomeruli. Since similar changes were observed in renal tissue from controls but less frequently, these alterations may be artifacts. Hematoxylin and eosin stain; x100.

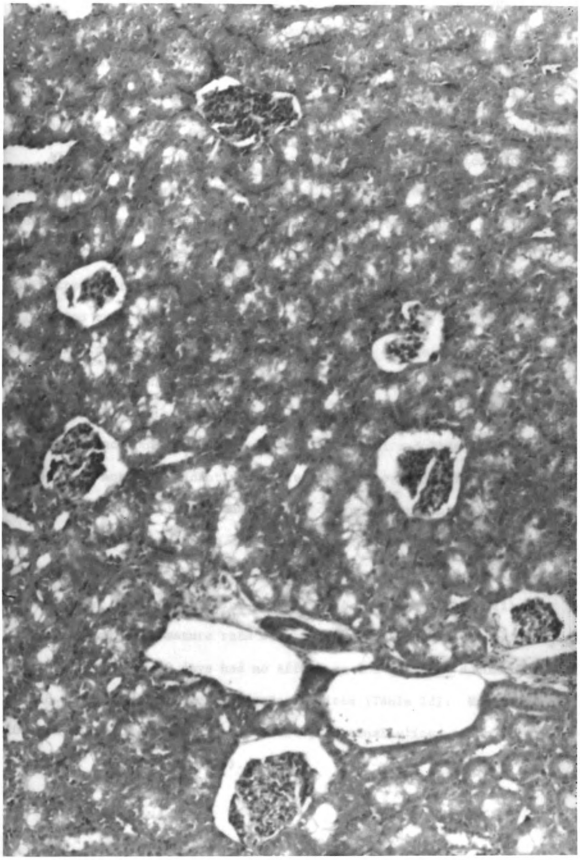

Figure 13

Bowman's membrane, while not thickened, was diminished in proportion to the shrinking tuft. A single focus of lymphocytes was seen in one kidney. Similar renal histopathological changes were seen in control animals but less frequently. Therefore, these alterations may be artifacts.

Although glomerular changes may have been observed microscopically, BUN was not affected by 30 or 90 days exposure to 100 ppm PBBs (Table 8). Similarly, the clearance of inulin (glomerular filtration rate) and the clearance of PAH (effective renal plasma flow) were unaffected by treatment with 100 ppm PBBs (Table 9). Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were not different from controls before or after volume expansion (Table 9). Filtration fraction (GFR/ERPF) was unaffected by PBBs. Urine flow rates (µl/min) were not different between control and PBBs treated animals. Fractional sodium excretion in control and PBBs exposed animals was not significantly different before or following volume expansion (Figure 14). Perinatal and continuous exposure to 100 ppm PBBs had no effect on serum calcium concentration or bone mineral mass at 112 days of age (Table 10). Perinatal exposure to 100 ppm PBBs had no effect on the concentration of vitamin A in kidney (Table 11).

Treatment of immature rats with 150 mg/kg PBBs or adults with 100 ppm PBBs for 30 or 90 days had no effect on the *in vitro* accumulation of PAH and NMN by thin renal cortical slices (Table 12). Exposure to 100 ppm PBBs did not affect the ability of renal slices to produce ammonia or glucose (Table 13).

TABLE 8

Effect of PBBs on Blood Urea Nitrogen (BUN)[a]

Treatment	Duration	BUN (mg urea nitrogen/100 ml/blood)
Control	30 days	17.28±2.75
100 ppm PBBs	30 days	22.19±1.92
Control	90 days	19.06±1.86
100 ppm PBBs	90 days	18.59±1.52

[a]Values are means ± S.E.M. for 4 animals. Adult female rats were fed 0 or 100 ppm PBBs for 30 or 90 days.

TABLE 9

Effect of PBBs on Glomerular Filtration Rate[a] (GFR) and Effective Renal Plasma Flow (ERPF)[a]

Period[b]	GFR (ml/min)		ERPF (ml/min)	
	Control	100 ppm PBBs	Control	100 ppm PBBs
I	2.46±0.16	2.34±0.21	10.87±0.63	8.77±0.97
II	2.86±0.51	3.14±0.43	10.95±1.94	10.67±0.78
III	2.77±0.59	2.51±0.17	9.61±1.54	9.05±1.73
IV	2.40±0.24	1.95±0.27	10.39±1.77	10.66±1.95

[a] Values are means ± S.E.M. for 4 animals. Adult female rats were maintained on diet containing 0 or 100 ppm PBBs for 90 days.

[b] Rat plasma-saline (4% of body wt) was infused after period I.

Figure 14. Effect of PBBs on fractional sodium excretion. Rats were fed diet containing 0 or 100 ppm PBBs for 90 days. After control determination, animals were volume expanded with 1:4 rat plasma-saline (4% body weight) and three additional determinations made. Values are means ± S.E.M. for 4 animals.

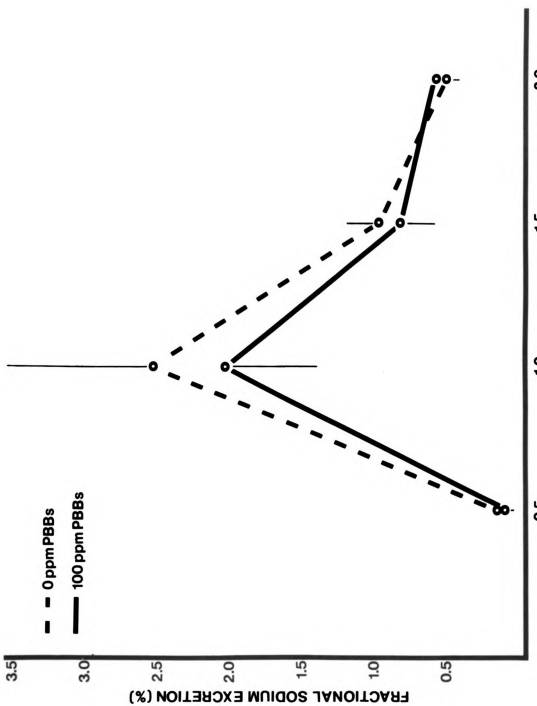
FRACTIONAL SODIUM EXCRETION (%)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.5
1.0
1.5
2.0
TIME(hrs)
0 ppm PBBs
100 ppm PBBs

Figure 14

TABLE 10

Serum Calcium Concentration and Bone Mineral Mass in Female Rats Exposed to PBBs[a]

Treatment	Serum Calcium Concentration (μg/ml)	Bone Mineral Mass (g/cm^2)
Control	88±6	0.220±0.029
100 ppm PBBs	86±10	0.209±0.028

[a]Values are means ± S.E.M. for 5 (serum calcium concentration) or 10 (bone mineral mass) animals. Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation until 16 wks postpartum, when measurements were made.

TABLE 11

Effect of Perinatal Exposure to PBBs on the Concentration of Vitamin A in Kidney of Rats[a]

PBBs (ppm)	Vitamin A Concentration (μg/g)
0	4.66±0.83
100	4.93±0.67

[a]Values are means ± S.E.M. for at least 4 animals. Rats were treated with 0 or 100 ppm PBBs from day 8 of gestation until 28 days postpartum at which time vitamin A was quantified.

TABLE 12

Effect of PBBs on the Accumulation of PAH and NMN[a]

	Treatment	S/M Ratio	
		PAH	NMN
30 Days[b]	Control	10.17±1.46	5.71±0.40
	100 ppm PBBs	9.84±0.87	5.59±0.22
90 Days[b]	Control	10.49±0.43	5.79±0.10
	100 ppm PBBs	10.16±0.76	5.20±0.17
Acute[c]	Control	8.29±0.81	5.10±0.31
	150 mg/kg PBBs	8.56±0.33	5.63±0.17

[a]Values are means ± S.E.M. for at least 3 animals.

[b]Adult female rats were fed 0 or 100 ppm PBBs for 30 or 90 days.

[c]Two week old rats were treated with PBBs 72 hrs prior to sacrifice.

TABLE 13

Effect of PBBs on Ammoniagenesis and Gluconeogenesis[a]

Treatment	Net Production (μmole/mg wet tissue wt/hr)	
	Ammonia	Glucose
Control	3.39±0.09	0.03±0.01
100 ppm PBBs	3.45±0.09	0.03±0.01

[a]Values are means ± S.E.M. for 4 animals. Adult female rats were fed 0 or 100 ppm PBBs for 90 days.

Activity of renal microsomal enzymes increased with age in control and PBBs treated rats (Figure 15). Administration of 150 mg/kg PBBs to 7 day old pups increased the activity of both AHH and EROD in kidney (Figure 15). Following 150 mg/kg PBBs, renal AHH and EROD activities were highest after 63 days (450% and 1800% of control values, respectively). Renal EH activity was not increased by 150 mg/kg PBBs (Figure 15). BP-2-OH, BP-4-OH and Hex-OH activities were not detectable in kidney.

Renal EH activity also was not affected by neonatal treatment with 150 or 500 mg/kg PBBs or perinatal exposure to 100 ppm PBBs (Figure 16). However, renal AHH was stimulated at both 28 and 56 days of age by all treatments with PBBs (Figure 16). Activity of AHH in kidney was increased to the greatest extent following 100 ppm PBBs and was greater at 56 than 28 days of age (approximately 1900% and 700% of control values, respectively). Renal AHH activity was higher following 500 than 150 mg/kg PBBs (approximately 200% and 150% of control value, respectively, at 28 days of age). Activity of AHH in kidney following 500 or 150 mg/kg PBBs was greater at 56 than 28 days of age (approximately 650% and 150% of control values, respectively, at 56 days of age).

D. Heart

The heart weight-to-body weight ratio was not affected by perinatal and continuous exposure to 100 ppm PBBs (Figure 17). Exposure to 100 ppm PBBs also had no effect on the *in vitro* accumulation of (^{3}H)-d,l-metaraminol by ventricular slices when determined at 14, 28, 56, 84 and 98 days of age (Table 14). Left atria from 70 day old rats

Figure 15. Renal postmitochondrial supernatant protein and enzyme activities in rats at various times after treatment with 0 or 150 mg/kg PBBs on day 7 postpartum. Each point represents the mean ± S.E.M. for at least 3 rats. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

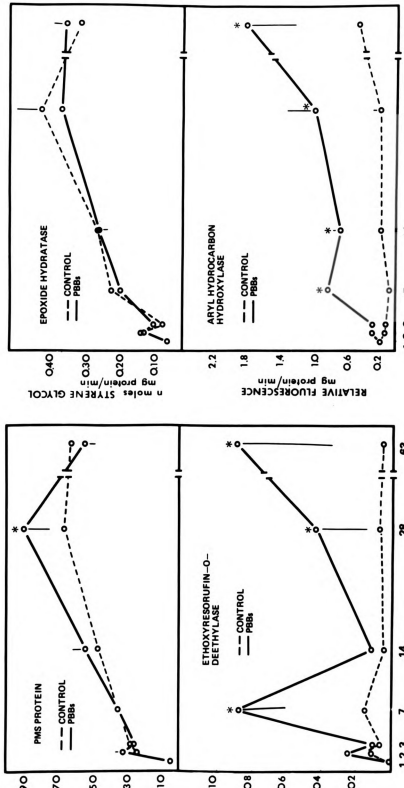
PMS PROTEIN
CONTROL
PBBs
mg/gm WET TISSUE WT.
ETHOXYRESORUFIN-O-DEETHYLASE
n moles RESORUFIN mg protein/min
DAYS AFTER ADMINISTRATION
EPOXIDE HYDRATASE
n moles STYRENE GLYCOL mg protein/min
ARYL HYDROCARBON HYDROXYLASE
RELATIVE FLUORESCENCE mg protein/min

Figure 15

Figure 16. Effect of neonatal or perinatal treatment with PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on the day after birth (day 1) or with 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

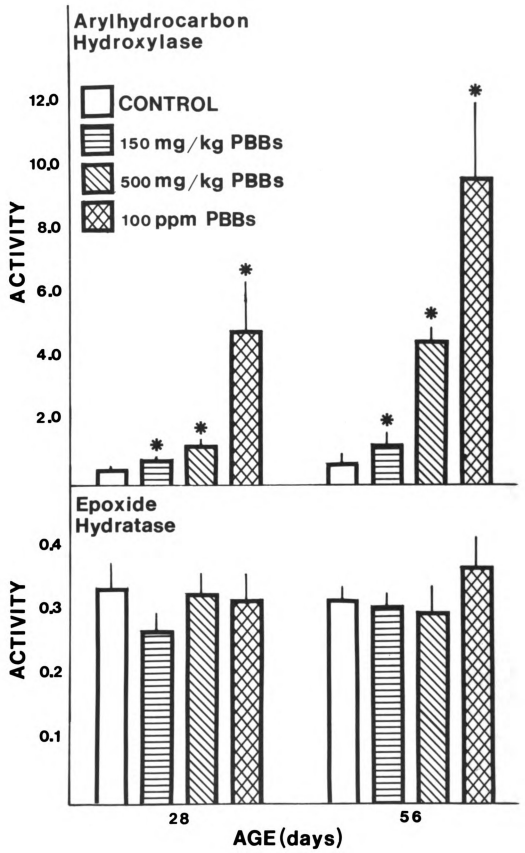
Arylhydrocarbon Hydroxylase
CONTROL
150 mg/kg PBBs
500 mg/kg PBBs
100 ppm PBBs
ACTIVITY
12.0
10.0
8.0
6.0
4.0
2.0
Epoxide Hydratase
ACTIVITY
0.4
0.3
0.2
0.1
28
56
AGE (days)

Figure 16

Figure 17. Effect of PBBs on the heart weight-to-body weight ratio. Rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for at least 3 animals.

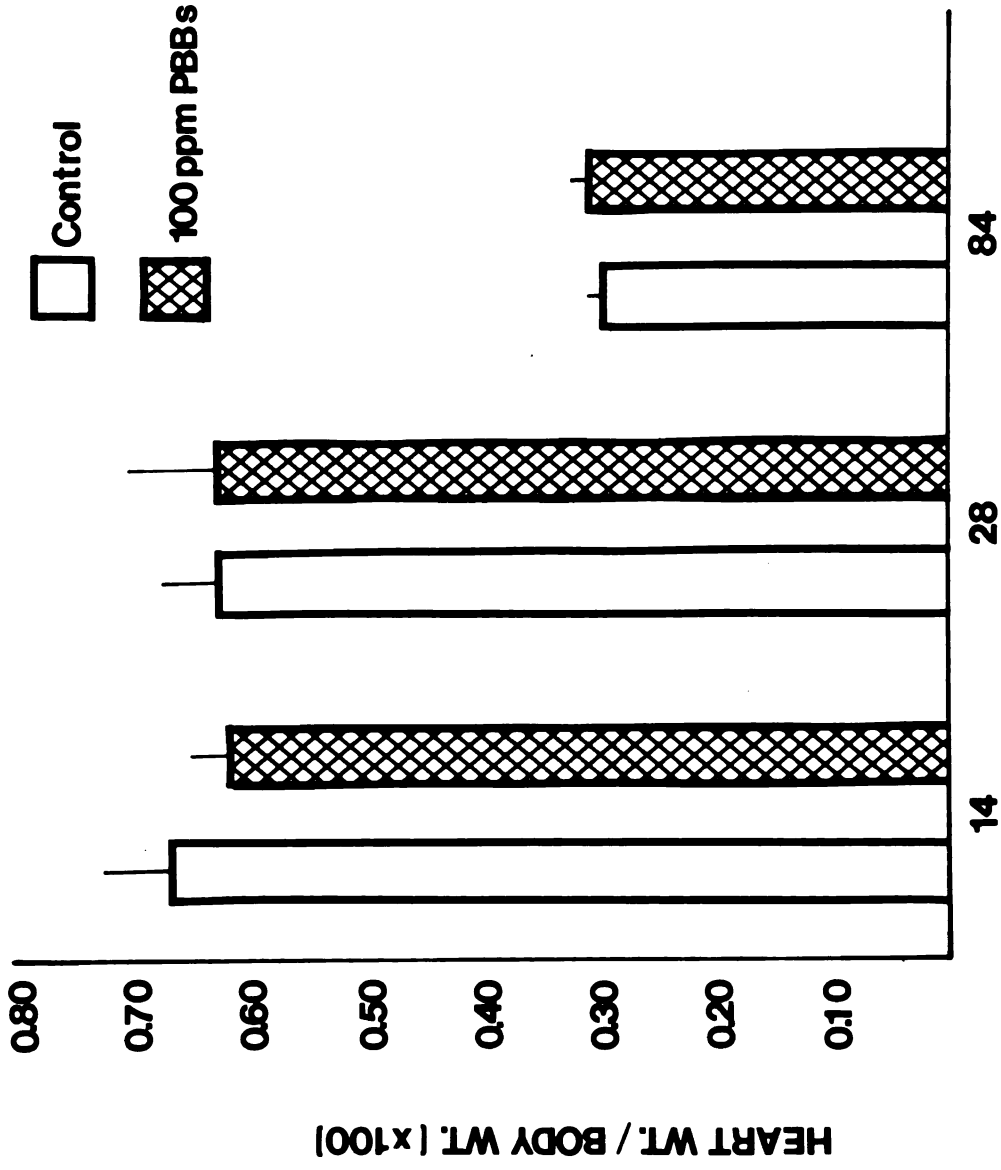
Control
100 ppm PBBs
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
HEART WT. / BODY WT. [x100]
14
28
84
AGE [days]

Figure 17

TABLE 14

Effect of PBBs on the Accumulation of (^{3}H)-d,l-Metaraminol[a]

Age (days)	Treatment	S/M Ratio
14	Control	1.79±0.04
	100 ppm PBBs	1.94±0.10
28	Control	2.68±0.24
	100 ppm PBBs	2.83±0.13
56	Control	3.04±0.08
	100 ppm PBBs	2.95±0.10
84	Control	3.44±0.26
	100 ppm PBBs	3.40±0.24
98	Control	4.01±0.47
	100 ppm PBBs	3.93±0.45

[a]Values are means ± S.E.M. for 4 animals. Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation until experiments were performed.

perinatally and continuously exposed to 100 ppm PBBs did not differ from controls in susceptibility to ouabain-induced arrhythmias, control twitch tension or maximum inotropic response to ouabain (Table 15). However, the maximum inotropic response to calcium was greater in atria from PBBs exposed rats (approximately 200% of control value) (Table 15). Systolic arterial pressure did not differ between controls and adult rats dietarily exposed to 100 ppm for 90 days (Table 16).

E. Lung

The lung weight-to-body weight ratio was not affected by perinatal and continuous exposure to 100 ppm PBBs (Figure 18). When determined at 28 days of age, the clearance of AI and 5HT by isolated perfused lungs from rats perinatally exposed to 100 ppm PBBs was reduced (85% and 89% of control values, respectively) (Table 17). Metabolism of 5HT by isolated perfused lungs was also reduced by PBBs (85% of control value) (Table 17). However, perinatal exposure to PBBs had no effect on MAO, ACE or EH activities in pulmonary homogenates (Figures 19 and 20). Pulmonary AHH activity was increased above controls at 28 and 56 days of age by perinatal and continuous exposure to 100 ppm PBBs (approximately 300% and 550% of control values, respectively) (Figure 20).

F. Testis and Ovary

The testis weight- or ovary weight-to-body weight ratios were not affected at 100 days of age by perinatal and continuous exposure to PBBs (Figure 21). Perinatal and continuous exposure to 100 ppm PBBs did not produce histopathological changes in testis, ovary or uterus

TABLE 15

Effect of PBBs on Left Atrial Susceptibility to Ouabain-Induced Arrhythmia, Twitch Tension and Inotropic Response to Quabain or Calcium[a]

	Control	100 ppm PBBs
Quabain concentration for onset of arrhythmia	$1x10^{-5}M$	$5x10^{-4}M$
Control twitch tension	1.16±0.25 g	1.11±0.21 g
Inotropic response to ouabain (% change from control) quabain, $1.5x10^{-6}M$	3.56±1.70	5.02±2.74
Inotropic response to calcium (% change from control) calcium, $1.0x10^{-2}M$	12.14±2.71	24.66±4.08[b]

[a]Values represent means ± S.E.M. for 5 animals. Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation until 70 days postpartum when experiments were performed.

[b]Significantly different from control value, $p<0.50$.

TABLE 16

Effect of PBBs on Mean Systolic Blood Pressure[a]

Treatment	Unanesthetized	Anesthetized
Control	122.0±3.0	97.5±4.3
100 ppm PBBs	121.5±2.4	94.5±5.3

[a]Values are means ± S.E.M. for 4 animals. Adult female rats were maintained on diet containing 0 or 100 ppm PBBs for 90 days. Units are mmHg.

Figure 18. Effect of PBBs on the lung weight-to-body weight ratio. Rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for at least 3 animals.

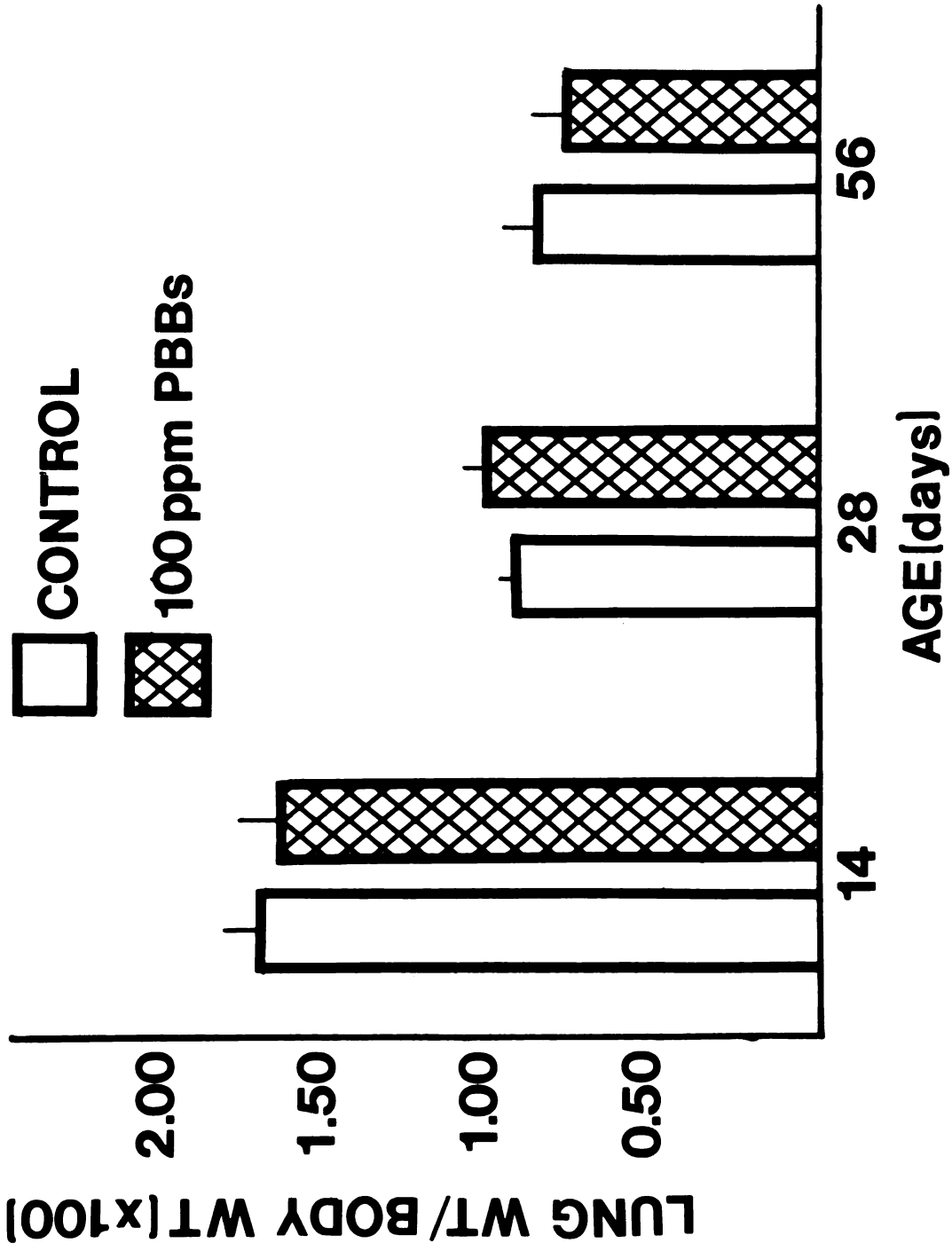
CONTROL
100 ppm PBBs
LUNG WT/ BODY WT (x100)
2.00
1.50
1.00
0.50
14
28
56
AGE (days)

Figure 18

TABLE 17

Effect of Perinatal Treatment with Polybrominated Biphenyls on the Clearance of Angiotensin I (AI) and 5-Hydroxytryptamine (5-HT) by Isolated Perfused Rat Lungs[a]

	AI	5-HT	
	% Removal[b]	% Removal[b]	% Metabolized[c]
Control	53.16±2.64	79.72±2.09	29.69±1.33
PBBs[d]	45.30±2.17[e]	70.84±2.22[e]	25.30±1.33[e]

[a]Values represent mean ± S.E.M. of 9 determinations in 28 day old rats.

[b]Calculated from the transpulmonary difference in perfused AI (1 ng/ml) or 5-HT (0.1 μM/L).

[c]Calculated from the fraction of radiolabel appearing as metabolite in the effluent medium.

[d]Dietary PBBs (100 ppm) were administered to the dam from day 8 of gestation to 28 days postnatally.

[e]Significantly different from control ($p<.05$).

Figure 19. Effect of perinatal treatment with PBBs on the activity of angiotensin-converting enzyme and monoamine oxidase in lung. Rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until they were killed at day 28 postpartum. Values are means ± S.E.M. for at least 4 animals.

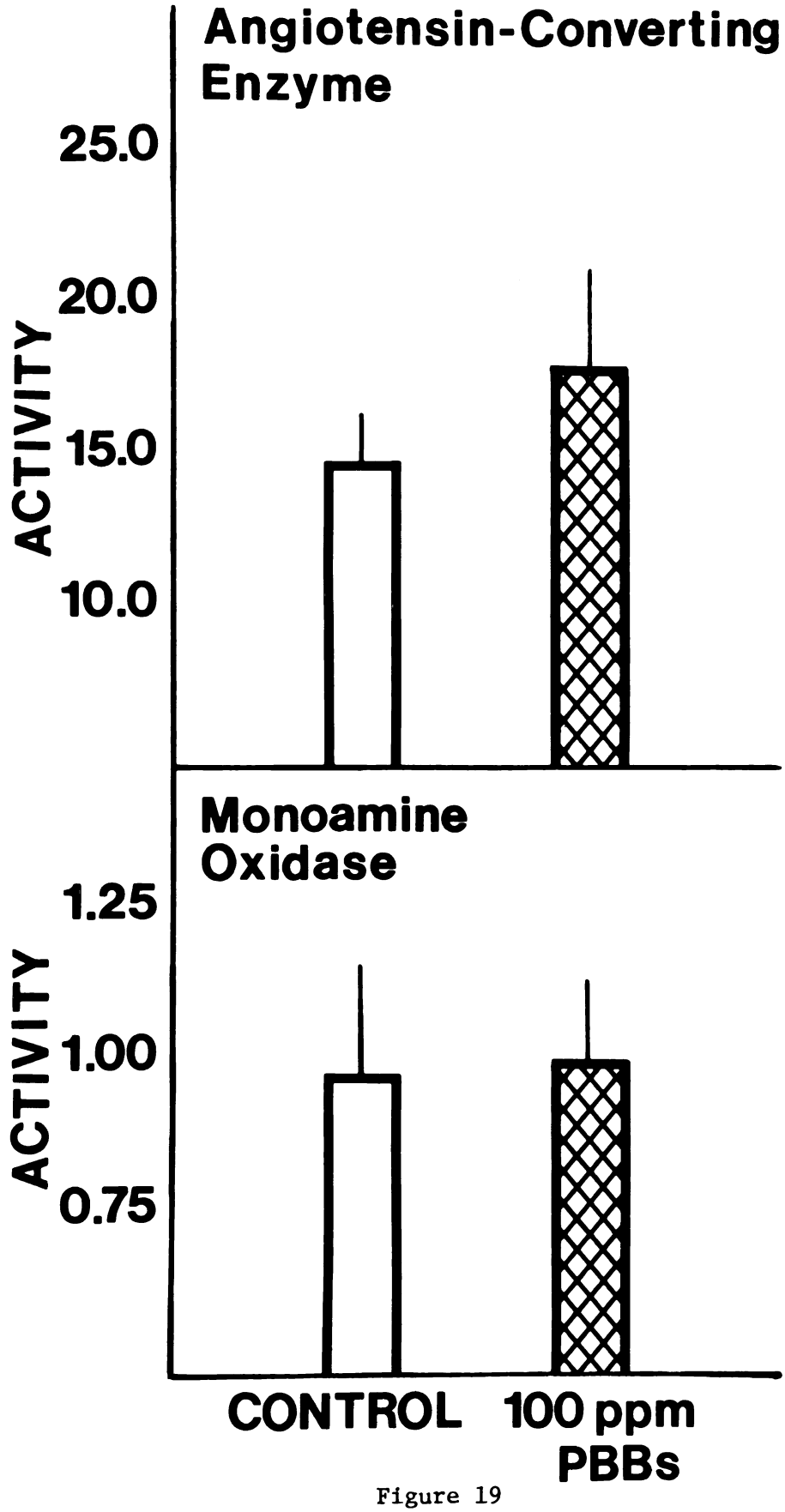
Angiotensin-Converting Enzyme
25.0
20.0
15.0
10.0
ACTIVITY
Monoamine Oxidase
1.25
1.00
0.75
ACTIVITY
CONTROL
100 ppm PBBs

Figure 19

Figure 20. Effect of PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in lung. Rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for at least 3 animals. Asterisk indicates a statistically significant difference from control value, $p<0.05$.

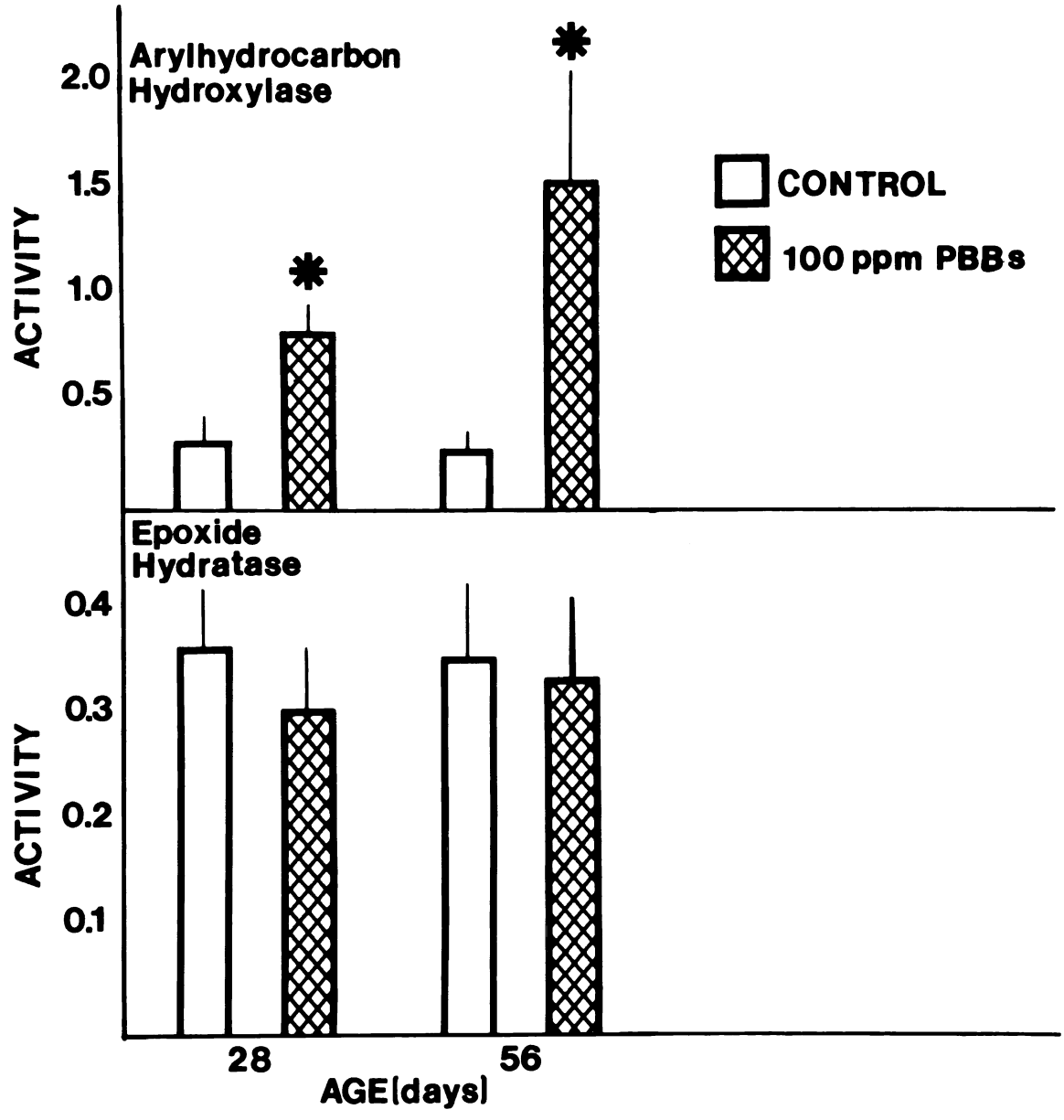
Arylhydrocarbon Hydroxylase
2.0
1.5
1.0
0.5
ACTIVITY
CONTROL
100 ppm PBBs
Epoxide Hydratase
0.4
0.3
0.2
0.1
ACTIVITY
28
56
AGE(days)

Figure 20

Figure 21. Effect of PBBs on the testis weight- or ovary weight-to-body weight ratios. Rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until they were killed. Values are means ± S.E.M. for at least 4 animals.

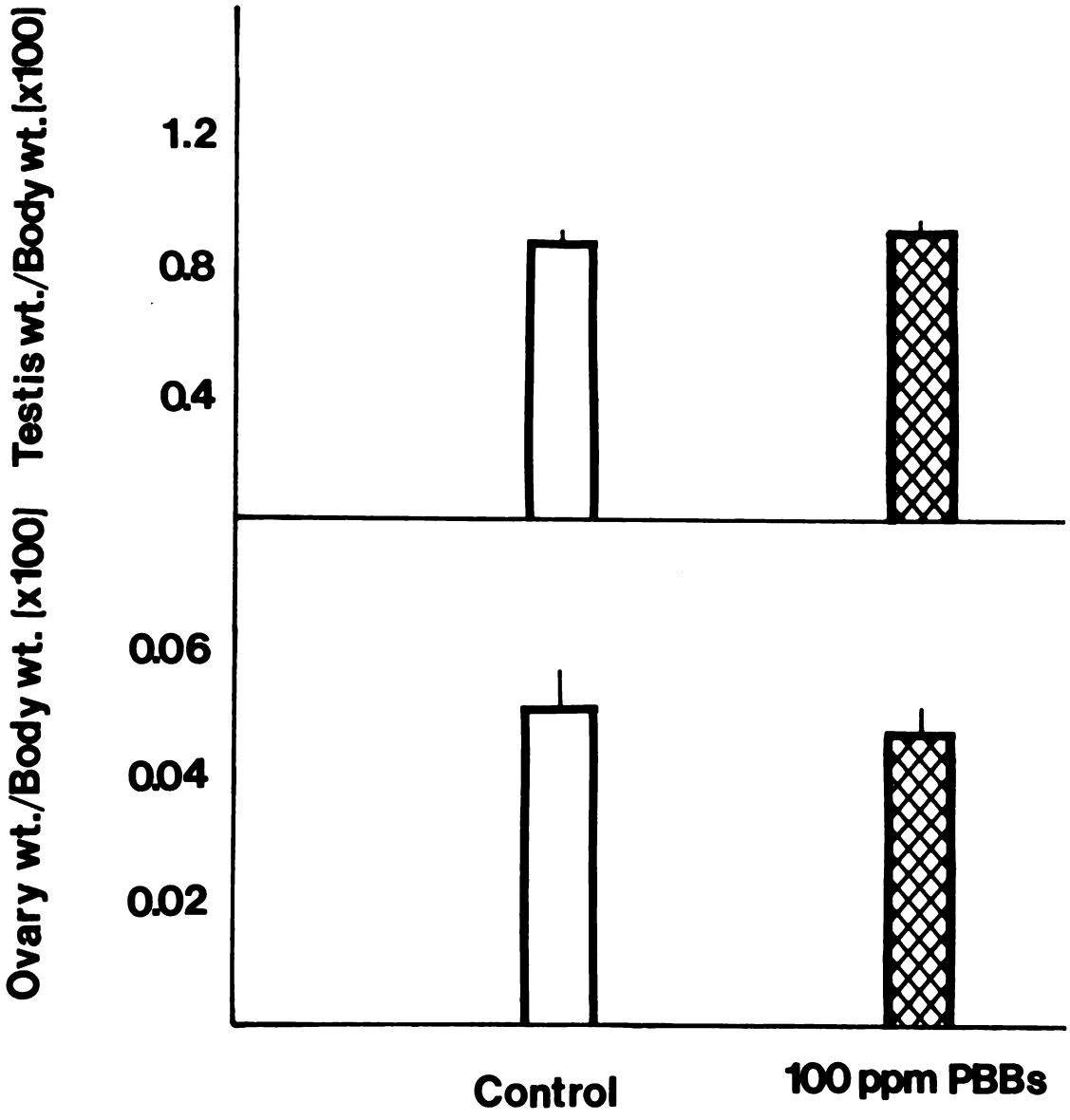
Ovary wt./Body wt. [x100]
Testis wt./Body wt. [x100]
1.2
0.8
0.4
0.06
0.04
0.02
Control
100 ppm PBBs

Figure 21

when examined at 56 days of age. At 100 days of age, testosterone and progesterone concentrations were not affected by PBBs (Table 18). Perinatal exposure to 100 ppm PBBs also had no effect on age of testes descent, vaginal perforation or estrus cycle length (Table 19).

G. Response to Xenobiotics

Duration of anesthesia following pentobarbital was decreased at 84 days of age by neonatal treatment with 150 or 500 mg/kg PBBs or perinatal exposure to 100 ppm PBBs (Figure 22). Sleeping time was shortest after pretreatment with 100 ppm PBBs (10% of control value). Sleeping time was reduced more by 500 than 150 mg/kg PBBs (25% and 75% of control values, respectively).

Duration of anesthesia following diethyl ether was not affected by PBBs. At 90 days of age, sleeping time after diethyl ether for control rats and animals perinatally and continuously exposed to 100 ppm PBBs did not differ (Figure 23).

Bromobenzene lethality was enhanced by pretreatment with PBBs. At 49 days of age, the median time to death (LT_{50}) of animals given approximately an LD_{85} of bromobenzene was reduced by neonatal treatment with 150 or 500 mg/kg PBBs (74% and 62% of control value, respectively) (Table 20).

Digitoxin lethality was decreased by pretreatment with PBBs. At 90 days of age, the LT_{50} of animals given approximately an LD_{85} of digitoxin was increased by perinatal and continuous exposure to 100 ppm PBBs (130% of control value) (Table 21).

TABLE 18

Concentration of Testosterone and Progesterone in Serum from Rats Exposed to PBBs[a]

Treatment	Testosterone	Progesterone
Control	3.77±1.54	366.28±11.10
100 ppm PBBs	4.17±0.37	323.82± 8.31

[a]Values represent ng/ml, expressed as means ± S.E.M. for at least 5 animals. Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation until 100 days postpartum when serum was collected from males (testosterone) and females (progesterone).

TABLE 19

Vaginal (Estrus) Cycle Length and Age of Vaginal Opening and Testicular Descent in Rats Exposed to PBBs[a]

Parameter	Treatment	
	Control	100 ppm PBBs
Testicular Descent	24.6±0.4	25.0±0.7
Vaginal Opening	37.4±1.7	39.1±2.1
Vaginal Cycle Length	4.6±0.3	4.8±0.4

[a]Values are means ± S.E.M., in days, for at least 4 litters (testicular descent and vaginal opening) or 10 animals (vaginal cycle length). Animals were exposed to 0 or 100 ppm PBBs from day 8 of gestation through examination periods.

Figure 22. Effect of neonatal or perinatal treatment with PBBs on the duration of anesthesia produced by pentobarbital. Female rats were treated with 0, 150 or 500 mg/kg PBBs in peanut oil on day after birth or with 0 or 100 ppm PBBs from day 8 of gestation until 84 days of age at which time 40 mg/kg pentobarbital was administered i.p. Duration of anesthesia was recorded as time of injection until animals regained righting reflex. Values are means ± S.E.M. for 5 animals. Asterisk indicates a statistically significant difference from the control response, $p<0.05$.

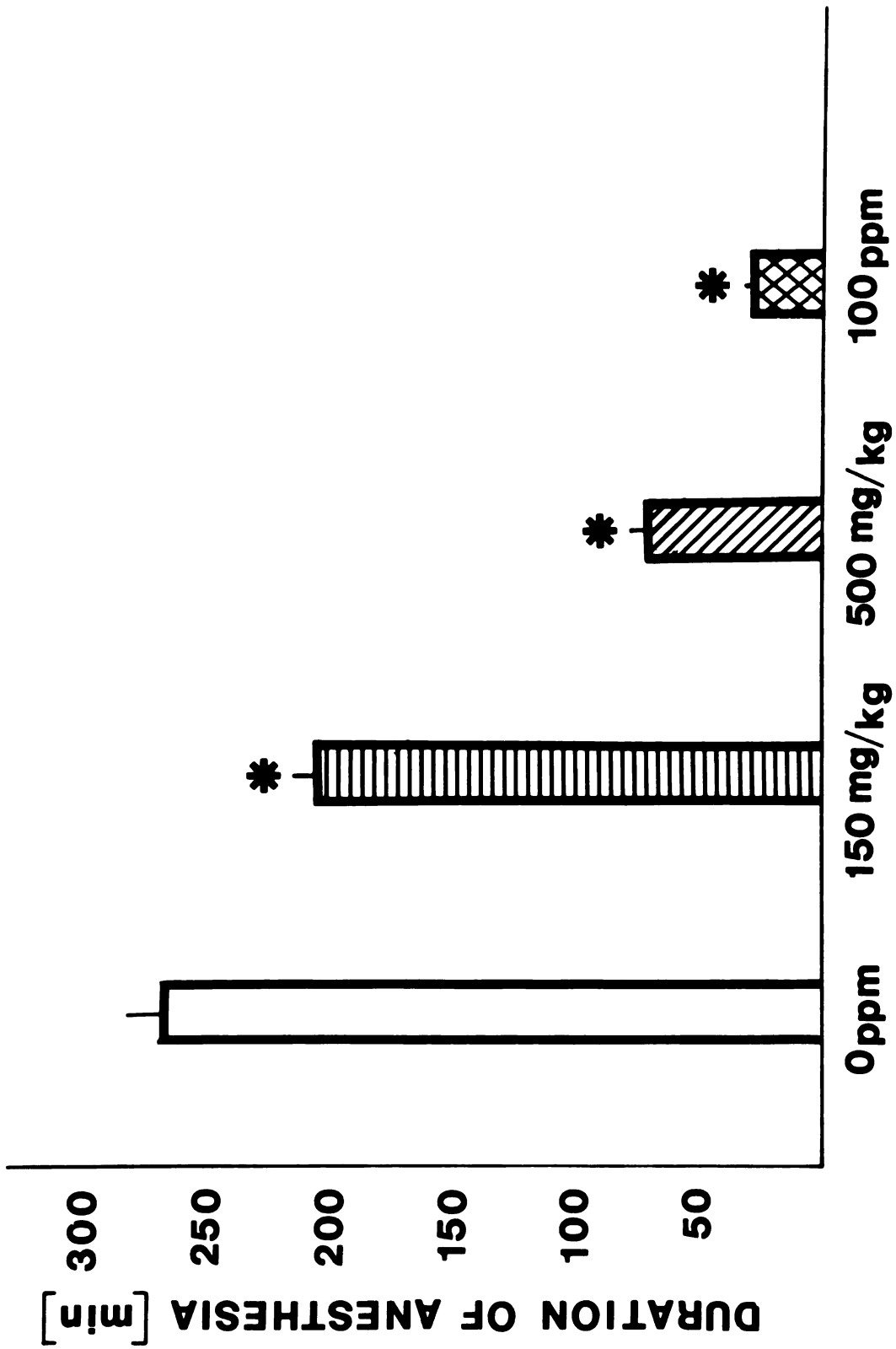
DURATION OF ANESTHESIA [min]
300
250
200
150
100
50
0ppm
150 mg/kg
500 mg/kg
100ppm
PBBs
*
*
*

Figure 22

Figure 23. Effect of PBBs on the duration of anesthesia produced by diethyl ether. Female rats were exposed to 0 or 100 ppm PBBs from day 8 of gestation until 90 days of age at which time animals were exposed to diethyl ether for 90 secs. Duration of anesthesia was recorded as time of removal from dessicator until the righting reflex was regained. Values are means ± S.E.M. for 4 animals.

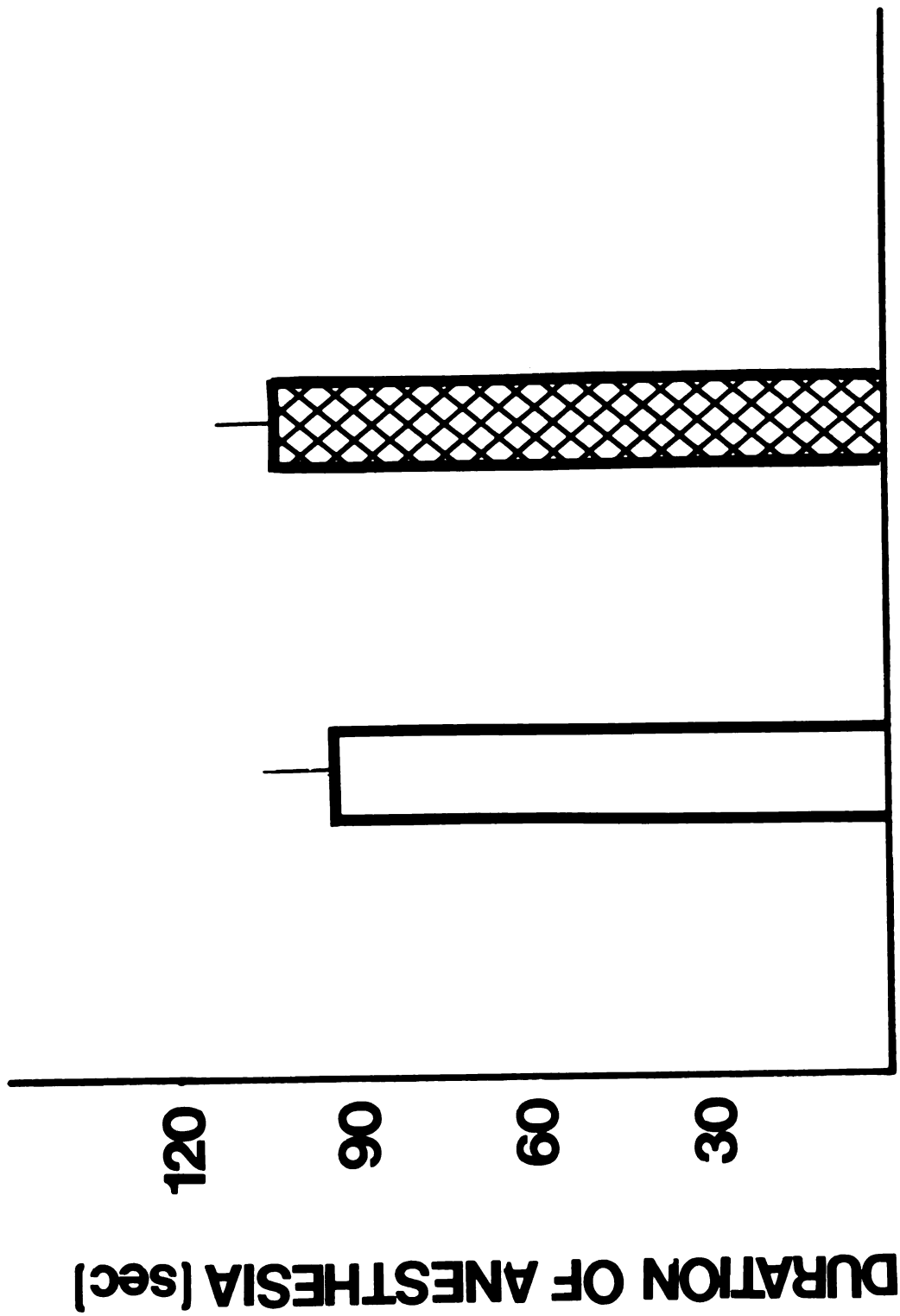

Figure 23

TABLE 20

Median Time to Death After Administration of Bromobenzene (2820 mg/kg) in 49 Day Old Male Rats Neonatally Treated with Polybrominated Biphenyls[a]

Pretreatment[b]	LT_{50} (95% confidence limit)
Control	7.2 (6.1-8.5)
150 mg/kg PBBs	5.3 (4.7-5.9)[c]
500 mg/kg PBBs	4.5 (3.8-5.5)[c]

[a]Values are in hr for 8 (500 mg/kg PBBs) or 10 (control and 150 mg/kg PBBs) animals.

[b]Animals were treated with peanut oil, 150 mg/kg PBBs or 500 mg/kg PBBs in peanut oil on the day after birth (day 1).

[c]Significantly different from control, $p<0.05$.

TABLE 21

Median Time to Death After Administration of Digitoxin[a] in Female Rats Pretreated with PBBs

PBBs (ppm)[b]	LT50 (95% confidence limit)
0	326.1 (287.4-366.8)
100	424.5 (389.7-472.4)[b]

[a]Values are means in min for 9 animals. Female rats were treated with 0 or 100 ppm PBBs from day 8 of gestation until 90 days postpartum at which time 10 mg/kg digitoxin in dimethyl sulfoxide was administered i.p.

[b]Significantly different from the control value, $p<0.50$.

H. Response to Exogenously Administered Steroid Hormones

Duration of anesthesia following 150 mg/kg progesterone was reduced by perinatal exposure to 10 or 100 ppm PBBs (27% and 11% of control values, respectively) (Figure 24). Radioactivity was decreased in whole brain homogenates, HEX and EA extracts 3 hr after (^{14}C)progesterone by 100 ppm PBBs (22%, 13% and 24% of control values, respectively) (Figure 25). The ratio of EA to HEX extractable radioactivity was increased after 3 hr by 100 ppm PBBs (183% of control value). Serum radioactivity was also reduced 3 hr following progesterone by 100 ppm PBBs (56% of control value) (Figure 26). At waking, radioactivity in brain was similar regardless of pretreatment. When determined at waking, EA to HEX extractable radioactivity in brain and radioactivity in serum also were not affected by PBBs.

Although PBBs did not alter seminal vesicle weight-to-body weight ratio (SVW/BW), the increase in SVW/BW 72 hr following 20 mg/kg testosterone was diminished by perinatal exposure to 100 ppm PBBs (79% of control) (Figure 27). Radioactivity in testis 24 hr after (^{3}H)-testosterone was reduced by pretreatment with 10 or 100 ppm PBBs (79% and 70% of control value, respectively) (Figure 28). Radioactivity in serum 24 hr after (^{3}H)testosterone was also reduced by exposure to 10 or 100 ppm PBBs (81% and 64% of control values, respectively) (Figure 29).

Although PBBs did not modify uterus weight-to-body weight ratio (UW/BW), the increase in UW/BW 4 hr following 1 or 3 μg/kg estradiol-17β was reduced by perinatal exposure to 100 ppm PBBs (86% and 78% of control values, respectively) (Figure 30). Radioactivity in uterus 4

Figure 24. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the duration of anesthesia produced by progesterone. Rats were treated with 150 mg/kg progesterone i.p. at 28 days of age. Duration of anesthesia was recorded as time of injection until animal regained righting reflex. Values are means ± S.E.M. for at least 6 animals. Asterisk indicates a statistically significant difference from the control response, $p<0.05$.

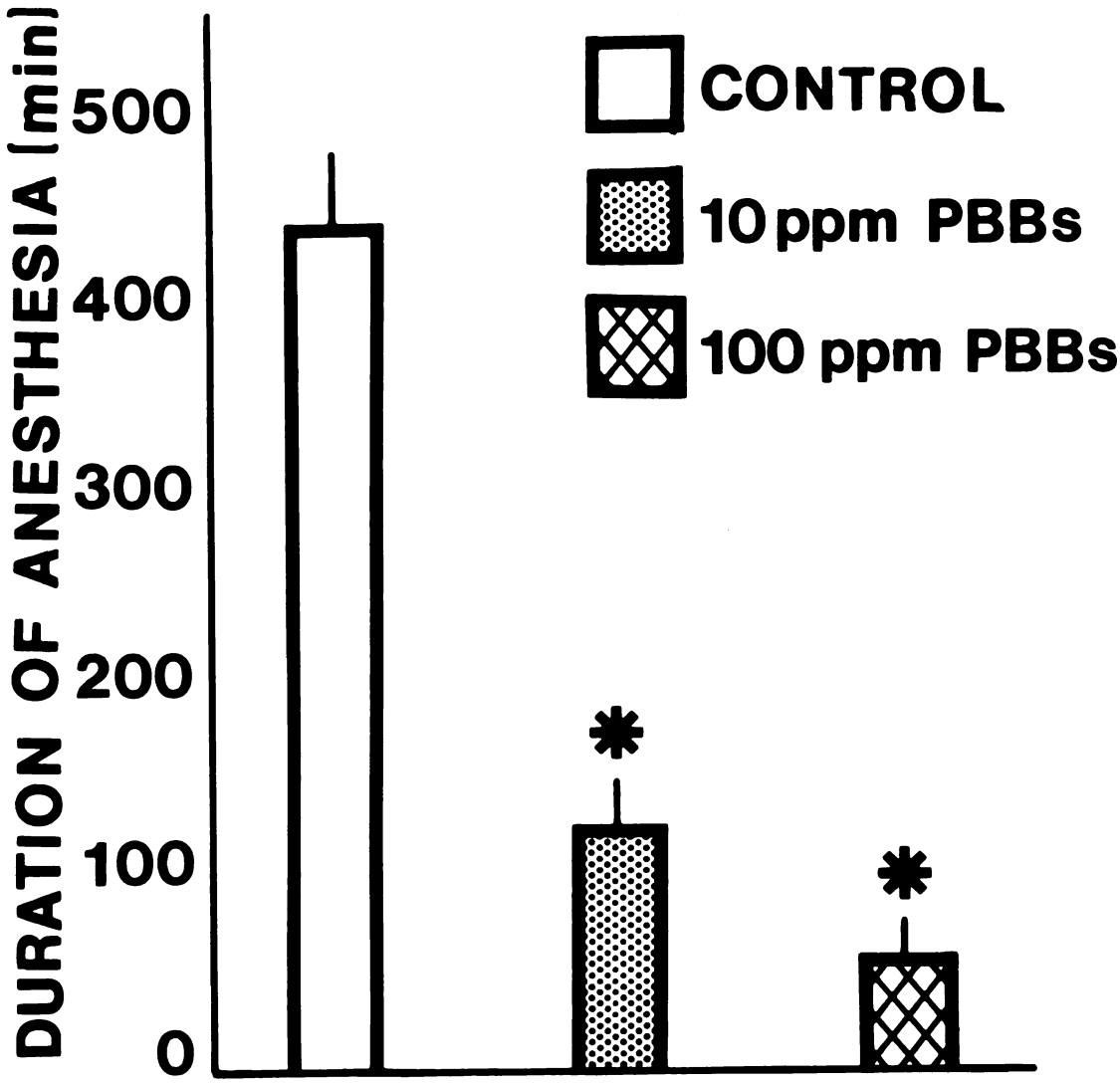
DURATION OF ANESTHESIA (min)
500
400
300
200
100
0
CONTROL
10 ppm PBBs
100 ppm PBBs
*
*

Figure 24

Figure 25. Effect of perinatal exposure to 0 or 100 ppm PBBs on the concentration of equivalents of progesterone-^{14}C in brains of rats treated with progesterone-^{14}C. Rats were treated with 150 mg/kg progesterone-^{14}C i.p. at 28 days of age. Whole brains were excised 3 hr later. Radioactivity was determined in brain homogenates and n-hexane and ethyl acetate extracts of homogenates. Values are means ± S.E.M. for at least 3 animals. Asterisk indicates a statistically significant difference from control value, $p<0.05$.

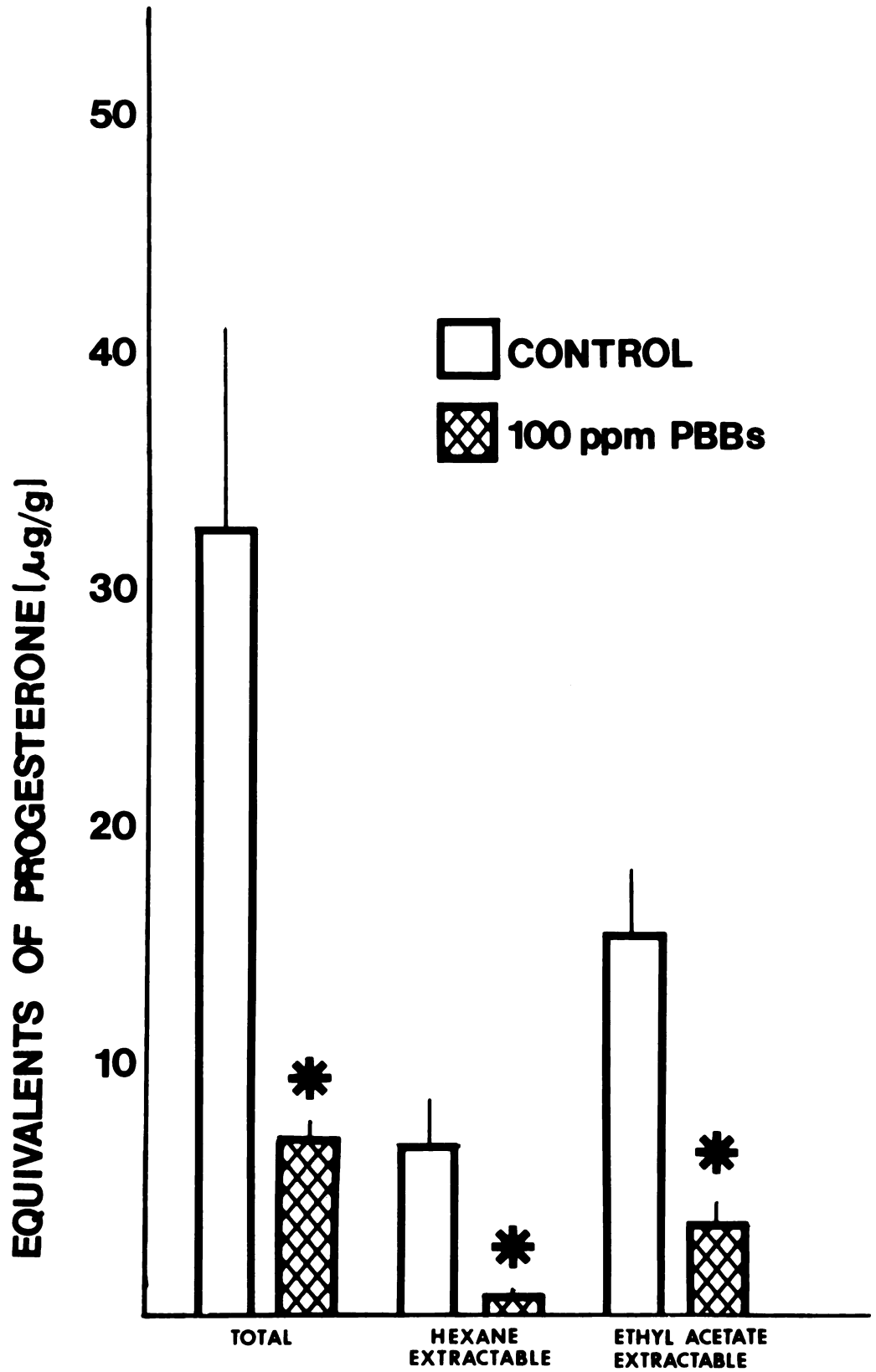
50
40
30
20
10
EQUIVALENTS OF PROGESTERONE (µg/g)
CONTROL
100 ppm PBBs
TOTAL
HEXANE EXTRACTABLE
ETHYL ACETATE EXTRACTABLE

Figure 25

Figure 26. Effect of perinatal exposure to 0 or 100 ppm PBBs on the concentration of equivalents of progesterone-^{14}C in serum of rats treated with progesterone-^{14}C. Rats were treated with 150 mg/kg progesterone-^{14}C i.p. at 28 days of age. Serum was collected 3 hr later. Values are means ± S.E.M. for at least 3 animals. Asterisk indicates a statistically significant difference from control value, $p<0.05$.

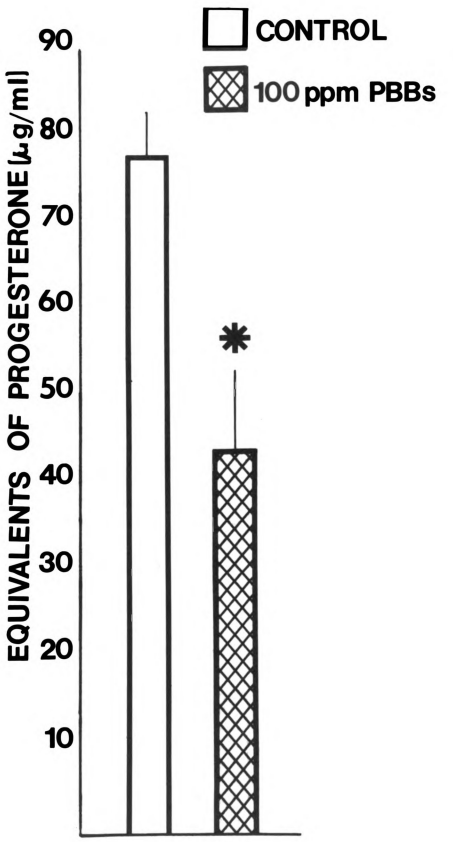
CONTROL
100 ppm PBBs
EQUIVALENTS OF PROGESTERONE (µg/ml)
90
80
70
60
50
40
30
20
10
*

Figure 26

Figure 27. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the increase in seminal vesicle wt-to-body wt ratio produced by testosterone. Rats were treated with 0 or 20 mg/kg testosterone s.c. at 25 days of age. Seminal vesicles were excised and weighed 72 hr later. Values are means ± S.E.M. for at least 6 animals. Asterisk indicates a statistically significant difference from the control response, $p<0.05$.

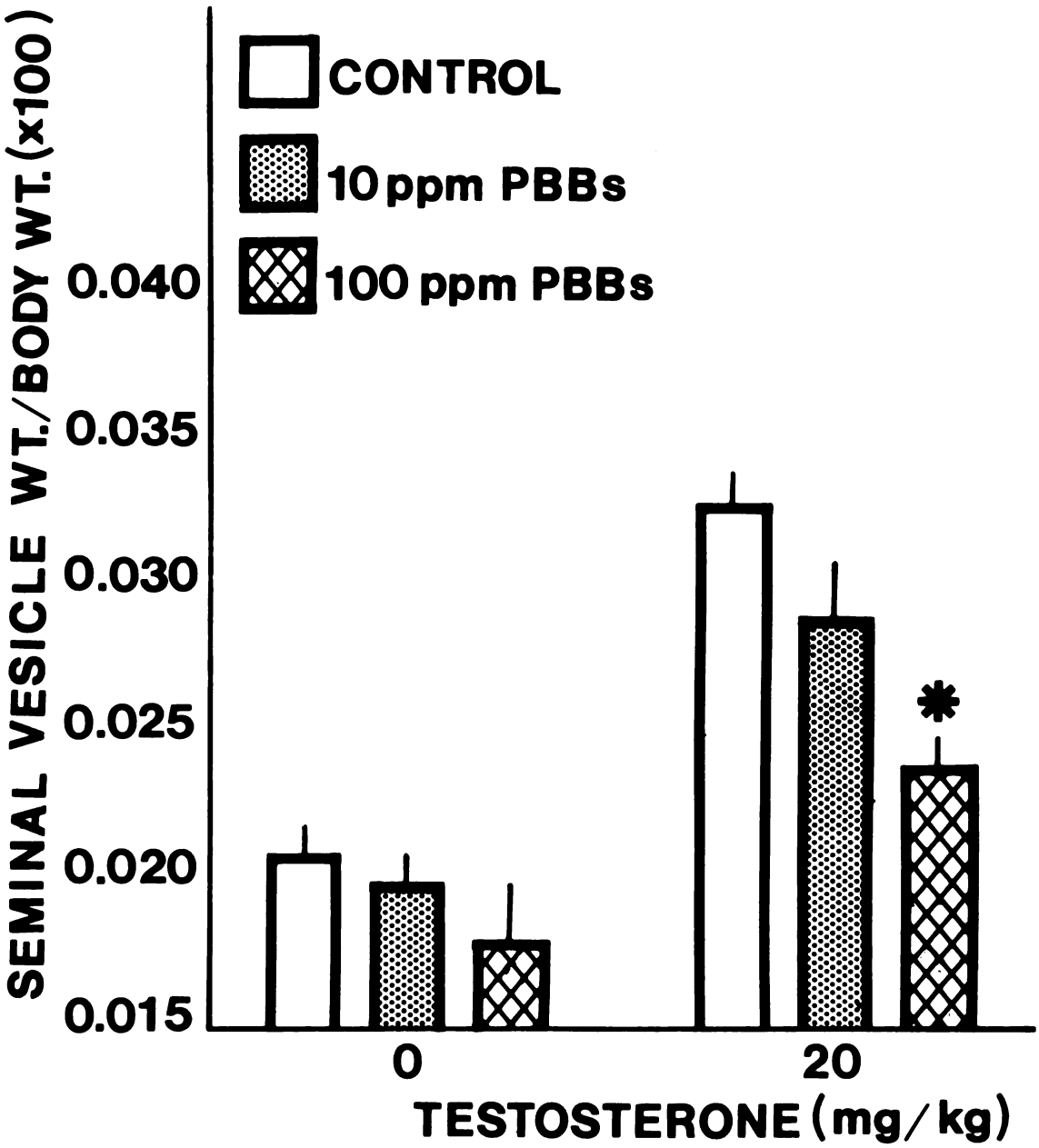
CONTROL
10 ppm PBBs
100 ppm PBBs
SEMINAL VESICLE WT./BODY WT. (x100)
0.040
0.035
0.030
0.025
0.020
0.015
*
0
20
TESTOSTERONE (mg/kg)

Figure 27

Figure 28. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of testosterone-^{3}H in testis of rats treated with testosterone-^{3}H. Rats were treated with 20 mg/kg testosterone-^{3}H s.c. at 25 days of age. Testes were collected 24 hr later. Values are means ± S.E.M. for at least 6 animals. Asterisk indicates a statistically significant difference from control value, $p<0.05$.

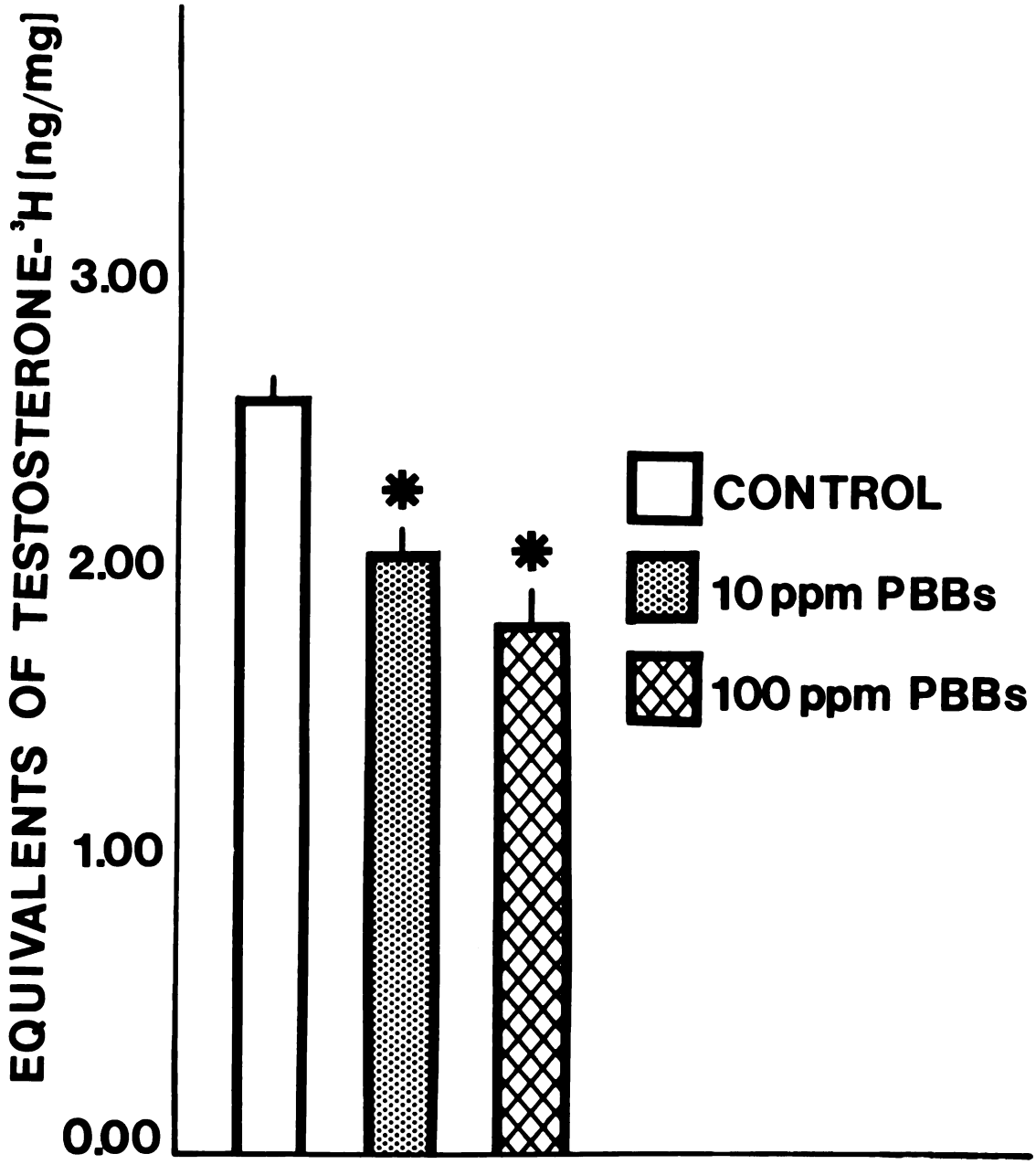
EQUIVALENTS OF TESTOSTERONE-³H (ng/mg)
3.00
2.00
1.00
0.00
*
*
CONTROL
10 ppm PBBs
100 ppm PBBs

Figure 28

Figure 29. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of testosterone-^{3}H in serum of rats treated with testosterone-^{3}H. Rats were treated with 20 mg/kg testosterone-^{3}H s.c. at day 25 postpartum. Serum was collected 24 hr later. Values are means ± S.E.M. for at least 6 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

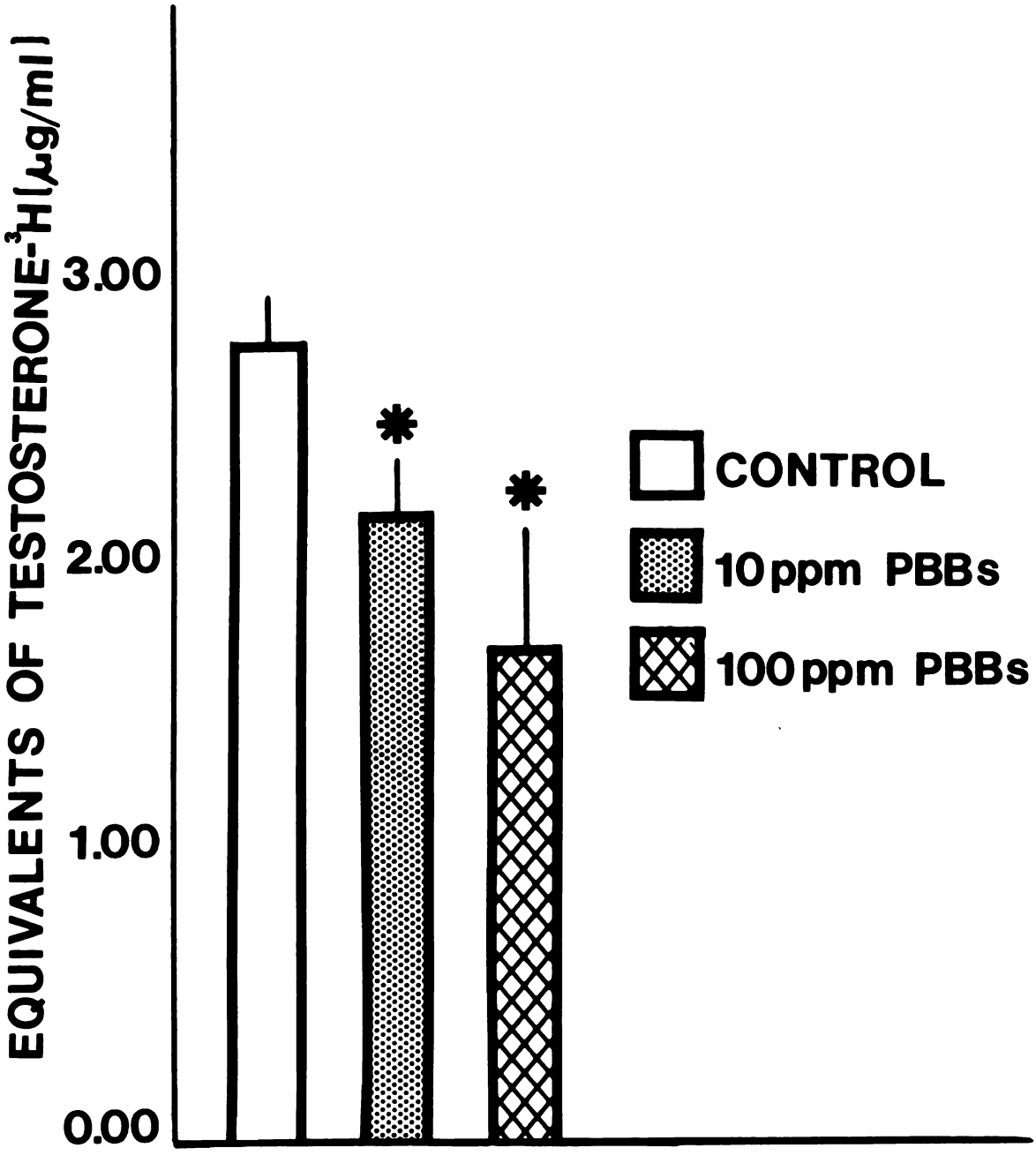
EQUIVALENTS OF TESTOSTERONE-^{3}H(µg/ml)
3.00
2.00
1.00
0.00
*
*
CONTROL
10ppm PBBs
100ppm PBBs

Figure 29

Figure 30. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the increase in uterus wt-to-body wt ratio produced by estradiol-17β. Rats were treated with 1.0 or 3.0 μg/kg estradiol-17β i.p. at day 28 postpartum. Uteri were excised and weighed 4 hr later. Values are means ± S.E.M. for 5 animals. Asterisk indicates a statistically significant difference from the control response, $p<0.05$.

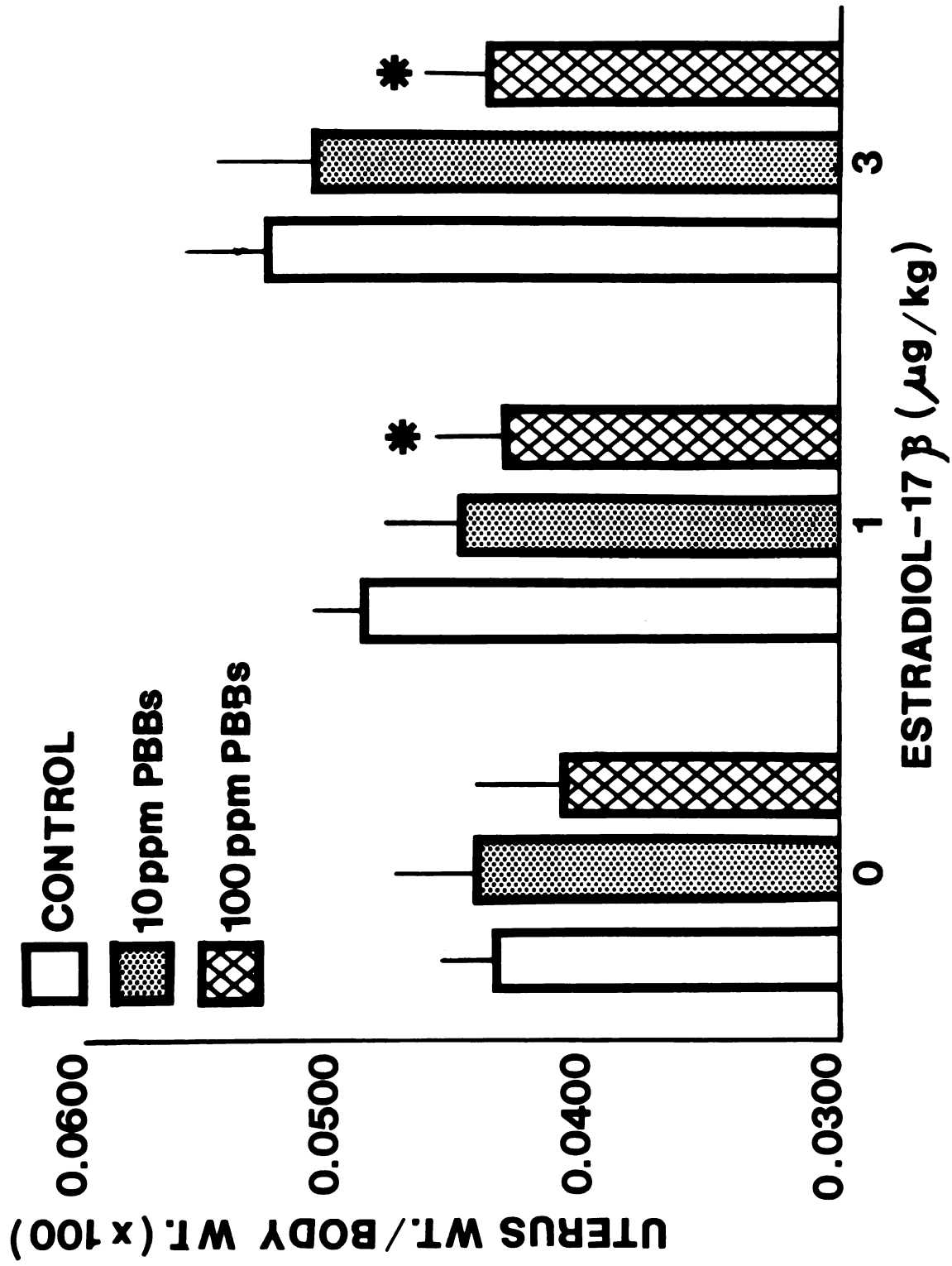
UTERUS WT./BODY WT. (x 100)
0.0600
0.0500
0.0400
0.0300
CONTROL
10 ppm PBBs
100 ppm PBBs
0
1
3
ESTRADIOL-17β (μg/kg)
*
*

Figure 30

hr after 1 or 3 μg/kg (^{3}H)estradiol-17β was reduced by exposure to 100 ppm PBBs (64% and 32% of control values, respectively) (Figure 31). Radioactivity in uterus following 3 μg/kg (^{3}H)estradiol-17β was also reduced by 10 ppm PBBs (57% of control value) (Figure 31). Radioactivity in serum following 1 or 3 μg/kg (^{3}H)estradiol was reduced by 100 ppm PBBs (62% and 21% of control values, respectively) (Figure 32). Radioactivity in serum following 1 μg/kg (^{3}H)estradiol-17β also was reduced by 10 ppm PBBs (76% of control value) (Figure 32).

I. Persistence of Effects

Microsomal enzyme activity was increased in liver and kidney even 63 days following a single i.p. injection of 150 mg/kg PBBs to 7-day old rats (Figures 8, 9, 15). Enzyme stimulation at 63 days following treatment was indicative of persistence of PBBs in tissues. Detectable concentrations of PBBs were found in various tissues at different times after administration of 150 mg/kg PBBs (Table 22). No sex dependent differences in tissue concentrations of PBBs were observed, so data from males and females were combined.

Concentration of PBBs in brain was low at all times and remained fairly constant until 28 days following treatment. By day 63, concentration of PBBs in brain had decreased considerably. Sufficient fat was obtained for analysis on days 28 and 63. Concentration of PBBs in fat increased markedly between days 28 and 63. Concentrations of PBBs in heart and liver were lower than fat and higher than brain. Similar to brain, PBBs in heart and liver decreased markedly between days 28 and 63. Muscle, skin and lung contained similar concentrations of PBBs through 63 days with concentrations in lung diminishing more rapidly than those in muscle and skin.

Figure 31. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of estradiol-17β-6,7-^{3}H in uterus of rats treated with estradiol-17β-6,7-^{3}H. Rats were treated with 1.0 or 3.0 μg/kg estradiol-17β-6,7-^{3}H i.p. at day 28 postpartum. Uteri were collected 4 hr later. Values are means ± S.E.M. for 5 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

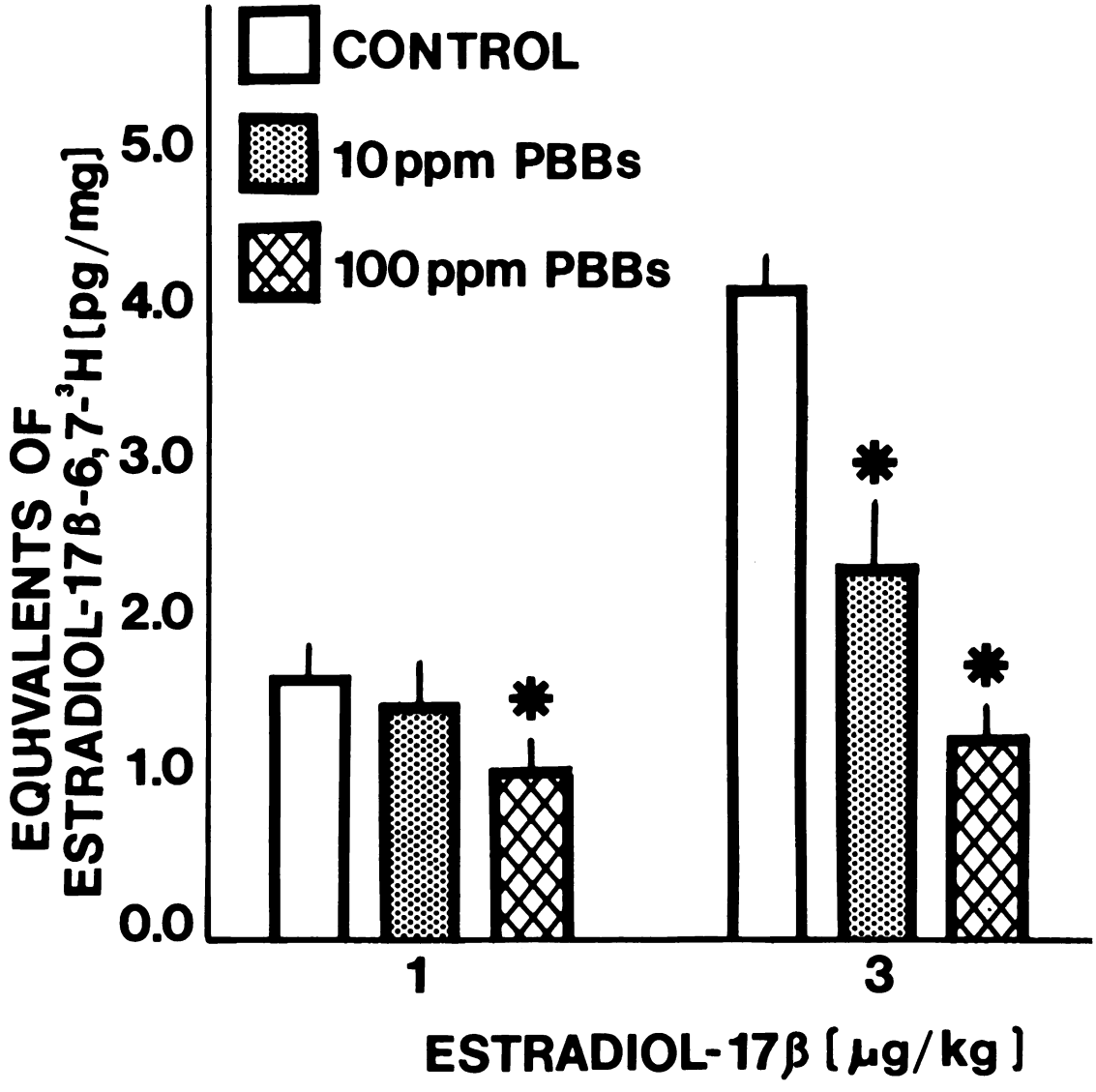
CONTROL
10 ppm PBBs
100 ppm PBBs
EQUIVALENTS OF ESTRADIOL-17β-6,7-³H [pg/mg]
5.0
4.0
3.0
2.0
1.0
0.0
1
3
ESTRADIOL-17β [μg/kg]

Figure 31

Figure 32. Effect of perinatal exposure to 0, 10 or 100 ppm PBBs on the concentration of equivalents of estradiol-17β-6,7-^{3}H in serum of rats treated with estradiol-17β-6,7-^{3}H. Rats were treated with 1.0 or 3.0 μg/kg estradiol-17β-6,7-^{3}H i.p. at day 28 postpartum. Serum was collected 4 hr later. Values are means ± S.E.M. for 5 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

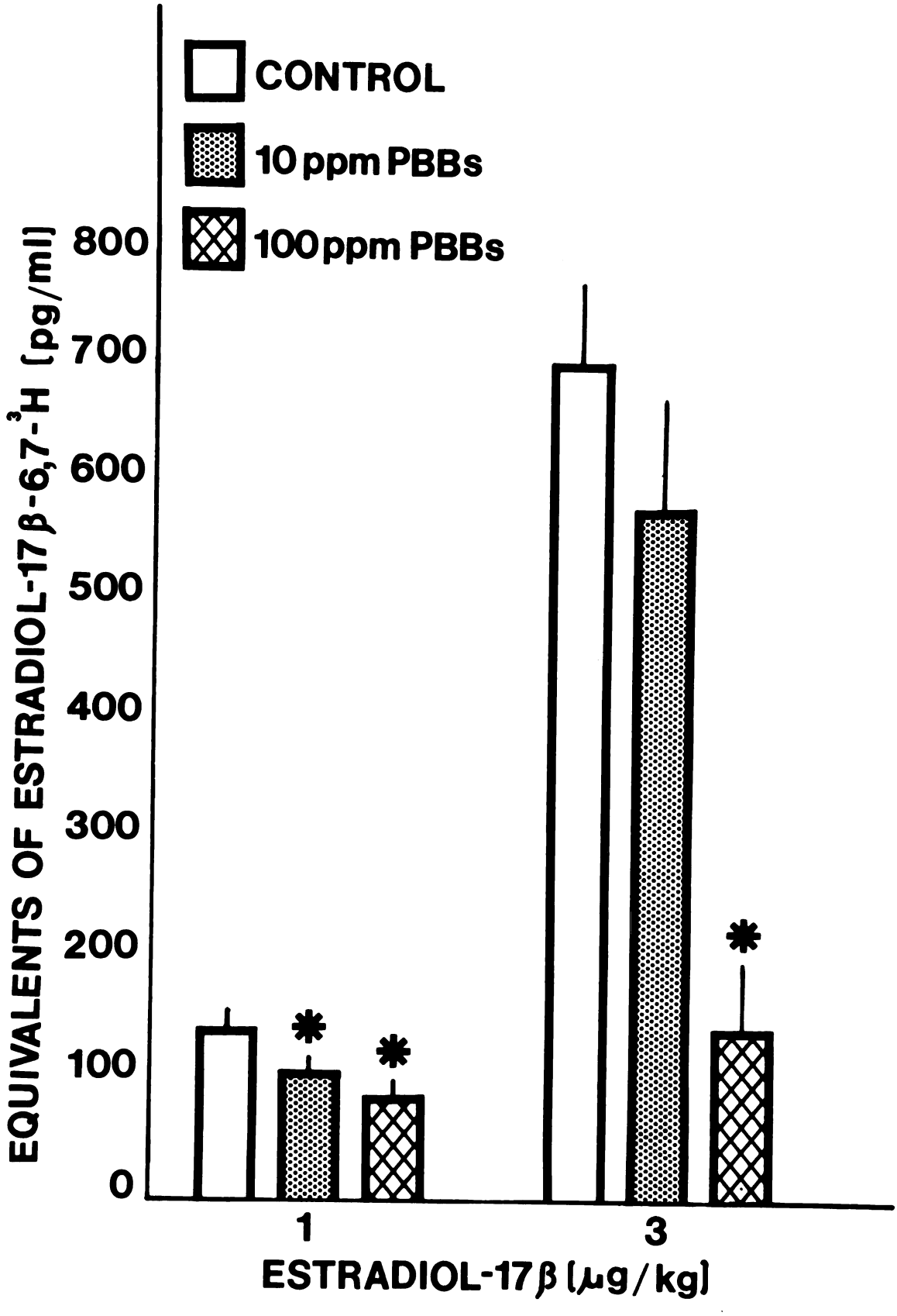
CONTROL
10 ppm PBBs
100 ppm PBBs
EQUIVALENTS OF ESTRADIOL-17β-6,7-³H (pg/ml)
800
700
600
500
400
300
200
100
0
1
3
ESTRADIOL-17β (μg/kg)

Figure 32

TABLE 22

Tissue Concentrations of PBBs Following a Single Injection[a]

Days After Injection	Tissue							
	Brain	Fat	Heart	Intestine	Liver	Muscle	Skin	Lung
1	1.3±0.2	---	5.4±0.5	110.2±12.4	---	8.0±1.1	15.2±3.6	19.3±2.1
2	2.0±0.2	---	9.2±1.1	85.6±15.9	2.0±0.2	10.3±0.9	13.9±1.7	17.9±1.7
3	0.6±0.1	---	2.8±0.6	78.1±12.6	---	7.2±0.9	20.0±3.2	15.2±1.0
7	0.8±0.2	---	3.0±0.4	67.0± 6.0	---	10.5±1.8	20.8±3.5	15.5±2.8
14	2.0±0.4	---	3.4±0.6	34.2±3.3	6.1±1.0	11.0±2.2	26.0±3.3	7.6±1.8
28	0.9±0.1	1.8± 0.4	1.9±0.4	11.7±1.1	4.7±0.3	1.9±0.2	15.1±1.6	7.3±1.6
63	0.1±0.1	69.5±10.0	0.2±0.1	7.1±1.2	4.6±1.1	5.4±1.4	10.0±2.0	2.5±0.5

[a]Values are means in µg PBBs/g wet wt tissue ± S.E.M. for 6 animals. Animals were treated with 150 mg/kg PBBs, i.p., on day 7 postpartum.

Concentration of PBBs in intestine was initially high, however, it decreased steadily with time and reflected the amount of PBBs in the peritonal cavity (Table 22; Figure 33). Disappearance of PBBs from the peritoneal cavity over the time period investigated appeared to follow first-order kinetics. Half-time for disappearance of PBBs from the peritoneal cavity following 150 mg/kg PBBs was approximately 9.3 days. Peritoneal cavity did not contain PBBs by 63 days after treatment. PBB congeners did not disappear at detectably different rates through day 7 after administration of PBBs; that is, the percentage of each congener quantified in peritoneal cavity was not different from that injected. However, preferential disappearance of hexa-congeners relative to hepta-congeners was observed 14 and 28 days after treatment.

Animals perinatally exposed to 100 ppm PBBs and weaned onto control diet had an increased liver weight-to-body weight ratio at weaning (day 28) (165% of control value) and 150 (111% of control value) but not 328 days of age (Figure 34). Hepatic histopathological alterations produced by PBBs were most severe at 28 days of age but persisted throughout the residual phase. As noted, the most prominent change was vacuolation which gave the cytoplasm a foamy appearance (Figure 6). Cellular swelling caused the sinusoids to become less apparent. At 150 days of age, vacuolation and cellular swelling were common but not as marked as at 28 days. Some necrosis, retained bile, eccentric nuclei and pycnotic nuclei were observed at 28 days. At 328 days of age, hepatic cellular changes were similar in type and severity to those found at 150 days. However, vacuolation, necrosis

Figure 33. Disappearance of PBBs from the peritoneal cavity of rats after a single intraperitoneal injection. Rats were treated with 150 mg/kg PBBs, i.p., on day 7 postpartum. Each point represents the mean ± S.E.M. for 6 animals.

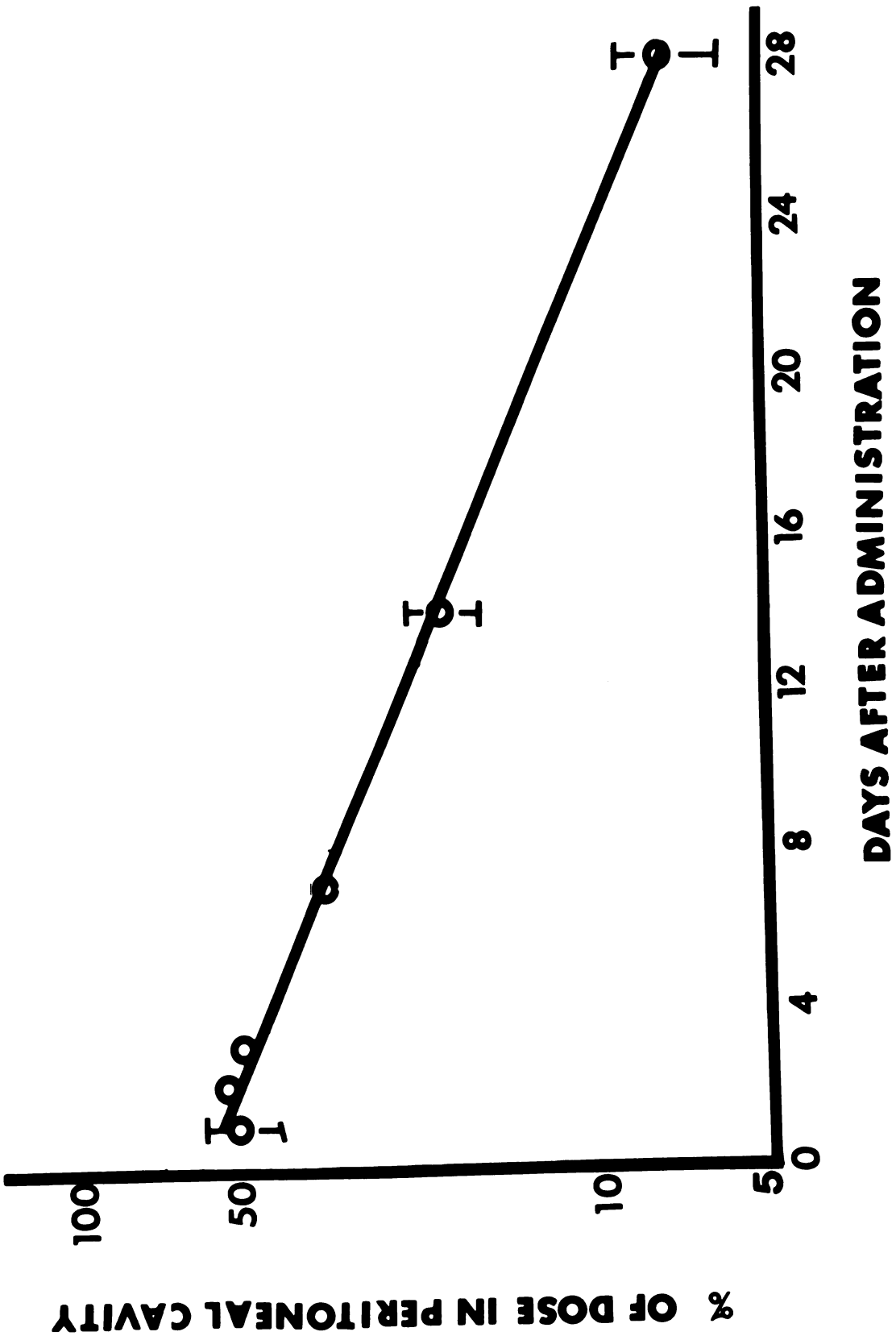
% OF DOSE IN PERITONEAL CAVITY
100
50
10
5
0
4
8
12
16
20
24
28
DAYS AFTER ADMINISTRATION

Figure 33

Figure 34. Residual effect of perinatal exposure to PBBs on liver wt-to-body wt and kidney wt-to-body wt ratios. Rats were born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 postpartum. All animals were weaned onto control diet at 28 days of age. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

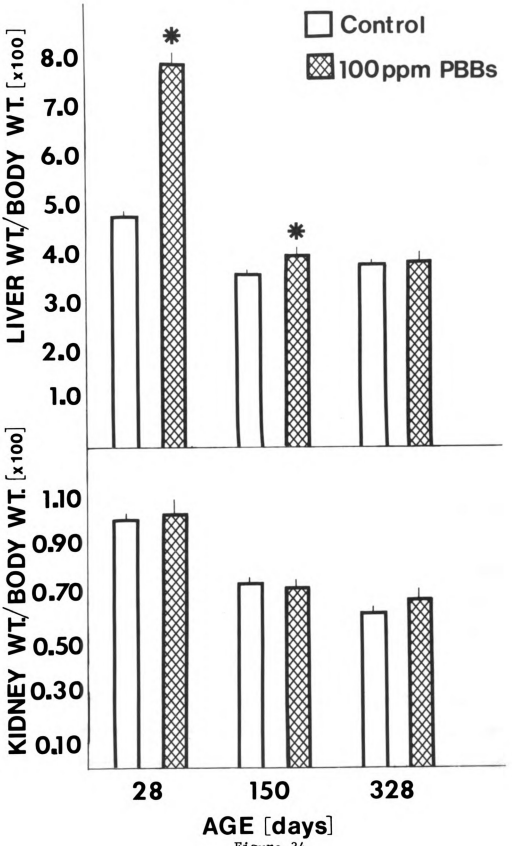
Control
100ppm PBBs
LIVER WT./BODY WT. [x100]
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
KIDNEY WT./BODY WT. [x100]
1.10
0.90
0.70
0.50
0.30
0.10
28
150
328
AGE [days]

Figure 34

and pycnotic nuclei were also seen in some liver sections from 328 day old control animals.

Microsomal enzymes were stimulated in liver by PBBs at all ages investigated (Figure 35). Hepatic AHH activity was increased above controls by PBBs at 28, 150 and 328 days of age (approximately 1400%, 650% and 750% of control values, respectively). Hepatic EH activity was also increased above controls by PBBs at 28, 150 and 328 days of age (approximately 200%, 160% and 180% of control values, respectively).

Kidney weight-to-body weight ratio was not affected by PBBs (Figure 35). Perinatal exposure to PBBs did not result in renal histopathological alterations. Exposure to 100 ppm PBBs increased renal AHH activity at 28, 150 and 328 days of age (700%, 400% and 550% of control value, respectively) (Figure 36). However, renal EH activity was not modified by PBBs (Figure 36).

Following treatment with 100 ppm PBBs, no elevation of BUN was detected at either 28 or 328 days of age (Table 23). Similarly, PBBs did not alter SGPT at these ages (Table 23).

Pentobarbital sleeping time was reduced at both 28 and 328 days of age by perinatal exposure to PBBs (Figure 37). This effect was more pronounced at 28 than 328 days of age (4% and 58% of control values, respectively).

Tissue concentrations of PBBs decreased with time following termination of exposure to PBBs (Table 24). At 328 days of age (10 months after weaning animals onto diet free of PBBs) the concentration of PBBs in liver, kidney, ovary and fat were 15%, 33%, 49% and 35% of PBBs concentrations at 28 days of age, respectively.

Figure 35. Residual effect of perinatal exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver. Rats were born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 postpartum. All animals were weaned onto control diet at 28 days of age. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

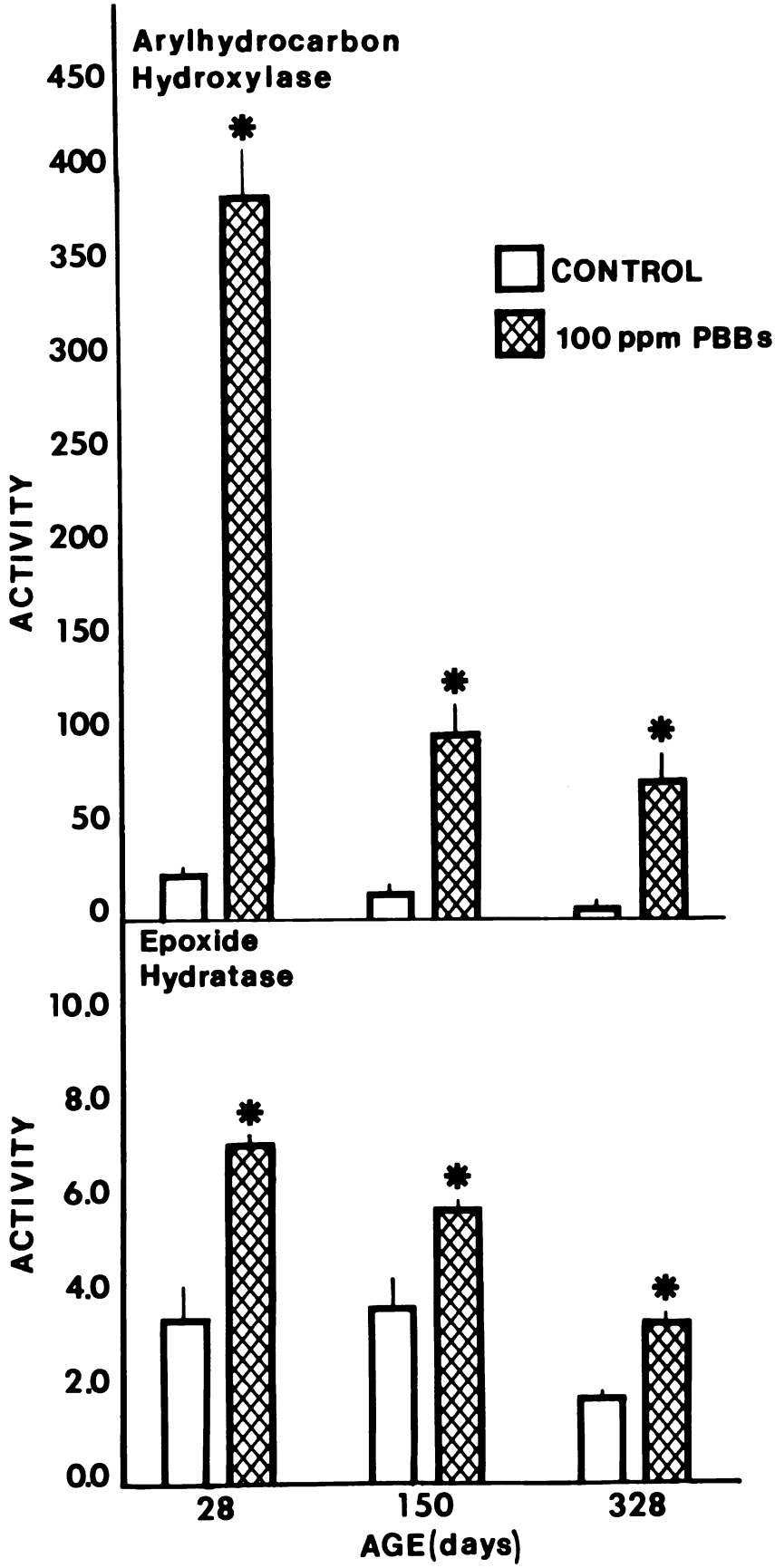
Arylhydrocarbon
Hydroxylase
450
400
350
300
250
200
150
100
50
0
ACTIVITY
CONTROL
100 ppm PBBs
Epoxide
Hydratase
10.0
8.0
6.0
4.0
2.0
0.0
ACTIVITY
28
150
328
AGE(days)

Figure 35

Figure 36. Residual effect of perinatal exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney. Rats were born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 postpartum. All animals were weaned onto control diet at 28 days of age. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

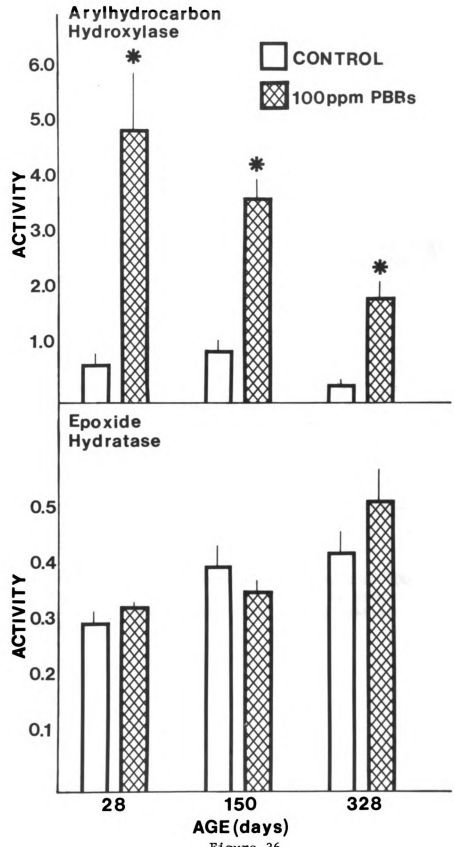
Arylhydrocarbon
Hydroxylase
CONTROL
100ppm PBBs
ACTIVITY
6.0
5.0
4.0
3.0
2.0
1.0
*
*
*
Epoxide
Hydratase
ACTIVITY
0.5
0.4
0.3
0.2
0.1
28
150
328
AGE (days)

Figure 36

TABLE 23

Residual Effect of PBBs-Exposure on Blood Urea Nitrogen (BUN) and Activity of Serum Glutamic Pyruvic Transaminase (SGPT)[a]

Treatment	Age[b] 28 Days	328 Days
	BUN (mg urea nitrogen/100 ml blood)	
Control	26.5±0.2	25.3±0.9
100 ppm PBBs	26.6±0.8	24.5±0.5
	SGPT (units/ml serum)	
Control	48.0±3.8	59.7±1.7
100 ppm PBBs	57.7±1.7	74.0±9.0

[a]Values are means ± S.E.M. for 3 animals.

[b]Rats born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 post-partum. All animals were weaned onto control diet at 28 days of age.

Figure 37. Residual effect of perinatal exposure to PBBs on duration of anesthesia produced by pentobarbital. Rats were born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 postpartum. All animals were weaned onto control diet at 28 days of age. At 28 and 328 days of age male rats were treated with 40 mg/kg pentobarbital i.p. Duration of anesthesia was recorded as time of injection until animals regained righting reflex. Values are means ± S.E.M. for 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

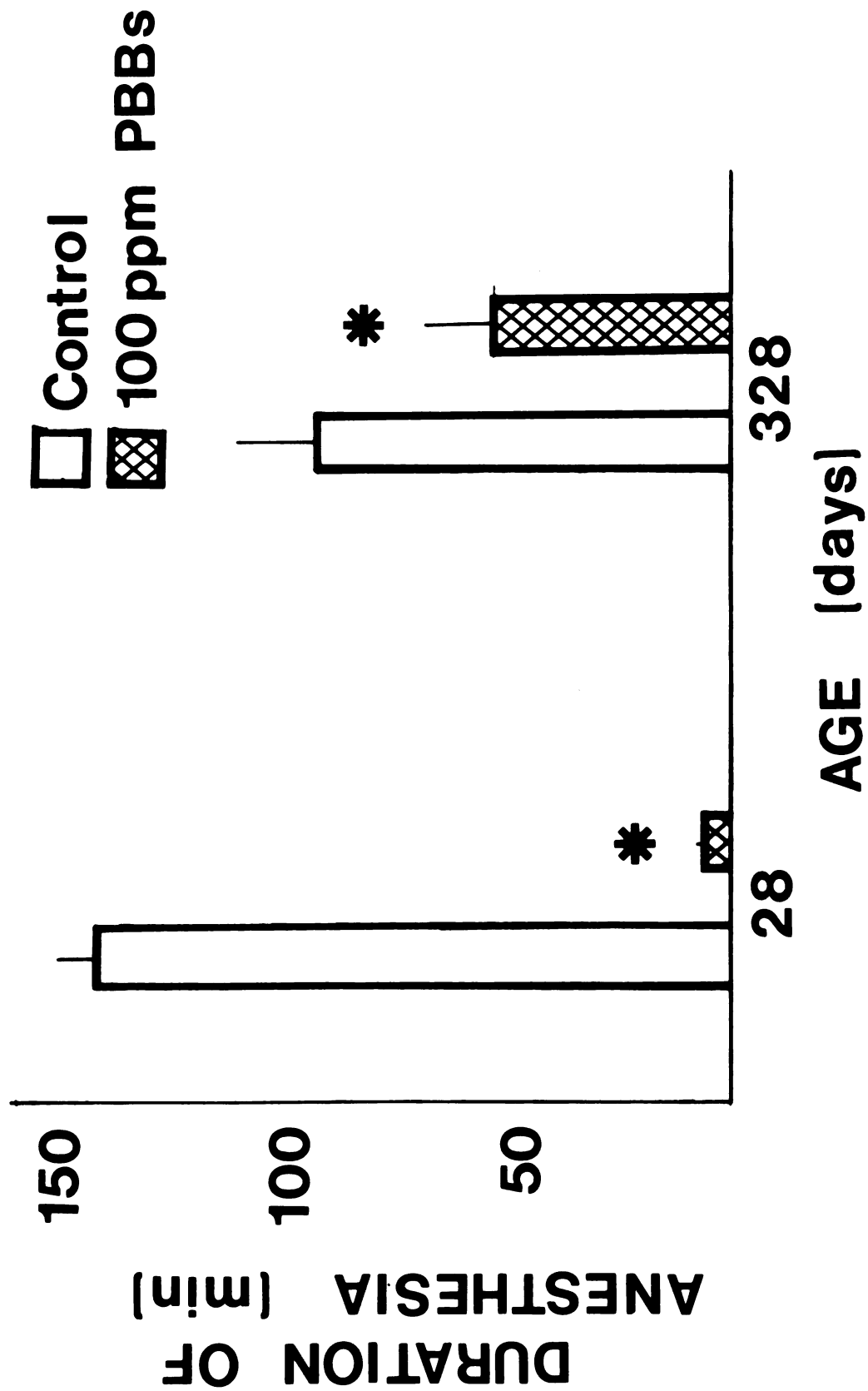
DURATION OF
ANESTHESIA (min)
150
100
50
28
328
AGE (days)
Control
100 ppm PBBs
*
*

Figure 37

TABLE 24

Tissue Concentrations of PBBs Following Perinatal Exposure to PBBs[a]

Treatment	Tissue			
	Liver	Kidney	Ovary	Fat
	28[b]			
Control	0.0	0.0	0.0	0.0
100 ppm PBBs	27.2±6.9	16.5±4.2	117.4±16.9	282.2±17.3
	328[b]			
Control	0.1	0.0	0.0	0.3
100 ppm PBBs	4.2±1.4	5.4±1.7	57.1± 8.2	98.1±25.1

[a]Values are means in μg/g wet wt tissue ± S.E.M. for at least 3 animals. Rats born to and nursed by dams which received 0 or 100 ppm PBBs from day 8 of gestation through day 28 postpartum at which time all pups were weaned onto diet free of PBBs.

[b]Age of animals in days.

Body weight at weaning (28 days postpartum) was lower in animals exposed to 100 ppm PBBs from day 8 of gestation (F_1-100) than controls (80% of control value) (Figure 38). Survival-to-weaning of F_1-100 was also less than controls (87% of control value) (Figure 39).

The liver weight-to-body weight ratio was increased at weaning in F_1-100 rats, their progeny (F_2-100) and F_1-10 (exposed to 10 ppm PBBs from day 8 of gestation) (165%, 128% and 126% of control values, respectively) (Figure 39). Activity of AHH was increased at this age in liver from F_1-100, F_2-100, F_1-10 and F_2-10 (approximately 1400%, 600%, 400% and 130% of control values, respectively) (Figure 40). Activity of EH was increased in liver from F_1-100, F_2-100 and F_1-10 (approximately 250%, 125% and 125% of control values, respectively) (Figure 40).

Renal AHH activity was increased in F_1-100 and F_1-10 (approximately 700% and 200% of control values, respectively) (Figure 42). Activity of EH in kidney was not affected by PBBs (Figure 41).

Duration of anesthesia following 150 mg/kg progesterone was reduced in F_1-100, F_2-100, F_1-10 and F_2-10 (10%, 16%, 25% and 50% of control values, respectively) (Figure 42). Pentobarbital sleeping time was reduced in F_1-100, F_2-100, F_1-10 and F_2-10 (4%, 16%, 8% and 75% of control values, respectively) (Figure 43). Progeny of F_2-100 (F_3-100) did not exhibit a difference from controls in any response quantified in this investigation.

Dose and generation related effects were correlated with hepatic concentrations of PBBs (Table 25). The concentration of PBBs in liver was highest in pups born to and suckled by dams fed diet containing

Figure 38. Effect of exposure to PBBs on body weight and the liver wt-to-body wt ratio in subsequent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually, and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. Values are means ± S.E.M. from at least 7 litters. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

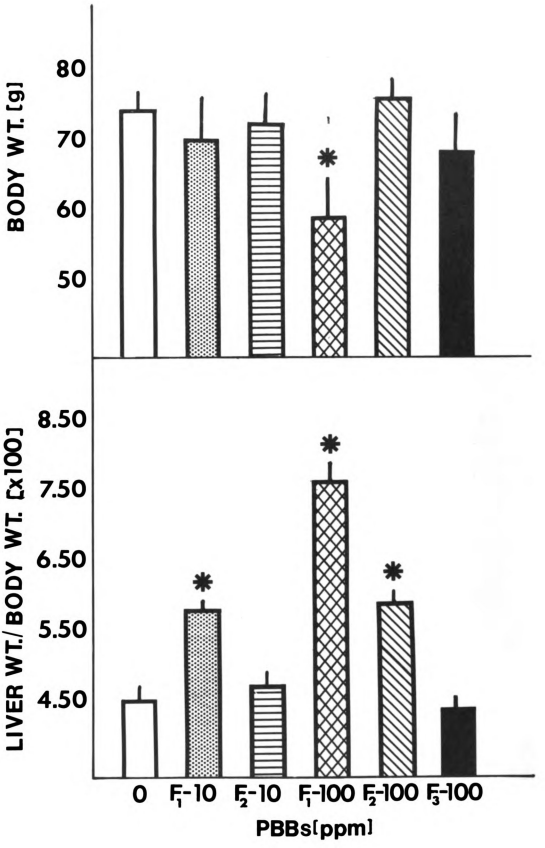
BODY WT.[g]
80
70
60
50
LIVER WT./BODY WT. [x100]
8.50
7.50
6.50
5.50
4.50
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs[ppm]

Figure 38

Figure 39. Effect of exposure to PBBs on percent survival-to-weaning in subsequent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually, and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. Values are means ± S.E.M. from at least 7 litters. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

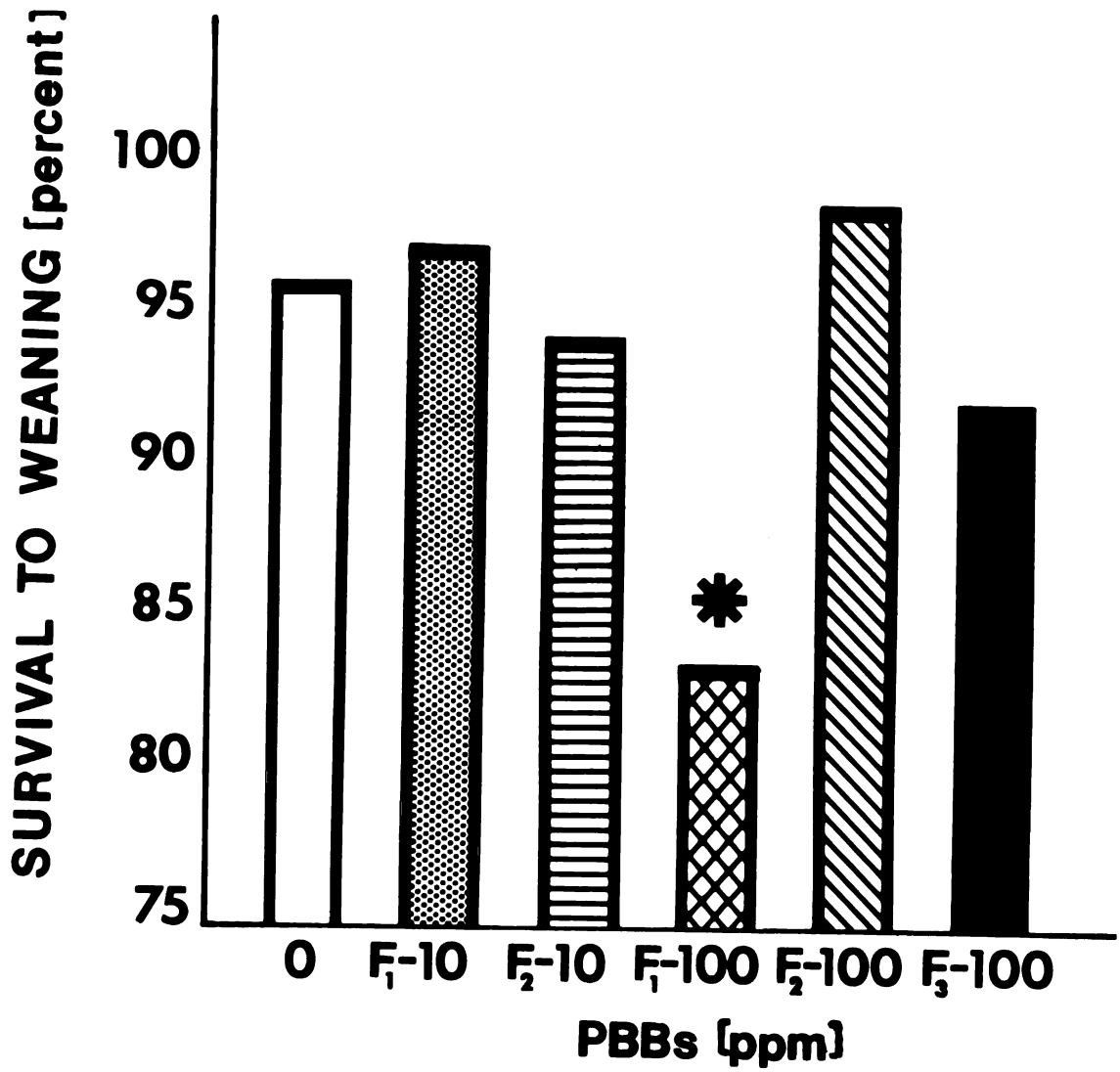
SURVIVAL TO WEANING [percent]
100
95
90
85
80
75
*
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs [ppm]

Figure 39

Figure 40. Effect of exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in liver in subsquent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually, and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. Values are means ± S.E.M. from at least 7 litters. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

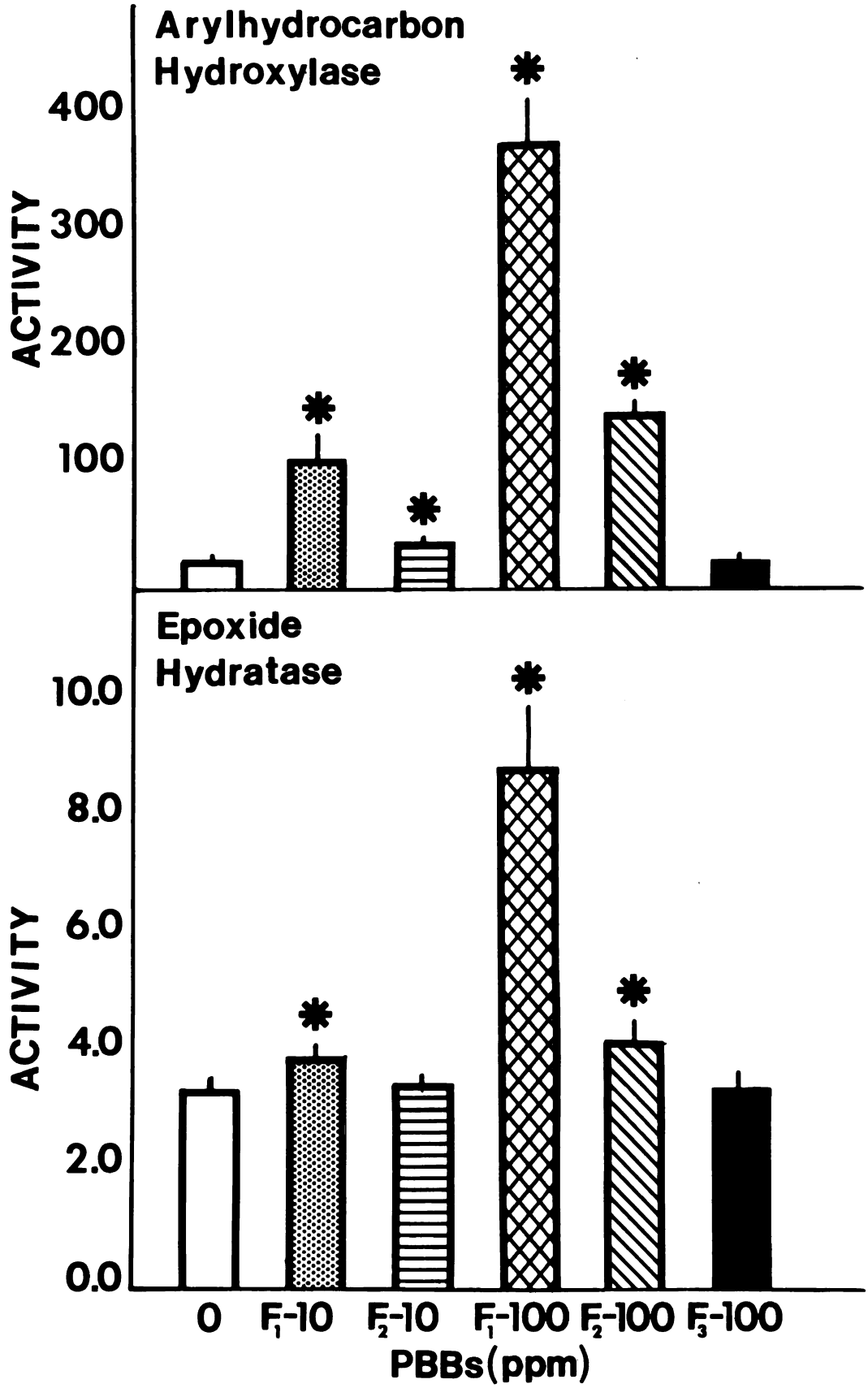
Arylhydrocarbon
Hydroxylase
400
300
200
100
ACTIVITY
Epoxide
Hydratase
10.0
8.0
6.0
4.0
2.0
0.0
ACTIVITY
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs(ppm)

Figure 40

Figure 41. Effect of exposure to PBBs on activity of arylhydrocarbon hydroxylase and epoxide hydratase in kidney in subsequent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually, and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. Values are means ± S.E.M. from at least 7 litters. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

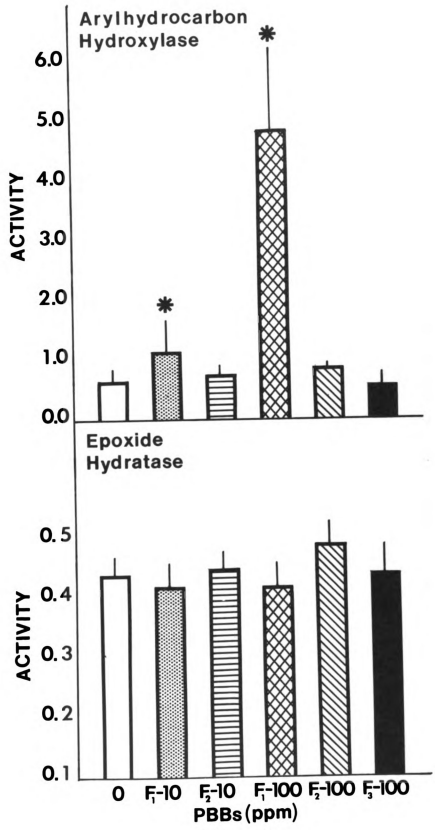
Arylhydrocarbon
Hydroxylase
ACTIVITY
6.0
5.0
4.0
3.0
2.0
1.0
0.0
*
*
Epoxide
Hydratase
ACTIVITY
0.5
0.4
0.3
0.2
0.1
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs (ppm)

Figure 41

Figure 42. Effect of exposure to PBBs on duration of anesthesia produced by progesterone in subsequent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. At 28 days of age, female rats were treated with 150 mg/kg progesterone, i.p. Duration of anesthesia was recorded as the time of injection until animals regained righting reflex. Values are means ± S.E.M. for at least 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

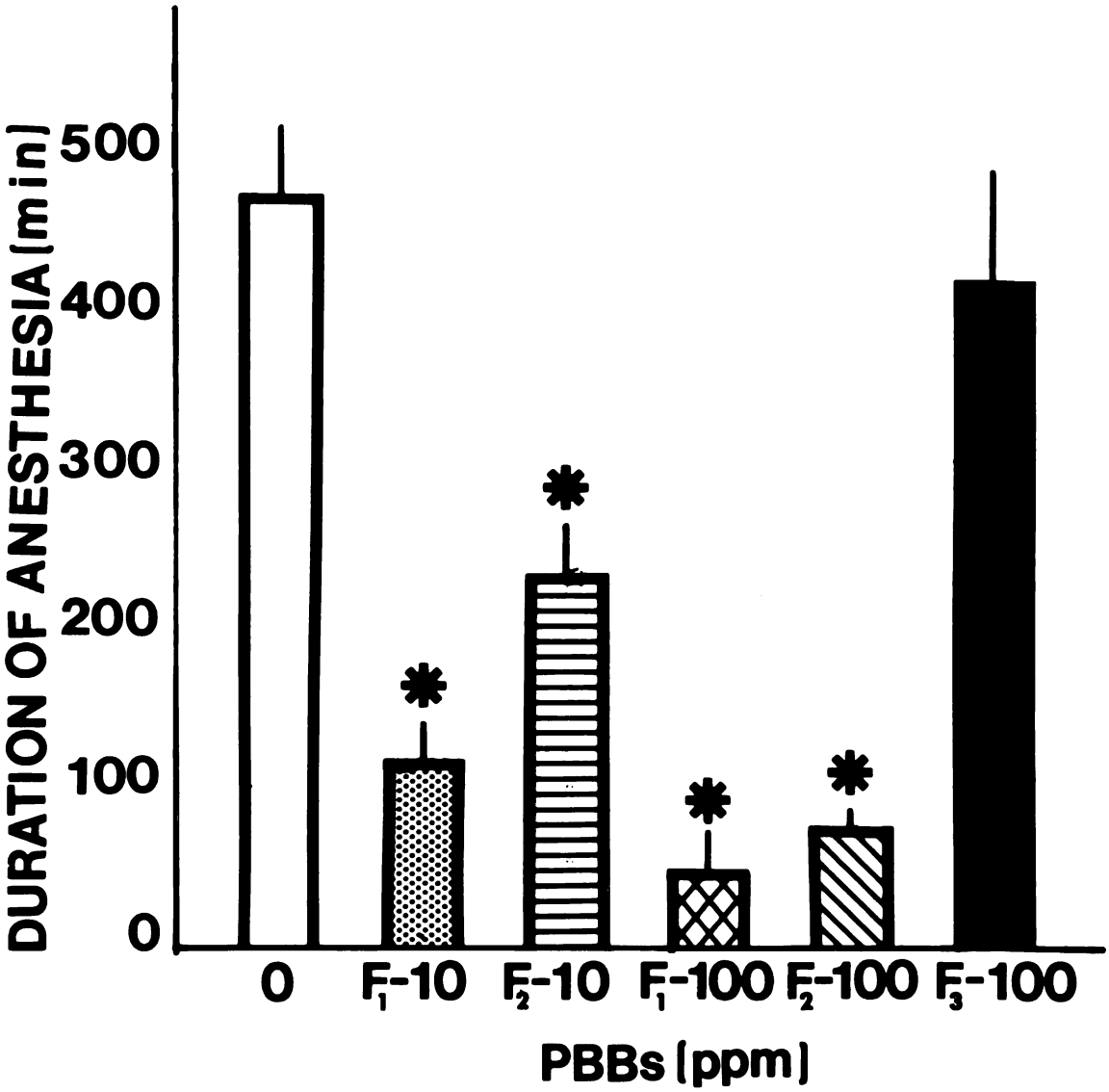
DURATION OF ANESTHESIA (min)
500
400
300
200
100
0
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs (ppm)

Figure 42

Figure 43. Effect of exposure to PBBs on duration of anesthesia produced by pentobarbital in subsequent generations. Rats (F_0) were fed 0, 10 or 100 ppm from day 8 of pregnancy until 28 days postpartum at which time all pups (F_1) were weaned onto control diet, allowed to mature sexually and bred with littermates to produce F_2. F_2 generation from 100 ppm PBBs was bred with littermates to produce F_3. Duration of anesthesia was recorded as the time of injection until animals regained righting reflex. Values are means ± S.E.M. for at least 4 animals. Asterisk indicates a statistically significant difference from the control value, $p<0.05$.

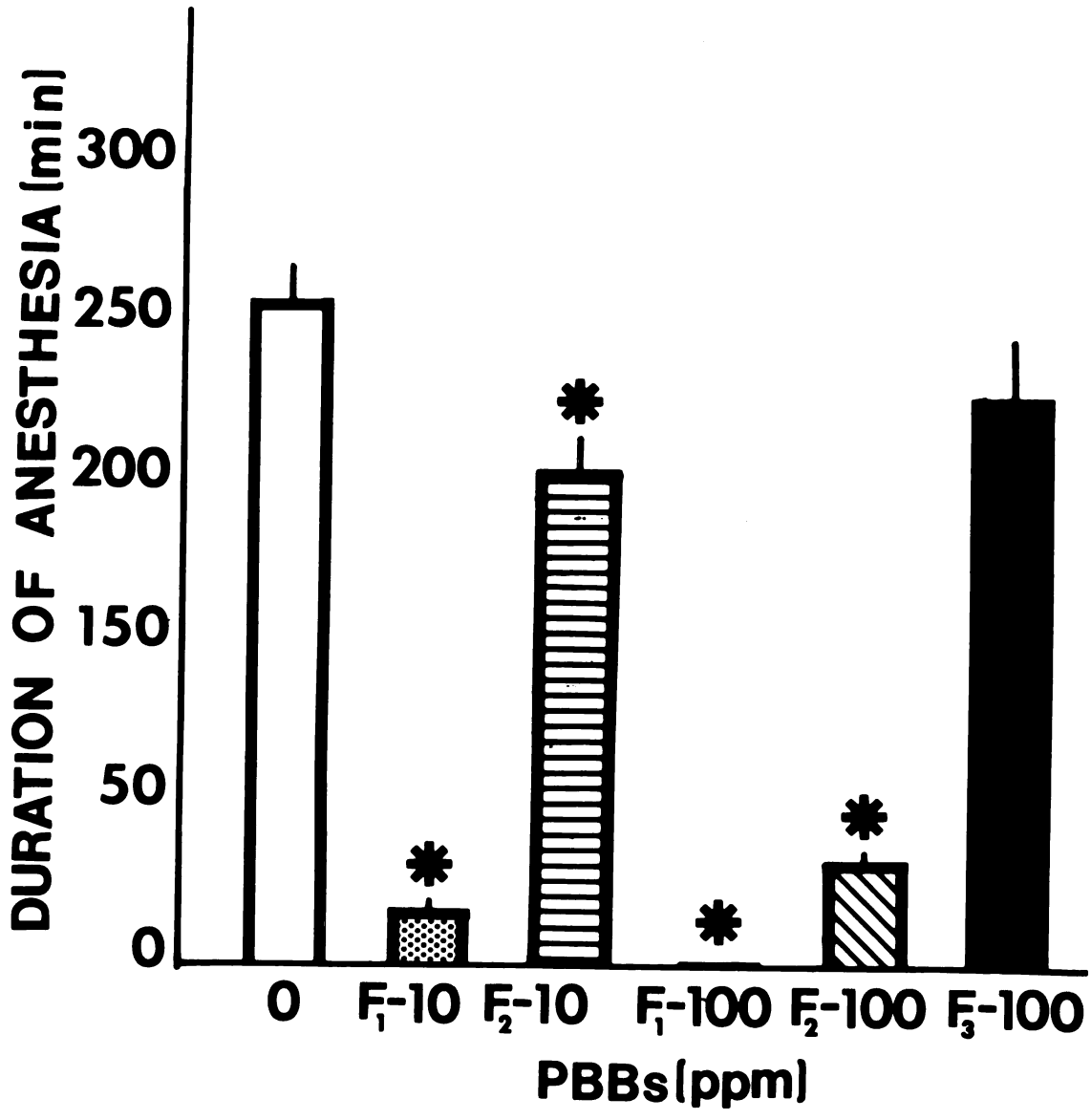
DURATION OF ANESTHESIA (min)
300
250
200
150
50
0
0
F1-10
F2-10
F1-100
F2-100
F3-100
PBBs (ppm)

Figure 43

TABLE 25

Concentration of PBBs in Liver Following Perinatal Exposure to PBBs[a]

Treatment	Concentration of PBBs (µg/g wet tissue)
Control	0.0
F_1-100	33.6±7.9
F_2-100	4.1±0.6
F_3-100	<0.1
F_1-10	2.5±0.4
F_2-10	<0.1

[a]Values are means ± S.E.M. for at least 3 animals. PBBs were quantified at 28 days of age.

100 ppm PBBs. Their pups, F_2-100, and a hepatic concentration of PBBs similar to that of F_1-10. Liver from either F_3-100 or F_2-10 had only traces of PBBs.

DISCUSSION

A. Survival, Growth and Development

Concern about the hazardous effects of PBBs on prenatal and neonatal human development has increased since PBBs were found to undergo transplacental movement and excretion into milk (Kay, 1977). Although the teratogenic potential of PBBs appears to be low, a variety of anomalies including hydronephrosis, exencephaly and cleft palate have been observed in fetal rodents following administration of PBBs in the maternal diet during organogenesis (Corbett et al., 1975; Beaudoin, 1977). Pregnant cows offered a diet containing high concentrations of PBBs came back into heat suggesting embryo resorption (Jackson and Halbert, 1974). Animals had an aversion to feed containing high concentrations of PBBs and markedly decreased food consumption (Jackson and Halbert, 1974; Kimbrough et al., 1978). Stressful stimuli, such as maternal food deprivation, have increased the embryolethality and teratogenicity of certain hormones and herbicides, possibly by altering their disposition and/or metabolism (Kalter, 1960; Rosenzweig and Blaustein, 1970; Preache and Gibson, 1975).

In the present study although dietary exposure of pregnant rats to 100 ppm PBBs or maternal food deprivation for 48 hr alone did not produce embryotoxic effects, embryolethality was increased and fetal body weight decreased following combined treatment with PBBs and

maternal food deprivation (Table 1). Fat depots are mobilized during food restriction which has resulted in altered disposition and increased toxicity of certain lipophilic xenobiotics including organohalogens (Dale et al., 1963; Sanders et al., 1977; Villeneuve et al., 1978). Since the whole fetal carcass concentration of PBBs was not different in stressed and nonstressed fetuses exposed to PBBs, increased embryotoxicity may not have been attributable to an increased body burden of PBBs in stressed animals (Table 1). However, the concentration of PBBs was not determined in stillborn or partially resorbed fetuses or individual fetal tissues. Thus, it is possible that maternal food deprivation affected the disposition of PBBs.

In response to stressful food restriction, a variety of metabolic adjustments are made including accelerated oxidation of triacylglycerols and catabolism of amino acids. Circulating concentrations of gonadotropins and steroid sex hormones are reduced following food restriction (Piacsek and Meites, 1967; Howland, 1971, 1972). Circulating concentrations of steroid hormones are also altered after exposure to certain mixtures of PCBs, and concomitant food restriction has intensified these effects (Wasserman et al., 1973; Sanders et al., 1974; Sanders and Kirkpatrick, 1975; Sanders et al., 1977). Results from this investigation suggest that steroid metabolism may be accelerated following treatment with PBBs. Metabolic alterations resulting from food deprivation may have interacted in an undetermined manner with metabolic changes produced by PBBs to increase embryolethality and decrease fetal body weight.

Body weight gain and rate of physical maturation were not affected by neonatal treatment with 150 or 500 mg/kg PBBs, however, after 1 wk

postpartum both were retarded in pups born to and suckled by dams fed 100 ppm PBBs (Figure 1, Table 2). Only perinatal exposure to 100 ppm PBBs reduced survival rate-to-weaning (Figure 2). Pup mortality was not affected by 100 ppm PBBs until after 1 wk postpartum. These treatment differences were correlated with tissue concentrations of PBBs which were highest in pups exposed to 100 ppm PBBs (Table 3). A latent period of approximately 1 wk apparently existed for manifestation of retarded body weight gain and physical maturation and increased pup mortality in pups exposed to 100 ppm PBBs. This delay may have been related to the time required for PBBs to accumulate to concentrations in tissues sufficient to produce such effects, as PBBs are excreted with milk fat. Concentrations of PBBs were 3-4 times higher in milk from rats fed PBBs than in maternal diet (Figure 3). At 14 days postpartum, whole carcass concentrations of PBBs were approximately 25 times higher in rat pups born to and suckled by dams fed 50 ppm PBBs from day 8 of gestation than in pups born to PBBs exposed dams (50 ppm) but suckled by control dams (Rickert et al., 1978).

Since milk consumption was not monitored and nutritional value of milk produced by PBBs-exposed dams not assessed in these investigations, the mechanism(s) by which body weight gain and physical development were retarded is not known. Animals may have had an aversion to milk containing high concentrations of PBBs and subsequently reduced milk intake. Reduced body weight gain in adult rats exposed to PBBs in diet has also been attributed, at least in part, to decreased food efficiency (i.e., weight gain/food consumed) (Sleight and Sanger,

1976; Garthoff et al., 1977). Numerous metabolic factors may contribute to decreased food efficiency including alterations in energy metabolism at the subcellular level. Mitochondrial ultrastructural degenerative changes have been observed following PBBs (Corbett et al., 1978). In this investigation, activity of hepatic monoamine oxidase, an enzyme localized in the outer mitochondrial membrane, was reduced after perinatal exposure to 100 ppm PBBs (Figure 12). Dietary treatment of adult rats with PBBs altered concentrations of adenine nucleotides in hepatocytes and inhibited respiration in isolated mitochondria (Garthoff et al., 1977). Alterations in mitochondrial electron transport systems have resulted from treatment with PCBs (Pardini, 1971; Sivalingan et al., 1973; La Rocca et al., 1975).

Many other factors may have contributed to retarded body weight gain and physical maturation in rats exposed to 100 ppm PBBs. The effect of perinatal treatment with PBBs on production, secretion and action of growth hormone or absorption and metabolism of nutrients, including vitamins, vital for normal growth and development, has not been determined (Mason, 1939; Thompson, 1969). Decreased growth rate is one of the earliest and most sensitive indices of vitamin A deficiency (Corey and Hayes, 1972). Concentration of vitamin A in liver, where vitamin A is stored, was reduced in rats perinatally exposed to PBBs (Table 4). However, the concentration of vitamin A in serum and kidney was not affected by PBBs. Whether or not the reduction in hepatic vitamin A concentration following 100 ppm PBBs was sufficient to account for retarded growth and development has not been determined. Body weight gain and concentration of vitamin A in liver have

been reduced by PCBs (Cecil et al., 1973; Innami et al., 1976). Rats fed PCBs and high amounts of vitamin A gained more weight than those fed PCBs and only standard amounts of vitamin A (Innami et al., 1976). Thus, vitamin A may protect against certain effects, including retarded growth rate, produced by polyhalogenated biphenyls.

B. Liver

The liver weight-to-body weight ratio was increased following perinatal exposure to 10 or 100 ppm PBBs and after neonatal treatment with 150 or 500 mg/kg PBBs (Figures 4 and 38). Liver enlargement was dose dependent and directly related to hepatic concentrations of PBBs (Tables 3 and 25). Increased protein content may have contributed to liver enlargement produced by PBBs. Total liver protein synthesis has been increased in association with liver weight gains in adult rats exposed to PBBs (Garthoff et al., 1977). In this investigation, PBBs produced an increase in hepatic microsomal protein (Figure 8). Increased liver weight may also have been due at least in part to lipid deposition. All liver lipid fractions, including total fat, phospholipid, neutral lipid and cholesterol were elevated in rats fed PBBs (Garthoff et al., 1977). Increased liver cholesterol produced by PBBs may have been due to increased activity of β-hydroxy-β-methylglutaryl (HMG)-Co-A reductase which is rate-limiting in cholesterol biosynthesis. Although activity of HMG-Co-A reductase was not quantified in this investigation, it has been induced by phenobarbital and PBBs exhibit certain enzyme stimulating properties which are similar to phenobarbital (Gibbons and Mitropoulos, 1973; Dent et al., 1976a,b).

Microscopic morphologic alterations were also produced by PBBs. Compared to liver from control animals, liver from rats perinatally exposed to 100 ppm PBBs had vacuolation which was so extensive that the cytoplasm appeared foamy (Figures 5 and 6). Histopathological alterations following 100 ppm PBBs also included hepatocyte swelling to an extent that sinusoids appeared only as small, clear spaces with an occasional Kupffer cell visible, focal necrosis, absent or pycnotic nuclei and myelin inclusion bodies. Some of these cytoplasmic inclusion bodies were uniform in appearance while others had a lighter, central mass surrounded by a wide, darker band (Figure 7). Consistent with increased microsomal protein, electron microscopic changes in liver following PBBs included massive proliferation of smooth endoplasmic reticulum (Corbett et al., 1978). Such alterations are typical manifestations of exposure to halogenated aromatic hydrocarbons (Kimbrough, 1974, 1978). Proliferated membranes are suggestive of reorganization of structural and enzymatic proteins and may provide sites for storage, isolation and contact of membrane enzymes (microsomal) with lipophilic halogenated aromatic hydrocarbons (Norback and Allen, 1972). The subcellular distribution of PBBs has not been determined. However, following oral administration of ^{3}H-PCBs to rats, microsomal membranes had the highest amount of radioactivity of any subcellular fraction from liver indicating preferential localization of PCBs in that organelle (Fujita et al., 1971).

Microsomal MFO activity has been increased in liver by PBBs (Farber and Baker, 1974; Dent et al., 1976a,b). PBBs belong to a class of xenobiotics termed "mixed inducers"; that is, PBBs exhibited

stimulating characteristics of both phenobarbital and 3MC, two agents which induce distinct types of hepatic microsomal enzymes (Sladek and Mannering, 1969a,b). Several PBB congeners such as the two major components of the mixture, 2,4,5,2',4',5'-hexa- and 2,3,4,5,2',4',5'-heptabromobiphenyl may be responsible for phenobarbital-like action and others, possibly a minor PBB congener or trace contaminant, may be responsible for 3MC-like effects (Moore et al., 1978, 1979). In addition, one or more congeners, such as 2,4,5,3',4',5'-hexabromobiphenyl, may produce both types of enzyme stimulation (Dannan et al., 1978).

Following acute administration of PBBs to adult rats and mice, the pattern of hepatic microsomal enzyme stimulation changed from phenobarbital-like initially (activity of EH, Hex-OH, BP-4-OH increased) to 3MC-like (activity of AHH, EROD, BP-2-OH increased) with time following treatment (Dent et al., 1976a,b). Changes in the ethylisocyanide difference spectra also indicated that the character of cytochrome P450 changed with time following administration of PBBs to adult rodents (Dent et al., 1976a,b). The ratio of peaks at 428 and 455 in the ethylisocyanide difference spectra reached a maximum soon after treatment with PBBs (i.e., closest to values observed after phenobarbital administration) and subsequently declined toward values observed in hepatic microsomes from control or 3MC-treated animals (Dent et al., 1976, 1977).

In this investigation, PBBs also stimulated microsomal enzymes in developing rats. Activities of microsomal and cytosolic enzymes in liver were elevated as long as 63 days following a single i.p.

injection of 150 mg/kg PBBs to 7-day-old rats (Figures 8 and 9). Hepatic microsomal enzymes regarded as sensitive to 3MC treatment (BP-2-OH, EROD, AHH) were stimulated above controls within 2 days following treatment with PBBs. Maximal stimulation of these enzymes was observed 7-14 days after PBBs. Activity of hepatic microsomal enzymes regarded as sensitive to phenobarbital treatment (EH, Hex-OH, BP-4-OH) was increased by 7 days but did not reach maximum values until 28 days after PBBs. Thus, in contrast to what has been observed in adult rodents, the pattern of hepatic enzyme stimulation in developing rats following PBBs appeared to more closely resemble a 3MC-like stimulation initially with a phenobarbital-like effect occurring at later times.

Liver weight-to-body weight ratio and hepatic microsomal protein were increased in adult rodents as early as 12 hr after acute treatment with PBBs (Dent et al., 1976, 1977). However, increases in these parameters were not observed in developing rats until 7 days following treatment. Since phenobarbital produces hepatocyte hypertrophy and proliferation of smooth endoplasmic reticulum while 3MC leads to enzyme induction without hypertrophy of liver, these observations were also consistent with the contention that there are age differences in the pattern of hepatic MFO stimulation following acute treatment with PBBs (Parke, 1975). The mechanism for age-related differences in the pattern of stimulation of hepatic MFOs, following an i.p. injection of PBBs, is not known. However, this difference may be a function of the low basal activity of these enzymes in immature animals, an unexplained age dependent differential sensitivity toward enzyme inducers

and/or age-related differences in absorption, distribution and/or excretion of certain PBB congeners (Dickerson and Basu, 1975; Basu et al., 1971).

Alterations in microsomal enzyme activity can result in altered susceptibility to subsequently administered xenobiotics. For example, bromobenzene lethality was increased in adult mice by pretreatment with phenobarbital or 3MC (Roes et al., 1977). In mice, phenobarbital treatment shifted the bromobenzene time lethality curve without altering its slope. In contrast, 3MC pretreatment altered the slope and decreased the median time to death, suggesting both an enhancement of lethality and a change in the mechanism by which lethality occurred (Roes et al., 1977).

Adult mice pretreated with a single injection of PBBs showed a time-dependent change in susceptibility to bromobenzene toxicity (Roes et al., 1977). At times soon after administration of PBBs, the pattern of bromobenzene lethality was similar to that in 3MC-pretreated animals. The change in effect of PBBs on bromobenzene lethality from phenobarbital-like initially to 3MC-like at later times coincided with changes in the pattern of hepatic MFO stimulation following PBBs (Dent et al., 1977). The major bromobenzene metabolite produced following pretreatment with phenobarbital differs from that formed after 3MC (Zampaglione et al., 1973; Gillette, 1976). Thus, this change in pattern of enzyme stimulation may account for the time-dependent alterations in bromobenzene lethality following PBBs.

Multiple metabolic pathways appear to exist for other xenobiotics such as carbon tetrachloride. Similar to bromobenzene, carbon

tetrachloride hepatotoxicity is modified in a different manner by phenobarbital than by 3MC (Suarez et al., 1972; Maling et al., 1974; Carlson, 1975). Thus, subsequent treatment with chemicals like bromobenzene or carbon tetrachloride might result in a time course of toxicity in immature animals different than that of adult animals pretreated with PBBs because of differences in the pattern of microsomal enzyme stimulation. Although carbon tetrachloride time-course studies following pretreatment with PBBs have not yet been performed in either adult or immature animals, dietary exposure of rats to PBBs has increased carbon tetrachloride hepatotoxicity (Kluwe et al., 1978). Similarly, dietary exposure of rodents to PBBs has increased the hepatotoxicity of chloroformm which is more toxic following pretreatment with either phenobarbital or 3MC (Lavigne and Marchand, 1974; Kluwe et al., 1978).

Toxicity of chemicals metabolized in liver and/or excreted by liver into bile may be affected by changes in other hepatic functions. The role of hepatic function in limiting the toxicity of many of these compounds was demonstrated when bile duct ligation resulted in enhanced drug toxicity (Klaassen, 1973). Stimulation of hepatic blood and bile flow and biliary excretion has resulted from treatment with phenobarbital-like agents but not from 3MC-like agents (Klaassen, 1970). Increased hepatic excretory function following phenobarbital-like agents has been associated with more rapid elimination and decreased toxicity of chemicals, such as ouabain, that do not require biotransformation for hepatic excretion (Klaassen, 1974; Damm et al., 1973). Age-dependent alterations in excretion of drugs, including

ouabain, via liver have also coincided temporally with the pattern of microsomal enzyme stimulation following PBBs (Cagen et al., 1977a,b).

Therefore, alterations in hepatic function and microsomal enzyme activity following PBBs depend on the degree and timing of exposure to PBBs and developmental status. Changes in hepatic microsomal enzyme activity produced by PBBs may affect metabolism and, consequently, the toxicity of certain subsequently administered xenobiotics.

Increased microsomal enzyme activity can also result in accelerated metabolism of endogenous compounds including fat-soluble vitamins and steroid hormones (Conney, 1967). Although 3MC has little or no effect on metabolism of steroid hormones, hydroxylation of testosterone, estrogens and progesterone has been increased following treatment with phenobarbital or PCBs (Conney and Klutch, 1963; Levin et al., 1968, 1969; Nowicki and Norman, 1972). In this investigation, activity of 16αPH and 6βPH was increased in liver from rats 28 days of age that had been perinatally exposed to 100 ppm PBBs (Figure 11). Increased hepatic progesterone hydroxylase activity in vitro was reflected in vivo by a reduced response to a pharmacologic dose of progesterone and a reduced concentration of progesterone equivalents in serum and target tissue (Figures 24-26).

Vitamin A is essential for normal growth and maintenance of specific organ functions such as vision and reproduction (Mason, 1939; Thompson, 1969). This fat-soluble vitamin takes part in a dynamic shift between liver, serum and vitamin A requiring tissues (Ganguly, 1960; Olson, 1968). Vitamin A ester, the storage form in liver is continuously hydrolyzed to vitamin A alcohol and then excreted into

serum, binding with retinol-binding protein (Goodman, 1974). Vitamin A concentration in serum is held constant as long as liver stores are not exhausted (Goodman, 1974).

In this investigation, hepatic vitamin A, expressed per g liver or per liver, was reduced at 28 days in a dose-dependent manner by perinatal exposure to 10 or 100 ppm PBBs (Table 4). When animals were maintained on diet containing 100 ppm PBBs, vitamin A in liver continued to be lower than controls at 56 and 100 days of age. However, the reduction in hepatic vitamin A following 100 ppm PBBs was greatest at 28 days of age suggesting an age-related increase in ability to compensate for this effect.

The mechanism by which the vitamin A concentration in liver was reduced by PBBs has not yet been determined. Vitamin A is hydroxylated by hepatic microsomal enzymes and conjugated with glucuronic acid prior to its excretion into bile (Olson, 1969; De Luca and Roberts, 1969). Activity of hydroxylating and conjugating enzymes has been increased and bile flow stimulated by PBBs (Dent et al., 1976a,b; Cagen and Gibson, 1978; McCormack et al., 1979). Thus, PBBs may decrease the hepatic concentration of vitamin A by increasing hydroxylation and/or conjugation of vitamin A and/or by increasing biliary excretion of vitamin A.

Cows and monkeys fed PBBs developed dermal lesions including hyperpigmentosis and hyperkeratosis which are suggestive of vitamin A deficiency (Jackson and Halbert, 1974; Moorhead et al., 1978; Allen et al., 1978). However, in this investigation PBBs did not affect the concentration of vitamin A in serum or kidney (Tables 4 and 11).

Therefore, it is not clear whether or not hepatic stores of vitamin A were sufficiently depleted to contribute to retarded growth and development observed in rats perinatally exposed to 100 ppm PBBs (Tables 4 and 11).

Heme formation, which is essential for production of cytochromes and hemoglobin, may occur in all aerobic cells but is particularly active in erythroid components of liver and bone marrow (DeMatteis, 1975). Under normal conditions heme biosynthesis is efficiently regulated, usually by end product inhibition of the regulatory enzymes Δ-aminolevulinic acid (ALA) synthetase (major) and ALA dehydratase (minor), with little waste of intermediates (Lehninger, 1975). However, in porphyria, control of heme biosynthesis via these mitochondrial enzymes is affected such that far more precursors are synthesized than are utilized in heme production so that they accumulate and are excreted in excess (DeMatteis, 1975).

Perinatal and continuous exposure to PBBs resulted in porphyria. The concentration of coproporphyrin (COPRO) and of uroporphyrin (URO), intermediates in the heme biosynthetic pathway, was increased in liver following PBBs (Table 4). The concentration of COPRO was also increased in urine after exposure to PBBs (Table 6). Increased accumulation and excretion of these intermediates and previous reports of increased concentrations of cytochromes in liver after PBBs suggest that heme biosynthesis may be altered in rats exposed to PBBs (Dent et al., 1976a, 1977; Moore et al., 1978). The mechanism(s) by which PBBs affect heme biosynthesis has not been determined. Inhibition of uroporphyrin decarboxylase may account, at least in part, for increased accumulation and excretion of porphyrins following PBBs. URO

decarboxylase has been inhibited by hexachlorobenzene and PCBs which also produce porphyria (DeMatteis, 1975; Elder et al., 1976). In contrast to previous reports following PCBs (Goldstein et al., 1974), the concentration of URO in rat urine was not affected by PBBs.

Although urine from animals in this investigation fluoresced slightly on gross examination under ultraviolet light, no macroscopic fluorescence was detected in feces, liver, kidney, intestine or bone marrow. This suggests that PBBs may be only slightly porphyrigenic in rats compared to PCBs which increased tissue porphyrin concentrations to the extent that gross fluorescence was detected (Vos et al., 1970, 1971; Vos and Notenboom-Ram, 1972; Kimbrough et al., 1972). However, since compensation may have occurred during perinatal exposure to PBBs, valid comparison of the porphyrigenic potential of PBBs vs. PCBs would require identical treatment regiments.

Hemoglobin and packed cell volume have been reduced by PCBs (Vos and Beems, 1971; Bruckner et al., 1974). In this investigation, PBBs had no effect on packed cell volume at either 28 or 112 days of age (Table 7). Since anemia was not produced, PBBs may not affect the overall rate of hemoglobin synthesis in rats. Comparison of these results to those obtained in rats following PCBs may be only suggestive as the treatment regimen used in this investigation was unique (i.e., treatment from gestational day 8).

Porphyria has been produced in other species by PBBs. Japanese quail treated with PBBs had increased fluorescence of tissues including liver, kidney and intestine which correlated with accumulation of 2-, 4- and 8-carboxyporphyrins in liver and 4-, 6-, 7- and 8-carboxyporphyrins in kidney (Strik, 1978). Activity of ALA synthetase

was also increased in Japanese quail following PBBs (Strik, 1978). On the basis of macroscopic fluorescence of urine, total porphyrin values of 100 µg/l or greater and abnormal thin-layer chromatography porphyrin patterns it was estimated that 1/4 to 1/2 of Michigan farm family members exposed to PBBs had coproporphyrinuria and chronic hepatic porphyria (Strik, 1978). Collectively, these effects and previously discussed effects of PBBs on cytochrome P-448, AHH activity and the immune system suggest that certain PBB congeners may bind to the TCDD receptor.

C. Kidney

The kidney weight-to-body weight ratio was not altered by treatment with PBBs in this investigation (Figure 4). Although glomerular degenerative changes were observed following 90 days exposure of adult rats to 100 ppm PBBs, similar changes were seen in control animals but less frequently (Figure 13). The significance of this quantitative difference in histopathological alterations is not readily apparent and these alterations may have been artifacts. Since kidney enlargement and more severe renal degenerative changes have been observed in dairy cows after Firemaster and rats after octabromobiphenyl, nephrotoxicity of PBBs may be dependent on species and chemical form of PBBs administered (Jackson and Halbert, 1974; Norris et al., 1974; Moorhead et al., 1978). The paucity of prominent renal histopathological alterations in immature and adult female rats after PBBs correlated with a lack of effect of PBBs on renal function in rats.

Blood urea nitrogen (BUN) is used as a standard estimate of glomerular function; as glomerular filtration diminishes BUN

increases. Exposure of adult rats to 100 ppm PBBs for 90 days had no effect on BUN, which was consistent with its lack of effect on GFR prior to or following volume expansion (Tables 8 and 9). Similarly, ERPF and filtration fraction (GFR/ERPF) were unaffected by PBBs (Table 9).

Nephrotoxic agents often modify the ability of the kidney to concentrate urine and also affect the volume of urine excreted (Berndt, 1976). Suggestive of a concentrating defect were findings of polyuria and urine with low specific gravity in cows following PBBs (Mercer et al., 1976). Sodium transport might also be altered by a chemical that affects renal function. In this investigation, urine flow rate and fractional sodium excretion in control and PBBs-exposed rats did not differ before or after volume expansion (Figure 14). These results are also indicative of a species difference between cows and rats in the nephrotoxicity of PBBs.

The in vitro renal slice technique may be more sensitive than in vivo whole body methods for determination of nephrotoxic effects on renal transport processes because blood flow effects are eliminated (Berndt, 1976; Hirsch, 1976). This in vitro technique has been routinely employed to determine the effect of chemicals on renal organic acid (PAH) and organic base (NMN) transport as well as on renal gluconeogenesis and ammoniagenesis (Hirsch, 1976). Treatment of immature or adult rats with PBBs had no significant effect on any of these in vitro parameters of renal function concordant with in vivo results (Tables 12 and 13).

D. Heart

Perinatal and continuous exposure to 100 ppm PBBs did not affect the heart weight-to-body weight ratio and had little effect on cardiac function (Figure 17). Arterial pressure (systolic) and postnatal development of the sympathetic neuronal transport system were unaffected by PBBs (Tables 14 and 16). Although pretreatment with PBBs had no effect on the maximum inotropic response to ouabain, the maximum inotropic response to calcium was greater in atria from PBBs-exposed rats (Table 15). The mechanism by which PBBs affected the inotropic response to calcium has not been determined. However, altered calcium homeostasis may be responsible, at least in part for this effect. Although serum calcium concentrations and bone density were not altered in this investigation, changes in serum and urinary calcium concentrations have been observed in other species following PBBs (Willett and Irving, 1976; Mercer et al., 1976). Calcium homeostasis may be modified by effects of PBBs on hormones including parathyroid hormone, calcitonin and adrenal glucocorticoids and/or modifications in metabolism and/or action of vitamin D.

E. Lung

Perinatal and continuous exposure to 100 ppm PBBs had no effect on the lung weight-to-body weight ratio (Figure 18). The clearance of AI and 5HT and metabolism of 5HT by isolated perfused lungs from rats perinatally exposed to 100 ppm PBBs was reduced (Table 17). However, PBBs had no effect on activity of ACE or MAO in pulmonary homogenates even though MAO was quantified using 5HT as substrate. These results suggest that exposure to PBBs decreased delivery of substrate to

enzymes in the isolated perfused lung. Although the mechanism(s) for these effects has not been determined it may be related to perfusion deficiencies possibly secondary to alterations in pulmonary lipid metabolism such as have been reported following PCBs (Shigematsu et al., 1978).

F. Extrahepatic Microsomal Enzyme Stimulation

As previously discussed, in this investigation PBBs did not have a direct effect on renal function and had little effect on pulmonary function. However, microsomal enzyme activity was altered in kidney and lung following PBBs (Figures 15, 16 and 20). Although in most instances liver is quantitatively the dominant metabolic organ, extrahepatic organs may, in certain circumstances play a major role in determining the physiological, pharmacological and/or toxicological action of an agent. For example, extrahepatic organs may play a significant role in metabolism when liver is damaged, its metabolic activity inhibited or when the rate of metabolism of a compound by liver is low; when extrahepatic sites receive the compound before liver; and when an extrahepatic site produces an active metabolite especially if different from metabolites produced in liver (Lake et al., 1973).

Pretreatment with PBBs has increased the nephrotoxicity of subsequently administered CCl_4 and $CHCl_3$ (Kluwe et al., 1978). These short-chain chlorinated hydrocarbon solvents may be more nephrotoxic following PBBs because of enhanced production of a chemically-reactive intermediate by MFOs (Kluwe et al., 1978). Since these metabolites, the ultimate toxicants, are highly reactive and unstable they may not

be transported from liver to extrahepatic tissues. Thus, if metabolic activation of CCl_4 and $CHCl_3$ to ultimate toxicants is an essential step in nephrotoxicity produced by these solvents it may occur in kidney (Kluwe et al., 1978).

In this investigation, activity of MFOs (enzymes which may mediate hepatotoxicity and possibly nephrotoxicity of CCl_4, $CHCl_3$ and numerous other chemicals) in kidney was altered by PBBs. Following acute administration of PBBs to 7-day old rats, renal microsomal enzymes sensitive to 3MC-treatment (AHH and EROD) exhibited patterns of stimulation similar to those observed in immature liver (Figure 15). However, PBBs did not increase activity of renal microsomal enzymes (EH and Hex-OH) which in liver are sensitive to phenobarbital treatment (Figure 15). This observation is consistent with a previous report that when administered to rats, phenobarbital did not increase MFO activity in kidney (Uehleke and Greim, 1968). Treatment with phenobarbital increased the hepatotoxicity but not the nephrotoxicity of $CHCl_3$ in mice (Kluwe et al., 1978). Since PBBs increased hepatic and renal MFO activity as well as both the hepatotoxicity and nephrotoxicity of subsequently administered $CHCl_3$, the nephrotoxic $CHCl_3$ metabolite may have been produced in kidney (Kluwe et al., 1978).

Stimulation of microsomal MFO system, reflected by an increased activity of AHH, without a concomitant increase in activity of EH following PBBs was not a phenomenon limited to immature kidney or to a particular treatment regimen. This pattern of enzyme stimulation was observed in adult kidney, and immature and adult lung following a variety of acute or subacute treatments with PBBs (Figures 16 and

20). If binding to a cytosolic receptor (TCDD receptor) is essential for stimulation of AHH, these results suggest that the TCDD receptor exists in kidney and lung. Biosynthetic control of EH activity in these organs may be different from that in liver (Schmassman et al., 1978). By analogy, kidney and lung may not possess a phenobarbital receptor. Increased MFO activity without stimulation of EH in extrahepatic tissues following PBBs may be of toxicological concern. Many relatively inert compounds, such as aromatic polycyclic hydrocarbons, require metabolic conversion to electrophilic intermediates (epoxides) which may subsequently react with cellular nucleophiles including protein, DNA and RNA to produce deleterious effects (Miller, 1970; Daly et al., 1972; Oesch, 1973; Sims and Grover, 1974). Activity of AHH may represent the potential for formation of such reactive intermediates. Such arene oxides or epoxides may, in some cases, be detoxified by spontaneous rearrangement to phenols, conjugation with glutathione by cytosolic transferases and/or subsequent metabolism to dihydrodiols by membrane bound EH (Daly et al., 1972; Oesch, 1973; Jerina and Daly, 1974). Particularly important may be EH, since it is localized in the same cell compartment as the epoxide generating MFO system and since metabolically formed arene oxides, which are lipophilic, preferentially remain in the membrane and may reach the nucleus by lateral diffusion (Oesch and Daly, 1971; Schmassmann et al., 1978).

Although the relative balance in activity of an activating (AHH) and often inactivating (EH) enzyme may be altered in extrahepatic organs following PBBs, it is not yet possible to accurately predict the hazard associated with this effect. Exposure to PBBs may increase

the quantity of arene oxides available in extrahepatic tissues for initiation of a toxic response. However, the role of EH in overall metabolism in vivo of potentially carcinogenic polycyclic hydrocarbons to ultimate carcinogens has not been established. Evidence obtained using hepatic microsomes suggests that EH may act as an inactivating enzyme or as a coactivating enzyme, as in the case of benzo(a)pyrene metabolism which was previously noted (Sims et al., 1974; Wood et al., 1976). The role of extrahepatic EH in subsequent metabolism of arene oxides has not been determined. Furthermore, little is known about activity of cytosolic transferases in extrahepatic tissues of rat following exposure to PBBs.

G. Response to Xenobiotics

Alterations in microsomal enzyme activity can result in modifications in duration of action of certain subsequently administered compounds. Pentobarbital sleeping time was inversely related to microsomal enzyme activity and body burden of PBBs (Figure 4, Table 3). The central depressant action of pentobarbital, an intermediate-acting barbiturate, is terminated primarily by biotransformation in reactions catalyzed by microsomal MFOs (Cooper and Brodie, 1957). Digitoxin is also a substrate for MFOs and inducers, such as phenylbutazone and phenobarbital, have been demonstrated to accelerate metabolism of this cardiac glycoside (Bigger and Strauss, 1972). In this investigation, perinatal and continuous exposure to 100 ppm PBBs reduced the toxicity of digitoxin suggesting that PBBs increased digitoxin metabolism and/or excretion (Table 21). Although

pentobarbital sleeping time and digitoxin toxicity were reduced following PBBs, duration of anesthesia after exposure to diethyl ether was not affected by pretreatment with PBBs (Figure 23). In contrast to pentobarbital and digitoxin which are extensively metabolized, more than 90% of absorbed diethyl ether is excreted unchanged in expired air (Van Dyke et al., 1964). These results suggest that the duration of action of therapeutic agents, such as pentobarbital and digitoxin as well as the anticoagulants bishydroxycoumarin and warfarin, which require biotransformation for excretion may be decreased following pretreatment with PBBs.

As previously discussed, alterations in activity of microsomal MFOs can result in altered susceptibility to subsequently administered toxic compounds. Treatment of rats with phenobarbital increased bromobenzene hepatotoxicity, whereas pretreatment of rats with 3MC protected against bromobenzene toxicity (Ried et al., 1971; Jollow et al., 1974). It has been postulated that in phenobarbital-treated rats formation of the putative hepatotoxicant, bromobenzene-3,4-epoxide is stimulated (Zampaglione et al., 1973). In rats, 3MC pretreatment enhances formation of the possibly less toxic 2,3-epoxide of bromobenzene (Gillette, 1976). Bromobenzene lethality was increased in 49 day old rats by neonatal treatment with PBBs (Table 20). At this age, phenobarbital-sensitive enzymes were also stimulated by PBBs suggesting predominance of the pathway for production of bromobenzene-3,4-epoxide. Treatment with PBBs also has increased the toxicity of subsequently administered carbon tetrachloride and chloroform (Kluwe et al., 1978). Thus, the potential exists for multiple toxic chemical interactions following exposure to stimulating agents such as PBBs.

H. Response to Exogenously Administered Steroid Hormones

Xenobiotics are not the only substrates for microsomal enzymes. Fat-soluble vitamins and steroid hormones are endogenous substrates for the microsomal MFO system (Conney, 1967). Actually, the apparent Km values for various steroid hormones have been demonstrated to be lower than those for many xenobiotics suggesting that endogenous compounds may be preferred substrates *in vivo* (Chung *et al.*, 1975). Metabolism of steroid hormones by NADPH-dependent hepatic microsomal enzymes has been accelerated *in vitro* following pretreatment with other inducing agents such as PCBs and phenobarbital (Kuntzman *et al.*, 1964; Conney *et al.*, 1966; Nowicki and Norman, 1972). Metabolism of steroid hormones has also been enhanced *in vivo* following PCBs or phenobarbital and has been correlated with modified action of exogenously administered steroids including progesterone, testosterone and estradiol (Conney *et al.*, 1966; Levin *et al.*, 1968, 1969; Orberg and Ingvast, 1977; Derr, 1978).

Effects of certain mixtures of PCBs suggest that these compounds may modify endogenous steroid hormone metabolism to the extent that physiological sequelae are noted. Adrenal weights and plasma corticosterone concentrations have been modified in mice, rats and rhesus monkeys by PCBs (Wasserman *et al.*, 1973; Sanders *et al.*, 1974; Barsotti and Allen, 1975). Urinary androgen in boars and seminal vesicle weight and spermatogenesis in mice have been reduced by pretreatment with PCBs (Platonow *et al.*, 1972; Sanders *et al.*, 1974; Sanders and Kirkpatrick, 1975). Certain mixtures of PCBs cause uterine atrophy, reduced plasma progesterone concentrations, ovarian stromal changes

and lengthened estrus cycle in rodents (Vos and Beems, 1971; Jonsson et al., 1976; Orberg and Kihlstrom, 1973; Kimbrough et al., 1978). Rhesus monkeys consuming diet containing PCBs had lengthened menstrual cycles (Allen and Barsotti, 1976). Women with Yusho disease, caused by consumption of rice oil contaminated with a mixture of PCBs and traces of other chlorinated compounds, also had mentrual cycle irregularities, dysmenorrhea and altered serum concentrations of ketosteroids (Kuratsune et al., 1972).

In this investigation, metabolism and/or excretion of exogenously administered steroid hormones was accelerated in vivo following perinatal exposure to PBBs. Responses to steroid hormones were generally modified in a manner directly related to dose of PBBs and were correlated with changes in serum and target tissue concentrations of steroids and metabolites (Figures 24-32). Such alterations were consistent with the increased activity of microsomal enzymes, including steroid hormone hydroxylases, in hepatic microsomes prepared from rats perinatally exposed to PBBs (Figures 10 and 11).

Increased catabolism of progesterone following PBBs was reflected by reduced duration of anesthesia produced by a pharmacological dose of progesterone (Figure 24). Shorter sleeping time for rats pretreated with PBBs was correlated with decreased concentration of progesterone equivalents (total radioactivity) in brain and serum 3 hr following progesterone when rats exposed to PBBs were awake and controls asleep (Figure 26). No differences in brain radioactivity were seen when the comparison was made at the time of waking. Ratio of EA to HEX extractable radioactivity, which may be a gross index of polar vs. nonpolar metabolites and parent progesterone, in brain was

increased at 3 hr, but not waking, by PBBs, further suggesting that PBBs increased progesterone hydroxylase activity. Stimulation of steroid-hydroxylases by PBBs was also reflected in vivo by reduced response to exogenously administered testosterone and estradiol-17β (Figures 27 and 30). Diminished steroid action was associated with reduced concentrations of testosterone and estradiol-17β equivalents in serum as well as testis and uterus, respectively (Figures 28, 29, 31 and 32).

Although perinatal exposure to PBBs reduced serum concentrations of exogenously administered steroid hormones, exposure to 100 ppm PBBs from day 8 of gestation had no effect on the concentration of endogenous progesterone or testosterone in serum when determined at 100 days of age (Table 18). Perinatal and continuous exposure to PBBs also did not affect testis weight- or ovary weight-to-body weight ratios when determined at 100 days of age (Figure 21). Similarly, PBBs had no effect on age of testes descent, vaginal perforation, or estrus cycle length (Table 19). Thus, compensation for accelerated steroid hormone catabolism apparently occurred in rats following perinatal exposure to 100 ppm PBBs.

The compensatory mechanism(s) involved in maintaining normal steroid hormone concentrations in serum following PBBs has not yet been identified. Alterations in hypothalamic function such as increased secretion of gonadotropin-releasing hormone (GnRH) may be mediated by decreased steroid feedback. Increased GnRH may then act on the pituitary to increase secretion of gonadotropins including follicle-stimulating hormone (FSH), luteinizing hormone (LH) and/or

prolactin. These gonadotropins would stimulate the gonads to produce additional steroid hormones to compensate for increased steroid metabolism and/or excretion produced by PBBs.

Compensation for increased steroid hormone catabolism may not always be adequate. Harris et al. (1978) reported that prenatal administration of PBBs (10 mg/kg PBBs/day on days 7-15 of gestation via oral gavage) to rats delayed vaginal opening. Expression of estrogen positive-feedback is critical for initiation of puberty in female rats (Ying and Greep, 1971; Caligaris et al., 1972; Parker and Makesh, 1976). Therefore, increased catabolism of estrogens produced by PBBs may have resulted in estrogen concentrations insufficient for positive feedback. Dietary exposure to PBBs has produced endocrine alterations in other species further suggesting that PBBs enhance endogenous steroid metabolism. Monkeys had lengthened menstrual cycles after consuming PBBs and this effect was associated with flattened and lengthened serum progesterone peaks (Lambrecht et al., 1978). Monkeys exposed to PBBs had hypoactive seminiferous tubules (Allen et al., 1978). Young bulls fed high doses of PBBs had testicular atrophy and reduced spermatogenesis (Jackson and Halbert, 1974; Kimbrough et al., 1978).

Certain transient effects result from rapid changes in quantities of steroid hormones at target tissues. If a latency period exists before accelerated steroid hormone catabolism produced by PBBs is compensated for, these effects may be reduced in magnitude or not elicited. For example, following exposure to PBBs the rise in corticosterone following acute stress may be insufficient for adaptation to the stressful stimulus.

The potential for decreased response to stress or diminished reproductive capacity following PBBs can not yet be accurately predicted as effects of PBBs on steady-state concentrations of endogenous steroid hormones in serum and target tissues and endocrine and neuroendocrine compensatory mechanisms have not been fully determined. However, ability to respond to stress and fertility may be reduced following PBBs as a consequence of enhanced steroid hormone metabolism. Since oral contraceptive use is prevalent, the possibility also exists that fertility may be increased following exposure to PBBs as a consequence of accelerated steroid catabolism.

I. Persistence of Effects

A single i.p. injection of 150 mg/kg PBBs to 7-day old rats produced measurable tissue concentrations of PBBs for at least 63 days (Table 22). Similarly, neonates treated with a s.c. injection of PBBs had detectable concentrations of PBBs in tissues at 56 days of age (Table 3). This investigation demonstrated that retention of PBBs after i.p. administration was due, at least in part, to very slow disappearance of these compounds from the peritoneal cavity (Figure 33). The rate at which PBBs were eliminated could not be determined from these data. However, another study in rats with 2,4,5,2',4',5'-hexabromobiphenyl, the major component of Firemaster BP-6, suggested that although approximately 90% of a single oral dose was absorbed less than 10% of the total dose would ever be excreted (Matthews et al., 1977).

Hepatic microsomal enzymes were stimulated for at least 14 days in adult rats treated with an i.p. injection of PBBs (Dent et al.,

1976). Treatment of 7-day old rats with 150 mg/kg PBBs increased activity of EH, AHH, Hex-OH and GSH-transferase in liver and EROD and AHH in kidney for at least 63 days (Figures 8, 9 and 15). Treatment of neonates with 150 or 500 mg/kg PBBs resulted in similar alterations in enzyme activity at 56 days of ages which were correlated with decreased duration of pentobarbital anesthesia, an *in vivo* index of microsomal enzymes activity (Figure 22). Since PBBs disappeared slowly from the peritoneal cavity and were detected in tissues even 63 days following administration, constant exposure of liver and kidney to slowly absorb and retain PBBs may account, at least in part, for persistence of these effects.

The apparent half-time for disappearance of PBBs from the peritoneal cavity was approximately 9-10 days. Only 61.5% of the administered dose was in the peritoneal cavity 24 hr after administration suggesting that an initial phase with a shorter half-time contributed prominently to disappearance of PBBs during the first 24 hr. Although material remaining in the peritoneal cavity during the first 7 days after an i.p. injection was the same as that administered, preferential disappearance of hexa-congeners relative to hepta-congeners was observed 14 and 28 days after treatment. Pharmacokinetic studies to determine relationships between structure and bioaccumulation factors for PBB congeners have only recently been initiated as pure congeners were not previously available. However, these data are consistent with previous investigations using PCBs which suggest that biological half-life, which is affected by rates of accumulation, metabolism and excretion, is directly related to chlorine content (Matthews and Anderson, 1975; Report, 1976).

Concentrations of PBBs in most tissues decreased markedly between days 28 and 56 or 63 following a single injection as did the quantity of PBBs remaining in the peritoneal cavity (Tables 3 and 22). Several factors including enhanced elimination of PBBs, increased total organ mass and redistribution of PBBs to developing fat deposits may have contributed to reductions in tissue concentrations of PBBs between these times. Redistribution of lipophilic PBBs to fat may have been a major factor following i.p. administration since most tissue concentrations of PBBs decreased while fat concentrations of PBBs increased. Formation of fat deposits may have also accounted for the final disappearance of PBBs from the peritoneal cavity.

Dietary exposure to PBBs also resulted in their accumulation in tissues. High concentrations of bromine were detected in rat tissues, particularly fat, 18 wk following termination of 4 wk dietary exposure to 10, 100 or 1000 ppm octabromobiphenyl (Lee et al., 1975). In this investigation, tissues from rats exposed to PBBs transplacentally and via mothers milk (100 ppm PBBs in dam's diet) and weaned (28 days of age) onto diet free of PBBs had detectable concentrations of PBBs at 328 days of age (Table 24). At 10 months after terminating exposure to PBBs (328 days of age), the concentration of PBBs in liver, kidney and fat were approximately 15%, 49% and 35% of respective tissue concentrations at weaning, indicating slow excretion of PBBs.

Activity of AHH in liver and kidney and EH in liver remained increased above control values throughout the 10 month residual phase (Figures 35 and 36). Diminutions in AHH stimulation with time after exposure to PBBs were similar to declines in tissue concentrations of

PBBs. Stimulation of AHH in liver and kidney at 328 days was 19% and 40%, respectively, of that observed at 28 days of age suggesting that AHH activity was dependent on tissue concentrations of PBBs. Increased MFO activity was correlated with decreased duration of anesthesia following pentobarbital (Figure 37). Since perinatal exposure to PBBs increased MFO activity for at least 300 days following treatment, the potential for toxic interactions between secondary compounds may be increased for a long time and exaggerated in an environment containing many xenobiotics. Furthermore, persistent stimulation of microsomal enzymes by PBBs may produce long-lasting subtle alterations in endogenous compounds that may be manifest as physiological sequelae during stressful episodes or in individuals with deficiencies in compensation.

Hepatic histopathological lesions were observed throughout the PBBs residual phase. However, effects of PBBs were most severe at weaning and decreased with time following exposure as did MFO activity and tissue concentrations of PBBs. Perinatal exposure to PBBs increased liver weight-to-body weight ratio at 28 and 150 but not 328 days of age (Figure 34). Nevertheless, persistence of PBBs in tissues and effects on microsomal enzyme activity and liver morphology throughout the 300 day residual phase emphasize the long-lasting effects of perinatal exposure to PBBs. These results suggest that PBBs, which undergo transplacental movement and excretion into milk, are efficiently absorbed from the gastrointestinal tract and are only very slowly eliminated from the body.

Subsequent generations may be affected by stable, lipophilic PBBs as assimilated PBBs are released with body fat which is mobilized during lactation (Fries et al., 1978). In this investigation, rates of body weight gain and survival were reduced only in pups (F_1-100) born to and suckled by dams maintained on diet containing 100 ppm PBBs (Figures 38 and 39). However, the liver weight-to-body weight ratio was increased in progeny (F_2-100) of F_1-100 rats and in offspring (F_1-10) of dams fed 10 ppm PBBs (Figure 38). Liver enlargement was associated with increased activity of microsomal enzymes. Activity of AHH was increased in liver from both F_1 and F_2 descendants of rats treated with 10 or 100 ppm PBBs (Figure 40). Similarly, hepatic EH was increased in F_1-100, F_2-100 and F_1-10. By the third generation (F_3-100), activity of hepatic AHH and EH did not differ from controls (Figure 40). Stimulation of hepatic AHH in F_1 and F_2 generations was correlated with a reduced duration of anesthesia following treatment with either pentobarbital or progesterone (Figures 42 and 43). Enzymatic and morphologic changes were correlated with hepatic concentrations of PBBs (Table 25).

These results demonstrate that PBBs can be transferred from one generation to the next to produce alterations in liver size and ability to metabolize xenobiotic and endogenous compounds. Therefore, the health hazard associated with exposure to PBBs may not be limited to a single generation.

SUMMARY AND CONCLUSIONS

This series of experiments was designed to determine if functional and metabolic alterations are produced by prenatal and/or postnatal treatment with PBBs. In this investigation, numerous biochemical and physiological sequelae to perinatal exposure to PBBs were identified. Effects of PBBs occurred over many levels of biological organization from retarded body weight gain and physical development to macroscopic changes in organ morphology to microscopic changes in tissue architecture to submicroscopic changes in enzyme activity. Doses of PBBs used to produce these effects in rats were chosen to approximate maximal human exposure. The highest reported concentrations of PBBs detected in human milk were between 90 and 100 ppm (Eyster, 1976; Cordle et al., 1978). However, the median concentration of PBBs in milk from Michigan women may be much lower (0.07 ppm) (Brilliant et al., 1978).

Dietary exposure of dams to 100 ppm PBBs from day 8 through day 20 of gestation, when caesarean sections were performed, did not alter litter size, resorption rate, fetal body weight or length or incidence of gross, soft tissue or skeletal anomalies. Food deprivation in combination with this treatment increased fetal resorption rate and decreased fetal body weight. Treatment with 150 or 500 mg/kg PBBs on day 1 postpartum had no effect on growth, development or mortality. However, after 1 wk postpartum growth and development were retarded

and mortality increased in pups born to and suckled by dams fed 100 ppm PBBs. Consistent with these effects, concentrations of PBBs in tissues from animals exposed to PBBs transplacentally and via mother's milk were higher than concentrations in tissues from rats neonatally treated with a single injection of PBBs.

At birth, milk from dams maintained on diet containing PBBs from the 8th day of pregnancy had a concentration of PBBs that was 3-4 times higher than the dietary concentration. However, by 14 days postpartum concentrations of PBBs in milk and diet were similar. Therefore, pups suckling dams fed PBBs received higher doses of PBBs during the period shortly after birth than at later times. The latent period for manifestation of effects postnatally may have been related to the time required for PBBs to accumulate to concentrations in tissues sufficient to retard growth and development and increase mortality.

The liver weight-to-body weight ratio was increased following perinatal exposure to 10 or 100 ppm PBBs and after neonatal treatment with 150 or 500 mg/kg PBBs. Liver enlargement was dose dependent and directly related to hepatic concentrations of PBBs. Increased liver weight may have been due, at least in part, to elevated protein content as hepatic microsomal protein was increased by PBBs. Microscopic morphologic alterations were also produced by PBBs. Liver from rats perinatally exposed to 100 ppm PBBs had vacuolation, hepatocyte swelling, necrosis, absent or pycnotic nuclei and myelin bodies.

Perinatal exposure to PBBs produced porphyria. The concentration of coproporphyrin and uroporphyrin, intermediates in the heme biosynthetic pathway, was increased in liver following PBBs. The

concentration of coproporphyrin was also increased in urine after PBBs. However, PBBs had no effect on packed cell volume. Therefore, although PBBs altered heme biosynthesis the overall rate of hemoglobin synthesis may not have been affected by PBBs.

Kidneys from rats exposed to PBBs did not have prominent, if any, macroscopic or microscopic morphological changes. The paucity of renal structural alterations was correlated with a lack of effect of PBBs on renal function. Similarly, perinatal exposure to PBBs did not affect the lung weight- or heart weight-to-body weight ratios and had little effect on pulmonary or cardiac function.

Activity of microsomal enzymes in liver and extrahepatic organs was altered by PBBs in a manner that was dependent on dose, age and time following administration. These enzymatic changes produced by PBBs were correlated with modifications in the toxicity and/or duration of action of certain subsequently administered therapeutic agents and environmental chemicals. Thus, even animals that exhibited no visible expression of PBBs toxicity were more susceptible to drug interactions after exposure to PBBs. However, effects of PBBs may depend on a variety of factors including: the mechanism by which the secondary chemical elicits its effects, site at which the chemical is metabolized, degree and timing of exposure to PBBs and developmental status. These factors add to the dilemma of predicting drug interactions after exposure to PBBs.

Alterations in microsomal enzyme activity may also affect the physiological action of fat-soluble vitamins and steroid hormones which are metabolized in reactions catalyzed by microsomal enzymes. Although the concentration of vitamin A in liver was reduced by

perinatal exposure to PBBs, the concentration of vitamin A in serum and kidney was not affected by PBBs. Therefore, it is not clear whether or not hepatic stores of vitamin A were sufficiently depleted to contribute to retarded growth and development, symptoms of vitamin A deficiency, observed in rats perinatally exposed to 100 ppm PBBs.

Activity of progesterone hydroxylases was increased in hepatic microsomes prepared from rats perinatally exposed to 100 ppm PBBs. Accelerated metabolism of progesterone *in vitro* was reflected *in vivo* by a reduced duration of anesthesia following a pharmacologic dose of progesterone. Similarly, responses to exogenously administered estradiol-17β and testosterone were diminished by perinatal exposure to PBBs. Radioactivity in serum and target tissues following administration of labeled steroid hormones was also reduced by PBBs. Despite increased steroid catabolism, endogenous concentrations of steroids and rate of maturation of secondary sexual characteristics were not altered by exposure to PBBs. Thus, compensation for accelerated steroid hormone catabolism apparently occurred in rats exposed to PBBs.

The potential for diminished reproductive capacity, decreased response to stress or production of fat-soluble vitamin deficiencies following PBBs can not yet be accurately predicted. Effects of PBBs on steady-state concentrations of endogenous steroid hormones and fat-soluble vitamins in serum and target tissues and endocrine and neuroendocrine compensatory mechanisms have not been fully determined. However, ability to respond to stress and fertility may be reduced and vitamin deficiencies produced by PBBs as a consequence of enhanced metabolism.

Perinatal exposure to 100 ppm PBBs modified microsomal enzyme activity for at least 300 days following treatment termination. Therefore, the potential for toxic interactions between secondary compounds may be increased for a long time after exposure to PBBs and exaggerated in an environment containing many xenobiotics. Persistent stimulation of microsomal enzymes by PBBs, which remained in tissues throughout the residual phase, may also produce long-lasting subtle alterations in endogenous compounds. Accelerated catabolism of fat-soluble vitamins and steroid hormones by PBBs may be manifest as physiological sequelae during stressful episodes or in individuals with deficiencies in compensation.

Effects of PBBs may not only persist for long periods of time in directly exposed individuals but may also be produced in their descendants. PBBs were transferred from one generation to the next via transplacental movement and excretion into milk to produce alterations in liver morphology and ability to metabolize xenobiotic and endogenous compounds. Therefore, the health hazard associated with exposure to PBBs may not be limited to a single generation.

Perinatal exposure to PBBs resulted in a variety of biochemical and physiological sequelae. Many effects or potential effects of PBBs were consequences of persistent and complex alterations in activity of microsomal enzymes. Complete demonstration of the mechanism of a particular effect would require study of discrete PBB congeners and trace contaminants in the Firemaster BP-6 mixture. While the active component(s) of PBBs have not been identified, it remains that the Firemaster BP-6 mixture was the source of environmental contamination.

Therefore, the data obtained in this investigation will assist assessment of the potential health hazard posed by PBBs to developing mammals.

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