This is to certify that the

dissertation entitled

EFFECT OF POLYBROMINATED BIPHENYL TREATMENT
ON TARGET ORGAN ANDROGEN METABOLISM
AND BRAIN SEXUAL DIFFERENTIATION

presented by

Donald Leroy Wilke

has been accepted towards fulfillment
of the requirements for

doctoral degree in Pharmacology/
Toxicology

W. Emmett Bradley Jr.
Major professor

Date June 12, 1985

O-12771

EFFECT OF POLYBROMINATED BIPHENYL TREATMENT ON TARGET ORGAN ANDROGEN METABOLISM AND BRAIN SEXUAL DIFFERENTIATION

By

Donald Leroy Wilke

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Pharmacology

1985

ABSTRACT

EFFECT OF POLYBROMINATED BIPHENYL TREATMENT
ON TARGET ORGAN ANDROGEN METABOLISM
AND BRAIN SEXUAL DIFFERENTIATION

By

Donald L. Wilke

Hypothalamic androgen aromatization is an important intermediate step regulating steroid feedback and sexual behavior. Initial studies determined that hypothalamic aromatase (HA) activity is regulated by adult testosterone titer, and is unaffected by perinatal hormone treatments which interfere with brain sexual differentiation. Treatment of adult rats with 150 mg/kg of the PBB mixture fireMaster BP-6 decreased hypothalamic aromatase activity, and reduced circulating testosterone titers 14 days after treatment. Both HA and plasma testosterone titers returned to control values by 90 days after treatment.

Adult administration of 150 mg/kg PBB decreased ventral prostate weights but did not affect 5a-reductase activity *in vitro*. Perinatal PBB treatment (100 ppm in food from day 8 of gestation until 28 days postnatal), failed to affect adult testosterone titer, or testicular testosterone synthesis.

Perinatal PBB treatment resulted in transplacental induction of testosterone hydroxylation beginning just before birth on day 22 of gestation. PBB treated neonates

had decreased blood testosterone titers beginning on day 3 postnatally.

Perinatal PBB treatment demasculinized male copulatory behavior, but did not increase feminine lordosis behaviors in castrated, estrogen primed adult males. Rats treated with PBB perinatally also showed feminization of the plasma LH response to gonadectomy.

These results show that PBB treatment of adult rats lowers blood testosterone and affects neuroendocrine androgen metabolism. Perinatal treatment of rats increases testosterone metabolism and decreases blood testosterone titer during brain differentiation. This effect was associated with impaired male sexual behavior, and feminized adult LH dynamics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION TO THESIS

GENERAL INTRODUCTION

The accidental contamination of cattle feed with a flame retardant mixture during 1973 resulted in the exposure of over 90% of the population of Michigan to the mixture of polybrominated biphenyls known as "fireMaster". Since the discovery of the contamination by a Michigan dairy farmer, the "Poisoning of Michigan" has become the subject of intense scientific investigation, as well as the inspiration for a television "docudrama", the impetus for the reorganization and sale of a chemical company, and has resulted in increased public awareness of the far-reaching consequences of environmental contamination.

During the twelve years following the accident, hundreds of abstracts and papers covering various aspects of the toxicologic and environmental properties of polybrominated biphenyls (PBB) have been presented. Soon after the discovery of PBB contamination of Michigan farms questionaire type surveys of exposed farm families revealed dozens of health complaints associated with PBB exposure. Follow-up studies in affected individuals have confirmed a variety of problems, but have failed to establish a

definite causal link to PBB exposure. One certain result of the accident has been the staggering economic loss suffered by those involved, and estimates of from $25,000,000 to $45,000,000 have been suggested (Dunkel, 1975) for the losses of the farmers alone, without including funds expended in the study, clean-up and litigation which followed the disaster.

One of the first toxic effects of PBB noted was shrinking udders and increased numbers of abortions in contaminated cattle (Jackson and Halbert, 1974). Early work on the contamination problem established that PBBs were potent inducers of the mixed-function oxidase enzymes which normally metabolize xenobiotics and steroid hormones. Animals fed PBB had a variety of reproductive effects associated with treatment, including regression of seminal vesicles and prostates in males and irregular estrus cycling in females (Johnston et.al., 1980). In general, the reproductive effects of PBBs have been blamed on increased MFO activity in treated animals. The primary objective of the work presented here has been to discover additional ways in which PBB may interact with mammalian reproductive systems.

History, Chemistry, and Ecology of the Accident

The contamination of Michigan cattle feed with PBB occured during the summer of 1973 when the Michigan Chemical Corporation (St. Louis, MI), was unable to obtain the red bags in which the flame retardant mixture fireMaster FF-1 was normally distributed. For a period of time, fireMaster was bagged in brown bags similar to those used for the distribution of nutriMaster MgO, a magnesium oxide feed supplement also manufactured by Michigan Chemical. NutriMaster was used as a feed additive by the Michigan Farm Bureau for the purpose of sweetening acidic feeds (Carter, 1976) and as part of a mineral pre-mix to buffer rumen contents, allow the use of high protein feeds to increase milk production, and prevent the development of hypomagnesic tetany (Bekesi et.al., 1979). Because of a warehousing error, Michigan Chemical accidentally shipped at least one pallet of between ten and twenty 50 pound bags of fireMaster to the Michigan Farm Bureau in place of nutriMaster, and the PBB mixture was subsequently incorporated into several lots of feed.

During September of 1973, the Halbert farm began feeding PBB contaminated feed to milk cattle. Within two weeks, Halbert noted decreased appetite and a 40% decrease in milk production among cattle receiving the magnesium supplemented feed. Because of these symptoms the suspect feed was withdrawn 16 days after supplementation was

started. Among the affected cattle Halbert noted symptoms such as increased lacrimation, skin and hoof abnormalities, increased numbers of abortions, and shrinking udders among recently freshened cattle (Jackson and Halbert, 1974). Halbert suspected chemical contamination of his feed, and confirmed his suspicions by feeding some of the suspect feed to previously unaffected calves. Halbert also noted that the pelleted feed "eradicated rodents", and placed some feed in a neighbor's barn, where the normal population of barn rodents also disappeared. When the suspected chemical contaminants in Halbert's feed could not be identified locally, samples were sent to several laboratories across the country in an effort to determine the cause of the symptoms seen in exposed cattle. The USDA laboratories at Ames Iowa tested the feed and failed to find mycotoxins, heavy metals, lindane, or DDT, but did locate some unidentified late=eluting peaks by gas chromatography. In April of 1974, a feed sample was sent to George Fries at the USDA laboratory in Beltsville Maryland, and he identified the contaminants by gas chromatography/mass spectroscopy as polybrominated biphenyls. In May of 1974, the FDA established action levels for PBB of 1 ppm in milk and meat, and 0.3 ppm in feed. The initial action levels were established by comparison with the toxicity of polychlorinated biphenyls (PCB), and 30 herds of cattle were quarantined in May 1974

(Cordle and Kolbye, 1979). Contaminated farms were identified by sampling milk from collection centers. If PBBs were detected in milk from collection centers, then individual farms were tested, and quarantined if necessary. Many farms were probably missed in this initial screening due to dilution of contaminated milk by good milk in collection centers. In November of 1974 as more toxicity data became available and analytical methods improved, the FDA lowered action levels to 0.3 ppm for milk and meat, and 0.05 ppm for feed. Ultimately, 29,800 cattle, 5920 hogs, 1,500,000 chickens and 1470 sheep were destroyed along with 865 tons of feed, 17,990 lb. cheese, 2630 lb. butter, 34,000 lb. milk and 5,000,000 eggs (Kay, 1977).

Although initially at least three lots of feed produced by the Michigan Farm Bureau were contaminated by the direct addition of PBBs to the mix, the long delay between contamination and identification of the problem lead to significant indirect contamination also. Kay (1977) has identified at least three routes of indirect contamination including the use of dead animals as food, contaminated mixing and storage facilities and feed swapping between farms. Contamination of feed mills persisted even following steam and high pressure cleaning of mixing and storage areas. Contaminated feed ingested during the incident contained PBB concentrations ranging from 4000 to 13,500 ppm (Kay, 1977; DiCarlo et. al.,

1978). Cattle and poultry had tissue PBB levels as high as 2700 and 4600 ppm respectively, and milk and eggs contained as much as 595 and 59.7 ppm respectively.

The chemical complexity of the fireMaster mixture has made toxicological study of the contamination difficult. The fireMaster FF-1 originally mixed into cattle feed was compounded from a mixture of PBBs called fireMaster BP-6 to which 2% of calcium trisilicate had been added as an anti-caking agent. FireMaster contains at least 60 distinct compounds, most of which are PBB congeners of different structures and degrees of bromination. The mixture as a whole contains 75% bromine by weight, and is composed of approximately 2% tetra, 4% penta, 63% hexa, and 30% hepta-bromo biphenyls (Kay, 1977; DiCarlo, 1978), along with small amounts of heavier congeners. FireMaster contains twelve distinct peaks which can be resolved by conventional gas chromatography as well as at least 50 other peaks which can be resolved only by capillary gas chromatography (Orti et. al., 1983). The major constituents of fireMaster are 2,4,5,2',4',5-hexabromobiphenyl (HBB) (55%) and 2,3,4,5,2',4',5'-heptabromobiphenyl (23.5%). Nineteen other PBB congeners have been identified, and are listed in Table 1 (Orti et.al., 1983; Moore and Aust, 1978; Robertson et.al., 1984; Kay, 1977; DiCarlo et.al., 1978; Moore et.al., 1980; Dannan et.al. 1983).

Table 1 Structure, concentration and activity of identified polybromobiphenyl congeners in fireMaster FF-1.

congener #	configuration	percent composition	enzyme induction
B	2,3,6,2',5'	.02	
1	2,4,5,2',5'	1.54	PB
1b	2,4,5,2',4'	~.06	
1c	3,4,3',4'	~.01	MC
2	2,4,5,3',4'	.80	PB + MC
3	2,3,6,2',4',5'	.78	
4	2,4,5,2',4',5'	55.2	PB
4a	3,4,5,3',4'	<.01	MC
5	2,3,4,2',4',5'	5.23	PB + MC
6	2,4,5,3',4',5'	3.37	PB + MC
7	2,3,4,5,3',4'	.37	PB + MC
7a	2,3,4,3',4',5'	.05	
7b	2,3,4,5,2',3',6'	.24	
7h	2,3,4,5,2',3',5'	<.30	
7i	3,4,5,3',4',5'	<.30	MC
8	2,3,4,5,2',4',5'	23.5	PB
9	2,3,4,5,2',3',4'	1.66	PB + MC
9b	2,3,4,5,3',4',5'	.51	
10	octa-Br	.30	
11	octa-Br	.31	
12	2,3,4,5,2',3',4',5'	1.65	PB

In addition to the polybrominated biphenyls present in fireMaster, the mixture has been shown to contain between 70 - 150 ppm hexabromonaphthalene (Birnbaum et.al., 1983; Huss et.al., 1978). Hexabromonaphthalene is a potent inducer of mixed-function oxidase enzymes (MFO) and produces toxic symptoms similar to those seen with PBBs, PCBs, and dioxins. Although present in fireMaster, bromonaphthalenes are probably not a major factor in the chronic toxicity, since the most toxic bromonaphthalene congeners are readily metabolized and excreted (Birnbaum et.al., 1983). The fireMaster mixture has <u>not</u> been found to contain the highly toxic polybromodibenzofurans (PBDF) or polybromodibenzodioxins (PBDD) (detection limit 0.5 ppm; Huss et.al., 1978; O'Keefe, 1979), although O'Keefe (1978) has reported that PBDD's may be formed from PBB during high temperature pyrolysis. Other compounds present in the fireMaster mixture include: biphenyl ether, naphthalene, fatty acids, substituted anthracenes and phenols, biphenyl ketone, tris(2,3-dibromopropyl)phosphate, lead, arsenic, and mercury (Huss et. al., 1978; Wallach, 1978). The absence of PBDF and PBDD from the fireMaster mixture is important since the corresponding chlorodibenzofurans and chlorodibenzodioxins have greatly complicated the understanding of the Yusho PCB contamination incident.

Physically, fireMaster is a solid material, light grey to light pink in color, which has a tendency to form lumps

unless anti-caking compounds are added. PBBs begin melting at 72°C, and decompose between 300°-400°C (Dunkel, 1975). FireMaster is virtually insoluble in pure water (11 ppb), but its solubility is increased as the organic content of water increases (Simmons and Kotz, 1982). Since PBBs are highly soluble in non-polar organic solvents (up to 75% in benzene), they are also highly fat soluble and tend to bioaccumulate (Kay, 1977).

PBBs are subject to photodecomposition, and this is the primary degradative pathway in soil. Environmental PBB tends to stay in top soil with minimal leaching and little microbial degredation. Plants grown in contaminated soil do not absorb PBB, and PBBs should not be spread through vegetation grown in contaminated soil (DiCarlo et.al., 1978; Damstra et.al., 1982). Incident ultraviolet light forms photodecomposition products only when PBB is present at high levels in soil. In highly contaminated soils photodecomposition of the 2,4,5,2',4',5'-hexabromo congener forms measurable quantities of highly toxic pentabromo congeners which are present only in small quantities in the original fireMaster mixture (Hill et.al., 1982; Damstra et.al., 1982).

Michigan, New Jersey, and Texas all have measurable quantities of polybromobiphenyls present in the environment. Michigan Chemical Corp. alone produced over 10,000,000 lbs of PBB between 1970 and 1974 when production

was halted. Although the majority of PBB produced in the U.S. found its way into polyurethane foams and ABS (acrylonitrile/butadiene/styrene) plastics, thousands of pounds are present in environmental sinks. Because of their low water solubility and tight binding to soils, PBBs move very slowly in the environment, and with few exceptions should not pose a significant threat to ground water. PBB contaminated carcasses and feed were buried in clay lined pits in Kalkaska County, Michigan, and there is little chance of PBB entering the environment from that source. Gratiot County, Michigan contains a landfill which currently holds at least 160,000 lbs of PBB in a chemically concentrated form. Hydrogeologic surveys have shown the presence of a saturated leachate-groundwater mound in the Gratiot County landfill, and the entry of measurable amounts (up to 2.5 ppb) of PBB into groundwater in the area. Unfortunately, large amounts of PBB are buried in a "hydrogeologically delicate" region of the landfill (i.e. the clay lining of the pit was removed down to the level of the water table; Shah, 1978), and the potential exists for measurable contamination of local drinking water. The Pine River which flows past the site of Michigan Chemical's St. Louis plant contains 0.01 to 0.07 ppb PBB in the water, and up to 6200 ppb in the sediment as far as twelve miles downstream from the plant. Fish and ducks collected from the Pine River had levels of PBB as high as 2.7 ppm

(Damstra et.al., 1982; Hess and Powers, 1978). At this time the Gratiot County landfill and old Michigan Chemical plant in St. Louis Michigan are the major sites of PBB entry into the environment.

Although low level PBB contamination is fairly widespread, especially in Michigan, there is currently little potential for further major environmental insult. PBB production was halted in the United States in 1974, and the majority of the 13,000,000 lbs of PBB produced in the U.S. to date are physically immobilized within ABS and polyurethane plastic products. The Gratiot county landfill is probably the most significant source of further entry of PBB into the environment at this time, and the low water solubility and low leachability of PBB should prevent all but local problems with groundwater contamination.

Exposure, Persistence and Toxic Effects of PBB

Although PBB containing materials are fairly widespread, there is probably little human exposure except through direct consumption of PBB contaminated foodstuff. The feed implicated in the poisoning of cattle on the Halbert farm was found to have PBB levels of up to 13,500 ppm. Halbert and Jackson (1974) estimated that the most severely exposed cattle may have consumed as much as 0.5 lb of fireMaster, even though the contaminated feed was

withdrawn after only 16 days because of anorexia. Other lots of contaminated feed had PBB levels as high as 3500-4000 ppm (Kay, 1977), but may have been fed to cattle for longer periods of time. Exposure of cattle may also depend on additional feeds presented at the time of contamination, since the presence of high fat content in other supplemental feeds may modify the absorbtion of PBB (Wallach, 1978).

Kay (1977) estimated that probably at least 8000 Michigan residents were directly exposed to PBB during the later part of 1973 by eating products from contaminated farms. By 1976, when the epidemiological studies of the PBB disaster began in ernest, up to 95% of the population of Michigan had measurable blood levels of PBB (Brilliant et.al., 1978; Selikoff and Anderson, 1979). Studies of lactating Michigan women revealed that 96% of lower peninsula women had detectable PBB in their breast milk, with levels up to 1.2 ppm (Brilliant et.al., 1978). Women living in other states had no detectable PBB in breast milk. In 3683 Michigan individuals tested as part of the Michigan Department of Public Health survey the highest level of PBB found in serum was 3.15 ppm, although median PBB titer was only 4 ppb (Kreiss et.al., 1982). Health surveys of Michigan Chemical Corporation workers involved in the manufacture of PBB showed mean PBB serum levels of 603.9 ppb, with the highest individual tested having 1.73

ppm. Chemical workers had high levels of PBB in body fat, with an average fat level of 196 ppm and a fat:serum ratio of 287:1 (Wolff et.al., 1979). In general, chemical workers and individuals living on quarantined farms had higher levels of PBB in serum and fat than did individuals from non-quarantined farms.

Animal studies have indicated that fireMaster is well absorbed from the gut. Over 90% of an oral dose of 2,4,5,2',4',5'-hexabromobiphenyl is absorbed in rats, and PBBs may be detected in serum from cattle as soon as 4 hours following an oral dose (Matthews et.al., 1977; Matthews et.al., 1978). It has been shown that the more highly brominated congeners of PBB are less well absorbed from the GI tract than is 2,4,5,2',4',5'-HBB (Damstra et.al., 1982; Matthews et.al., 1978), but highly brominated biphenyls are more slowly metabolized and excreted than are lighter congeners. Structure/metabolism studies have shown that para bromination slows metabolic degradation in mammals, although some congeners may be slowly hydroxylated and excreted (Hill et.al., 1982; Damstra et.al., 1982). In general, metabolic degradation of PBB requires 2 adjacent unsubstituted carbons, and proceeds more rapidly in the absence of para substitution (Damstra et.al., 1982).

Once in the body the distribution and deposition of PBBs depends at least in part on the nutritional status of the animal. In normal animals, PBBs tend to rapidly move

into adipose tissue, and establish a fat:serum equilibrium ratio of approximately 170:1 (Poland and Cohen, 1980). Distribution depends partly on the route of exposure, and chemical workers had average fat:serum ratios of over 280:1 (Wolff et.al., 1979). In addition, 2,4,5,2',4',5'-HBB distributed itself differently depending on the amount of body fat, with obese animals retaining a greater proportion of PBB than did other animals (Domino et.al., 1982).

Removal of PBB from the body occurs very slowly in most mammalian species. Excretion of PBB in male animals occurs mainly through the bile-fecal route, and only very slowly. It has been estimated that a rat will excrete less than 10% of its body burden by this route over its entire life-time (Matthews et.al., 1977). Female animals may excrete PBBs more quickly through lactation or egg production. Lactation is the major route of PBB elimination in healthy mammals (Damstra et.al., 1982). Fat mobilization due to lactation or food restriction results in increased mobilization and excretion of PBBs. Food restriction and the administration of cholestipol increased PBB elimination in poultry (Polin and Leavitt, 1984). Rozman and coworkers (1982) found that administration of cholestyramine and mineral oil increased fecal excretion of PBB almost two fold in monkeys.

Both animal and human studies have established that PBBs cross the placenta into the fetus. In humans, the ratio of maternal:fetal serum PBB is approximately 7:1 (Jacobsen et.al., 1981; Eyster et.al., 1983), i.e. the placenta slows the passage of PBB to the fetus, but does not stop it. PBBs cross the placenta in rats and cattle also (Rickert et.al., 1978). Although PBBs are transferred transplacentally, the major route of transmission from mother to offspring is through the milk. In humans the milkfat:serum ratio is between 61:1 (Jacobsen et.al., 1984) and 80:1 (Brilliant et.al., 1978). Eyster et.al. (1983) have found human milk levels as high as 92 ppm PBB on a fat basis, although mean human milkfat levels were only 0.3 ppm. In experiments conducted in rats, McCormack and coworkers (1981) studied the transmission of PBB through three generations. The exposed generation (F1) was fed a PBB containing diet until 28 days old. Offspring of the exposed generation (F2) had measurable levels of PBB in serum and fat as well as symptoms of PBB toxicity such as hepatomegaly and MFO induction. Offspring of the F2 generation (F3) had measurable levels of PBB in tissue, although they had never been directly exposed. These studies also established that lactational exposure may not be the same as direct ingestion of fireMaster. McCormack et.al., (1981) discovered that in rats, the F1 generation retained more of the relatively non-toxic

2,4,5,2',4',5'-HBB congener while transmitting the more toxic 2,4,5,2',5'-penta, 2,4,5,3'4'-penta, and 2,3,4,2',4',5'-hexabromo congeners on to offspring. From the above, it can be seen that PBBs may represent a danger to growth and development of offspring from exposed individuals, since both fetuses and neonates can acquire significant body burdens of PBB from the mother.

Once exposed, an organism will retain PBBs throughout the remainder of its life. Non-nursing or non-egg-laying animals will never clear their bodies of PBB (Damstra et.al., 1982). Tuey and Matthews (1980) have estimated that the half-life of PBB in humans is at least 6.5 years, and it has been shown that less than 10% of an oral dose of PBB will be excreted over the lifetime of a rat (Matthews et.al., 1977). In lactating cattle the half-life of PBB in milk is 58 days (Kay, 1977). Many cattle were destroyed because it was not economically feasable to keep the contaminated animals until PBB levels dropped below FDA action levels. In rats, animals fed PBBs until day 28 still had measurable levels in kidney, liver and fat at 328 days of age. These animals exhibited MFO induction in liver and kidney, as well as decreased pentobarbital anesthesia time (McCormack et.al., 1980). Fasting may increase the rate of PBB elimination, but does not completely clear it from the body. Food restriction in rats virtually eliminated PBB stores in fat, and up to 65%

of the body burden was excreted, however the remaining PBB (35%) was redistributed, primarily into skin (Johndorf et.al., 1983). Domino et.al. (1982) have estimated that the half-life of 2,4,5,2',4',5'-hexabromobiphenyl is 60 days in an emaciated rat, 145 days in a "normal" rat, and 311 days in an obese rat. While food restriction can increase the rate of PBB excretion, elimination kinetics dictate that measurable contamination in animals or humans will persist throughout a normal lifetime.

The PBB contamination of the Halbert cattle farm was discovered as weight gain, feed efficiency and milk production dropped off in the herd. Jackson and Halbert (1974) have identified the primary symptoms of PBB toxicosis in cattle, and described two phases of the toxic response. Phase I includes anorexia, decreased milk production, excessive lacrimation, shrinking of just freshened udders and fetal resorption. After PBB contaminated feed was withdrawn, the cattle continued to have decreased milk production and weight gain. Phase II of PBB toxicosis includes the development of hematomas and abcesses on the legs, abnormal hoof growth, alopecia, and thickening of the skin (hyperkeratosis). Necropsy of dead cattle from Halbert's herd showed liver and kidney pathology as well as atrophied testes and non-motile sperm in some bulls. In addition to the above symptoms, experimental feeding of PBBs to cattle has caused diarrhea,

dehydration, excess salivation and proteinuria (Durst et.al., 1977; Mercer et.al., 1978). Dietary supplementation with vitamin A was able to reverse some signs of PBB toxicosis, but feeding PBB at levels of 25 g/day resulted in significant morbidity within 60 days (Jackson and Halbert, 1974; Durst et.al., 1977). Blood chemistry testing of PBB exposed cattle showed elevated serum glutamate-pyruvate transaminase (SGPT), elevated lactate dehydrogenase (LDH) and blood urea nitrogen (BUN), and hyperbilirubinemia (Durst et.al., 1978). Other bovine symptoms included decreased hematocrit, increased abortion and fetal death, and difficulty in birthing (Jackson and Halbert, 1974; Willett et.al., 1980).

PBB toxicity has been extensively evaluated in laboratory rodents, as well as in non-human primates. Although the acute LD_{50} of PBB in the rat is only 21.5 g/kg, PBB is more toxic in smaller repeated doses. The 90 day LD_{50} in male rats is 149 mg/kg/day, and in female rats it is 65 mg/kg/day (Gupta and Moore, 1979). Feeding of 100 ppm PBB to rats for 30 days resulted in decreased weight gain with decreased feed efficiency, even though feed consumption remained constant. Necropsy of animals fed 100 ppm for 90 days revealed hepatomegaly, thymic and splenic atrophy, bile duct hyperplasia and hepatic necrosis (Gupta and Moore, 1979). Microscopic tissue examination of rats fed 100 ppm for 30 days revealed swelling and

vacuolation of liver cells, proliferation of smooth endoplasmic reticulum, centrilobular fatty change and intracellular myelin inclusion bodies (Sleight and Sanger, 1976)

Administration of 10 mg/kg/day PBB to rats resulted in appearance of pathologic changes within six months; these included hepatomegaly, decreased thymus weight and body weight, and decreased hemoglobin and hematocrit with no change in red blood cell number. In addition, animals exposed for six months showed decreased serum thyroxine and triiodothyronine as well as decreases in serum triglycerides coupled with increased serum cholesterol and SGPT. Necropsy of animals treated for six months found hepatic porphyria, hepatic necrosis coupled with fatty infiltration, bile duct hyperplasia, and increased numbers of hepatic altered foci (Gupta and Moore, 1979; Gupta et.al., 1983). Lifetime treatment of rats with 10 mg/kg/day decreased longevity by 150 days in male, but not in female rats, and necropsy of the animals revealed the presence of hepatocellular carcinomas and cholangic carcinomas (Gupta et.al. 1983b). Other investigators have found that PBB is histotoxic to the thyroid gland, and PBB treatment results in significant follicular dysgenesis with cellular distention, vacuolated mitochondria, and the presence of intracellular colloid (Kasza et.al., 1978).

Despite the well described pathology of PBBs in cattle

and experimental animals, the presence of toxic effects in humans has been controversial. Although humans exposed to PBB display a variety of symptoms, it has been difficult to establish a causal link to PBB exposure. The primary reason for the difficulty has been the absence of a good dose-response relationship for symptoms among exposed individuals. This difficulty has led one observer to remark that "there exists no relationship between PBB levels and physical or laboratory findings...[exposed individuals have]...few objective findings, and reactive depression may be responsible for the high incidence of constitutional symptoms." (Stross et.al., 1981).

The problem with establishing the human health effects of PBB has been compounded by the fact that there is an overall lack of association between blood PBB levels and most symptoms. In fact, those who believed they were suffering PBB related symptoms had overall lower PBB values (Anderson et.al., 1979). In addition, the most highly exposed individuals were those who were employed by Michigan Chemical, and they reported fewer subjective complaints than did any other non-control group. Arthralgia, myalgia and elevated SGPT and SGOT were the only consistent findings in both farm and chemical workers (Selikoff, 1979; Stross et.al., 1981). In addition, individuals from quarantined farms did not show increased numbers of complaints when compared with non-quarantined

farms, in spite of much higher average blood PBB levels (Kay, 1977). Symptoms occurring in exposed farmers which were not correlated with blood PBB levels included: tiredness, weakness, memory loss, abdominal pain, frequent infections, rashes, alopecia, GI disturbances, altered cognative function, hypersomnolence, nausea, headache, dizziness, nervousness and back or leg pain (Kay, 1977; Stross et.al., 1981; Anderson et.al., 1979). Perhaps unsurprisingly, the self-referred group had a higher incidence of symptoms than did any other group (Anderson et.al., 1979). Because the occurrence of symptoms appeared to be unrelated to the extent of PBB exposure, several investigators have suggested that the majority of symptoms seen have been the result of reactive depression (Selikoff et.al., 1979; Stross et.al., 1981). Other researchers have pointed out that depression could not be the sole cause of the symptoms since some non-farm consumers of contaminated dairy products had symptoms similar to those seen in farm families (Anderson et.al., 1979).

Confirmed findings in exposed individuals have been in four basic areas: gastrointestinal and hepatic, dermal, immunological and neurobehavioral. The most consistent clinical finding in exposed humans has been altered hepatic physiology. Stross et.al.,(1979) found hepatomegaly in 72% of Michigan farmers from quarantined farms, and elevated SGPT, LDH, and alkaline phosphatase activities in 80% of

exposed individuals. It must be pointed out however, that even though many individuals showed elevated SGPT titers, only those individuals who were also heavy drinkers had SGPT levels as high as twice normal. (Stross et.al., 1979; Valciukas et.al., 1979; Selikoff, 1979; Anderson et.al., 1979) Elevated serum trigycerides and serum iron were also discovered in approximately 30% of exposed individuals. Even though there are increased numbers of Michigan farmers with elevated serum enzymes, these effects seem to point to only mild hepatic toxicity. Studies in laboratory animals have confirmed the hepatic toxicity of PBBs, and biochemical studies in PBB treated rats have found decreases in serum retinal and microsomal a-tocopherol in treated animals. In addition to the hepatic necrosis and neoplasias seen at high dose levels in rats, short term biochemical studies have found increased levels of hepatic malon dialdehyde, a possible sign of increased lipid peroxidation in exposed animals (Bernert et.al., 1983; Bernert and Grace, 1984; Kimbrough et.al., 1977).

Dermal symptoms have been noted in both humans and animals exposed to PBB. Jackson and Halbert noted the occurance of skin and hoof abnormalities among exposed cattle (Jackson and Halbert, 1974). Some type of skin abnormality was noted in approximately 28% of individuals from quarantined farms (Stross et.al., 1979), although these were largely common skin problems such as

uncomplicated acne. Exposed farm families also reported increased hair loss, itching and erythema, although halogen acne was not reported among farmers (Chanda et.al., 1982). Thirteen percent of Michigan Chemical workers had halogen acne or hyperkeratotic symptoms. This is not surprising since fireMaster contains PBB congeners which have been found to be hypoerkeratotic on rabbit ears (Hill et.al., 1982; Orti et.al., 1983), and MCC workers were directly dermally exposed.

The immune system is also a target for the toxic action of PBBs. Although the reports have not been clinically verified, PBB exposed families reported increased illness among young children, and an especially high incidence of respiratory infections (Weil et.al., 1981; Selikoff, 1979). It is well established that in laboratory animals PBB administration results in thymic atrophy (Gupta et.al., 1983; Kay, 1977), although the thymic toxicity of PBBs may be partially reversed by the administration of dietary vitamin A (Darjono et.al., 1983). Fraker (1980) has reported that mice exposed to PBB experience a variety of immunologic effects even before overt toxicity is manifest. In laboratory rodents, PBB administration results in B-cell and helper-T-cell impairment, as well as dramatic effects on humoral immune responses (Fraker, 1980). Loose et.al. (1981) have reported decreased antibody production with decreases in

IgM and IgG, and dogs treated with PBB have been found to have thymic involution and altered plasma cell function (Farben, 1978). Allen and Lambrecht (1978) have found decreased numbers of B and T cells in rhesus monkeys treated with PBB. Conflicting reports of PBB immunotoxicity in humans have been made. Bekesi and coworkers (Bekesi et.al., 1978; Bekesi et.al., 1979) found decreased T-cell function and number, as well as small but measurable alterations in B-cell function in exposed humans. In addition, Bekesi has also reported increased numbers of null cells in exposed humans, but has been unable to demonstrate any effects on the delayed hypersensitivity response. Other human studies have failed to duplicate the findings of Bekesi. Silva et.al. (1979) and Stross et.al. (1981) have been unable to find alterations in either lymphocyte number or function in PBB exposed humans. Landrigan et.al. (1979) have suggested that the findings of Bekesi may be artifacts related to different timing of collection of control and exposed blood samples, and have noted that although PBB exposed men had lowered indices of immune function, even within the PBB exposed group, all values were still within the normal range.

The majority of human subjective symptoms can be classed as neurological (i.e. headaches, tiredness, weakness, fatigue, memory loss, etc.). When Michigan

farmers were compared to Wisconsin farmers, the Michigan groups were found to have a variety of neurological symptoms which developed in a progressive fashion. Within three months of the PBB disaster, Michigan farmers began to experience depression, nervousness, tiredness, and excessive sleepiness. Between three and six months, nausea, headaches and dizziness began to occur. From six months to one year, exposed farmers began to develop blurred vision, loss of balance, muscle weakness, difficulty walking and increased perspiration (Valciukas et.al., 1979). Although most of the symptoms listed above are subjective complaints, and therefore could have been caused by reactive depression, some of the exposed non-farm groups (who should not have been affected by reactive depression) had similar symptoms. Some symptoms such as tiredness, headaches, loss of balance, and muscle weakness were more prevalent in individuals with lower serum PBB levels however, and there appeared to be no relationship between PBB level and the appearance of symptoms (Stross et.al., 1981). Neurobehavioral testing using the Minnesota Multiphasic Personality Inventory (MMPI) showed that the most common measurable findings were reactive depression, anxiety, irratibility, "somatic preoccupation" and "somatic delusions" (Stross et.al., 1981; Brown and Nixon, 1979). The studies by Brown and Nixon (1979) found that cognitive deficits reported by others were eliminated after the

results were adjusted for education. Although measurable neurological changes have been reported in PBB exposed individuals, these have been primarily peripheral neuropathies such as abnormal electromyograms and slowed sensory nerve latency (Stross et.al., 1979). In 1983 Seagull reported that testing two to four year old children with the McCarthy Scale of Children's Abilities revealed a decrease in test score associated with increased body burdens of PBBs. Others have since criticized her study based on the absence of control subjects and the small number (18) of children tested (Nebert et.al., 1983). Follow-up studies in the same children have revealed the PBB exposed children were in the "normal range" in all areas tested, and have suggested that the results of Seagull's testing may have been skewed as a result of additional physical and psychological testing performed on the children at the same time (Schwartz and Rae, 1983; Nebert et.al., 1983).

In summary, the existance of human symptoms which can be related unequivocally to PBB exposure is still controversial. It seems clear that there is an association of PBB exposure with hepatic effects such as increased serum enzyme levels and hepatomegaly. Serious dermal complaints were seen only in highly exposed workers from Michigan Chemical, and the presence of immunologic dysfunction is somewhat questionable. Neurobehavioral

symptoms, although present in exposed individuals, may be psychological in origin, and related either to reactive depression or conscious knowledge of PBB exposure, rather than to neurotoxic effects of fireMaster.

Mixed Function Oxidase Induction and Reproductive Effects of PBB

PBBs are known potent inducers of hepatic and extrahepatic mixed-function oxidase (MFO) enzymes (McCormack et.al., 1978; Dent, 1978; Kluwe et.al., 1978; Goldstein et.al., 1979; Dent et.al., 1978; Robertson et.al., 1981; Dent et.al., 1976; Babish and Stowesand, 1977). The PBB mixture fireMaster FF-1 has been established as a "mixed" inducer of MFOs, with effects similar to those seen with the simultaneous administration of both phenobarbital (PB) and 3-methylcholanthrene (3MC). Phenobarbital treatment results in increased quantities of hepatic cytochrome P-450, increased cytochrome c reductase, and increased bile flow and biliary excretion. Treatment with 3MC results in increased hepatic content of cytochrome P-448, without the effects of phenobarbital on bile flow or cytochrome c reductase (Damstra et.al., 1982). PBB or PCB treatment results in a pattern of MFO induction similar but not identical to that seen with simultaneous administration of PB and 3MC (Dent, 1978). It has been estimated that

PBBs are three to five times more potent as MFO inducers than are equimolar quantities of PCBs (Damstra et.al., 1982)

PBBs induce MFOs in liver, lung, kidney and mammary gland, but not in testes (Damstra et.al., 1982; Kluwe and Hook, 1978; Dent et.al., 1977; McCormack et.al., 1982; Kluwe and Hook, 1981). In mice, P-450 induction occurs within 24 hours, and P-448 induction within 96 hours (Damstra et.al., 1982). In rats, maximal P-450 induction occurs by 48 hours after PBB administration, while maximal P-448 induction requires up to 28 days (Dent et.al., 1976). Hepatic enzyme induction has been shown to occur with PBB doses as small as 0.3mg/kg/day (Damstra et.al., 1982). In liver, PBB induces a wide variety of MFO-type enzymes including: ethoxycoumarin O deethylase (ECOD), aminopyrine N demethylase, ethoxyresorufin O deethylase(EROD), arylhydrocarbon hydroxylase (AHH), epoxide hydrase (EH), glutathione S transferase (GST), UDP glucuronyl transferase (UDPGT), and various steroid hydroxylases (McCormack et.al., 1982; McCormack et.al., 1979; Dent et.al., 1978; Sleight and Sanger, 1976). PBB treatment increased EROD and AHH activity in kidney microsomes, but not EH or GST activity (McCormack et.al., 1979). In lung, PBB not only induced AHH and EROD activity, but also decreased clearance of 5-HT, probably by decreasing 5-HT uptake (McCormack et.al., 1982).

Although MFO induction is usually measured in microsomes *in vitro*, PBB exerts effects on biotransformation which are also measurable *in vivo*. PBB treatment decreases pentobarbital sleep time and the duration of progesterone anesthesia in rats (McCormack et.al., 1981; McCormack et.al., 1980). PBB pretreatment increases the sensitivity of liver and kidney to chloroform toxicity, an effect due to increased metabolism of chloroform (Kluwe and Hook, 1978; Kluwe et.al., 1979). PBB induction of mammary mixed function oxidases (Dent et.al., 1977) protects female rats against 2-fluoroacetamide induced mammary carcinogenicity (Schwartz et.al., 1980). Both indocyanine green and ouabain are more rapidly cleared from plasma in PBB treated animals (Cagen et.al., 1977) and PBB treatment decreases the time to death following bromobenzene administration (Roes et.al., 1977).

The study of MFO induction with PBBs is complicated by the fact that fireMaster is a complex mixture of compounds, each with different specificities for enzyme induction. Of the congeners present in the fireMaster mixture, some induce only PB type enzymes, some are pure 3MC inducers, and some induce both P-450 and P-448 (Robertson et.al., 1982; Dannan et.al., 1983; Orti et.al., 1983). Table 1 (p. 7) lists the relative abundance and MFO inducing properties of the PBB congeners which have been identified to date (Orti et.al., 1983; Dannan et.al., 1983). The study of MFO

induction by various PBB congeners has been complicated by the fact that pure congeners are difficult to obtain since some PBB congeners are not well separated even by capillary GC. The structure activity rules for MFO induction by PBBs are similar, but not identical to those already established for PCBs. In general, PBBs are at least three times more potent in inducing enzyme activity than are PCBs (Robertson et.al., 1982). Studies of MFO inducing activity using polyhalobiphenyls substituted at the same sites with either chlorine or bromine have revealed that increasing the Br/Cl ratio increases the degree of AHH induction (Robertson et.al., 1982; Andres et.al., 1983). The same studies have established that increasing bromination also increases toxicity, the extent of thymic atrophy, and binding to the Ah receptor. Study of induction patterns of PBB congeners reveal the following structure/activity rules:

1. P-448 type induction requires at least two para and one meta bromine, or the presence of 2,3,4 substitution on one side.

2. P-450 type induction occurs with ortho substituted congeners.

3. ortho substitution diminishes but does not always eliminate P-448 type induction (Robertson et.al., 1982; Dannan et.al., 1982; Dannan et.al., 1983; Parkinson et.al., 1983).

Several studies have attempted to relate MFO induction

and toxicity. All PBB congeners with known cytotoxic effects are 3MC inducers. Compounds which are most toxic are approximate isostereomers of 2,3,7,8-tetrachloro dibenzodioxin, and these same compounds are the most potent of the 3MC type inducers (Andres et.al., 1983). In the PBB congeners studied, toxicity has paralleled AHH induction and Ah receptor binding. Structure/activity studies have established that ortho- bromination decreases both cytotoxicity and AHH induction (Tsushimoto et.al., 1982). Comparison of 3,4,5,3',4',5'-HBB with 2,4,5,2',4',5'-HBB has shown that 3,4,5,3',4',5'-HBB causes anorexia and emaciation as well as decreased thymus weight and overt liver pathology. Tumor promotion by 3,4,5,3',4',5'-HBB occurs only at doses which produce toxic effects. Treatment with 2,4,5,2',4',5'-HBB results in proliferation of smooth endoplasmic reticulum, and causes tumor promotion at doses far below those required to produce overt cytotoxicity (Render et.al., 1982; Jensen et.al., 1983). 3,4,5,3',4',5'-HBB is a potent 3MC type inducer and 2,4,5,2',4',5'-HBB is a PB type inducer. Similar results have been found when comparing 3,4,3',4'-TBB and 2,5,2',5'-TBB; 3,4,3',4'-TBB is an overtly toxic 3MC type inducer, whereas 2,5,2',5'-TBB is a nontoxic PB type inducer (Robertson et.al., 1983). Research done to date shows that PB type inducers are tumor promotors, but are not frankly cytotoxic at doses which result in tumor

promotion. All known cytotoxic congeners are 3MC type inducers and induce cell and organ toxicity at doses below those required for tumor promotion. It has been pointed out that although all toxic PBB congeners are 3MC type inducers, not all 3MC type inducers are toxic; this indicates that toxicity and induction may occur through different mechanisms (Parkinson et.al., 1983).

Treatment of animals with other proven inducers of MFOs such as phenobarbital or PCBs has lead to increased microsomal metabolism of steroid hormones (Nowicki and Normal, 1972; Orberg and Kihlstrom, 1973; Orberg and Lundberg, 1974; Derr, 1978; Sanders et.al., 1974). Administration of phenobarbital during development results in permanent reproductive deficits in rats including lifelong reductions in circulating testosterone titer (Gupta et.al., 1980; Gupta et.al., 1982). In rodent systems, PCBs have produced uterine atrophy, reductions in plasma progesterone concentration, and lengthened estrous cycles (Jonsson et.al., 1976; Kimbrough et.al., 1978). Increased microsomal steroid metabolism may be responsible for the alterations in reproductive function seen in PCB treated animals.

The MFO inducing properties of PB and PCBs are shared by PBBs. Several endocrine-related alterations observed in animals treated with PBBs suggest that these compounds modify steroid metabolism to the extent that reproductive

function is diminished. Bulls exposed to PBB had testicular atrophy with aspermia or azoospermia (Jackson and Halbert, 1974; Cook et.al., 1978). Roosters treated with PBBs had decreased testicular and comb weight (Damstra et.al., 1982). PBB treatment of hens caused decreased laying and hatchability, although treatment with 2,4,5,2',4',5'-HBB alone did not result in altered egg production (Cecil and Bitman, 1977; Polin and Ringer, 1978; Polin et.al., 1979; Bursian et.al., 1983). Rhesus monkeys fed as little as 0.3 ppm PBB in their diet had prolonged menstrual cycles, increased post-implantation bleeding, and flattened serum progesterone peaks. Male rhesus monkeys treated with PBBs had hypoactive seminiferous tubules (Allen et.al., 1978; Allen et.al., 1979; Damstra et.al., 1982).

Neonatal treatment of female rats with PBB resulted in delayed vaginal opening, and increased numbers of five and six day estrus cycles relative to control rats (Johnston et.al., 1980; McCormack et.al, 1981). Male rats treated with PBB had decreased seminal vesicle and prostate weights as well as attenuated seminal vesicle response to injections of exogenous testosterone (McCormack et.al., 1979; Johnston et.al., 1980). Both progesterone and testosterone were more rapidly cleared from plasma in PBB treated rats and PBB treatment decreased the duration of progesterone anesthesia (McCormack et.al., 1981; Harris

et.al., 1981; McCormack et.al., 1979). The uterotropic action of exogenously administered estradiol was decreased by PBB treatment, and estradiol binding to cytosolic receptors was blunted (Bonhaus et.al., 1981).

In vitro metabolism of estradiol, progesterone and testosterone is increased by PBB pretreatment (Bonhaus et.al., 1981; Newton et.al., 1982; Arneric et.al., 1980). PBB treatment increased 7a-, 6ß- and 16a-hydroxy testosterone production in hepatic microsomes. The PBB induced increase in testosterone hydroxylation was also associated with decreased formation of 5a-dihydrotestosterone (Newton et.al., 1982). Although PBBs increase metabolism of steroid hormones and attenuate responsiveness to exogenous steroids, previous research has failed to produce evidence that PBB treatment alters levels of endogenous steroid hormones in rats or cattle (Castracane et.al., 1982; Johnston et.al., 1980; Willet et.al., 1983). Semen testing of men exposed to PBBs as adults has not found any PBB-related effects on sperm count, motility or morphology (Rosenman et.al., 1979).

Administration of PBBs to pregnant animals has demonstrated that the fireMaster mixture is only weakly teratogenic. Administration of 100 mg/kg/day of fireMaster has resulted in increased fetal death, but failed to produce terata in rats (Kay, 1977; Damstra et.al., 1982). PBB doses of 400 mg/kg/day or higher have produced cleft

palate and diaphragmatic hernia in rats, and mice show cleft palate and exencephaly when exposed to dietary PBB levels of 1000 ppm (Damstra et.al., 1982; Corbett et.al., 1975). Cattle fed 5000 ppm fireMaster in food showed increases in fetal death and abortions, but there was no evidence of fetal malformation (Kay, 1977).

Although PBBs are not potent teratogens, their MFO inducing properties may engender more subtle developmental effects. MFO induction and concommitant increases in steroid metabolism during the critical period of brain differentiation could result in lifelong effects on reproductive performance.

Reproductive Physiology and Sexual Differentiation

Steroids such as testosterone may affect reproduction in two different ways: by direct effects on reproduction in adult animals, and by "organizational" effects resulting from sexual differentiation of target tissues during fetal or neonatal life.

Many steroid target tissues undergo differentiation or "imprinting" as a result of perinatal androgen or estrogen exposure. Both imprinting and adult effects of androgens may require metabolic activation of testosterone to one of two active forms: 5a-dihydrotestosterone (DHT) or 17ß-estradiol (Figure 1). Reduction of testosterone to

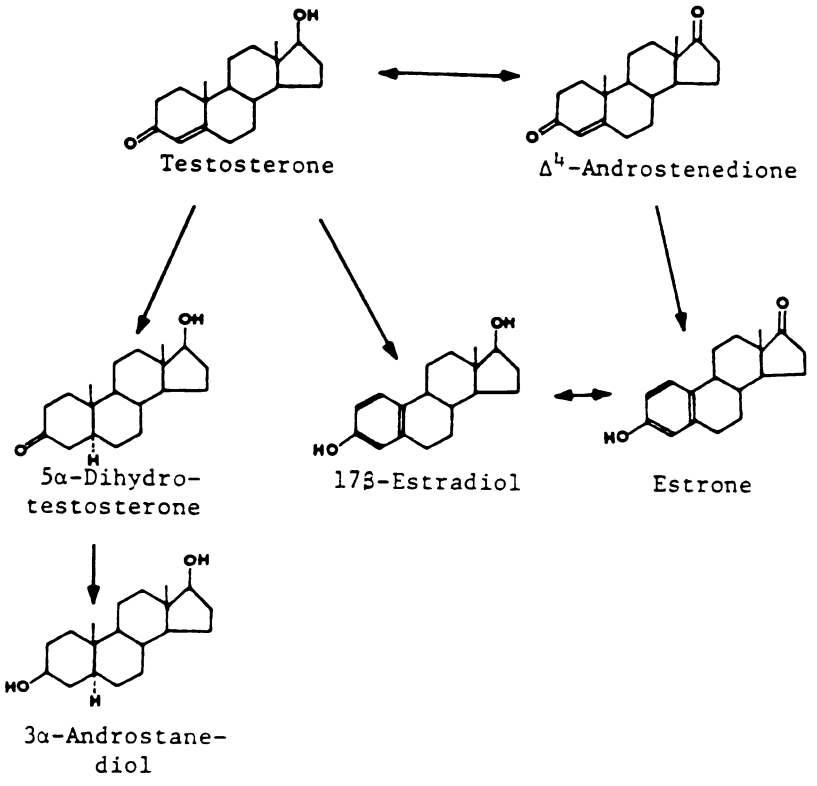

FIGURE 1 Formation of active metabolites of testosterone

5a-DHT is an essential step in masculinization of peripheral structures such as external genetalia. Even in tissues directly affected by testosterone, such as Wolffian ducts, DHT exhibits greater potency (Bardin and Catterall, 1981). Aromatization of testosterone to estradiol is required for the male differentiation of the brain, the establishment of male sexual behavior patterns and masculinization of hypothalamic neuroendocrine control mechanisms (MacLusky and Naftolin, 1981).

Although 5a-reduction of testosterone to DHT may play a role in central neuroendocrine events, 5a-reduced metabolites are of greatest importance in peripheral tissues such as prostate and seminal vesicle. In androgen sensitive tissues such as prostate DHT has greater affinity for the cytoplasmic androgen receptor than does testosterone, and serves as the "active androgen" (Liang et.al., 1984). In prostate, testosterone is first reduced to 5a-DHT, and then metabolized by 3a or 3ß hydroxysteroid dehydrogenase to either 5a-androstane-3a,17ß-diol (3a-Diol) or 5a-androstane- 3ß,17ß-diol (3ß-Diol). 3a-Diol serves as a "storage" form of 5a-reduced androgen, and may be rapidly back-converted to DHT (Ofner et.al., 1982). 3ß-Diol acts as an intermediate form only, and is not converted back to DHT, but rather is rapidly hydroxylated and eliminated as a 6a, 6ß, or 7a-triol (Ofner et.al., 1979). In prostate, 5a-reduction is regulated by plasma androgens, with

castration decreasing and testosterone treatment increasing 5a-reductase activity (Celotti et.al., 1979; Tenniswood et.al., 1982). Formation of 3a-Diol from DHT is unrelated to endocrine status (Shimazaki et.al., 1972). The polyhydroxylation of 3ß-Diol occurs through an inducible P-450 type mixed function oxidase. The formation of 5a, 3ß-reduced triols may be increased by treatment with mixed-function oxidase inducers such as phenobarbital, ß-napthoflavone (ßNF), or TCDD (Haaparanta et.al., 1984; Lee et.al., 1981; Soderkvist et.al., 1982). 5a-Reduction of testosterone in prostate is an important activation step, and increased formation of DHT from testosterone may be partially responsible for the increased tissue growth associated with benign prostatic hyperplasia (Hudson et.al., 1983).

Conversion of testosterone to DHT is of great importance in the development of a male phenotype in fetal and neonatal mammals. Fetal testes are needed for male morphological differentiation and either the absence of testes or gonadal dysgenesis results in the development of a female habitus. Fetal testes are under the control of the placenta and fetal pituitary, and begin to secrete two hormones needed for male differentiation about the time the spermatic chords and Leydig cells develop (day 13 to 16 of gestation in the rat). The first hormone secreted by fetal testis is known as Mullerian Regression Factor (MRF), a

poorly characterized peptide hormone of approximately 70,000 daltons. MRF causes the atrophy and disappearance of the Mullerian ducts which would develop into uterus and fallopian tubes in female animals. Mullerian regression is an active process, as demonstrated by the existance of a "persistant Mullerian duct syndrome" an X-linked recessive genetic disorder characterized by the absence of MRF, and persistance of Mullerian structures in male animals (Wilson et.al., 1981).

Virilization of male fetuses begins to occur immediately following the secretion of testosterone. Testosterone secretion begins around day 16 with the appearance of 20,22 desmolase activity in fetal testis. In Wolffian ducts (internal male structures which develop into the epididymus, vas deferentia and spermatic cord), testosterone alone is required for development, whereas the differentiation of external structures such as the penis and scrotum require the metabolic activation of testosterone to DHT. Julienne Imperato-McGinley and co-workers have identified several cohorts of individuals deficient in 5a-reductase activity. In one village in the Dominican Republic congenital 5a-reductase deficiency is common. Affected individuals are born with female external genetalia and raised as girls, but at puberty undergo virilization, with the growth of the clitoris into a penis, testicular descent, and elongation and rugation of the

scrotum. Of 18 individuals affected with this syndrome, 15 were living with women, although none had fathered children because of the severe hypospadias resulting from incomplete fetal masculinization (Rubin et.al., 1981; Imperato-McGinley et.al., 1985). Wolffian ducts differentiate normally in affected individuals, as shown by the fact that they may have sperm bearing ejaculations from the perineal urethra. Treatment of rats with 4-aza steroids which act as 5a-reductase inhibitors has verified that testosterone alone is necessary for masculinization of internal Wolffian ducts, but conversion to DHT is required for virilization of external genitalia (Imperato-McGinley et.al., 1985). The reason testosterone virilizes internal structures in fetuses, but is ineffective in masculinizing external genitalia prior to puberty is unknown (Wilson, et.al., 1981).

Although DHT is more active than testosterone in mediating morphological sexual differentiation and in maintaining peripheral structures, it is much less important in the differentiation and control of central neuroendocrine structures. 5a-Reductase activity has been found in a variety of CNS structures including hypothalamus, midbrain, amygdala, hippocampus, cerelbellum and cerebral cortex (Martini et.al., 1979), and has been identified in the brain of rat, mouse, dog, monkey, cattle, guinea pig, humans and birds (Martini et.al., 1982).

Despite its wide distribution, 5a-reductase is of major physiological importance only in the pituitary gland. The 5a-reductase activity in pituitary gland is secondary only to activity in the prostate. In pituitary, 5a-reduction occurs mainly in gonadotroph cells (Denef, 1983; Celotti et.al., 1979) and is regulated by circulating steroids. Castration of adult males results in proliferation of pituitary gonadotrophs and increased 5a-reductase activity, while treatment with androgens or estrogens causes decreased conversion of testosterone to DHT. Manipulation of the endocrine environment does not affect 5a-reductase activity in the hypothalamus, although it has been reported that treatment with melatonin, reserpine, or methamphetamine may increase hypothalamic testosterone reduction (Martini, 1982). As in prostate, both pituitary and hypothalamus form 3a-Diol and 3ß-Diol from DHT, and 3a-Diol may be back-converted to DHT (Samperez and Jouan, 1979; Kubli-Gorfias, 1984).

In pituitary, DHT is six-fold more effective than testosterone in supressing LH release, although testosterone administered in conjunction with a 5a-reductase inhibitor can also decrease LH secretion. 3a-Diol is also effective in supressing pituitary LH secretion, although neither DHT nor 3a-Diol are particulary effective in modulating FSH release (Liang et.al., 1984; Martini 1982). DHT does not appear to affect LHRH release

from the hypothalamus. Neither DHT nor 3a-diol are effective in reversing the castration induced depletion of hypothalamic LHRH (Martini, 1982).

The role of 5a-reduction in brain differentiation and control of sexual behavior is controversial. Although most authors agree that aromatizable androgens are more potent masculinizing agents, there may be some effects of 5a-reduced androgens on sexual differentiation and behavior (Yahr, 1979; Whalen and DeBold, 1974; Parrott, 1974). Although DHT is much less potent in maintaining male sexual behavior in castrated rats than is testosterone, DHT will maintain sexual behavior if given in high doses for long periods of time, or when implanted directly into the pre-optic area of the hypothalamus (Martini, 1982). It has been reported that flutamide, an androgen receptor antagonist, blocks mounting behavior when administered to male or female rats (Gladue and Clemens, 1980), and that it can increase lordosis in female rats (Erskine, 1983). Studies using androgen antagonists must be interpreted with care, however, since the peripheral effects of DHT may contribute to masculine sexual behavior by a non-central action. DHT has a potent effect on the growth of penile spines (papillae), and long-term treatment with androgen antagonists may impair copulatory behavior as a result of impaired sensation (Parrott, 1974). Beyer has postulated the existance of an "arousal mechanism" and a "copulatory

mechanism", and it has been suggested that aromatizable androgens affect arousal, and reduced androgens insure copulatory ability (Sodersten et.al., 1980; Parrott, 1974; Beyer 1976). Therefore, although DHT is clearly less potent than testosterone in maintaining sexual behavior in male rats, it appears that both aromatized and reduced androgens are required to maintain the full spectrum of male sexual behavior (Yahr, 1979; Parrott, 1974).

Evaluation of steroid actions by assessing sexual behavior is complicated somewhat by the two different but related modes of brain behavioral differentiation; masculinization and defeminization. Masculinization of the brain may be assessed by measuring male copulatory behavior patterns. Defeminization of rat sexual behavior is measured by noting the occurance (or absence) of typically feminine behaviors such as lordosis. The role reduced androgens play in mediating brain differentiation may also be different in different species. McEwen (1980) reports that rats and hamsters are demasculinized and feminized by aromatizable androgens, guinea pigs are masculinized by androgens and defeminized by estrogens, and that primates do not undergo defeminization, but are masculinized by androgens. Other reports concerning androgenic control of brain differentiation suggest that the picture is even less simple than McEwen proposes. Although several reports have shown that DHT administration in neonatal rats does not

masculinize or defeminize behavior in rats (Stewart et.al., 1979; Sodersten and Hansen 1978; Whalen and Rezek, 1974; Olsen, 1983) it has been shown that administration of R-1881, a non-aromatizable androgen completely defeminizes adult behavior (Olsen, 1983). In addition, neonatal administration of cyproterone acetate, a potent androgen antagonist, does feminize sexual behavior in male rats (Ward and Renz, 1972) and flutamide treatment of neonates demasculinized male behaviors (Clemens et.al., 1978). Studies with cyproterone acetate must be interpreted with caution, since cyproterone functions both as an aromatase inhibitor (Schwartzel et.al., 1973) and as a potent synthetic progestogen (Goodman et.al., 1980). Studies with other progestins have shown that progesterone can attenuate the defeminizing effects of testosterone or estradiol (McEwen et.al., 1979; Meyer-Bahlber and Ehrhardt, 1980) and block the effects of estradiol on LH feedback when administered to adults (Brown-Grant, 1976). Cyproterone, then, may function as an anti-estrogen as well as an anti-androgen, and studies using cyproterone must be interpreted cautiously.

Although 5a-reduced metabolites of testosterone play the major role in morphological development, brain sexual differentiation depends on the production of estrogens (17ß-estradiol and estrone) from circulating androgens (testosterone and androstenedione). In addition,

aromatization of androgens to estrogens in the brain is requisite for many effects of testosterone in adults, including control of gonadotropin secretion and regulation of sexual behavior.

The chemistry of aromatization has been studied primarily in human placental microsomes, since the enzyme is present in large quantities. The aromatization of testosterone to estradiol requires 3 moles of NADPH and 3 moles of molecular oxygen for each mole converted (Thompson and Siiteri, 1974), and proceeds through three successive hydroxylation reactions (Figure 2). The first hydroxylation occurs at C-19, resulting in the formation of 19-hydroxy-testosterone. The second hydroxylation at C-19 forms the gem-diol in equilibrium with the 19-oxo- compound (Thompson and Siiteri, 1974; Braselton et.al., 1974). The site of the third oxidation step has been in question, with 4,5-epoxidation, 1ß- or 2ß-hydroxylation, and C-19 peroxidation suggested as possible sites. Recently, Hahn and Fishman (1985) have presented compelling evidence that 2ß-hydroxylation of the A ring is the final step in aromatization of testosterone (Miyari and Fishman 1985; Goto and Fishman, 1977). Following 2ß-hydroxylation of the androgen, a rapid, non-enzymatic rearrangment results in the loss of the C-19 carbon, aromatization of the A ring, and reduction of the 3-keto group to an alcohol. Since the

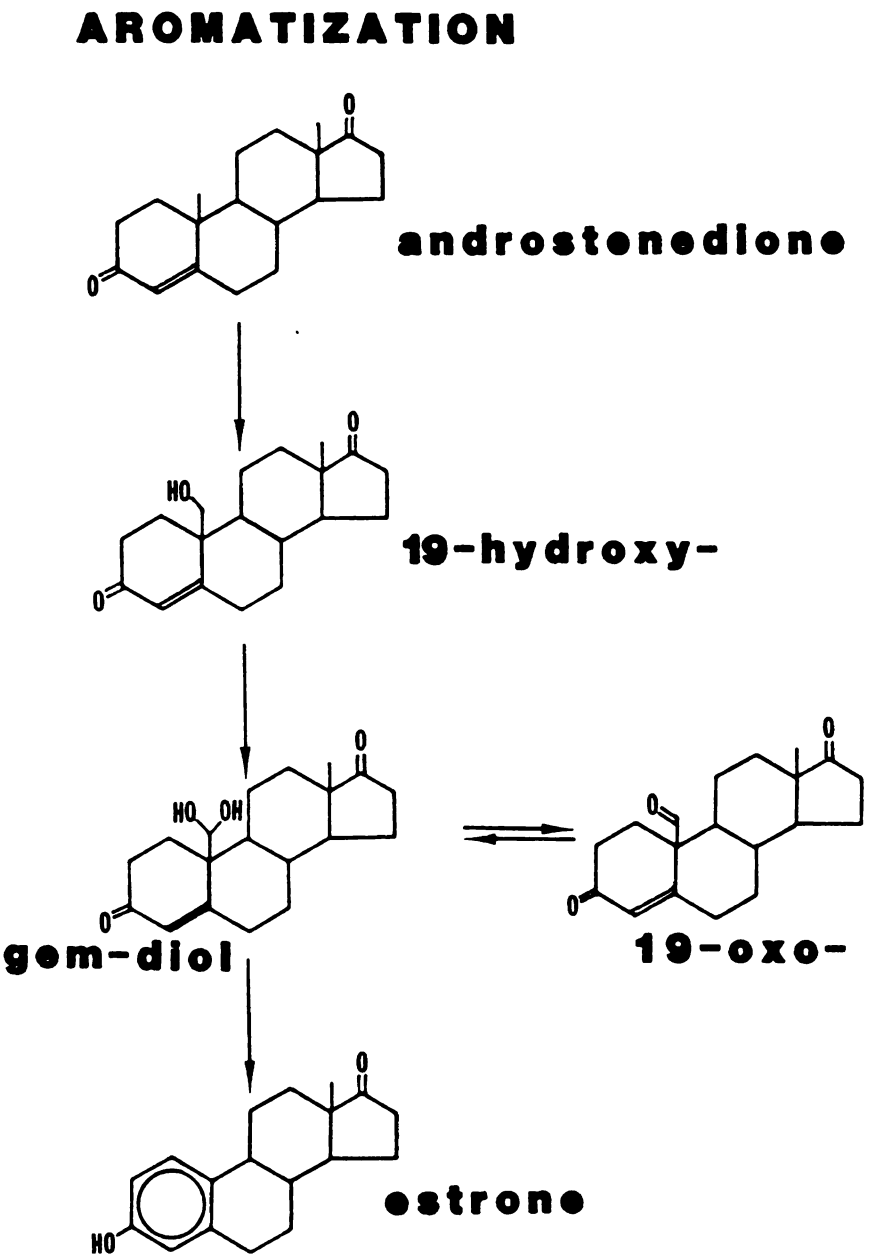

FIGURE 2 Intermediates formed during the aromatization of androstenedione.

final step in the aromatization pathway is non-enzymatic, estrogens do not act as aromatase inhibitors (Schwartzel et.al., 1973), and the reaction is non-reversable.

Aromatization of androgens to estrogens occurs in the brain of many species including: man, rat, rabbit, bat, cow, horse, pig, monkey, snake, sea turtle, mudpuppy, sculpin, shark, skate, dove, quail, wallaby, dog and hamster (Reddy et.al., 1973; Naftolin et.al., 1971; Callard et.al., 1983; Callard et.al., 1979; Callard et.al., 1982; vanKrieg et.al., 1983; Steimer and Hutchison, 1980; Flores et.al., 1973; Worgul et.al., 1981; Callard et.al., 1978). In adult rats, aromatization is a necessary intermediate step in the regulation of LH secretion and the control of sexual behavior. The role of aromatization in androgenic control of reproductive processes is generally demonstrated in one of several ways:

1. if testosterone is active, and DHT or another non-aromatizable androgen is not,

2. if 19-hydroxy testosterone (an intermediate in aromatization) is more effective than testosterone, and estrogen is more effective than 19-hydroxy testosterone,

3. if the action is blocked by the administration of an anti-estrogen such as MER-25 or tamoxifen, or

4. if the action is blocked by the administration of an aromatase inhibitor such as androsta-1,4,6- triene-3,17-dione (ATD) or 4-hydroxy androstenedione.

The presence of the aromatase enzyme and estrogen receptors in the hypothalamic nuclei involved in the regulation of reproduction is additional evidence that aromatization may play a role in reproductive control.

There is evidence that aromatization plays a role in regulation of LH secretion from several species. Clinically, the estrogen antagonist clomiphene citrate is used to increase LH and testosterone titers in infertile men. Administration of the aromatase inhibitor aminoglutethimide to dogs results in increased plasma LH and testosterone titers (Worgul et.al., 1981). ATD treatment of male rhesus monkeys results in increased plasma LH and testosterone, while the simultaneous administration of estradiol and ATD does not affect plasma LH titer (Ellinwood et.al., 1984). In addition, the administration of testosterone or estradiol (but not DHT) reverses the castration induced rise in plasma LH in rats, while simultaneously increasing the content of LHRH in medial basal hypothalamus (Kalra and Kalra, 1983).

Aromatization also plays a role in adult regulation of male and female copulatory behavior in the rat and other rodent species. Although both estradiol and DHT must be administered to maintain a full spectrum of copulatory behavior in castrated male rats, it is believed that DHT acts mainly in the maintainance of peripheral mechanisms, while estradiol plays the major role in arousal and

motivation (Whalen and DeBold, 1974; Parrott, 1974). Estrogens are clearly more effective than androgens in maintaining copulatory behavior in castrated male rats (Whalen and DeBold, 1974) and administration of ATD blocks testosterone induced mounting behavior in male rats (Christensen and Clemens, 1975). Lesioning and deafferentation studies have established that the medial pre-optic area of the brain is important in male copulatory behavior, and autoradiography studies have confirmed that estrogen binding occurs in the pre-optic hypothalamus (McEwen, 1981). In addition, studies on the distribution of aromatase activity in brain have established that the medial pre-optic hypothalamus (MPON) has the highest levels of aromatase activity in brain (Table 2) (Selmanoff et.al., 1977; Kobayashi and Reed, 1977).

Many experiments have established that aromatization plays an important role in brain sexual differentiation. Naftolin et.al. (1971) established the existance of aromatase activity in the diencephalon of human fetal brain, and other labs showed that aromatase was present in the brain of rodents as well (Weisz and Gibbs, 1974). Since the mid-70's, the role of aromatase in brain differentiation has been studied in several species. Ruppert and Clemens (1981) established that ATD inhibited testosterone but not estradiol induction of behavioral

Table 2
Aromatase activity in various brain regions

region	aromatase activity pmol/100mg/hr
medial pre-optic + anterior hypothalmic	30
arcuate + ventro-medial	5
lateral preoptic	3
medial amygdaliod	2
lateral hypothalamic	1
cortex	low

adapted from Selmanoff et.al., 1977

masculinization in the hamster. Administration of ATD to male rats in utero feminized their behavior as adults, but estradiol administration mimicked the masculinizing effects of testosterone (Stewart et.al., 1979; Clemens and Gladue, 1978). Estrogens are more potent masculinizing agents than testosterone (Gorski and Jacobson, 1981; Sodersten and Hansen, 1978), and the administration of either ATD or estrogen antagonists such as MER-25 will block the masculinizing effects of testosterone (Gladue and Clemens, 1980).

Aromatase activity in both male and female fetal brain peaks during the critical period of brain differentiation which begins on day 17 of gestation (George and Ojeda, 1982; Weisz et.al., 1982). Fetal testosterone titers also peak on day 18 of gestation, and brain estrogen receptors begin to appear on day 17 of gestation (Orth et.al., 1981; Weisz and Ward, 1980; Vito and Fox, 1982; Ward and Weisz, 1980). Since aromatase activity, blood testosterone and brain estrogen receptors are all necessary for estrogenic differentiation of fetal brain to take place it is important for all three to be in place during the critical period of brain differentiation (Figure 3).

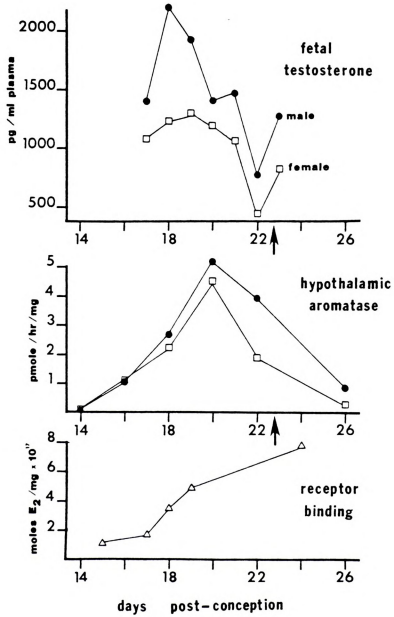

FIGURE 3 Simultaneous occurance of fetal testosterone surge, hypothalamic aromatase, and estrogen receptor binding in fetal rat hypothalamus (from Orth et.al., 1981, George and Ojeda, 1982 and Vito and Fox 1982)

Aromatase mediated masculinization of perinatal rat brain can be detected in adulthood by testing four different parameters:

1. masculinization of hypothalamic/pituitary control of LH secretion,

2. masculinization and defeminization of copulatory behavior,

3. morphological differentiation of the sexually dimorphic nucleus of the pre-optic area and

4. differentiation of hepatic drug and steroid metabolic patterns.

Each of the four parameters above are masculinized or defeminized by perinatal aromatization of testosterone in the rat. It must be pointed out that although aromatization occurs in a variety of species, the same control mechanisms that operate in rats do not necessarily operate in other species. Primates in particular do not seem to undergo perinatal masculinization of the hypothalamic control of LH secretion in the same way as rodents do (Karsch et.al., 1973), and direct extrapolation of data from rodent experiments to human behavior and physiology is unwarranted.

Perinatal aromatization of testosterone to estrogen results in the defeminization of the hypothalamic/ pituitary control of LH secretion. Normal female rats respond to estrogen administration in a biphasic fashion;

i.e. low doses of estradiol result in negative feedback to the hypothalamic/ pituitary system and low levels of plasma LH, while high doses of estradiol given after negative feedback is established can induce an LH surge, which is normally associated with ovulation. Normal male rats do not produce an LH surge when exposed to high doses of estradiol, although castration and very high doses of estradiol may occasionally result in a small female-like LH surge (Dorner et.al., 1975). The LH "surge mechanism" can be defeminized in female rats by the perinatal administration of as little as 10μg of TP, (DaMassa et.al., 1983) and results in delayed anovulatory sterility (DAS). Testosterone induction of DAS in female rats can be blocked by the administration of the estrogen antagonist MER-25 (Hayashi, 1979). More recent studies have demonstrated that administration of estradiol, catechol estrogens or the synthetic estrogen DES will all result in the development of DAS in female rats (Nass et.al., 1984; MacLusky et.al., 1983).

Other studies have shown that male rats castrated at birth can respond with an LH surge following estradiol priming in adulthood. Either castration or the administration of testosterone antibodies to perinatal male rats will prevent the defeminization of the LH surge mechanism (Gogan et.al., 1981). Recently, it has been shown that the hour of castration on the day of birth has a

potent influence on the ultimate development of the LH surge mechanism in castrated male rats. Castration 6, 12, or 24 hours after birth resulted in the development of the LH surge mechanism in only a few animals, and the LH surge produced in adulthood was small in magnitude (Gogan et.al., 1980; Corbier, 1985). Normal male neonates produce a marked surge of testosterone within 2 hours following birth, and this perinatal testosterone peak is associated with high levels of hypothalamic estrogen (Corbier, 1985; Rhoda et.al., 1984). Castration within 2 hours of birth not only abolishes the hypothalamic testosterone and estradiol surge, but also prevents the defeminization of the LH surge mechanism (Corbier, 1985; Gogan et.al., 1980). If the perinatal testosterone surge is allowed to occur, adult LH response to estradiol priming is masculinized.

In addition to the presence or absence of the LH surge mechanism, perinatal androgen exposure is also associated with differences in the rate of rise of plasma LH following gonadectomy. Male rats, when castrated as adults have a rapid increase (within 12 hours) in plasma LH titer, whereas female rats require 48 hours or longer to increase plasma LH levels after ovariectomy (Johnson and Gay, 1983; Blackwell and Ames, 1971; Gay and Hauger, 1977). Recent work has shown that administration of 10μg of TP to 3,4, or 5 day old female rats results in a rapid increase of plasma LH when these animals are ovariectomized as adults (DaMassa

et.al., 1983). Similarly, neonatal castration of male rats slows the rise of plasma LH following removal of testosterone capsules implanted in adults (Johnson and Gay, 1983). These results suggest that the LH response to castration is affected by neonatal androgens, although it must be noted that long-term treatment of adult males with estradiol may slow the LH response to castration in a few animals (Justo and NegroVilar, 1979).

In rats and other rodents, sexually dimorphic behaviors may be imprinted by the presence or absence of circulating androgens during the critical period of brain differentiation. As discussed above, the aromatization of androgens to estrogens is necessary for the masculinization of adult copulatory behavior. Administration of testosterone or estradiol to neonatal female rats masculinizes and defeminizes adult copulatory behavior (Gorski and Jacobson, 1981; Naftolin et.al., 1975; Whalen and Olson, 1981). In both rats and mice, female fetuses may experience behavioral masculinization in utero. Clemens et.al. (1978) showed that female rats located between males in utero had increased mounting behavior when adult, and increased ano-genital distances when born. Female mice located between two male littermates in utero have lengthened ano-genital distances when born and show masculinized aviodance responding, prolonged estrus cycles, and increased agressive behavior as adults (vomSaal et.al.,

1980; Hauser and Gondelman, 1983). Masculinization of female mice located near male littermates was associated with elevated levels of serum testosterone in utero (vomSaal, et.al., 1980). As suggested above, perinatal masculinization affects non-copulatory behaviors such as openfield activity, avoidance responding, agression, rough and tumble play, scent marking, feeding patterns, and learning (Beatty, 1979; Meyer-Bahlburg, 1982).

In addition to masculinization of adult behavior and neuroendocrine physiology, perinatal aromatization also mediates the morphological differentiation of the "sexually dimorphic nucleus of the pre-optic area" (SDN) first reported by Gorski et.al. (1978). The SDN is a region of the pre-optic hypothalamus which stains darker in male rats than in female rats. Measurements of the SDN have verified that the volume of this area is three times greater in males than in females. The volume of the SDN is unaffected by adult hormone manipulation, but perinatal castration of males feminizes the SDN and administration of DES or androgens to females masculinizes the volume of the SDN (Gorski et.al., 1978; Gorski et.al., 1980; Jacobson et.al., 1980; Dohler et.al., 1982). Further evidence that the volume of the SDN is related to estrogen and not androgen action is the observation that the SDN develops normally in mice with the tfm mutation (testicular feminization; absence of androgen receptors) (Gorski and Jacobson, 1981).

Further work with the SDN has shown that neonatal endocrine treatments such as tamoxifen which feminize male sexual behavior also result in decreased volume of the SDN (Gorski and Jacobson, 1981; Dohler et.al., 1984). The volume of the SDN has also been found to correspond to the vigor of male sexual behavior, but is unrelated to parameters of female sexual behavior (Gorski and Jacobson, 1981). A sexual dimorphism also exists in the size of the ventro-medial nucleus of the brain which is larger in males, smaller in females, and sensitive to neonatal testosterone administration (Matsumoto and Arai, 1983).

Regulation of hepatic drug and steroid metabolism lies within the sexually dimorphic pre-optic area of the hypothalamus (Gustafsson et.al., 1981). Parameters of drug metabolism which differ between male and female rats include:

1. male rats have increased amounts of cytochrome P-450 relative to female rats (Kato, 1974),

2. blue-shifted spectral maximum in female P-450 relative to P-450 from male rats (Kahl et.al., 1976),

3. more rapid turnover of P-450 in female rats (Levin and Ryan, 1975),

4. more rapid metabolism of substrates with type I binding in male rats (Kitada et.al., 1980) and

5. sex differences in the response of the P-450 system to MFO inducers such as phenobarbital or ßNF (Theron

et.al., 1981).

The role of the hypothalamus and pituitary in sex differences in hepatic metabolism was discovered in 1974 by Denef (Denef, 1974). Previously, Einarsson et.al. (1973) had proposed that liver steroid hydroxylases could be divided into three classes: Class I hydroxylases are androgen dependant(e.g. 6ß-hydroxylase), class II hydroxylases are more active in males and are not androgen inducible in females (e.g. 16a-hydroxylase), and class III hydroxylases are not distribulted differently between male and female rats (e.g. 7a-hydroxylase). The discovery in 1974 that hypophysectomy abolished the sex difference in class II hydroxylations suggested the role that brain sexual differentiation was later found to play. Studies using perinatal testosterone and estradiol showed that the control of hepatic drug metabolism is masculinized by aromatizable androgen during late pre-natal and early post-natal life. Lesioning and deafferentation studies demonstrated that the pre-optic region of the hypothalamus was involved in the regulation of hepatic metabolism (Gustaffson et.al., 1981). Recently it has been established that sex differences in the hypothlamic/pituitary release of growth hormone are responsible for the sex differences in hepatic drug metabolism (Gustaffson et.al., 1983; Mode et.al., 1983). The hypothalamic- pituitary axis, therefore, not only

regulates reproductive physiology directly, but may further mediate reproductive events through control of hepatic steroid metabolism (Figure 4).

Central effects which rely upon the aromatization of androgens to estrogens include: control of gonadotropin secretion (Worgul et.al., 1981; Hayashi, 1979), prolactin secretion, learning, circadian rhythmns (MacLusky and Naftolin, 1981), reproductive behaviors (Clemens and Pomerantz, 1982; Whalen and Olson, 1978), agression and play, morphological differentiation of the hypothalamus (Gorski et.al., 1980), and regulation of hepatic drug and steroid metabolism (Gustaffson et.al., 1981). Aromatization of testosterone to estradiol, therefore, is extremely important not only in the differentiation of the brain, but also in mediation of neuroendocrine events in adult animals. It has been established that endocrine manipulation can alter the hypothalamic aromatase activity in rabbits (Reddy et.al., 1973) and in rats (Roselli et.al., 1984), but the role of brain differentiation in regulating adult aromatase activities has not been fully investigated.

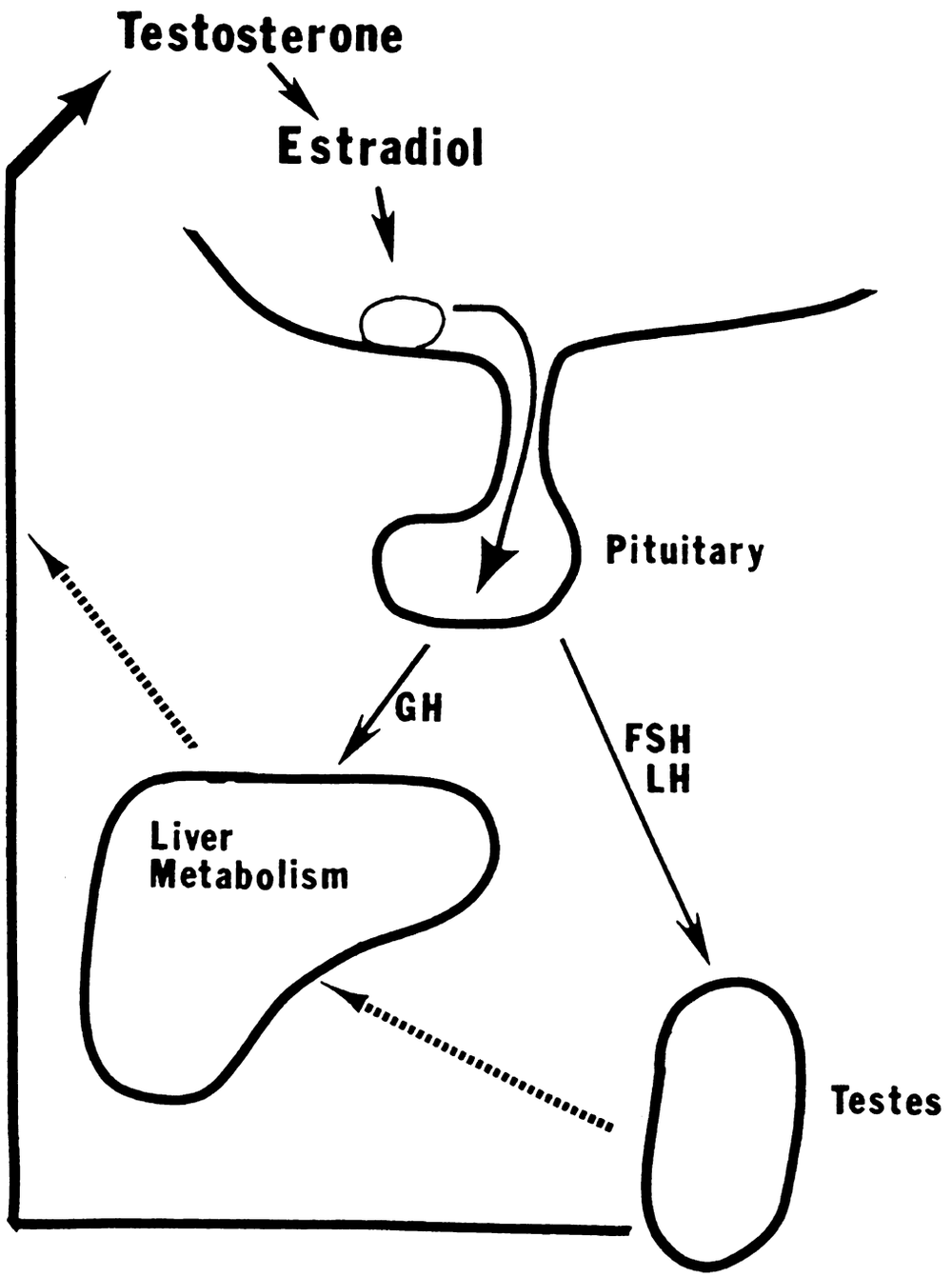

FIGURE 4 Interactions of the hypothalamic-pituitary system, gonadal steroid synthesis, and hepatic androgen metabolism (Gustafsson et.al., 1981)

Experimental Aims and Objectives

Mixed-function oxidase inducers such as PCBs, phenobarbital and PBBs have been shown to adversely affect reproductive capability in experimental animals. Induction of MFOs by PBBs results in decreased efficacy of exogenously administered steroids, and reduces the effects of endogenous compounds as well. Succecssful reproduction requires the coordination of a complex array of neuroendocrine and metabolic processes. Interruptions of control pathways in brain, alterations in hypothalamic or pituitary secretory processes or altered peripheral metabolic patterns could all adversely affect reproduction.

The nuclei of the anterior hypothalamus regulate reproductive physiology and behavior, and are potential targets for the toxic effects of PBBs on reproduction. Endocrine insults during brain development have already been shown to alter central sexual differentiation, and adult patterns of sexual behavior and physiology. The hypothalamus is very sensitive to the presence of aromatizable androgens during development. Even short-lived changes in testosterone availability can result in permanent effects on reproduction (Ward and Weisz, 1980; Weisz and Ward, 1980). PBB induced alterations in perinatal steroid dynamics, therefore, may affect reproduction throughout the life of the animal. Because of

the important role hypothalamic aromatization plays in control of reproductive processes, it is also important to determine whether perinatal or adult PBB treatment is capable of altering hypothalamic aromatase (HA) activity.

The objectives of the research described herein are the following:

1. Develop a simple and accurate technique for the determination of hypothalamic aromatase activity.

2. Determine how hypothalamic aromatase activity is controlled in the rat, and the extent to which perinatal effects may alter adult HA activity.

3. Discover the effects of perinatal or adult PBB treatment on hypothalamic aromatization of androgens.

4. Determine whether PBB treatment can alter plasma testosterone titer.

5. Discover the extent to which alterations in synthetic or target organ metabolic pathways may alter the reproductive capacity of PBB treated animals.

6. Determine whether perinatal PBB treatment alters steroid metabolism or testosterone titer during the critical period of brain sexual differentiation.

7. Test adult sexual behavior and neuroendocrine physiology to assess alterations in brain differentiation.

CHAPTER 1

ASSAY DEVELOPMENT

CHAPTER 1

INTRODUCTION

In order to accurately determine the activity of the aromatase enzyme system in the hypothalamus, it was necessary to develop an improved assay. Although aromatizing activity is central to the normal control of reproduction, hypothalamic aromatase is active at very low levels, and radioactive tracer methods are necessary for its detection. In general, other published assays have relied on one of three methods: production of $^{3}H_2O$ from the aromatization of 1ß,2ß-^{3}H-androstenedione (Thompson and Siiteri, 1974), production of ^{3}HCOOH from 19-$^{3}H_3$-androstenedione (Miyari and Fishman, 1985), or isolation and purification of ^{3}H-estrogens by solvent partition, thin layer chromatography, derivatization, and reverse isotope dilution followed by recyrstallization to constant specific activity (Dessi-Fulgheri and Lupo, 1982: Selmanoff et.al., 1977: Naftolin et.al., 1972).

Aromatase assays relying on the production of $^{3}H_2O$ do not always agree quantitatively with assays which directly detect the production of tritiated estrogens

(George and Ojeda, 1982). Assays which measure the release of tritiated water during aromatization can yield spurious results due to reactions at C1 and C2 which are unrelated to aromatization (Hersey et.al., 1981; Miyari and Fishman, 1985). Recently, Miyari and Fishman (1985) have proposed measuring the formation of tritiated formic acid from $^{3}H_{3}$-C19 androgens to determine aromatase activity. Although this method does not have the potential for inaccuracy that the tritiated water method does, isotope effects connected with the tritiation of the C-19 methyl render this technique 3.2 times less sensitive than either of the other two methods (Miyari and Fishman, 1985).

Although methods which rely on the direct detection of tritiated estrogens are more accurate and potentially more sensitive than the other two methods, heretofor these methods have been extremely time consuming. Previously, methods for the direct detection of estrogens have involved solvent partition, derivatization, thin layer chromatography and multiple recrystallization, and recovery calculations have relied on dual isotope techniques. High Performance Liquid Chromatography (HPLC) offers the advantage of direct product isolation of estrogens, while offering the potential for much more rapid separation of the estrogenic products of aromatization than previous methods. In addition, recovery can be determined by U.V. absorbance during HPLC, eliminating the need for dual

isotope techniques.

A valid enzyme assay using HPLC isolation of estrogens requires incubation conditions which produce time and protein dependant aromatization of androgens to estrogens, HPLC conditions which allow the rapid separation of estrogens from other steroids and proof of the radiochemical purity of the estrogens isolated by HPLC. Incubation conditions for placental and hypothalamic aromatase have been well described in the literature (Naftolin et.al., 1975; Selmanoff et.al., 1977; Ryan, 1959) but existing methods for determination of the reaction products are not suitable for detailed studies of the enzyme. The inital objective was therefore the development of a suitable HPLC system and demonstration of its suitability for measurements of hypothalamic aromatase activity.

METHODS

Substrate Purification

Reference steroids for method development were purchased from Steraloids Inc. (Wilton, N.H.), and tritiated androstenedione (1,2,6,7-^{3}H-androst-4-ene-3,17-dione, 90 Ci/mmole) was obtained from New England Nuclear (Boston, MA). Two successive HPLC procedures were necessary to insure substrate purity and to remove all traces of androsta-1,4,6,-triene- 3,17-dione, a potent inhibitor of the aromatase enzyme system, and a contaminant of some lots of tritiated androstenedione. Radioactive androstenedione was purified by reverse phase HPLC using acetonitrile/water (40:60) on an Altex 5μ Ultrasphere ODS column, then rechromatographed on the same column using THF/water (35:65) as the eluent.

Hypothalamic Incubation and Sample Preparation

Adult Sprague-Dawley rats were killed by decapitation, and the entire brain rapidly removed. Anterior hypothalamus containing the medial pre-optic and anterior hypothalamic nuclei was removed using a dissection similar to that reported by Naftolin et.al. (1972) (Figure 5).

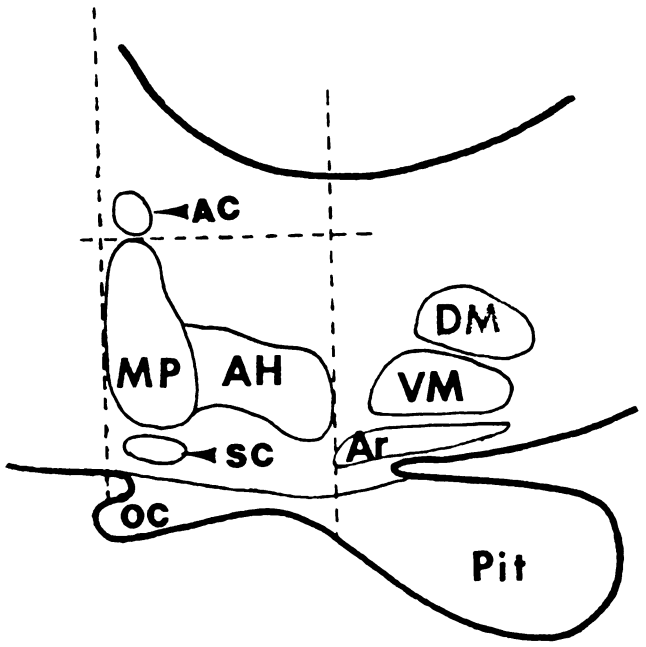

FIGURE 5. Sagittal view of the hypothalamus. Dotted lines show location of razor cuts used to remove the anterior hypothalamic nuclei. AC=anterior commissure, MP= medial preoptic, AH=anterior hypothalamic, SC=suprachiasmatic, Ar=arcuate, VM=ventral medial, DM=dorso-medial, OC=optic chiasm

The hypothalamus was homogenized in 0.05 M phosphate buffer (pH=7.0), and 10 µg of estrone was added as a product trap, and for recovery calculations at the end of the assay. An NADPH regenerating system was added, and the reaction was started by the addition of 10 µCi of 1,2,6,7-^{3}H-androstenedione, purified as described. Each assay tube contained between 2 and 10 hypothalami, 110 pmole ^{3}H-androstenedione, 2.4 µmole NADPH, 12 µmole glucose-6-phosphate and 0.4 U glucose-6-phosphate dehydrogenase in a final volume of 0.5ml. Assay tubes were incubated with agitation for between one and three hours at 37^{o}C. Reactions were terminated by the addition of 5ml ether/chloroform (3:1). Incubation blanks were prepared by the addition of ether/chloroform at zero time. Positive control incubations of human placental microsomes (prepared as described by Ryan, 1959), were carried out simultaneously with the incubation of hypothalamic homogenates.

Each incubation tube was extracted twice with 5ml of ether/chloroform and the combined extracts were dryed over anhydrous Na_2SO_4 and evaporated to dryness under N_2. Each sample was dissolved in 1N NaOH and extracted with toluene to remove non-polar lipids and unreacted androgens. The NaOH layer was then neutralized with HCl, re-extracted with ether/chloroform, and the extract evaporated to dryness and redissolved in 50µl of methanol prior to chromotography.

High Performance Liquid Chromotography

Two HPLC systems were developed for the assay (system A & B), and two additional systems used to confirm the purity of the peaks collected during the assay (system C & D)(Figure 6):

System A: acetonitrile water (70:30), 1.0ml/min, Waters 10μ radial-PAK (C-18) cartridge;

System B: THF/water (35:65), Waters 10μ radial-PAK C-18 cartridge;

System C: Acetonitrile/THF/water (55:5:40), 1.0ml/min, Altex 5μ Ultrasphere ODS column (15cm length);

System D: cyclohexane/n-octanol (95:5), 2.0ml/min, Waters 10μ radial-PAK normal phase cartridge.

The HPLC system consisted of a Waters model M6000A pump (Milford MA) with a Model 440 U.V. detector at 254nm (system A & B) or 280 nm (system C & D). Steroids were quantified, and recovery calculated using a Waters 760 Data Module recording integrator.

Following sample preparation as described above, the samples were chromatographed on system A, and the estrone peak collected and rechromatographed on system B. The estrone peak was collected during chromatography on system B, and the ^{3}H-estrone present was quantified by liquid

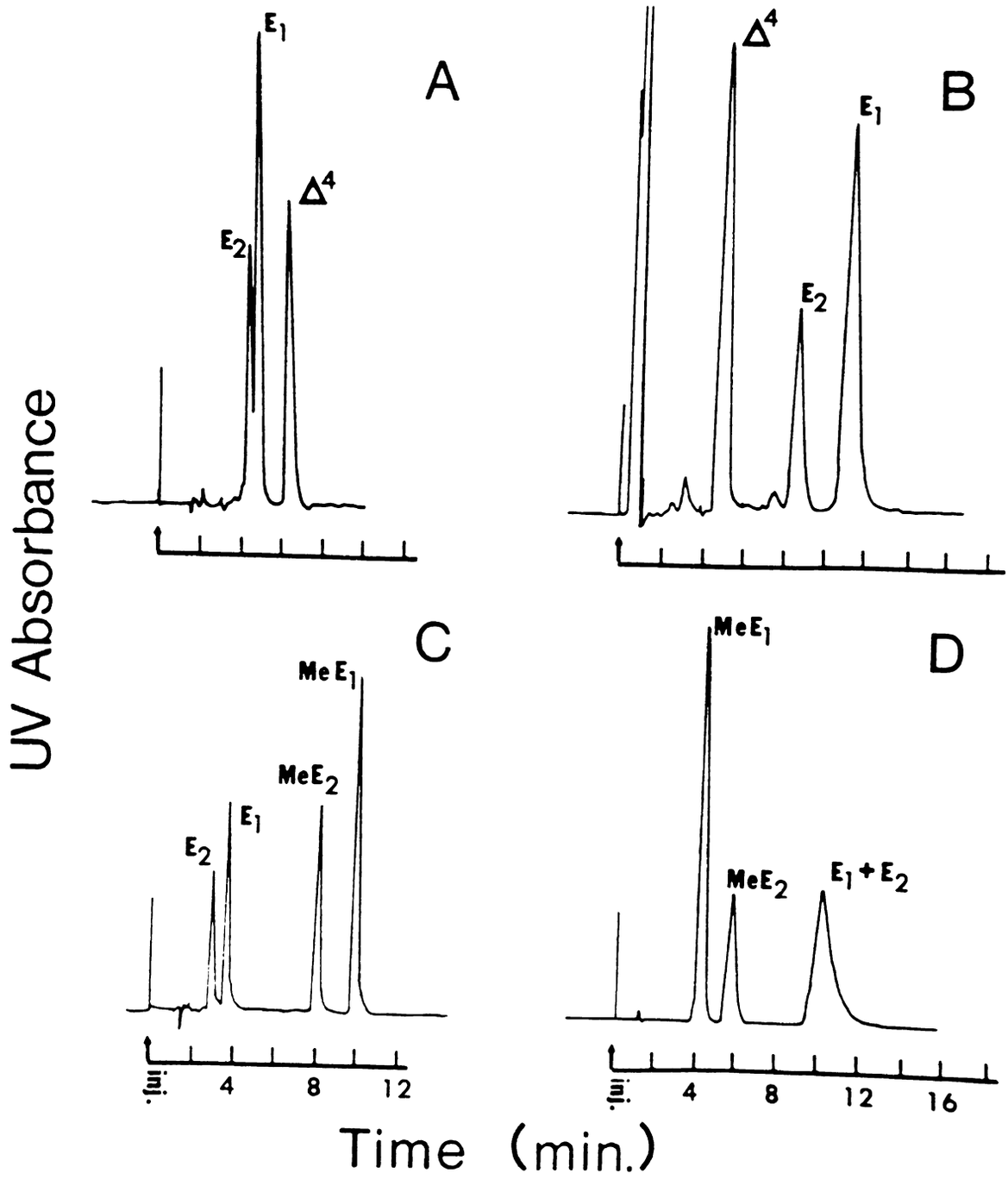

FIGURE 6. Chromatograms of reference steroids.
A) acetonitrile/water (70:30), C-18 column
B) THF/water (35:65), C-18 column
C) acetonitrile/THF/water (55:5:40), C-18, 5μ
D) cyclohexane/n-octanol (95:5), silica

scintillation in a Packard Tri-Carb (Model 460C). Recovery of estrone was calculated from U.V. absorbance during chromatography on system B.

Method Validation

To verify the purity of the estrone peaks collected following chromatography on system B, a series of test incubations were performed. Placental microsomes and hypothalamic homogenates (10 brains per tube) were incubated as described above. After incubation the estrogenic products were separated by chromatography on system A and B, then split into three aliquots. The first aliquot was counted, and the other two aliquots were derivatized to produce estrone 3-methyl ether as described by Brown (1955). Following methylation, the two remaining aliquots were chromatographed on either system C or D, and specific activity was compared with that obtained after chromatography on system A and B alone. In addition, time and protein dependancy of the hypothalamic incubations was verified using pooled hypothalamic homogenate equivalent to two to eight hypothalami, and for times up to three hours.

RESULTS AND DISCUSSION

Chromatography with system A was useful in separating the estrone produced from the highly radioactive ^{3}H-androstenedione substrate. Since the estrogens eluted first on system A, the chance of the androstenedione "tailing into" the estrogen peaks was eliminated. The second chromatographic step with system B then separated the estrogens from each other, and from any residual androstenedione which might remain. Calculation of estrone recovery following chromatography on system B showed recoveries which averaged 70 - 80% of the estrone added to the incubation tubes. Figure 7 shows a radiochromatogram (HPLC system B) of the estrone produced by incubation of ^{3}H-androstenedione with hypothalamic tissue in comparison with that seen in a blank incubation. Since hypothalamic tissue has been shown to contain little 17ß-dehydrogenase (George and Ojeda, 1982), and since only trace amounts of estradiol were observed following incubation of hypothalamic homogenates (Figure 7), the estradiol peak was not further characterized, and was not used in the calculation of hypothalamic aromatase activity.

An average purity of 95% or greater (Table 3) was

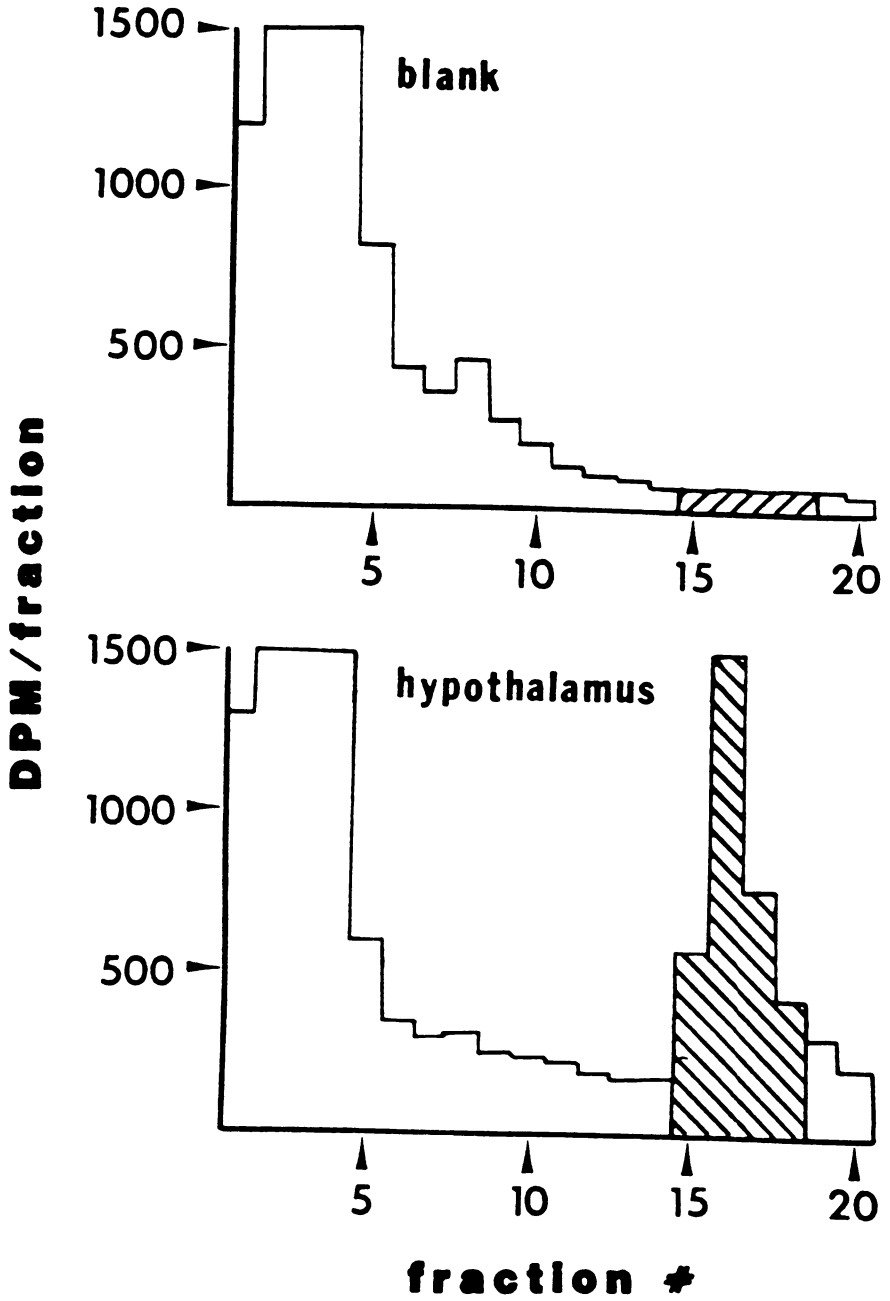

FIGURE 7 Radiochromatogram of fractions collected from system B chromatography of zero-time blank and hypothalamic incubation. Cross-hatch pattern corresponds to estrone peak by U.V.

TABLE 3.

Purity of Estrone Produced by Aromatization Following Reverse Phase Chromatography with 35% THF.

sample #	spec. activity after THF dpm/µg estrone	spec. activity following methylation reverse phase dpm/µg*	%THF	normal phase dpm/µg*	%THF
Hypothalamic homogenate					
1	213	224	105.%	221.	103.%
2	452	411	95.6%	371	86.3%
3	266	242	95.6%	247	97.6%
			98.7±3.1		95.6±5
Placental microsomes					
1	119	118	99.2%		
2	1050	1090	104. %		
3	793	752	94.8%		
4	191	174	91.1%		
5	686	684	99.7%		
6	402	363	90.3%		
			96.5±2.4		

* calculated on the basis of µg of original estrone added.

shown by collection of estrone following chromatography on system A, chromatography on system B, methylation, and rechromatography on system C or D. Since the criterion for purity using recrystallization is normally a 5% or smaller change in isotope ratio, the purity of estrone produced by dual HPLC separation is at least as good as that produced by repeated recrystallization.

Both time and protein dependancy were determined using placental microsomes (data not shown), and confirmed using hypothalamic incubations. This incubation technique showed linear time dependancy to 3 hours and was protein dependant with hypothalamic protein equivalent to 2 to 10 hypothalami (Figure 8).

These results indicate that hypothalamic aromatase may be measured following separation of estrogens on HPLC system A and isolation and quantification of ^{3}H-estrone on HPLC system B. The method offers good recoveries of 70 - 80%, is rapid and easily performed, produces products with a high degree of purity, and is sensitive, allowing the detection of less than 10 femtomoles of tritiated estrogen produced by aromatization. This method has the advantage of being more accurate than methods employing the measurement of tritiated water, and more sensitive than those measuring tritiated formic acid. In addition, it is

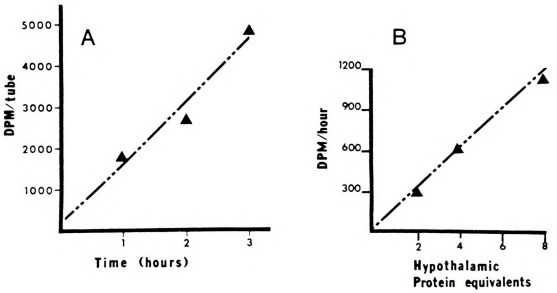

FIGURE 8 Time (A) and protein (B) dependance of incubation of hypothalamic homogenates.

more rapid, and at least as sensitive and accurate as previous techniques which directly measured the production of estrogenic products of hypothalamic androgen aromatization.

CHAPTER 2

CONTROL OF HYPOTHALAMIC AROMATASE ACTIVITY

CHAPTER 2

INTRODUCTION

The hypothalamic aromatizing (HA) enzyme system is important for the control of reproductive behavior and physiology in a wide variety of mammalian and non-mammalian species. Aromatization of circulating testosterone to estradiol during fetal and neonatal development results in the differentiation of critical brain regions which regulate sexual behavior, feedback control of steroid synthesis and hepatic drug metabolism in the adult. In adult rats, hypothalamic androgen aromatization mediates CNS control of reproductive behavior and physiology.

In mammalian species male animals have higher levels of hypothalamic aromatase activity than do female animals (Weisz and Gibbs, 1974; Naftolin et.al., 1975; Roselli et.al., 1984; Selmanoff et.al., 1977 Reddy et.al., 1973). Initial studies on the endocrine control of hypothalamic aromatization were performed in rabbits. Reddy et.al. (1973) showed that castration, testosterone treatment or estradiol treatment of adult rabbits resulted in increased levels of hypothalamic aromatase. These results are somewhat difficult to interpret, both since rabbits are

reflex ovulators and since one would normally expect castration and testosterone treatment to have opposite effects on steroid sensitive pathways.

Conflicting reports of the effects of castration on HA activity in rats have appeared. In 1977 an abstract appeared which reported that castration of male rats decreased the activity of HA in the pre-optic hypothalamus (Kobayashi and Reed, 1977), but no followup report has appeared in the primary literature. Dessi-Fulgheri and coworkers found that aromatase activity in the hypothalamus could be affected by olfactory stimuli, but failed to find any effects of castration or hormone treatment (Dessi-Fulgheri, personal communication; Larsson et.al., 1982; Dessi-Fulgheri and Lupo, 1982).

Shortly after the work described here was completed, Roselli et.al. (1984) reported that adult castration of male rats reduced the activity of hypothalamic aromatase to levels normally seen in female rats. Further, they showed that adult testosterone treatment increased HA activity in female rats to levels normally seen only in male animals. The results reported here extend and strengthen the report of Roselli et.al., by confirming the effects of adult hormone manipulation on HA activity, and demonstrating the absence of an effect of perinatal hormone treatment on adult hypothalamic androgen aromatization.

MATERIALS AND METHODS

Animals and Treatment

Experiment 1. Adult (60 day old) male and female Sprague-Dawley rats were obtained from Spartan Farms (Haslett, MI), and housed under 12:12 inphase light:dark conditions. Food and water were available ad lib.. Male rats were castrated or assigned to a control group. Two weeks following castration, hypothalamic aromatase activity was determined in males, castrate males and females as previously described (chapt 1; Wilke and Braselton, 1983). Hypothalami were pooled within groups, three per assay tube.

Experiment 2. Adult female Sprague-Dawley rats were obtained and housed as described above. Daily vaginal smears were taken from each rat for two weeks to establish the presence of a 4 day estrus cycle. Only rats having a four day cycle were used in the HA assay. Female rats were killed by decapitation, and hypothalami were pooled (3 per tube) according to stage of estrus cycle on day of sacrifice. HA activity was determined as previously described.

Experiment 3. Timed-pregnant females were obtained

as above, and treated so pups could be assigned to one of the following groups:

1. male, no treatment
2. female, no treatment
3. male, castrated on day of birth
4. male, castrated on day of birth, testosterone replaced with silastic implant (30 cm x 0.132 id x 0.183 od) when 60 days old
5. female, treated with 100μg testosterone propionate on day 3 postnatal
6. female, treated with silastic testosterone implant when 60 days old
7. male, treated with the aromatase inhibitor androsta-1,4,6-triene-3,17-dione from day 15 of gestation until day 5 postnatal (5mg/day to mothers, 0.5mg/day to pups)

Each pup from the above litters was kept until 90 days old, then sacrificed by decapitation. Hypothalamic aromatase activity was determined as previously described.

Statistical Analysis

Differences in hypothalamic aromatase activity between groups were assessed by analysis of variance followed by Duncan's New Multiple Range Test (significant if $p<0.05$).

RESULTS

Castration of adult male rats reduced hypothalamic aromatase activity to levels normally seen in female rats (Figure 9). Female rats killed at different stages of the estrus cycle (proestrus, estrus, diestrus day 1 and diestrus day 2) did not show differences in HA activity which were related to stage of the estrus cycle (Figure 10).

Male rats castrated on the day of birth had levels of HA equivalent to those present in normal females. Implantation of a 3cm silastic testosterone capsule in adult rats increased HA activity to normal male levels in both castrate males and in implanted females. Administration of 100µg testosterone propionate on day 3 of life had no effect on adult HA activity in female rats. A feminizing dose of the aromatase inhibitor androsta-1,4,6-triene- 3,17-dione administered to perinatal male rats had no effect on adult HA activity (Figure 11).

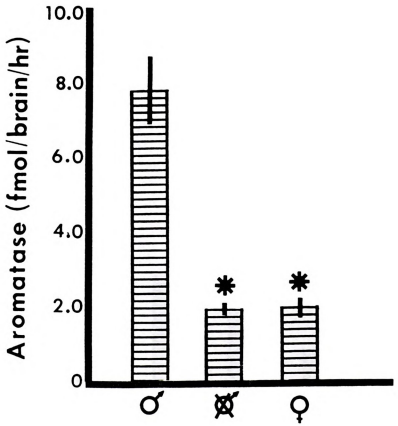

FIGURE 9. Effect of adult castration on hypothalamic aromatase activity. ♂=control male, ⚥=castrate male, ♀=control female
*Statistically different from control male by Duncan's Mew Multiple Range Test ($p<0.05$). Graph shows mean $\pm$ standard error (n=8).

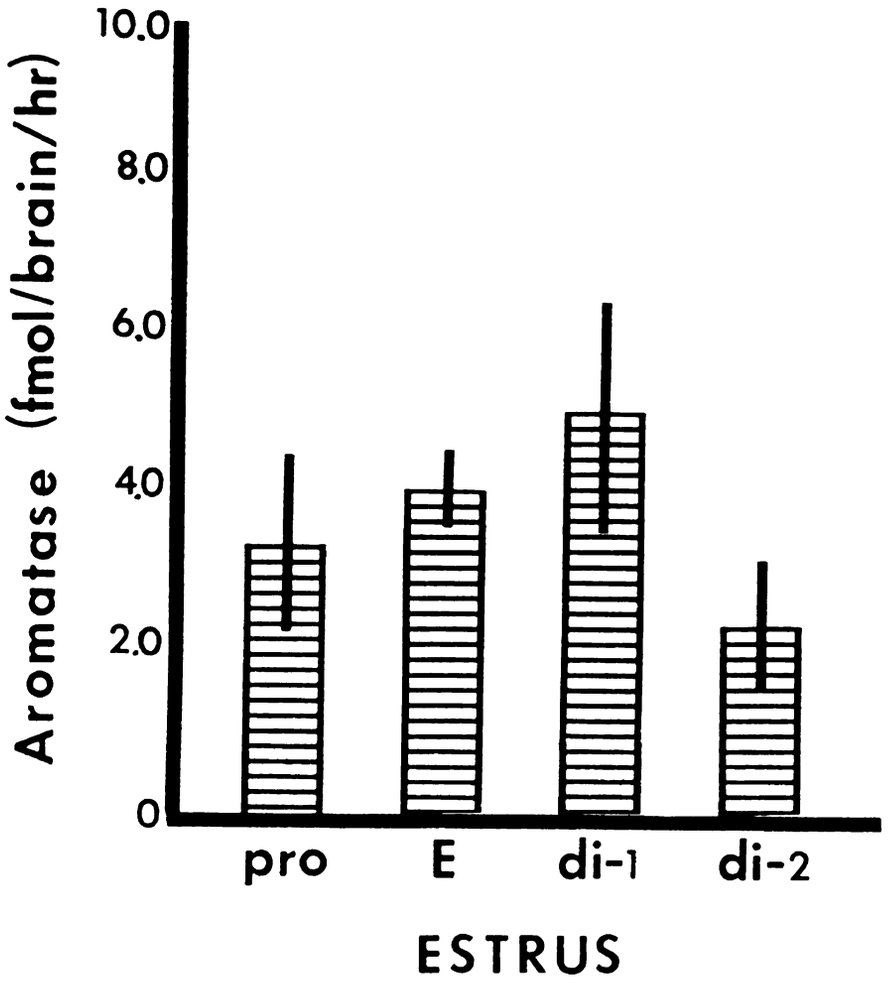

FIGURE 10. Hypothalamic aromatase activity throughout the estrus cycle in normal female rats. pro=proestrus, E=estrus, di-1=diestrus day 1, di-2=diestrus day 2. No significant differences between groups. Measures are mean ± standard error (N>6 for each group).

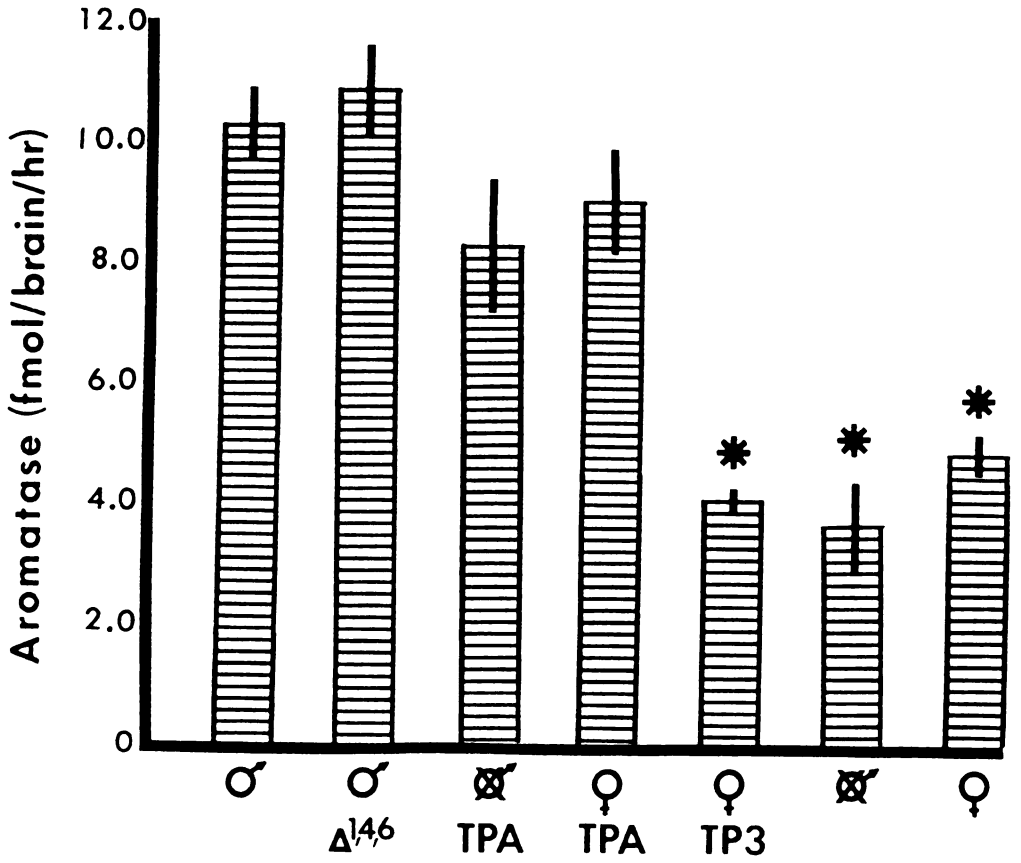

FIGURE 11. Effect of neonatal and adult endocrine treatments on hypothalamic aromatase activity in the rat.
♂=male
⊠=castrate male
♀=female
Δ^{146}=androsta-1,4,6-triene-3,17-dione administered perinatally
TPA=testosterone capsule implanted in adult
TP3=100μg testosterone propionate as neonate
*Statistically different from control male by Duncan's New Multiple Range Test ($p<0.05$)
Measures are mean ± standard error ($n>6$).

DISCUSSION

These experiments were designed to determine how hypothalamic aromatase activity is controlled in the rat. Although conflicting reports exist (Dessi-Fulgheri, 1982), these results confirm other studies which also found that adult castration decreases male hypothalamic aromatase activity to levels normally found in females (Kobayashi and Reed, 1977; Roselli et.al., 1984). Although hypothalamic aromatase activity is sensitive to circulating androgens, aromatase activity in amygdala and other brain regions does not change in response to endocrine manipulation (Roselli et.al., 1984; Kobayashi and Reed, 1977). In hypothalamus, aromatase activity is highest in pre-optic and anterior hypothalamic nuclei (Roselli et.al., 1984; Selmanoff et.al., 1977), sexually dimorphic brain areas involved in the regulation of reproductive behavior and physiology (Gorski et.al., 1978; Nordeen and Yahr, 1982).

Implantation of 3 cm testosterone capsules was chosen to test the ability of the HA system to respond to androgens since this dosage has already been shown to be effective in suppressing plasma LH in castrated male rats (DaMassa et.al., 1976). Testosterone treatment of females

or castrated males resulted in elevation of HA activity to levels normally seen in male rats. The ability of adult castration or testosterone treatment to affect hypothalamic aromatase activity suggests that it is under direct androgenic control.

Other evidence that rat HA activity is controlled through an androgen-receptor mediated mechanism is the report by Roselli and Resko (1984) that testosterone or DHT, but not estradiol will increase HA activity in the rat. Further, male tfm (testicular feminization mutation) mice, which lack functional androgen receptors, have hypothalamic aromatase activity no higher than female animals (Rosenfeld et.al., 1977). Blockade of the androgen receptor by the androgen antagonist flutamide reverses the testosterone and DHT induced increases in HA activity (Roselli and Resko, 1984). These reports suggest that hypothalamic aromatase is under the control of either testosterone, or its androgenic metabolite DHT.

Hypothalamic aromatase activity may also be influenced by stimuli other than circulating androgens. Brain catecholamines may be involved in regulation of HA activity. Catecholaminergic ß-agonists have been shown to decrease estrogen receptor occupancy following testosterone administration (Raum et.al., 1984), and both a- and ß-agonists reportedly decrease HA activity in fetal rats (Roselli et.al., 1984). The administration of

p-chlorophenylalanine (pCPA), an inhibitor of catecholamine and serotonin synthesis increases brain aromatase activity (Farabollini et.al., 1981). Olfactory stimuli also modulate aromatase activity. Male rats reared in the presence of female rats or bedding from female cages had elevated aromatase activity when compared with animals raised in the presence of males only (Dessi-Fulgheri and Lupo, 1982). This effect of female odors was abolished by olfactory ablation (Larsson et.al., 1982).

Although testosterone titer varies slightly throughout the estrus cycle in female rats (Rush and Blake, 1982), either the magnitude or the duration of the variation is insufficient to alter HA activity. Although female rats are responsive to alterations in plasma testosterone this study showed no change in HA activity throughout the estrus cycle. Identical results are also reported by Roselli et.al. (1984).

Perinatal hormone manipulation had no effect on the activity of hypothalamic aromatase in adult rats. Treatment of female neonates with testosterone propionate on day 3 has been shown to induce anovulatory sterility in adult females (DaMassa et.al.,1983). This treatment also defeminizes and masculinizes sexual behavior in rats (Gorski and Jacobsen, 1981; Stewart et.al., 1979). Although as little as 10 μg of testosterone propionate may cause behavioral and physiologic masculinization of

perinatal females, 100 μg of TP failed to affect adult aromatase activity. In addition, perinatal treatment with doses of androsta-1,4,6-triene-3,17-dione (ATD) similar to those employed here can demasculinize and feminize the behavior of male rats and hamsters (Ruppert and Clemens, 1981; Gladue and Clemens, 1980; Stewart et.al., 1979; Clemens and Gladue, 1978). ATD treatment failed to alter HA activity in adults, even though similar dosages demasculinize behavior in male rats.

The results discussed above suggest the following:

1. hypothalamic aromatase activity is regulated by adult androgen titer,

2. although HA exists in a sexually dimorphic region of the brain, it is unaffected by perinatal endocrine treatments which alter adult sexual behavior and physiology,

3. HA activity is inducible in both male and female rats by androgen treatment and

4. HA activity does not change throughout the estrus cycle in female rats.

CHAPTER 3

EFFECT OF PBB TREATMENT ON BLOOD TESTOSTERONE AND HYPOTHALAMIC AROMATASE

CHAPTER 3

INTRODUCTION

PBBs are potent inducers of the mixed function oxidase enzymes which metabolize steroid hormones. Previous research has already established that PBB treatment results in more rapid plasma clearance of testosterone and progesterone (McCormack et.al., 1981; Harris et.al., 1981; McCormack et.al., 1979). PBB treatment results in several effects which may be related to increased metabolism of endogenous steroids, including: decreased seminal vesicle and prostate weights, prolonged estrus cycles (Johnston et.al., 1980), infertility in cattle (Jackson and Halbert, 1974), and hypoactive seminiferous tubules (Allen et.al., 1978). Despite the evidence of impaired reproductive capability following PBB treatment, previous research has so far failed to find alterations in circulating steroid titers associated with PBB consumption (Castracane et.al., 1982; Johnston et.al., 1980).

The activity of the hypothalamic aromatase (HA) enzyme system is sensitive to the presence of circulating testosterone (chapt 2; Roselli et.al., 1984). The aromatization of androgens to estrogens is important in

brain sexual differentiation and the adult control of sexual behavior and reproductive physiology (Clemens and Gladue, 1978; Naftolin et.al., 1975; Worgul et.al., 1980; Christensen and Clemens, 1975). Preliminary studies with PBB showed that pre-pubertal PBB treatment caused a trend toward decreased HA activity in rats (Wilke and Braselton, 1983), a possible indication of PBB effects on testosterone titer. Because of the important role of hypothalamic aromatase in the control of reproduction, PBB effects on HA activity could have far-reaching reproductive consequences. It was therefore of interest to determine whether PBB treatment results in effects on HA activity, and whether those effects are associated with alterations in serum testosterone titer.

METHODS

Experiment 1

Weaned, 21 day old male Sprague-Dawley rats were obtained from Harlan (Haslett, MI) and assigned to PBB treated, control or castrate groups. PBB animals were given 150mg/kg fireMaster BP-6 (lot #6244-A, Michigan Chemical Corp. St.Louis MI) in peanut oil by i.p. injection when 22 days old. Castrate animals were orchidectomized trans-scrotally under light ether anesthesia when 22 days old. Control animals received 1ml/100g peanut oil by i.p. injection. Animals were maintained on a 12:12 inphase light:dark cycle with food and water available ad lib.. Animals were sacrificed when 75 days old, and hypothalamic aromatase activity was determined as previously described (Wilke and Braselton, 1982).

Experiment 2

Timed-pregnant Sprague-Dawley rats were obtained from Harlan (Madison, WI) and assigned to either the PBB or control group. On day 8 of gestation, dams in the PBB group began receiving ground Wayne Lab-Blox containing 100ppm fireMaster BP-6 in place of regular food. At birth, male pups were assigned to one of the following groups, and

treated as described:

1. control male;
2. male, castrated on day of birth;
3. male, castrated on day of birth, given 3.0cm testosterone implant when 60 days old;
4. PBB male, mother or pups fed 100 ppm of PBB in food from day 8 of gestation until sacrificed;
5. PBB male, PBBs as above, given testosterone supplementation; 50μg alternate days until 21 days old, 1.5 cm testosterone implant from 21 to 60 days, 3.0cm testosterone implant when 60 days old.

These rats were maintained as described above until 90 days old, then sacrificed by decapitation. Hypothalamic aromatase activity was determined, and prostate, seminal vesicle, and testicular weights were recorded. Blood testosterone was measured in control and PBB treated animals.

Experiment 3

Adult male Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) when 60 days old. Animals were castrated or assigned to a PBB or control group. PBB animals received 150mg/kg fireMaster BP-6 in peanut oil by i.p. injection. Control animals were given 2ml/kg peanut oil. Control and PBB treated animals were killed 14 or 90

days following PBB treatment. Castrate animals were killed 14 days after castration. On the day of sacrifice, hypothalamic aromatase activity was measured as previously described. Blood samples were taken for testosterone RIA.

Measurement of Blood Testosterone

Serum testosterone titer was determined as follows. Blood collected after decapitation was allowed to clot for 2 hours at room temperature. After clot removal, the serum was frozen at -20° C until RIA. Serum testosterone was determined using a direct solid-phase radio-immuno assay (Coat-A Count, Diagnostic Products, Los Angeles, CA). Sensitivity of the standard curve was 0.3ng/ml of testosterone using 25 µl serum samples. The standard curve was transformed by a logit/log regression technique (correlation coefficient of the standard curve =.987), and the intra-assay coefficient of variation was 4.7%).

Statistical Analysis

Statistical comparisons were made using analysis of variance, followed by Duncan's New Multiple Range Test ($p<0.05$).

RESULTS

Experiment 1

Castration of male rats at time of weaning resulted in decreased hypothalamic aromatase activity at 75 days of age. PBB treatment on day 22 resulted in a non-significant decrease in mean HA activity at 75 days of age (Table 4).

Experiment 2

Neonatal castration of male rats resulted in a marked decrease in HA activity at 90 days of age. Implantation of a testosterone capsule in castrated rats restored HA activity to levels seen in control males. Animals fed 100 ppm PBB throughout life showed a trend toward decreased aromatase activity when tested at 90 days old, but neither PBB rats nor PBB rats given testosterone had HA activity significantly different from control animals (Figure 12).

Neonatal castration resulted in non-detectable levels of serum testosterone in 90 day old animals. Animals treated with PBB showed a trend toward decreased serum testosterone relative to control males. Serum testosterone titers obtained from animals implanted with silastic capsules were unreliable, since decapitation often severed

TABLE 4
Effect of orchidectomy or PBB treatment at 21 days on the hypothalmic aromatase activity in adult male rats.

group	brain aromatase fmole/brain/hour
control male	6.8 ± 0.5 (3)
PBB male	5.2 ± 0.5 (3)
castrate male	4.0 ± 0.7 (3)*

expressed as mean ± s.e.m.(n)
* less than control activity by Duncan's New Multiple Range Test ($p<0.05$)

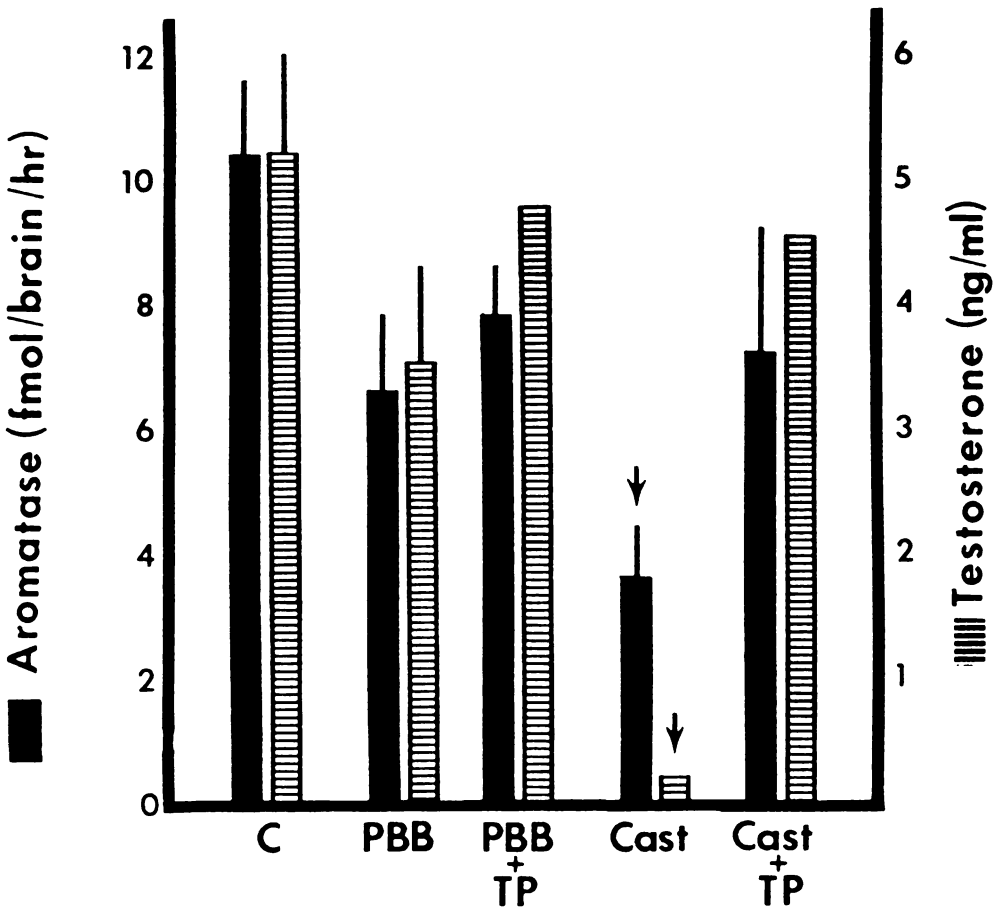

FIGURE 12. Effect of neonatal castration or lifelong PBB treatment on adult testosterone titer and hypothalamic aromatase activity; reversal of castration effects by adult testosterone treatment.
↓Statistically smaller than control male by Duncan's New Multiple Range Test ($p<0.05$). Measures are mean ± standard error ($n>6$, except testosterone values for TP; $n=2$).

the silastic casule, contaminating the blood sample. Average serum testosterone titers of three animals from each implanted group known to have intact silastic capsules are shown, and suggest that testosterone implantation resulted in near normal plasma testosterone in both PBB treated and castrate rats (Figure 12).

PBB treatment resulted in decreased body weight relative to control and castrate animals (Table 5). PBB animals given testosterone were heavier than PBB treated males, but smaller than castrates. Neonatal castration resulted in decreased body weight in 90 day old animals. Both PBB treatment and castration resulted in marked decreases in seminal vesicle and ventral prostate weight. Adult treatment of castrates with testosterone partially restored seminal vesicle and prostate weights. Testosterone supplementation of PBB rats resulted in increases in prostate and seminal vesicle weight to control levels or above (Table 5). PBB treated rats had testicular to body weight ratios greater than those in control rats. Testosterone treatment of PBB rats decreased testicular weights to below those seen in control animals.

TABLE 5
Effect of PBB treatment or orchidectomy on body weight and reproductive organs

group	body weight (g)	ventral prostate (mg/100gbw)	seminal vesicle (mg/100gbw)	testes (mg/100gbw)
control male	414±40(14)	136±26(14)	275±33(14)	916±82(14)
PBB male	243±56(12)*	33±16(12)*	177±66(12)*	1216±201(12)#
PBB male + T	276±34(8)*	134±57(8)	405±105(8)#	425±121(8)*
castrate male	344±37(14)*	4±1 (14)*	6±5 (14)*	-------
castrate + T	351±17(9)*	85±31(9)*	85±31(9)*	-------

all measures are mean ± standard deviation (n)
* smaller than control male by Duncan's New Multiple Range Test ($p<0.05$)
# larger than control male by Duncan's New Multiple Range Test ($p<0.05$)

Experiment 3

Acute dosing with 150 mg/kg PBB resulted in decreased hypothalamic aromatase activity and testosterone titer in male rats killed 14 days after dosing. Both hypothalamic aromatase and serum testosterone had returned to control levels by 90 days after PBB treatment. Castration decreased hypothalamic aromatase activity to below levels seen in PBB treated rats. Testosterone was not detectable in serum from castrate rats (Figure 13).

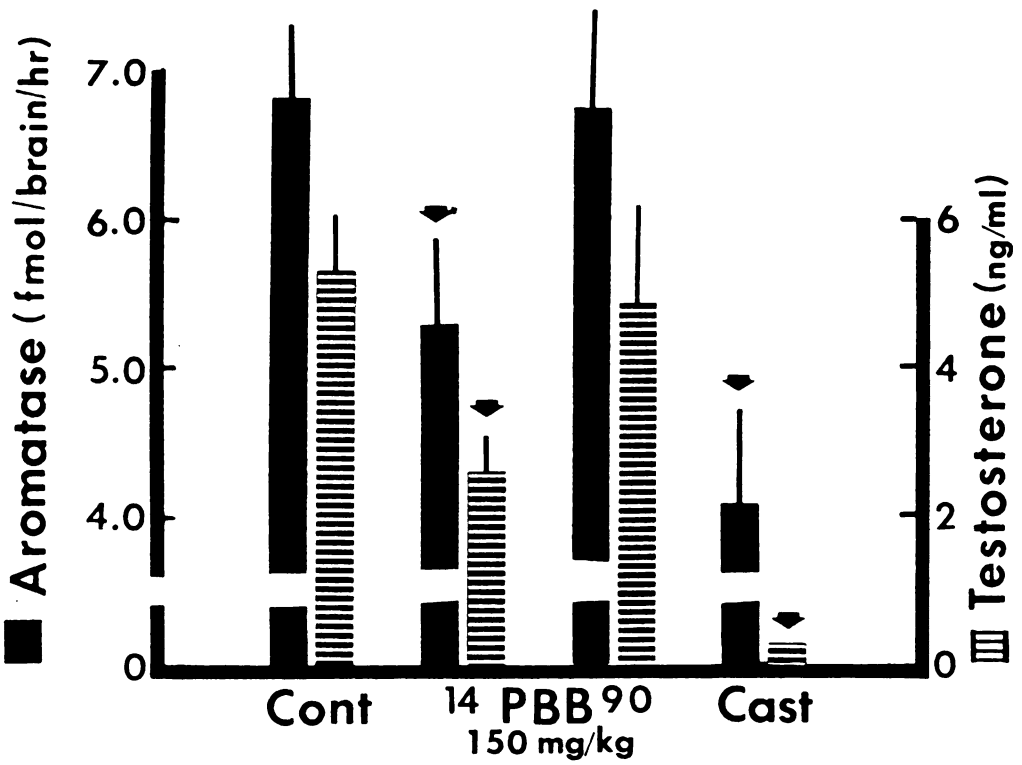

FIGURE 13. Effect of adult castration or PBB treatment on serum testosterone and hypothalamic aromatase activity. PBB treated rats were tested 14 or 90 days after treatment.
↓Statistically smaller than control male by Duncan's New Multiple Range Test ($p<0.05$).
Measures are mean $\pm$ standard error ($n=15$).

DISCUSSION

Because of the important role that hypothalamic aromatase plays in brain differentiation (McEwen et.al., 1980; Naftolin et.al., 1975; Whalen and Olson, 1978; Clemens and Pomerantz, 1982), regulation of adult sexual behavior (Popolow and Ward, 1976; Christensen and Clemens, 1975; Whalen and Rezek, 1977), and regulation of gonadotropin secretion (Selmanoff et.al., 1977; Kalra and Kalra, 1983; Worgul et.al., 1981) it is important to determine whether aromatase activity is affected by PBB treatment. In tissues where androgen derived estrogens are important, a PBB induced decrease in aromatase activity could worsen the effects of lowered serum androgens. PBB induced increases in tissue aromatase activity, however, could minimize the effects of PBB related reductions in testosterone titer.

Experiment 1 was a pilot study, designed to determine whether PBB treatment or castration could affect hypothalamic aromatase activity. PBB treated animals showed a trend toward decreased hypothalamic aromatase activity which was not statistically significant because of the small number of animals used. Pre-pubertal castration

decreased HA activity in adult rats. The small decrease in HA activity in PBB treated rats was suggestive of decreased testosterone titer, and further experiments were planned to study the PBB - HA interaction more carefully.

Using data derived from experiment one, statistical power analysis was performed to determine the number of animals needed for further studies on PBB - HA interactions. Experiment 2 was designed for a minimum of 15 replicate determinations of HA and plasma testosterone. Unfortunately, an outbreak of sendai virus in the rat colony killed 45% of the experimental animals, reducing the statistical power of our determinations. Data from experiment two (Figure 12) show that neonatal castration reduces HA activity in adult rats. The reduction of adult HA activity seen in neonatally castrated rats was reversed by adult administration of testosterone, confirming that adult androgen treatment is effective in regulating hypothalamic androgen aromatization. PBB treated animals showed a trend toward decreased HA activity that was associated with a trend toward decreased plasma testosterone titer. Neither trend achieved statistical significance (at $p<0.05$) due to insufficient numbers of animals. Implantation of testosterone capsules slightly increased plasma testosterone titer and HA activity in PBB treated animals. Increased HA activity in testosterone implanted rats was expected since hypothalamic aromatase

activity is regulated by plasma androgens (chapt 2; Roselli et.al., 1984).

Although a decrease in HA activity was associated with lowered plasma testosterone in PBB treated rats, a causal relationship cannot be definitely established. Both acute and chronic PBB treatment resulted in decreased plasma testosterone titer which was associated with decreased HA activity (Figures 12 and 13), but this experimental paradigm could not establish the absence of a direct effect of PBB on HA activity. Phenobarbital treatment of mice results in decreased brain aromatase activity which might be associated with decreased plasma testosterone, but this effect is also seen in vitro when phenobarbital is added directly to the incubation mixture (Weidenfeld et.al., 1983). Unlike PBBs, phenobarbital is metabolized by mixed-function oxidases, and therefore could be expected to compete for cofactors or P-450 binding with steroid substrates. Since PBB is not readily metabolized, it is unlikely that PBB would act directly to interfere with P-450 activity in the brain, but this hypothesis was not tested.

The decreased testosterone titer seen in PBB treated animals is believed to be due to increased metabolism of testosterone by mixed-function oxidases. Other studies (Castracane et.al., 1982) have failed to find decreases in serum testosterone titer after PBB treatment, but smaller

doses of PBB and longer post-treatment intervals may have obscured PBB related effects. Figure 13 shows that the decreased testosterone titer associated with acute PBB treatment is reversed by a post-treatment interval of 90 days, and both plasma testosterone and hypothalamic aromatase activity return to control levels.

Treatment of rats with the MFO inducer TCDD also results in decreased blood testosterone titers (Moore and Petersen, 1983). TCDD treatment causes rapid reductions in body weight gain as a result of decreased food intake, and food restriction alone was found to cause modest decreases in blood testosterone titer (Moore and Petersen, 1983; Becher, 1983). Since PBB treatment results in decreased weight gain due to lowered feed efficiency, it is possible that the decreased feed utilization of PBB treated rats may play a role in lowering blood testosterone titers. Generally, lowered blood testosterone arising from protein calorie malnutrition is associated with three things:

1. Decreases in caloric intake of at least 30%
2. Decreased serum LH and
3. Decreased testicular testosterone synthesis and decreased testicular response to hCG which persists even after refeeding.

Although reduced feed utilization may play a role in altering plasma steroid titers in PBB treated animals, it is not the major reason for decreased testosterone for the

following reasons:

1. Acute PBB treatment reduces serum testosterone titer without affecting body weight (c.f. Figure 13 and Table 6, chapter 4).

2. PBB treatment is not associated with alterations in serum LH titer (Johnston et.al., 1980; Table 10, chapter 6).

3. Rats treated with PBB prepubertally do not show alterations in gonadal responsiveness to hCG (Figure 15, chapter 4).

Decreased body weight in PBB treated animals has been reported in several species including the rat, and is due to decreased feed efficiency rather than decreased food intake (Sleight and Sanger, 1976; Gupta et.al., 1983). It has been suggested that the decreased feed efficiency is related to the inflammation of gastro-intestinal mucosa seen in PBB and PCB treated animals (Allen et.al., 1978; Kay 1977). Decreased seminal vesicle and prostate weight in PBB treated animals has been reported before (Johnston et.al., 1980; McCormack et.al., 1981), and is believed to be due to rapid testosterone clearance in PBB treated animals (McCormack et.al., 1981).

PBB treatment resulted in increased testicle/body weight ratios. This effect could be a result of compensatory testicular hypertrophy. In PBB treated animals, more rapid testosterone clearance (McCormack,

et.al., 1979) would necessitate increased testicular androgen production to maintain near-normal levels of circulating androgens. In fact, PBB treated animals did not have increased titers of pituitary gonadotropins (Johnston et.al., 1980), and PBB treatment resulted in decreased titers of serum testosterone. Raw testicle weights in PBB treated rats are actually smaller than those of control males. ($3.03 \pm .71$ g in PBB rats, $3.77 \pm .33$ g in control rats; PBB testes smaller by Duncans's Multiple Range test ($p<0.05$)). The increased testicle/body weight ratios in PBB treated rats are probably a result of the much smaller body weights seen in PBB treated animals. PBB treated rats treated with testosterone have testicle/body weight ratios much smaller than control animals, suggesting that the exogenous testosterone has suppressed endogenous androgen synthesis.

In summary, PBB treatment had several effects on reproduction including the following:

1. Chronic PBB treatment resulted in decreased weight gain, and decreased prostate and seminal vesicle weights.

2. Prostate and seminal vesicle weights in PBB treated rats were increased by the administration of exogenous testosterone.

3. Acute PBB treatment resulted in decreased serum testosterone and hypothalamic aromatase activity. The decreased testosterone and HA returned to normal levels by

90 days post-treatment.

4. Although decreased hypothalamic aromatase activity in PBB treated animals was associated with decreased serum testosterone, these experiments have not proved a cause and effect relationship.

CHAPTER 4

EFFECT OF PBB TREATMENT ON PROSTATIC ANDROGEN METABOLISM AND TESTICULAR ANDROGEN SYNTHESIS

CHAPTER 4

INTRODUCTION

Treatment of rats with a mixture of polybrominated biphenyls results in increased hepatic testosterone metabolism, and decreased circulating testosterone titer (chapt 3, Newton et.al., 1982). Associated with the increased androgen metabolism is a marked decrease in the weight of the ventral prostate and seminal vesicles. Both prostate and seminal vesicle are tissues sensitive to circulating testosterone titer throughout life. Prepubertal castration or treatment with estrogens permanently impairs the ability of the prostate to respond to plasma androgens (Chung and MacFadden, 1980; Rajfer and Coffey, 1979), and adult castration results in the regression of prostatic tissue and alterations in prostatic androgen metabolism (Celotti et.al., 1979; Tenniswood et.al., 1982). Lifelong PBB treatment has caused marked regression of prostate and seminal vesicle (Chapt 3, Johnston et.al., 1980; McCormack et.al., 1979) which was believed to result from decreased plasma androgen levels.

Administration of PBBs to rats resulted in increased hepatic testosterone hydroxylation *in vitro*, and

decreased reductive formation of 5a-DHT (Newton et.al., 1982). Other compounds possessing MFO inducing properties also decrease 5a-reductase activity in the liver; the list includes phenobarbital, TCDD, PCBs, and hexchlorobenzene (Kappas et.al., 1983). The formation of 5a-DHT from testosterone is an important activating step in prostate and seminal vesicle, and DHT may be as much as six-fold more active than testosterone in maintaining organ weight and secretory activity. Because of the importance of 5a-reduction as an activating step in androgen sensitive tissues, decreases in DHT formation could have marked effects on reproductive structures. The prostate has already been shown to be sensitive to the MFO inducing properties of TCDD, ßNF and phenobarbital. TCDD or ßNF treatment increases AHH, EROD and benz(a)pyrene metabolism (Haaparanta et.al., 1983; Lee et.al., 1981; Soderkvist et.al., 1982), and both ßNF and phenobarbital increase the polyhydroxylation of 5a-androstane-3ß,17ß- diol (Haaparanta et.al., 1984). Since PBB treatment has already been shown to decrease 5a-reductase activity in the liver, it is of interest to determine whether PBB treatment can alter the formation of DHT in the prostate, and thus interfere with androgen action.

Another possible site of PBB action on reproduction is the synthesis of testosterone by the testes. A cytotoxic effect of PBB treatment on leydig cells could alter

testosterone synthetic pathways, decreasing testicular androgen production. Reproductive senescence in rats is accompanied by decreased testicular androgen synthesis, and lowered sensitivity to the trophic effects of hCG (Simpkins et.al., 1981; Miller and Riegle, 1978). If PBB altered testicular androgen synthesis, or the sensitivity of the testes to pituitary gonadotropins, lowered testosterone titers could result. Acute PBB treatment results in lowered testosterone titer, and it is therefore of interest to determine whether PBB treatment alters testicular ability of synthesize testosterone.

The objectives of these experiments were therefore to:

1. determine whether PBB decreases 5a-reduction of testosterone in ventral prostate and

2. determine whether PBB treatment alters testicular testosterone synthesis.

METHODS

Effects of PBB on prostatic testosterone metabolism

Male 65 day old Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and housed under 12:12 inphase light:dark conditions with food and water available ad lib. Animals were assigned to a PBB treated, control, or castrate group. PBB animals were given 150mg/kg fireMaster BP-6 (lot#6244-A, Michigan Chemical Corp. St. Louis, MI) in peanut oil by i.p. injection. Castrate animals were orchidectomized trans-scrotally under light ether anesthesia. Control animals received 2ml/kg peanut oil i.p. Control and PBB animals were killed 14 or 90 days after treatment. Castrate animals were killed 14 days after castration. Prostate glands were removed from animals killed 14 days after treatment, and prostate and sub-mandibular glands removed from animals killed 90 days after treatment.

Prostate and submandibular glands were weighed and prepared for determination of 5a-reductase activity. Prostate or sub-mandibular gland were homogenized by polytron in 3 ml of ice-cold 66mM Tris buffer (pH=7.4), then centrifuged at 10,000 x g for 20 minutes. The

post-mitochondrial supernatant was used for the determination of 5a-reductase activity. Assay tubes were prepared containing 3ml of supernatant from prostatic homogenate to which was added an NADPH regenerating system and ^{14}C-testosterone (New England Nuclear, 52mCi/mmole). Each tube contained a final concentration of 4.5 μM glucose-6-phosphate, 0.3μM NADP, 0.3μM NADH, 0.1μM NADPH, 1 U glucose-6-phosphate dehydrogenase per ml and 3.8 μM testosterone. Each assay tube was pre-incubated at 37^{o} C. for 5 minutes, and the reaction was started by the addition of ^{14}C-testosterone in 30μl of methanol. The reaction mixture was incubated for 15 minutes, and the reaction stopped by the rapid addition of 5ml of ice-cold ether:chloroform (3:1). Each assay tube was extracted three times using the ether:chloroform mixture, and the combined extracts dryed over anhydrous sodium sulphate. The extracts were evaporated to dryness under N_2, re-suspended in 100 μl methanol and filtered prior to high performance liquid chromatography (HPLC).

HPLC with flow-through radioactivity detection was used to quantify the prostatic and submandibular 5a-reductase activity. Each sample was chromatographed on an Altex 3μ reverse phase HPLC column with a mixture of 18% THF, 22% methanol, 18% acetonitrile and 42% water as the eluent. Radioactivity of the column effluent was determined using a model Flow-One CU (Radiomatic Instuments

and Chemicals, Tampa, FL) equipped with a 0.5ml flow cell. ACS scintillation cocktail (Amersham, Chicago, IL) was used as the scintillant at a ratio of 2.5:1 of ACS:column effluent. Both 5a-DHT and 5a-androstane-3a,17ß-diol (Adiol) were well separated by the HPLC system (Figure 14). Total polar metabolites which eluted before testosterone were quantified as a group in prostatic homogenates 14 days post-treatment.

5a-Reductase activity was calculated based on the sum of 5a-DHT and 3a-Adiol produced during the incubation. Each sample was corrected for recovery based on total C14 eluting from the column compared against the amount added to the incubation. Sample results are expressed as pmole 5a-reduced metabolites formed per milligram of protein per hour. By expressing the results on a protein basis, enzyme activity is corrected for the different sizes of prostate in each treatment group.

Effect of perinatal PBB treatment on testosterone synthesis

Timed pregnant Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and housed under 12:12 inphase light:dark conditions with food and water available ad lib. On day 8 of gestation, one-half the animals were assigned to the PBB group, and their food replaced with ground Wayne Lab-Blox containing 100 ppm fireMaster BP-6. Control rats received ground Lab-Blox. Dams were allowed

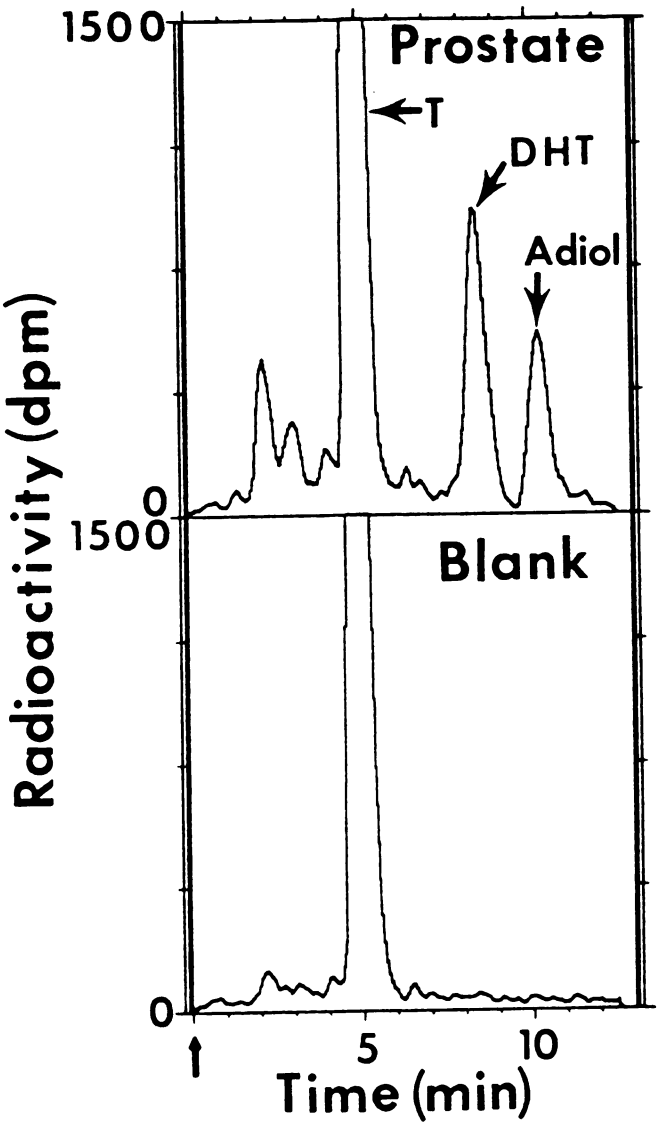

FIGURE 14 HPLC separation of ^{14}C-labelled testosterone metabolites. Conditions were 18% THF, 22% methanol, 18% acetonitrile, 42% water on and Altex 3μ C-18 column. HPLC flow rate was 1.0 ml/min., scintillant added at 2.5 ml/min.

to deliver normally, and maintained on PBB treated feed until pups were 28 days old. When pups were 28 days old, they were weaned onto the control diet, and maintained until 120 days old.

Testicular testosterone synthesis was evaluated in 120 day old adult males. Each rat was lightly anesthetized with ether, and a blood sample was taken by cardiac puncture. Still under ether anesthesia, each male received an injection of 2.5 IU of hCG (Sigma Chemical, St. Louis, MO) in 0.25 ml sterile saline in the dorsal penis vein. Repeat blood samples were taken from each male 45 and 90 minutes following hCG injection. Blood was allowed to clot at room temperature for 2 hours, and serum was collected and frozen at -20° C. until assayed for plasma testosterone. Blood testoserone values were determined by RIA as previously described (chapt 3).

Statistical Analysis

Statistical testing was done using Student's t-test ($p<0.05$). Multiple comparisons were made using analysis of variance followed by Duncan's New Multiple Range Test ($p<0.05$).

RESULTS

Prostatic 5a-reductase activity

Treatment of adult male rats with 150 mg/kg PBB resulted in unchanged body weights, but decreased prostatic weights 14 and 90 days after treatment. Submandibular gland was smaller 90 days after PBB administration (Table 6). Castration decreased prostatic weight 14 days after orchidectomy.

PBB treatment did not alter 5a-reductase activity in prostate or submandibular gland on any day tested. There was a small increase in the formation of polar testosterone metabolites 14 days after treatment in prostates from PBB treated animals (Table 7). Castration resulted in approximately a two-fold increase in the *in vitro* formation of 5a-reduced testosterone metabolites compared with control males.

Perinatal PBB treatment and adult testosterone synthesis

Injection of 0.25 ml of normal saline into control rats resulted in no change in blood testosterone titer by 90 minutes post-injection. Perinatal PBB treatment did not result in any change in the ability of the testes to synthesize testosterone. Both basal and post-hCG testosterone titers were similar in control and PBB treated rats.

TABLE 6

Effect of adult administration of 150 mg/kg of fireMaster BP-6 on body, prostate and submandibular gland (SMG) weights.

group	body weight (g)	prostate (mg/100gbw)	SMG (mg/100gbw)
14 days after treatment			
control	363±5.5 (15)	141±8.3	-------
PBB	367±6.8 (15)	126±8.2*	-------
castrate	361±7.5 (5)	24±6.9*	-------
90 days after treatment			
control	418±5.4 (15)	132±4.0	159±4.9
PBB	412±6.5 (14)	111±2.7*	148±4.5*

all measures are mean ± standard error (n)
*statistically different from control by Student's t-test (control vs PBB) or Duncan's New Multiple Range Test (control vs PBB vs castrate)($p<0.05$)

TABLE 7

Effect of adult administration of 150mg/kg of fireMaster BP-6 on reductive testosterone metabolism in rat prostate and submandibular gland.

group	Polar (pmol/mg/hr)	5a-DHT (pmol/mg/hr)	Adiol (pmol/mg/hr)	Total reduced (pmol/mg/hr)
Prostatic testosterone metabolism 14 days after treatment				
control	.032±.006(15)	.78±.05	.46±.04	1.24±.09
PBB	.050±.007(14)*	.78±.05	.41±.03	1.19±.06
castrate	.025±.010 (5)	1.59±.37*	2.41±1.2*	4.00±1.6*
Prostatic testosterone metabolism 90 days after treatment				
control	--------	.37±.02(15)	.15±.02	.52±.03
PBB	--------	.41±.04(15)	.18±.03	.59±.07
Submandibular gland 5a-reductase activity 90 days after treatment				
control	--------	1.81±.23(15)	.10±.03	1.91±.24
PBB	--------	1.72±.11(14)	.06±.01	1.79±.11

all measures are mean ± standard error (n)
*indicates statistically different from control by Student's t-test ($p<0.05$)

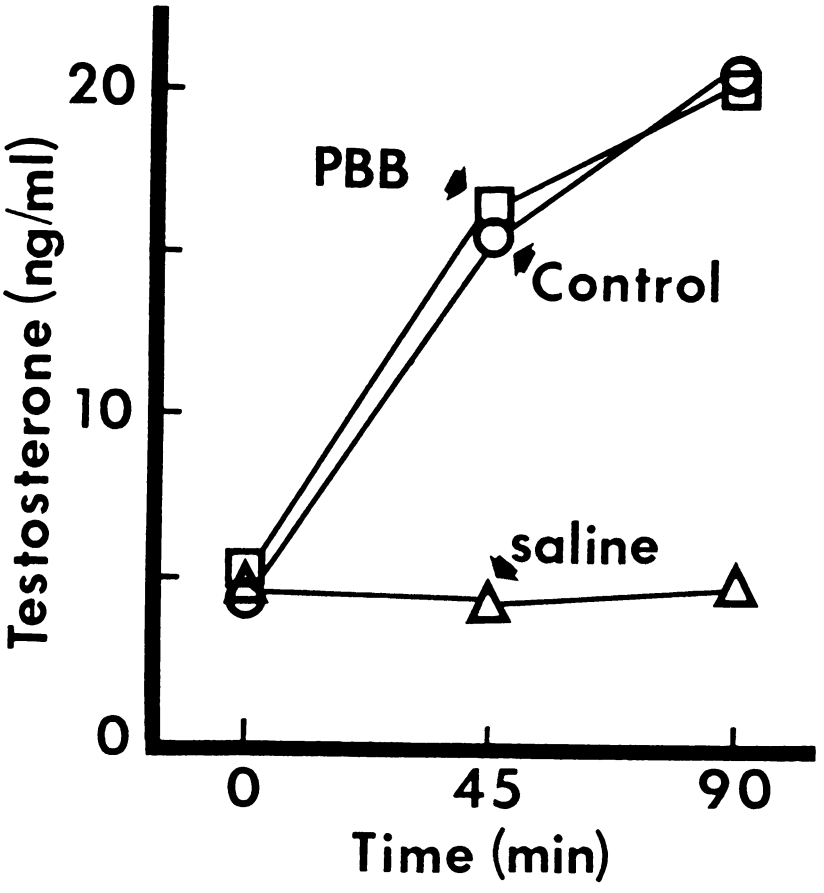

FIGURE 15 Effect of perinatal PBB treatment on testicular response to hCG in adults. Each rat received 2.5 IU of hCG at time 0. Each point represents at least 8 animals; error bars are within the symbol.

DISCUSSION

Reduction of testosterone to 5a-dihydrotestosterone (DHT) is an important activational step in many androgen target tissues. DHT is a more potent androgen than is testosterone, and in some tissues has a six-fold greater binding affinity for the androgen receptor (Liang et.al.,1984). In prostate, testosterone is first converted to DHT by 5a-reductase, then DHT may be further metabolized to either 5a-androstane-3a,17ß-diol (Adiol) or 5a-androstane-3ß,17ß-diol (3ß-Diol). Adiol acts as a storage form of DHT and may be converted back to DHT by 3a-hydroxysteroid dehydrogenase. 3ß-Diol, on the other hand is rapidly hydroxylated, and has little or no biological activity (Tenniswood et.al., 1982; Ofner et.al., 1982; Ofner et.al., 1979). Because Adiol may be converted to DHT, it has androgenic activity, and its formation should be included when total 5a-reductase activity is assessed. Although both DHT and testosterone act as androgens by binding to the cytoplasmic androgen receptor and being translocated to the nucleus, DHT is a more potent androgen. Since DHT is more active than testosterone in promoting growth and maintaining secretory activity of

androgen target tissues, defects in the 5a-reductase pathway may reduce the efficacy of circulating testosterone (Imperato-McGinley, et.al., 1985).

The 5a-reductase activity of the ventral prostate is believed to be under the control of circulating androgens. Celotti et.al., (1979) have demonstrated that by 5 weeks following castration prostatic 5a-reductase activity is reduced relative to control rats, and that treatment with either testosterone or DHT will restore the activity to control levels. Our results showed that castration actually increased 5a-reductase activity in the prostate. Two explanations are possible for the difference in effects between our results, and those reported by Celotti and co-workers (1979); first, our 5a-reductase determinations were carried out after only two weeks of castration, and second, we incubated with post-mitochondrial supernatants from tissue homogenates, while Celotti used fragments of whole tissue in his assay. It is possible that 5a-reductase activity exhibits a biphasic response to castration, with final activity of the enzyme lower only after prostatic regression is complete. In addition, whole tissue may be expected to show a different response to castration than microsomes in an <u>in vitro</u> incubation. Our <u>in vitro</u> technique used only the post-mitochondrial supernatant in the determination of 5a-reductase activity. Prostatic epithelial cells have the 5a-reductase present

not only in microsomes, but in virtually every cell fraction examined, including nucleus, cell membrane and cytosol fractions (Ofner et.al., 1982; Shimazaki et.al., 1972). Castration may result in decreased amounts of microsomal and cytosolic proteins, resulting in apparent increases in enzyme activity which are due in fact to lower amounts of non-active protein in the cell. It is important also to note that with in vitro techniques such as we employed here enzyme co-factors such as NADPH are delivered in high concentrations (approximately 0.3mM in our system). Prostate fragments from castrate animals are unlikely to have high co-factor concentrations to support androgen metabolism, since cellular growth is androgen regulated.

Despite the effect of castration on prostatic 5a-reductase activity, PBB treatment failed to alter the production of either DHT or Adiol at either time tested. Since PBB treatment decreases 5a-reductase activity in liver (Newton et.al., 1982), it was felt that a corresponding change might be manifest in prostatic tissue. These results show, however, that acute treatment of adult rats does not alter DHT or Adiol production in in vitro prostatic assays. Since Newton and co-workers (1982) used rats treated throughout development with PBB before finding decreased hepatic 5a-reductase activity, it is possible that more extensive PBB treatment may be required to alter prostatic testosterone reduction. It is apparent however,

that acute PBB treatment can interfere with prostatic growth (Table 6) even though it does not affect 5a-reductase activity. Since adult treatment with PBB lowers blood testosterone titer (Figure 13), it is most likely that the lower prostate weights seen in PBB treated rats are due largely to effects on blood androgen titers, rather than to effects on prostatic testosterone metabolism. It is interesting that prostatic weights are still reduced 90 days after PBB treatment (Table 6), even though blood testosterone titer has returned to control values (Figure 13). At least two explanations may be offered for this apparent discrepancy:

1. Prostatic weight values may recover more slowly than do blood testosterone titers from the effects of PBB treatment. This might be especially true if PBB exerts a direct cytotoxic effect on prostatic tissue, and the decreased prostatic weights are due to direct effects of PBB, rather than to the decreased testosterone titers seen in treated animals.

2. Our blood testosterone values were measured at 1100 hours, near the mid-morning circadian peak in testosterone titer (Simpkins et.al., 1981). Since instantaneous blood testosterone values cannot be used to tell what is happening throughout the circadian cycle, it is possible that peak values may be similar in PBB and control animals, yet PBB treated animals may have lower

average blood androgen titers due to more rapid metabolic clearance. If this is the case, prostate from PBB treated animals could be smaller due to lower average blood androgen activity, yet peak testosterone titers could be similar to those seen in control animals.

In order to determine which of the above explanations is likely to be correct, two additional experiments should be performed; first, blood testosterone titer should be assessed throughout the diurnal cycle in both PBB and control animals and second, controlled-release testosterone capsules might be implanted in control and PBB treated animals so that testosterone titer may be changed independantly of PBB treatment.

PBB treated animals did show a small increase in the prostatic production of polar testosterone metabolites. It is difficult to determine whether this effect is of physiological significance. Since the amount of polar metabolites we found was small relative to the amount of 5a-reduced metabolites produced, it may be that this effect is of minimal importance. However, Tenniswood et.al.(1982) have pointed out that polyhydroxylated androgens are not well extracted into common organic solvents, and our extraction technique may not have extracted polar metabolites quantitatively. For many years, it was believed that rat prostate did not form 3ß-reduced androstanediols because they were not found in prostatic

tissue incubations. Later it became evident that the 3ß-Diol was rapidly metabolized to highly polar androstanetriols, which had not been detected in earlier assays because they were not extracted from the incubation mixture by the method used (Tenniswood et.al., 1982; Haaparanta et.al., 1984). Since our extraction technique was not optimized for the recovery of polar metabolites, there may have been quantities of androstane poly-ols which were not recovered. Our results suggest that PBB treatment increases the production of polar metabolites from testosterone in the ventral prostate. Haaparanta and co-workers (1984) have shown that both phenobarbital and ßNF increase the production of androstanetriols in prostate when 3ß-Diol is used as the substrate. The increase in polar metabolites seen in PBB treated rats may represent increased hydroxylation of reduced androgens. The final resolution of this question must await experiments specifically designed to detect highly polar androgen metabolites.

Submandibular gland is an androgen sensitive target tissue which contains both 5a-reductase activity and androgen receptors (Koshika et.al., 1984). Submandibular gland 5a-reductase activity was studied in rats 90 days after PBB treatment because the gland is easily removed, and might provide another site for the determination of PBB effects on 5a-reductase activity. Our results show that as

in prostate, submandibular gland 5a-reductase activity is unaffected by PBB treatment.

These studies also showed that PBB treatment failed to alter the conversion of 5a-DHT to Adiol. Castration and hormone treatment do not affect the production of 3a-Adiol from DHT (Celotti et.al., 1979). PBBs could reduce the efficacy of DHT through rapid conversion to 3a-Adiol in prostate, but this apparently does not occur.

Another possible target for PBB effects on reproduction is the testicular synthesis of testosterone. These studies showed that animals treated perinatally with PBB have normal basal levels of plasma testosterone as adults(Figure 15), and that there is no deficit in testicular response to trophic hormone stimulation. Aged rats normally show decreased basal testosterone titer coupled with decreased testicular androgen synthesis following an hCG challenge (Simpkins et.al., 1982, Miller and Riegle, 1978). Rats affected by protein-calorie malnutrition also show decreased plasma testosterone and impaired response to hCG stimulation both during fasting, and during re-feeding (Becher, 1983). Our results suggest that protein-calorie malnutrition does not play a major role in PBB induced reproductive effects, since rats from the PBB treated group had normal testosterone production following hCG stimulation.

In summary, acute PBB treatment of adult rats does not

alter reductive metabolism of testosterone in ventral prostate or submandibular gland. PBB treated rats may have slight increases in the amount of polar testosterone metabolites produced by the ventral prostate, but the physiological significance of this is uncertain. Finally, perinatal PBB treatment alters neither basal blood testosterone titer nor testosterone synthesis following an hCG challenge.

CHAPTER 5

EFFECT OF PERINATAL PBB TREATMENT ON NEONATAL TESTOSTERONE HYDROXYLATION AND BLOOD TESTOSTERONE TITER

CHAPTER 5

INTRODUCTION

In rats, the critical period of brain sexual differentiation begins 7 days before birth (day 17 of gestation) and ends 5 days after birth (MacLusky and Naftolin, 1981). During the critical period the brain is exquisitely sensitive to even small alterations in circulating androgen titers. Influences such as prenatal stress which blunt the testosterone surge normally seen in male fetuses on day 18 of gestation, can have permanent effects on adult reproductive physiology and sexual behavior (Ward and Weisz, 1980; Ward, 1972; Rhees and Fleming, 1980; Orth et.al., 1981). In rats and mice, female fetuses located between two male fetuses *in utero* have masculinized morphology at birth, and masculinized behavior as adults when compared with littermates located between female fetuses *in utero* (Clemens et.al., 1978; vomSaal et.al., 1980). Doses of testosterone propionate as small as 10μg administered on day 5 postnatally can result in anovulatory sterility in female rats (DaMassa et.al., 1983). Because of the high sensitivity of fetal and neonatal brain to circulating androgens, alteration of the

endocrine environment during development has the potential to permanently alter adult reproductive behavior and physiology.

Treatment of animals with mixed-function oxidase inducers such as PCB and PBB results in increased microsomal metabolism of steroid hormones (McCormack et.al., 1981; Johnston et.al., 1980; McCormack et.al., 1979; Newton et.al., 1982). Increased steroid metabolism during the fetal and neonatal period has the potential to decrease circulating steroid titer, particularly since normal feedback mechanisms which regulate steroid synthesis may be poorly developed during this time. Other studies have established that prenatal treatment with PCB or TCDD increases hepatic drug metabolism in neonates (Lucier et.al., 1979; Dieringer et.al., 1979). These studies demonstrate that polyhalogenated hydrocarbons cross the placenta in sufficient quantities to induce hepatic MFO activity. It is known that PBB crosses the placenta in rat, as well as in other species (Rickert et.al., 1978), although PBB induction of fetal MFOs has not been previously demonstrated.

The purpose of these studies was to determine whether PBBs cross the placenta in sufficient quantities to induce fetal hepatic MFOs. In addition, we wished to investigate

the manner in which PBB treatment might alter fetal and neonatal steroid metabolism, and determine whether PBB treatment alters circulating testosterone titers during the critical period of brain sexual differentition.

METHODS

Animals and Treatment

Timed pregnant Sprague-Dawley rats were obtained from Harlan (Haslett, MI) and housed under 12:12 inphase light:dark conditions with food and water available ad lib.. On day 8 of gestation (Plug date= day 0) pregnant rats were assigned to either the control or PBB treated group. Dams in the PBB treated group received ground Wayne Lab-Blox containing 100ppm of the polybrominated biphenyl mixture fireMaster BP-6 (lot #6244-A, Michigan Chemical Corp. St.Louis MI). Control dams received ground Lab-Blox.

On days 21 and 22 (birth normally occurs on day 22) of gestation and on days 1 and 3 post-partum the fetal or neonatal rats were removed from the mother. Each pup was weighed and the crown-rump length and ano-genital distance were measured. Pups were then killed by decapitation and trunk blood was collected in heparinized capillary tubes. The pups were sexed by dissection, and livers and blood pooled according to sex. Each capillary tube containing blood was centrifuged, and tubes with hematocrit values of less than 35 were discarded to minimize dilution of pooled

blood samples by interstitial fluid.

Liver microsomes were prepared by the method of Dent et.al., (1976). Pooled livers were minced and homogenized in ice cold 20mM Tris-HCl buffer (pH=7.4) containing 1.15% KCl, and centrifuged at 10,000 x g for 20 minutes. The resulting supernatant was centrifuged at 105,000 x g for 60 minutes, and the microsomal pellet was resuspended in 66mM Tris-HCl buffer containing 250mM sucrose. The microsomes were frozen in 1ml aliquots at -70^{o}C until needed (less than 14 days).

Assays

Plasma testosterone in male control and PBB treated pups was determined using a solid-phase RIA kit purchased from Diagnostic Products Corp. (Los Angeles, CA). The kit was validated for use in rats with testosterone spiked rat serum from castrated/adrenalectomized rats. Cross reactivity of the antibody was 8% for DHT and 3% for androstenedione. Intra-assay coefficient of variation in our hands was 4.5%.

Activity of ethoxyresorufin-O-deethylase (EROD) was determined (in vitro) by the method of Johnson et.al. (1979). Each incubation tube contained approximately 0.5 mg of microsomal protein (as determined by the Lowry method) and an NADPH regenerating system (4.5 μM glucose-6-phosphate, 0.3μM NADP, 0.3μM NADH, 0.1μM NADPH, and 1U glucose-6-phosphate dehydrogenase) in a total volume

of 1.0ml. The reaction was started by the addition of 10µl of 0.125mM ethoxyresorufin (Pierce Chemical, Rockford, IL). The reaction mixture was incubated for 5 minutes at 37° C, and the reaction was stopped by the addition of cold acetone. The resorufin present was quantified by spectrofluorimetry; excitation = 510nm, emission = 586nm.

Benzphetamine-N-demethylase (BND) activity was determined by the method of Prough and Ziegler (1977). Hepatic microsomes were incubated with an NADPH regenerating system as described above, and the reaction was started by the addition of 1.5µmole benzphetamine (donated by the UpJohn Corp., Kalamzoo, MI). After incubation for 10 minutes at 37°C, the reaction was stopped by the addition of 1.0ml of 10% TCA. The formaldehyde produced by benzphetamine demethylation was measured using the acetate/acetoacetone method of Nash (1953), and the chromophore was measured by U.V. absorbtion at 415nm.

Microsomal testosterone hydroxylation was determined using the HPLC method of Newton et.al. (1982). Each incubation tube contained 0.8mg of microsomal protein and and NADPH regenerating system in a total volume of 1.0ml as described above. The reaction was started by the addition of 115µmole of testosterone in 10µl of DMSO. After incubation for 30 minutes at 37°C, the reaction was stopped by the addition of 5 volumes of chloroform/ether

(1/3), and the incubation mixture was extracted three times with 5 volumes of chloroform/ether mixture. The extract was evaporated to dryness, resuspended in 1.0ml ethanol, and filtered. The testosterone metabolites 7a-hydroxytestosterone, 6ß-hydroxytestosterone, and 16a-hydroxytestosterone were separated and quantified by HPLC using 24% THF as the eluent with an Altex 5µ Ultrasphere ODS column. Testosterone and its hydroxylated metabolites were quantified by U.V. absorbance at 254nm using a Waters 760 recording integrator.

Statistical Analysis

PBB and control groups were compared for each day tested using Student's t-test ($p<0.05$).

RESULTS

PBB treatment resulted in decreased ano-genital distance, crown-rump length and body weight in male fetuses and neonates on all days tested (Figure 16). Since decreased ano-genital distance can result from delayed maturation, ano-genital distance was scaled against crown-rump length using the formula:

$$\text{corrected A-G distance} = \frac{\text{ano-genital distance}}{\text{crown-rump length}}$$

After adjustment for crown-rump length, ano-genital distance in PBB treated male pups was smaller on day 21 and 22 of gestation, but not on day 1 or 3 postnatally (Table 8).

Preliminary statistical analysis demonstrated that male and female pups did not differ in rate of testosterone metabolism, EROD or BND activity. PBB treatment significantly increased formation of 7a-hydroxy testosterone on day 22, 23 and 25 post-conception. Formation of 16a-hydroxy testosterone was increased by PBB treatment on days 22 and 25 post-conception, and 6ß-hydroxylation was increased on day 22 only (Figure 17).

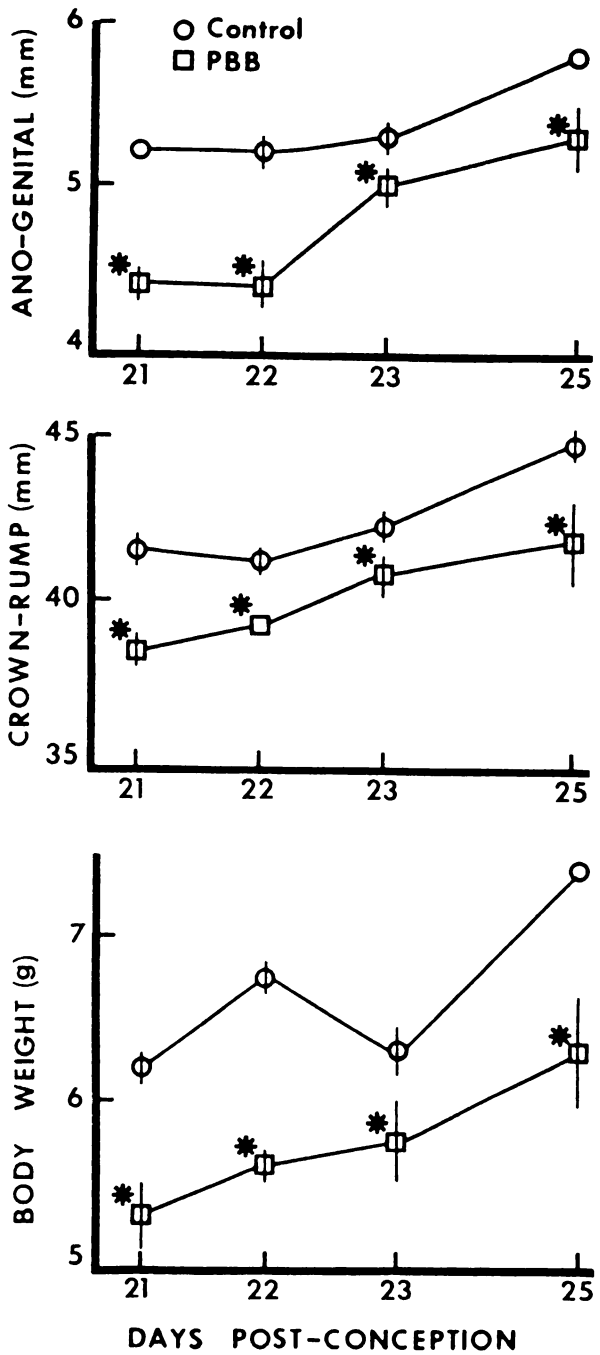

FIGURE 16 Effect of PBB treatment on fetal and neonatal growth and development. All measures are mean ± standard error; (n>6 for all points). * Statistically different from control by Student's t-test (<0.05).

Table 8

Ano-genital distance in male rat pups after correction for crown-rump length

group	Age of pups (days post-conception) 21	22	23	25
Control	.125±.002(22)	.126±.002(24)	.127±.002(12)	.129±.001(14)
PBB	.115±.003(20)+	.113±.004(11)*	.112±.004(7)	.126±.003(6)

All measures are mean ± standard error (n).
*Statistically different from control by Student's t-test ($p<0.05$).
Ano-genital distance was corrected using the formula:

$$\text{corrected value} = \frac{\text{ano-genital distance}}{\text{crown-rump length}}$$

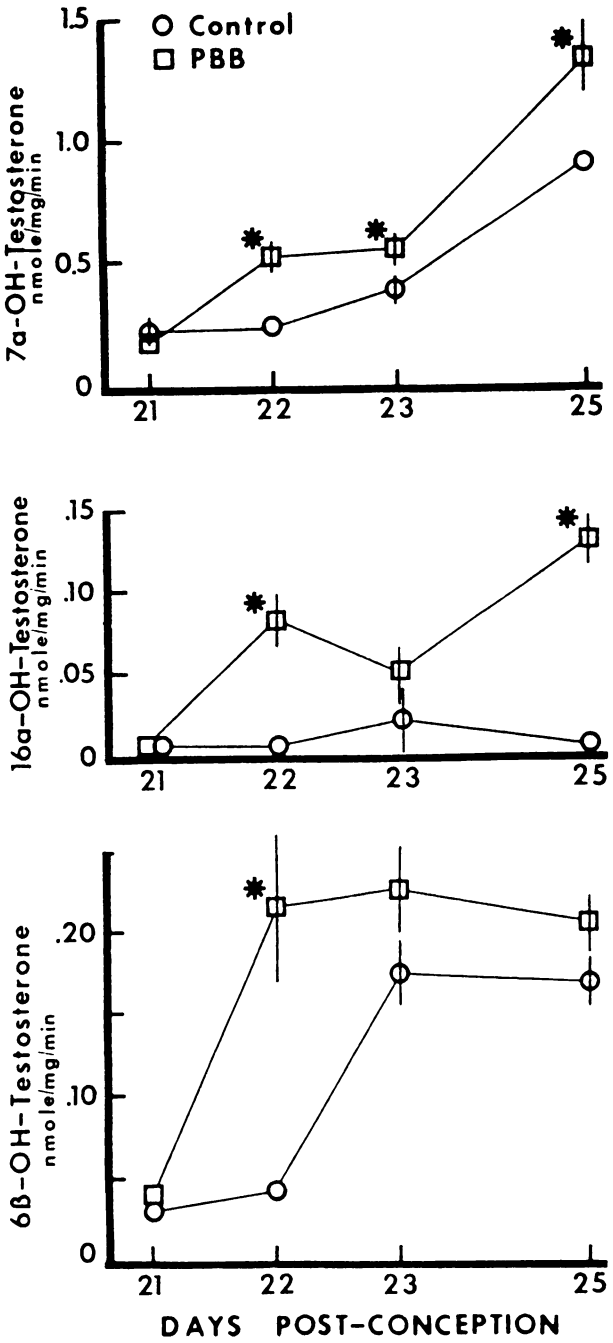

FIGURE 17. PBB induction of testosterone hydroxylation in fetal and neonatal liver microsomes. All measures are mean ± standard error (n>5). *Statistically different from control by Student's t-test ($p<0.05$).

Total hydroxylated testosterone formed was increased by PBB treatment on day 22, 23 and 25, but not on day 21 post-conception (Figure 18). PBB treatment increased EROD activity on all days tested, but benzphetamine demethylation was increased only on day 22, 23, and 25 post-conception (Figure 18).

Plasma testosterone titer of male pups was measured in plasma samples pooled by litter. Plasma testosterone titers showed great variability on each day tested, with some overlap between control and PBB distributions. Although mean plasma testosterone titers were lower in PBB treated rats on day 22, 23 and 25 post-conception, the extreme variability of the measurements resulted in a statistically significant difference only on day 25 post-conception.

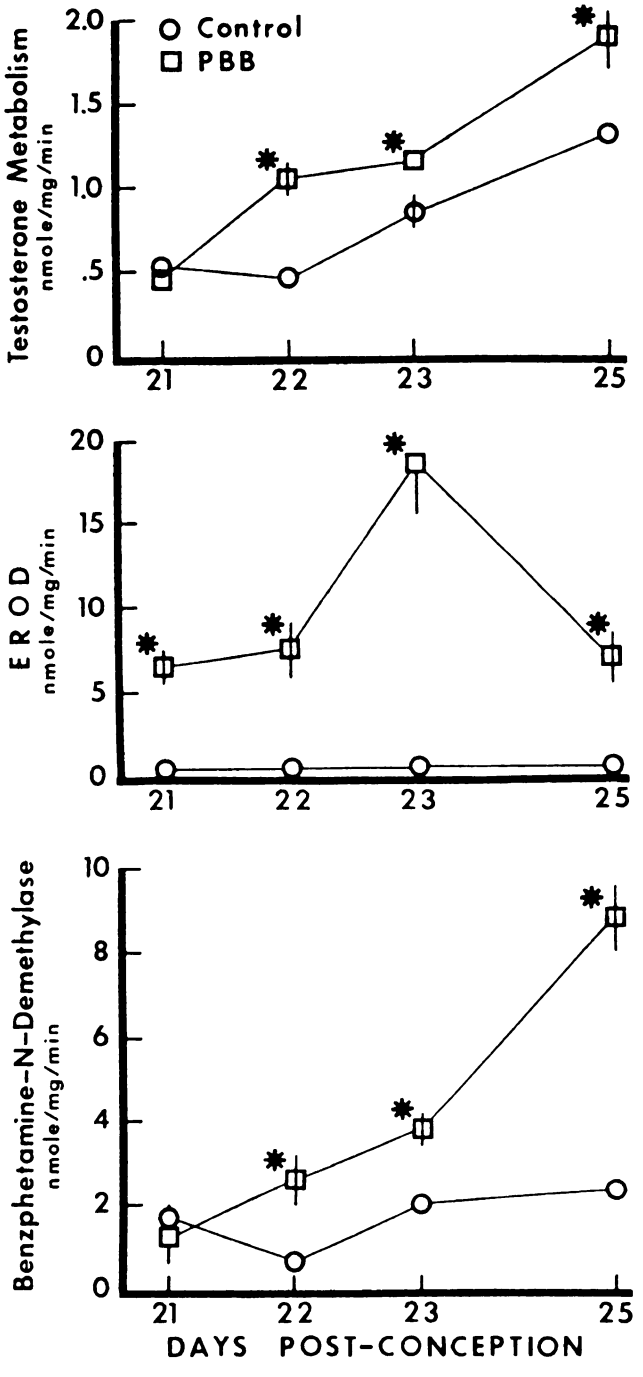

FIGURE 18 Effect of PBB treatment on hepatic mixed-function oxidase activity in ther perinatal rat. All measures are mean ± standard error (n>5 for each point).
*Statistically different from control by Student's t-test ($p<0.05$).

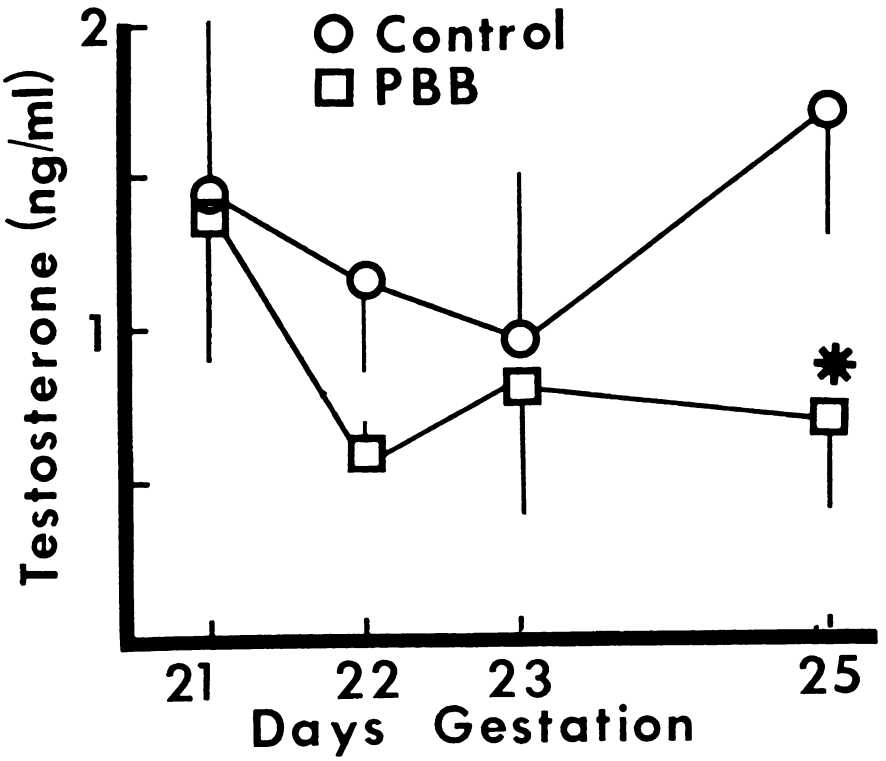

FIGURE 19 Effect of PBB treatment on blood testosterone titer in perinatal rats. All measures are mean ± standard error (n>10 for each point). *Statistically different from control by Student's t-test ($p<0.05$).

DISCUSSION

These results show that treatment of pregnant rats with 100 ppm PBB causes significant decreases in weight and development of fetuses and neonates. Although PBBs are only weak teratogens, chronic PBB treatment slows maternal weight gain, and delays fetal development (McCormack et.al., 1982; Johnston et.al., 1980; Kay, 1977; Damstra et.al., 1982). Ano-genital distance is a commonly used index of fetal masculinization (Dahlof et.al., 1978) and may be used to assess the development of the male reproductive system. PBB treatment resulted in decreased male ano-genital distance, a possible indication of decreased androgen efficacy. Although raw A-G distances were lower on each day tested, correction for fetal body size by computing AG/crown-rump ratios shows that on day 23 and 25 post-conception the decreased ano-genital distance in PBB pups may have been due to delayed development, rather than a specific effect on sexual differentiation.

Fetal hepatic testosterone hydroxylation was tested beginning on day 21 of gestation. Day 21 was chosen as the starting point because pilot studies showed no detectable androgen metabolism in fetal livers on day 20 (data not

shown). Total testosterone hydroxylation was increased in PBB treated pups on days 22, 23 and 25 post-conception. Previous studies on PBB induction of MFO activity have shown that trans-placental PBB exposure may increase hepatic drug metabolism as early as 9 days post-partum, but there are no reports in the primary literature of enzyme induction prior to 8 days of age (Dent, 1978; McCormack et.al., 1979). TCDD has been found to increase AHH activity as early as four days before birth (day 18 gestation) (Lucier et.al., 1979), proving that fetal liver MFOS are inducible given the proper stimulus. Treatment of pregnant rats with PCBs has resulted in increased testosterone 16a-hydroxylation as early as 6 days post-partum, but not during gestation (Deiringer et.al., 1979).

Rat pups tested on day 22 of gestation were surgically removed from the mother prior to delivery. This is important, since a major route of exposure of neonates to PBBs is through mothers milk. These results establish that transplacental exposure alone will induce hepatic steroid hydroxylation, prior to PBB exposure during lactation. Further, these results show that PBB treatment increases hepatic steroid metabolism before birth. A major masculinizing influence for male behavior and physiology is the testosterone surge which normally occurs within two hours of birth (Rhoda et.al., 1984). Rat neonates exposed

to PBB in utero have increased activity of testosterone catabolic pathways, and the MFO induction may alter the efficacy of the post-partum testosterone surge in brain masculinization.

Adult rats have different patterns of hepatic steroid metabolism depending on sex. Male rats normally show increased activity of testosterone 16a- and 6ß-hydroxylation relative to female rats, and female rats normally show greater testosterone 5a-reductase activity than do males (Dieringer et.al., 1979; Gustafsson et.al., 1983; Schriefers et.al., 1972). The differences in hepatic metabolism betwen sexes is related to differing amounts of androgens available (6ß hydroxylation) and to perinatal differentiation of the hypothalamic- pituitary- liver axis (16a-hydroxylation, 5a-reduction). Adult control of metabolic activity in the liver is under control of growth hormone released by the pituitary (Rumbaugh and Colby, 1980; Mode et.al., 1983). Patterns of testosterone metabolism in perinatal rats did not differ according to sex in this study. This is in accord with numerous other studies which have shown that sex differences in metabolism do not begin to appear until puberty (Pasleau et.al., 1981; Gustafsson and Skett, 1971; Rifkind et.al., 1979; ElDefrawy et.al., 1974). Similarly, male and female rats both showed similar responses to PBB induction of steroid metabolism. For these reasons, data from male and female pups were

merged for the statistical and graphical evaluation of PBB effects.

Mixed-function oxidase inducers such as phenobarbital and PCB are known to increase hepatic testosterone hydroxylation (Nowicki and Norman, 1972; Conney and Klutch, 1963). PBB treatment of pre-pubertal and adult rats results in increases in 16a, 7a- and 6ß- hydroxylation of testosterone (Newton et.al., 1982) which vary in an age and sex dependant manner. Testosterone hydroxylation at 16a, 7a, and 6ß is increased by phenobarbital pretreatment, indicating that these enzymes are coupled to cytochrome P-450 (Conney and Klutch, 1963). Treatment with the P-448 inducer TCDD increases testosterone 7a-hydroxylation (Gustafsson and Ingelman-Sundberg, 1979) as does treatment with 3-methylcholanthrene. Some compounds such as 3-MC and ßNF normally thought of as mixed-function oxidase inducers actually decrease the activity of testosterone hydroxylation at 16a- and 6ß- positions (Shiverick, 1981; Wood et.al., 1983) in adult rats. This study showed that prenatal PBB treatment increased total testosterone hydroxylation and 7a-hydroxylation beginning on day 22 of gestation. PBB treatment also increased 16a- and 6ß-hydroxylation in fetal rats on day 22 of gestation.

PBB treatment increased EROD activity on all days tested. TCDD treatment has been found to increase AHH activity in fetal rats as early as day 18 of gestation

(Lucier et.al., 1979). These results show that the genetic mechanisms to induce at least some MFOs are in place well before parturition, although neither the testosterone hydroxylases nor benzphetamine demethylase were inducible until day 22 of gestation (1 to 12 hours before birth). These results clearly show that PBBs cross the placenta in quantities sufficient to induce mixed-function oxidase activity, provided that the MFO in question is inducible during gestation. Dent (1978) has already shown that transplacental exposure alone is sufficient to increase MFO activity in 8 to 15 day old rat pups.

In adult rats, PBB administration accelerates testosterone metabolism sufficiently to decrease plasma testosterone titers (chapt 3), and decrease the efficacy of testosterone in maintaining prostate and seminal vesicle weight (McCormack et.al., 1979). Figure 19 shows that perinatal PBB administration markedly decreases plasma testosterone titer in 3 day old rats, and that mean testosterone titers are slightly decreased on day 22 of gestation as well. Fetal and neonatal rats normally show wide variations in plasma testosterone titer (Ward and Weisz, 1980, Rhoda et.al., 1984), and some females may have plasma testosterone titers higher than some male pups at a particular time. This wide variation in testosterone titers around the time of birth makes it difficult to statistically validate alterations in steroid concentration

which may be physiologically significant.

In summary:

1. PBBs cross the placenta in sufficient quantities to induce drug and steroid metabolism even before birth.

2. Total testosterone metabolism was increased by day 22 of gestation, and remained elevated on all days tested.

3. The induction of testosterone metabolism was associated with significant decreases in serum testosterone titer by day 3 post-partum.

4. Induction of testosterone metabolism and decreased serum testosterone associated with PBB treatment occured during the "critical period" of brain differentiation, and therefore have the potential to alter perinatal androgen imprinting of the brain.

CHAPTER 6

EFFECT OF PERINATAL PBB TREATMENT ON ADULT SEXUAL BEHAVIOR AND PHYSIOLOGY

CHAPTER 6

INTRODUCTION

The presence of adequate quantities of aromatizable androgens during fetal and neonatal life is important for normal differentiation of the central nervous system. In the rat, circulating androgens from the fetal testes are aromatized into estrogens in the anterior hypothalamus, and mediate the masculinization of adult behavior and physiology (Naftolin et.al., 1975; Weisz and Gibbs, 1974; George and Ojeda, 1982; Weisz and Ward, 1983). Interruption of the availability of estrogen to developing brain, either through decreases in circulating androgen titer (Orth et.al., 1981; Ward and Weisz, 1980) or blockade of hypothalamic aromatization (Gorski et.al., 1977; Whalen and Olson, 1981; Weisz and Ward, 1983) results in demasculinization of adult reproductive physiology and behavior (Ward, 1972; Gorski et.al., 1978; Ward and Weisz, 1980; Ruppert and Clemens, 1981; Gladue and Clemens, 1980; Gogan et.al., 1980; Corbier, 1985).

The critical period for brain sexual differentiation in the rat is between day 16 of gestation and day 5 post-natal (MacLusky and Naftolin, 1981). Maternal PBB

treatment increases hepatic androgen metabolism, and decreases circulating testosterone titer during this critical period (chapt. 5), and therefore has the potential to interfere with brain differentiation. To determine if the decreased testosterone titer seen in PBB treated perinatal rats alters brain sexual differentiation adult behavior and physiology were tested.

Two different but related modes of brain behavioral differentiation may be assessed; masculinization and defeminization. Masculinization is often assessed by measuring copulatory behavior in male rats. Defeminization of male rats may be evaluated by measuring the occurance of typically feminine behaviors such as lordosis. Severe impairment of brain masculinization may result in males which show an absence of intromittive or ejaculatory behavior patterns in adulthood (Ward, 1972; Rhees and Fleming, 1981; Gupta, 1978; Christensen and Clemens, 1975). Less severe effects on male behavior include prolonged intromission and ejaculation latencies, and decreases in mount and intromission frequency.

Defeminization of behavior and physiology in male rats is evidenced by very low levels of lordotic (feminine sexual) behavior in adults, and the absence of an LH surge following treatment of castrate rats with high doses of estradiol (Gogan et.al., 1980; Blackwell and Amos, 1971; Ward and Renz, 1972; Ward et.al., 1972; Clemens and Gladue,

1978). Female rats also have a slower post-castration rise in plasma LH following gonadectomy than do male rats. The difference in post-castration rate-of-rise of LH has been shown to depend on perinatal hormone exposure and could be used as a probe to determine the extent of brain defeminization(Justo and NegroVilar, 1979; DaMassa et.al., 1983; Johnson and Gay, 1983; Blackwell and Ames, 1971).

The extent of masculinization of PBB treated rats will be assessed by examining parameters of male copulatory behavior. Defeminization of behavior (lordotic responding) will be assessed in castrated and estrogen primed males, and defeminization of hypothalamic gonadotropin regulation will be determined by measuring the post-castration increase in plasma LH.

METHODS

Animals and Treatment

Timed pregnant Sprague-Dawley rats were obtained from Harlan (Indianapolis, IN) and housed under 12:12 inphase light:dark conditions with food and water available *ad lib.*. On day 8 of gestation, one-half the animals were assigned to the PBB group, and their food replaced with ground Wayne Lab-Blox containing 100ppm fireMaster BP-6. Control rats received ground Lab-Blox. Dams were allowed to deliver normally, and maintained on treated feed until pups were 28 days old. When pups were 28 days old they were weaned onto the control diet, and housed three per cage until 60 days old. When pups were 60 days old they were placed in individual cages in preparation for behavioral testing.

Masculine behavior testing

Male behavior testing began when pups were 75 days old. Ovariectomized females in behavioral estrus were used as stimuli. Two days before testing, stimulus females received 0.1mg estradiol benzoate; six hours before testing each female received 1mg of progesterone. Behavioral testing began one hour after the dark cycle

began and continued for three hours or less. The testing schedule was randomized so male rats were placed with different female partners for each test session.

After being removed from their cages, male rats were allowed 5 minutes to acclimate to the testing arena. Behavioral arenas were glass 10 gallon aquaria, having three sides covered with paper to reduce visual distraction. After males had acclimated to the arena, an estrus female was placed in the cage. Male behaviors were scored as mounts, intromissions, or ejaculations, and intromission and ejaculation latency were recorded. Each test was ended after 30 minutes, regardless of whether ejaculation had occured. Ejaculations were confirmed by the presence of sperm in a vaginal rinse. Testing occured once weekly for six consecutive weeks.

Ejaculations were reported on a cummulative basis, over the entire 6 week behavioral testing period. Ejaculatory latency was calculated as the time required after the first intromission before an ejaculatory behavior pattern was observed. Intromission latency and frequency was examined in detail for the last three weeks of behavior testing. Intromission frequency was calculated on the basis of the number of intromittive behaviors observed during the 30 minute test period. For purposes of analyzing intromission behavior, rats were divided into "ejaculating" and "non-ejaculating" groups based on whether

an ejaculatory pattern was seen during the last 3 behavioral tests. Males which did not show intromittive behaviors (8/35 PBB tests, 0/36 control) were excluded from calculations of intromission latency and frequency.

LH Response to Castration

Following the last male behavioral test, rats were allowed to recover for one week. Blood samples were taken by cardiac puncture under light ether anesthesia from PBB and control male rats and control female rats. The animals were then castrated or ovariectomized, and returned to their individual cages. Forty eight and ninety six hours after gonadectomy repeat blood samples were taken from each rat. Blood samples were allowed to clot for 2 hours at room temperature, and serum was removed, and stored at -20° C for six weeks until assayed for plasma LH.

Plasma LH was determined by RIA using the rat-LH kit supplied by the NIADDK. The assay used was a double anti-body method employing rabbit anti-LH at a final concentration of 1:50,000. The antigen-antibody complex was precipitated using goat anti-rabbit serum with a final concentration of 1:250. Sensitivity of the standard curve was 0.1ng/ml (rat LH-RP2) using 0.2ml serum samples. Total LH binding was 25.2%. LH values were computed using a logit/log transform of the standard curve (correlation coefficient = .994), and intra-assay coefficient of variation averaged 3.3%.

Feminine Behavior Testing

Two weeks following castration, male rats were tested for lordotic behavior. Prior to behavioral testing, rats were primed using 0.2mg estradiol benzoate (48 hours before) and 1mg progesterone (6 hours before). Female rats were also tested to provide baseline data, and to insure the continued vigor of the stud males used in the study.

Each castrate male was tested with a vigorous stud male until 10 good mounts had been achieved. The lordosis quality of the male was scored on a four point scale (Hardy and DeBold, 1971), from 0 (complete absence of lordosis) to 3 (complete dorsiflexon, with the stance held briefly after dismount). Lordosis quotient was computed for each run of ten mounts. Testing was carried out weekly for four consecutive weeks.

Statistical Tests

Behavioral count data (number of ejaculations and lordosis quotient) were evaluated using Chi-Square contingency tables ($p<0.05$). Student's t-test ($p<0.05$) was used to evaluate intromission and ejaculation latencies and intromission frequencies. Analysis of variance followed by Duncan's New Multiple Range Test was used to assess changes in post-castration LH dynamics ($p<0.05$).

RESULTS

PBB treatment did not affect the number of male rats ejaculating, nor the cumulative number of ejaculations per group (Figure 20). Perinatal PBB treatment did significantly increase ejaculatory latency in comparison to control rats. Among non-ejaculating rats, PBB treated animals had prolonged intromission latency and decreased numbers of intromissions in a 30 minute test. Intromission latency and intromission frequency in ejaculating rats did not differ between control and PBB treated animals (Table 9). It should also be noted that in the 71 behavioral tests examined in detail, 8 of 35 tests of PBB treated males showed a complete absence of intromittive behaviors, and in 36 tests of control males none failed to achieve intromissions. The failure of some PBB treated males to achieve intromission was significant ($p(X^2)>0.99$).

Radioimmunoassay of post-castration serum showed that female rats had a delayed LH response relative to control males. PBB treated males also had a feminized LH response to castration, i.e. plasma LH values 48 hours after castration were midway between control male and female

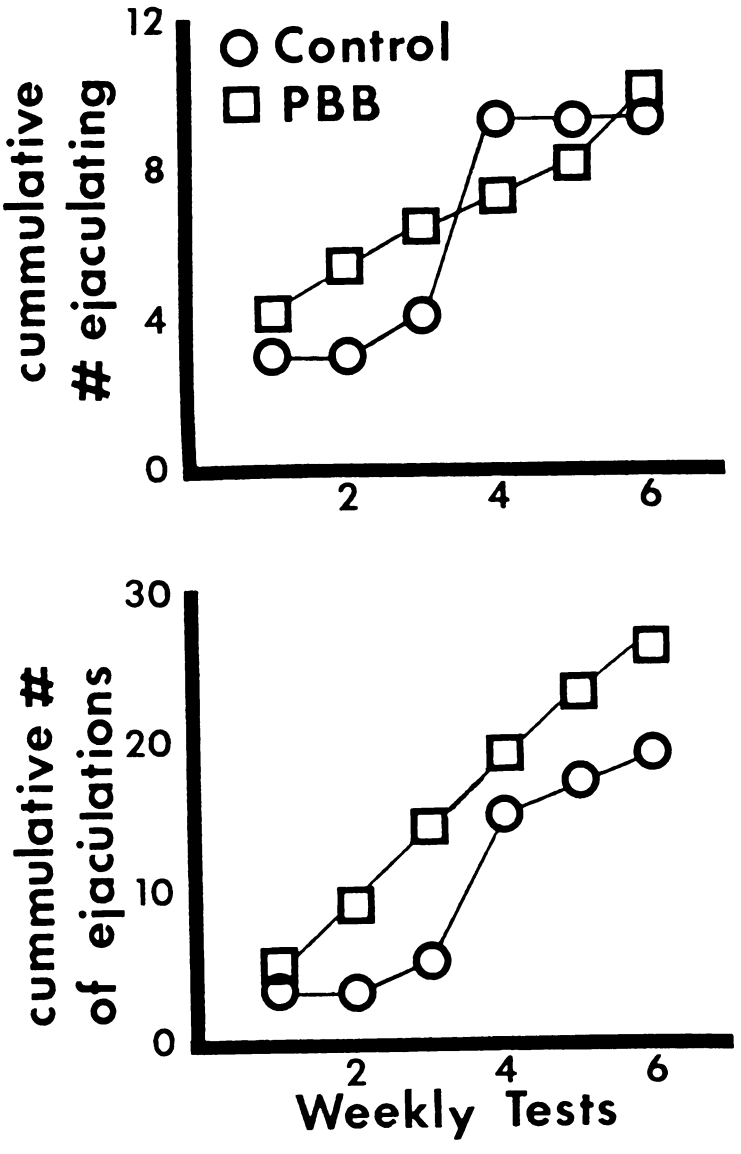

FIGURE 20 Perinatal PBB treatment does not affect adult ejaculatory frequency. Top shows male rats which ejaculated at least once during testing. Bottom shows total number of ejaculations of each group summed over the entire 6 week test. 12 male rats from each group were tested each week.

TABLE 9

Effect of perinatal PBB treatment on male sexual behavior in adult rats

	non - ejaculating		ejaculating		
	Intromission		Intromission		ejaculation
group	latency (min.)	number (per 30 min)	latency (min.)	number (per 30 min)	latency (min.)
control	5.1±.94(24)	33±3.4(24)	2.1±.72(12)	52±6.4(12)	14.3±1.8(16)
PBB	8.2±1.5(15)*	22±4.4(15)*	2.3±.52(12)	41±3.5(12)	17.6±1.2(22)*

Each measure is mean ± standard error (n).
* Statistically different from control by Student's t-test ($p<0.05$).
this table does not include data from rats which did not display intromission behavior during a particular test (8/35 of PBB group; 0/36 from control group)

values. Four days post-castration, PBB treated males had plasma LH values equivalent to those seen in control males while female LH titers remained lower than male (Table 10).

Perinatal PBB treatment did not affect adult feminine sexual behavior in male rats. PBB treated rats had lordosis quotients and lordosis quality scores which did not differ from those of control males (Figure 21).

TABLE 10

Effect of perinatal PBB treatment on LH response to castration in adult rats

group	pre-castration	48 hours post-castration	96 hours post-castration
control male	.16±.04 (11)	2.25±.23 (10)	2.22±.29 (10)
PBB male	.12±.03 (9)	1.21±.10 (9)*	2.28±.26 (9)
female	.11±.06 (6)	.59±.06 (5)*	1.56±.34 (4)*

Each measure is mean ± standard error (n).
*Statistically different from control male by Duncan's New Multiple Range Test ($p<0.05$).

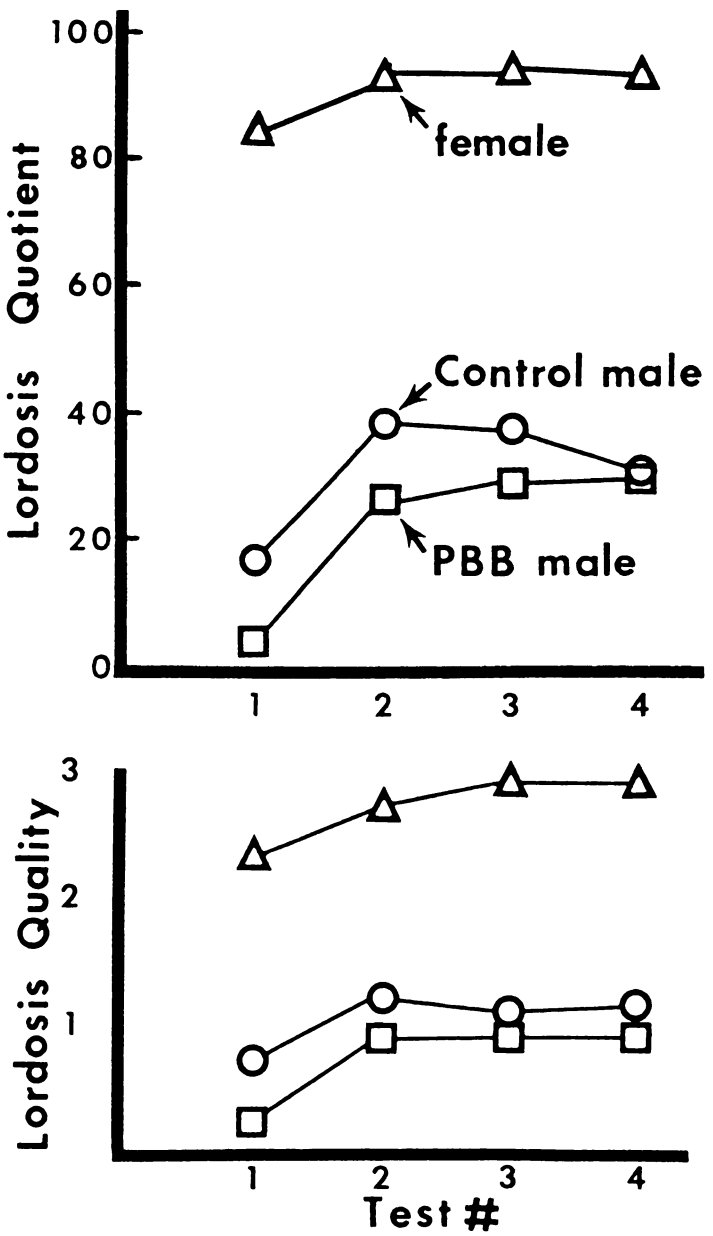

FIGURE 21. Perinatal PBB treatment does not increase lordosis quotient or lordosis quality in adult castrated, estrogen primed male rats. Each point represents 12 rats tested.

DISCUSSION

These results demonstrate an association of perinatal PBB treatment with impaired adult masculine copulatory behavior, and altered hypothalamic physiology. In the rat, masculinization and defeminization of behavior are two independant processes. In the control of gonadotropin secretion however, masculinization is the same as defeminization; i.e. the processes cannot be separated from each other as in behavior. When brain differentiation is assessed by testing the LH response to estradiol injection for example, there is no way to distinguish between a "masculinized" brain (tonic gonadotropin secretory patterns) and a "defeminized" brain (one which does not exhibit cyclic LH dynamics). Behavioral masculinization and defeminization, however, are independant processes which are not mutually exclusive. The independance of these two processes may be demonstrated by several lines of evidence. First, the appropriate combinations of endocrine or surgical intervention can produce either feminine or masculine behaviors in rats of either sex, indicating that the potential for heterotypical behavior exists in spite of the organizing actions of

perinatal hormones. Perinatal hormones appear to act by changing thresholds, rather than by qualitatively altering the fundamental neural circuits regulating behavior (Feder, 1982). Secondly, different hypothalamic nuclei are involved in the regulation of feminine and masculine behavior patterns, and some researchers have even suggested that right and left sides of the brain may play different roles in control of sexual behavior (Nordeen and Yahr, 1982). The pre-optic hypothalamus appears to control masculine sexual behavior, and the ventro-medial nucleus is involved in feminine sexual behavior. Both the pre-optic and ventro-medial nuclei are involved in the regulation of gonadotropin secretion (Nordeen and Yahr, 1982; Feder, 1982). Third, in some species, masculinization and defeminization may involve different organizing hormones (McEwen, 1981). In the guinea pig, behavior is masculinized by androgens, but defeminized by estrogens. Lastly, the period of maximum sensitivity for differentiation may be earlier for masculinization than for defeminization (Nordeen and Yahr, 1982; Feder, 1981). In spite of the differences between the masculinizing and defeminizing processes, they appear to proceed through similar mechanisms in the rat, and are often associated with similar endocrine treatments.

Many experimental treatments have been identified which alter normal sexual differentiation of rat brain.

Maternal stressors such as food restriction or restraint interfere with both masculinization and defeminization of behavior. Ward (1972) discovered that prenatal restraint/illumination stress resulted in impairment of male copulatory behavior, and increased lordosis quotients in males castrated and tested as adults. Maternal restraint stress was later found to abolish the peak of serum testosterone which normally occurs on day 18 and 19 of gestation in male fetuses (Ward and Weisz, 1980; Orth et.al., 1981). Maternal food restriction is another example of a stressor which results in demasculinization and feminization of adult sexual behavior (Rhees and Fleming, 1981). Endocrine manipulations such as neonatal castration or administration of testosterone antibodies also demasculinize adult behavior (Gupta, 1978; Corbier et.al., 1983), as does treatment with the anti-androgen flutamide (Clemens et.al., 1978). The masculinizing effects of neonatal androgens on behavior are mimicked by administration of estrogens (Rhoda et.al., 1984; Parsons et.al., 1984; Sodersten and Hansen 1978), and administration of anti-estrogens or aromatase inhibitors results in behavioral demasculinization (Ruppert and Clemens, 1981; Gladue and Clemens, 1980). These studies suggest that the estrogenic metabolites of androgen metabolism are important for the normal development of male copulatory behavior. Even exposure of neonatal rats to the

estrogenic pesticide chlordecone has resulted in behavioral masculinization, although it did not cause defeminization (Gray 1982).

The results reported here further support the concept that masculinization and defeminization of developing brain are not tightly coupled. The increased ejaculation and intromission latencies seen in males treated perinatally with PBB are evidence of mild demasculinization. Testing of feminine behavior, however, revealed no increases in either lordosis quotient (numbers of lordosis responses) or lordosis quality. PBB treatment then, results in demasculinization, but not feminization of adult sexual behavior. Further evidence of the demasculinizing effects of PBB is the absence of intromittive behavior patterns in 8 of 35 tests of PBB rats; all control rats showed some level of intromittive behavior during behavioral testing.

Endocrine manipulations which are extremely potent demasculinizing agents also alter adult control of gonadotropin secretion. Testing for demasculinization of the LH control pathways in adult rats is usually carried out by attempting to evoke an LH surge in a castrated male by administration of a high dose of estradiol benzoate. Recently Corbier (1985) has pointed out that if the neonatal testosterone surge (between 0 and 2 hours post-partum) is allowed to occur, the LH surge mechanism is masculinized, and it becomes difficult to evoke a

female-like LH response after estrogen treatment. These results (Corbier, 1985) imply that a potent demasculinizing treatment was needed to fully feminize the adult control of gonadotropin secretion. Since perinatal PBB treatment seemed likely to be a "mild" rather than a "potent" demasculinizing influence, it was decided to test the possible feminization of the LH control mechanism in another way. In 1971 Blackwell and Ames noted that the rise in plasma LH following gonadectomy occured more rapidly in male than in female rats. Male rats show an increase in plasma LH within 18 hours of castration, wherease female rats do not have a marked increase in LH for at least 36 hours. The study of LH dynamics following castration has been extended by Gay and co-workers (Johnson and Gay, 1983; Gay and Hauger, 1977), who noted that neonatal castration of males results in feminization of the post-castration LH surge, whereas females exposed to testosterone on day 2 have a masculinized LH response to castration. Exposure to as little as 10μg of testosterone propionate on post-natal day 3, 4 or 5 produced a male-like rapid rise in plasma LH following ovarectomy of female rats (DaMassa et.al., 1983). Other studies have demonstrated that adult steroids (testosterone vs. estradiol) do not have a strong influence on rate-of-rise of plasma LH after castration, and confirm that post-castration LH dynamics depend on perinatal brain differentiation (Justo and

NegroVilar, 1979).

These results show that perinatal PBB treatment demasculinizes the response of LH to castration (Table 10). Two days after castration, male rats treated perinatally with PBB had plasma LH titers midway between those seen in normal males and those of control females. By four days post-castration, PBB treated males had plasma LH titers equivalent to those seen in control males, while female LH titers were still somewhat lower. This shows that perinatal PBB treatment can affect hypothalamic control of adult reproductive physiology, even though there are no PBB related effects on basal LH titer (Figure 21, Johnston et.al., 1980).

The concept of PBB interfering with behavioral and physiological masculinization of the hypothalamus during the perinatal period is further supported by the observation that adult testosterone titers in PBB and control animals are the same (Figure 15, chapt. 4), and therefore the effects of PBB on behavior and LH dynamics are not mediated through differences in adult steroid titer. Although the demasculinization of behavior and physiology in PBB treated animals is associated with decreased testosterone titer in perinatal males, other endocrine and non-endocrine mechanisms may also affect adult behavior. It has been shown that maternal behavior during the perinatal period influences adult sexual

behavior patterns. Decreased maternal licking stimuli may result in impaired male copulatory behavior in adult rats (Moore, 1984). PBB induced disruptions of maternal nesting or nurturing behaviors therefore, could affect adult sexual behavior. In addition, decreased perinatal testosterone titer could alter morphological development, and interfere with the growth of structures such as penile papillae which are required to maintain erectile competance and normal copulatory sensation (Parrott, 1974). Since PBB treatment may have effects during the perinatal period which are not related to androgen induced brain differentiation, it is not possible to state unequivocally that the decreased masculinization seen in neonatally treated males is due to altered brain differentiation resulting from decreased perinatal testosterone titer. These results do show however, that perinatal PBB treatment results in demasculinization of adult sexual behavior and physiology.

In summary, perinatal PBB treatment results in increased hepatic testosterone oxidation and decreased plasma testosterone titer during the period of brain differentiation. These effects are associated with de-masculinization of brain behavioral and physiological control mechanisms. This is shown by the demasculinization of male copulatory behavior and the feminization of hypothalamic LH regulation in adult male rats.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

Polybrominated biphenyls (PBBs) are potent inducers of the mixed-function oxidase enzymes which metabolize steroid hormones. In vitro metabolism of estradiol, progesterone and testosterone is increased by PBB treatment (Bonhaus et.al., 1981; Newton et.al., 1982; Arneric et.al., 1980). Both progesterone and testosterone are more rapidly cleared from plasma following PBB administration, and the duration of progesterone anesthesia was decreased in PBB treated rats (McCormack et.al., 1981; Harris et.al., 1981; McCormack et.al., 1979). Male rats treated with PBB had decreased prostate and seminal vesicle weights as well as attenuated seminal vesicle growth following injections of exogenous testosterone (McCormack et.al., 1979; Johnston et.al., 1980).

Despite the evidence that PBB decreases the efficacy of reproductive steroids, previous research has failed to produce evidence that PBB treatment alters levels of endogenous steroid hormones in rats (Castracane et.al., 1982; Johnston et.al., 1980). There are many ways in which PBBs could act to modify reproduction:

1. PBB is known to increase plasma clearance of

steroid hormones (McCormack et.al., 1981), including testosterone. If testosterone synthesis did not change, increased plasma clearance would be expected to result in decreased basal androgen titers.

2. Testosterone synthesis could be affected by PBB. If PBB has a direct toxic effect on testicular leydig cells, it could reduce the amount of testosterone formed in response to pituitary gonadotropins. PBBs could also affect the hypothalamus or pituitary directly to decrease the amount of trophic hormones excreted, although no changes in plasma LH titer have been found in PBB treated animals (Johnston et.al., 1980).

3. PBB could alter the hypothalamic pituitary system in at least two ways. PBB present during development could alter steroid imprinting of the hypothalamus, either through direct toxic effects, or by altering the availability of circulating androgens. In adulthood, PBBs could act to impair the feedback control of hormone synthesis by altering central steroid metabolism, affecting binding of steroid hormones to CNS receptor sites, or directly attacking synthesis or release of hypothalamic or pituitary hormones.

4. PBB induced alterations in target organ steroid metabolism are also a possibility. PBBs are potent P-450 inducing agents, and alter hepatic steroid hydroxylation and reduction pathways (Newton et.al., 1982). Changes in

testosterone aromatization in the CNS, or alterations in 5a-reductase activity in peripheral tissues could greatly reduce the efficacy of circulating androgens.

5. PBBs could also interfere with the action of reproductive steroids by altering either steroid-receptor binding, or by interfering with the receptor-genome interaction. To date, this hypothesis has not been tested.

The purpose of the experiments which are described here was to determine whether PBB treatment altered target organ metabolism of testosterone in brain or prostate, and to determine if perinatal PBB exposure affected brain differentiation. The first studies were directed toward understanding the interactions of PBBs with hypothalamic androgen aromatase. The aromatization of androgens to estrogens in the hypothalamus is an important step in feedback regulation of gonadotropin secretion and in steroid regulation of male sexual behavior (Selmanoff et.al., 1977; Worgul et.al., 1981; Kalra and Kalra, 1983; Popolow and Ward, 1976; Christensen and Clemens 1975; Naftolin et.al., 1975). The aromatase enzyme system is coupled to cytochrome P-450, and thus could be a target for induction by PBB (Thompson and Siiteri, 1974). If PBB treatment resulted in increased hypothalamic androgen aromatization, lowered blood testosterone titers from increased plasma steroid clearance could be compensated for by increased hypothalamic production of estrogens. The

increased hypothalamic estrogen production would then prevent the activitation of feedback mechanisms which would normally increase pituitary LH release. Thus, PBB treatment could alter circulating steroid titers by interfering with normal steroid feedback control of pituitary LH release. In order to determine whether effects on hypothalamic androgen aromatization contribute to PBB reproductive effects, it was necessary to first develop an improved assay for hypothalamic aromatase (HA).

The assay of hypothalamic aromatase activity used tritiated androstenedione in an in vitro incubation to produce radiolabelled estrone. The radioactive estrone produced by aromatization was separated by two successive HPLC steps, and quantified by liquid scintillation counting. This technique offered good recoveries (70 - 80%), and was sensitive enough to detect less than 5 femtomoles of estrone. The estrone produced by this technique was of very high purity (>95%), and the assay technique represented a substantial improvement over existing methods with respect to accuracy and ease of use.

It was then necessary to determine how hypothalamic aromatase activity was normally controlled in the rat. Early studies using rabbits (Reddy et.al., 1973) established that HA activity is sensitive to endocrine manipulation, but since rabbits are reflex ovulators the results obtained could not be applied to rats. Initial

studies verified that HA activity is approximately three times greater in male than in female rats, and that castration of adult males reduced HA activity to levels normally seen only in females. Hypothalamic aromatase activity in female rats did not vary throughout the 4 days of the estrus cycle. Since the hypothalamic aromatase enzyme is concentrated in a sexually dimorphic region of the hypothalamus, there was reason to suspect that its activity might be determined during perinatal brain differentiation. Perinatal hormone manipulation failed to affect adult hypothalamic aromatase activity. A masculinizing dose of testosterone propionate administered to female rats on day 3 of life did not increase adult HA activity. Similarly, perinatal administration of a feminizing dose of the aromatase inhibitor androsta-1,4,6-triene-3,17-dione did not reduce adult aromatase levels in male rats. Hypothalamic aromatase activity was equally inducible in adult males which had been castrated at birth, and in adult females by the administration of testosterone in subdermal silastic implants. These results established that hypothalamic aromatase activity in the rat is regulated by circulating levels of androgens in the adult animal, and not by perinatal endocrine influences. Shortly after these results were obtained, Roselli et.al., (1984) published similar findings.

Studies carried out in PBB treated rats revealed that PBB administration decreases both blood testosterone titer and hypothalamic aromatase activity. Lifelong PBB treatment of male rats resulted in decreased prostate and seminal vesicle weights, and a trend toward decreased hypothalamic aromatase activity. Lifelong PBB treatment also resulted in a trend toward decreased plasma testosterone titer which was not statistically significant, possibly because of insufficient numbers of test animals. Rats given PBB throughout life also were significantly smaller than control rats. The decreased body weight seen in chronically treated rats is due to decreased feed efficiency in PBB fed animals (Sleight and Sanger, 1976). Although animals suffering from protein-calorie malnutrition may also display decreased blood testosterone titers, malnutrition is probably not a major factor in the decreased testosterone seen in PBB treated animals. This was shown by the fact that adult animals given PBB in a single dose show decreased blood testosterone titers 14 days after treatment even though there was no decrease in body weight. Animals treated with PBB had decreased blood testosterone titers and decreased hypothalamic aromatase activity 14 days after treatment, but not 90 days post-treatment. Although decreased hypothalamic aromatase activity was associated with decreased serum testosterone titer, these experiments have not established a certain

cause and effect relationship. Additional experiments in which PBB treatment and blood testosterone are varied independantly will be required to establish that lowered HA activity in PBB treated animals is a result of lowered blood testosterone. These experiments do show that PBB treatment is capable of affecting parameters of neuroendocrine reproductive control.

Animals given a single injection of 150mg/kg of PBB as adults show lowered blood testosterone titers 14 days but not 90 days after treatment. The same animals have decreased prostate weights both 14 and 90 days after treatment. In order to determine whether altered prostatic 5a-reductase activity might be involved in the prostatic regression seen in PBB treated animals, prostatic homogenates were incubated with ^{14}C-testosterone, and the formation of 5a-DHT and 3a- androstanediol was measured. PBB treated animals did not show any differences in the formation of 5a-DHT or 3a-androstanediol from control animals. Prostates from PBB treated animals did produce slightly higher levels of polar testosterone metabolites than did prostates from control animals, but this is probably not physiologically significant.

Pregnant rats were given 100 ppm PBB in food to determine whether it was possible to induce hepatic mixed-function oxidases in fetal and neonatal rats. TCDD increases AHH and EROD activity in fetal liver (Lucier

et.al., 1979). If PBB treatment increased fetal steroid metabolism it could interfere with brain and morphological sexual differentiation. Pilot studies established that fetal liver did not metabolize testosterone prior to day 21 of gestation. Beginning on day 21 of gestation, fetal liver metabolism and plasma testosterone levels were assessed. PBB treated rats showed increased levels of testosterone hydroxylation beginning on day 22 of gestation, and EROD activity was increased beginning on day 21 of gestation. These results show that transplacental exposure of rat fetuses to PBB can increase MFO activity even before birth. Blood testosterone titers were highly variable, and PBB treated animals showed a trend toward decreased levels of plasma testosterone on day 22 of gestation. Plasma testosterone was significantly lower in PBB treated rats by day 3 postnatal. These results show that PBB treatment can lower blood testosterone titer during the critical period of brain differentiation (day 17 of gestation to day 5 postnatal), and thus may potentially alter brain sexual differentiation.

The effect of perinatal PBB treatment on brain sexual differentiation was assessed in male rats treated with 100 ppm PBB in food beginning on day 8 of gestation and ending on day 28 postnatally. Male and female sexual behavior was tested in PBB treated and control males. PBB treatment did not affect either lordosis quotient or lordosis quality

(feminine sexual behavior) in castrated male rats. PBB treatment had no effect on number of male rats ejaculating in male behavior tests, however PBB treated males had significantly prolonged ejaculatory latencies. Non-ejaculating PBB-treated rats had prolonged intromission latencies and decreased intromission frequency relative to non-ejaculating control males. The impairment of male sexual behavior was seen in spite of the fact that PBB treated rats had adult blood testosterone titers identical to those seen in control males. Differentiation of the hypothalamic control of plasma LH dynamics was assessed in rats treated with PBB from day 8 of gestation until day 28 postnatally. Female rats normally have a slower rise in plasma LH following gonadectomy than do male rats. PBB treated males had a rate-of-rise of plasma LH following gonadectomy which was intermediate between control male and conrol female animals. These results indicate that the hypothalamic control of plasma LH was partially demasculinized by perinatal PBB treatment.

The major findings of these studies may be summarized as follows:

1. Acute PBB treatment results in decreased plasma testosterone titer, and decreased hypothalamic aromatase activity in adult rats. Since hypothalamic aromatase activity is normally regulated by circulating androgens, PBB induced reductions in plasma testosterone titer may be

responsible for the decreased hypothalamic androgen aromatization seen in treated animals. These results show that PBB treatment may affect some parameters important in the neuroendocrine control of reproduction.

2. PBB treatment resulted in decreased prostate and seminal vesicle weights. The decreased prostatic weights were not associated with changes in the 5a-reduction of testosterone to dihydrotestosterone.

3. PBB treatment of pregnant rats resulted in increased testosterone hydroxylation in fetal and neonatal liver. PBB treated neonates showed reductions in plasma testosterone titer relative to control animals as early as day 3 of life. Since PBB treatment results in lowered blood testoserone during the critical period of brain sexual differentiation, it may have effects on hypothalamic development.

4. Perinatal PBB treatment of male rats results in demasculinization but not feminization of sexual behavior. The impairment of male sexual behavior occured in spite of the fact that PBB and control males had no differences in serum testosterone titer at the time of testing. Post-castration plasma LH dynamics of rats treated with PBB perinatally are feminized. These results suggest that perinatal PBB treatment may interfere with androgen-dependant brain sexual differentiation.

BIBLIOGRAPHY

BIBLIOGRAPHY

AFTOSMIS JG, CULIK R, LEE KP, SHERMAN H, WARITZ R. (1972) Toxicology of brominated biphenyls i.) oral toxicity and embryotoxicity; ii.) skin, eye, and inhalation toxicity and an acute test method for evaluating hepatotoxicity and accumulation in body fat. Tox. Appl. Pharm. 22:316-317

ALLEN JR, BARSOTTI DA, LAMBRECHT LK, VAN MILLER JP. (1979) Reproductive effects of halogenated aromatic hydrocarbons on nonhuman primates. Ann. NY Acad. Sci. 320:419-425

ALLEN JR, LAMBRECHT L, BARSOTTI DA. (1978) Effects of polybrominated biphenyls in nonhuman primates. JAVMA 173:1485-1489

ANDERSON HA, WOLFF MS, LILIS R. (1979) Symptoms and clinical abnormalities folllowing ingestion of polybrominated-biphenyl-contaminated food products. Ann. NY Acad. Sci. 320:684-702

ANDRES J, LAMBERT L, ROBERTSON L, BANDIERA S, SAWYER L, LOVERING S, SAFE S. (1983) The comparative biologic and toxic potencies of polychlorinated biphenyls and polybrominated biphenyls. Tox. Appl. Pharm. 70:204-215

ARNERIC SP, MCCORMACK KM, BRASELTON WE, HOOK JB. (1980) Altered metabolism of progesterone by hepatic microsomes from rats following dietary exposure to polybrominated biphenyls. Tox. Appl. Pharm. 54:187-196

BABISH JG, STOWESAND GS. (1977) Polybrominated biphenyls: inducers of hepatic microsomal enzymes and type A cytochrome P-450 in the rat. J. Tox. Envir. Hlth. 3:673-682

BARDIN CW, CATTERALL JF. (1981) Testosterone: A major determinant of extragenital sexual dimorphism Science 211:1285-1293

BEATTY WW. (1979) Gonadal hormones and sex differences in nonreproductive behaviors in rodents: organizational and activational influences. Horm. Behav. 12:112-163

BECHER DJ. (1983) The endocrine responses to protein calorie malnutrition. Ann. Rev. Nutr. 3:187-212

BEKESI JG, ANDERSON HA, ROBOZ JP. (1979) Immunologic dysfunction among PBB-exposed Michigan dairy farmers. Ann. NY Acad. Sci. 320:717-729

BEKESI JG, HOLLAND JF, ANDERSON HA, FISCHBEIN AS, ROM W, WOLFF MS, SELIKOFF IJ. (1978) Lymphocyte function of Michigan dairy farmers exposed to polybrominated biphenyls. Science 199:1207-1209

BERNERT JT, GROCE DF. (1984) Acute response of rat liver microsomal lipids, lipid peroxidation, and membrane anisotropy to a single oral dose of polybrominated biphenyls. J. Appl. Envir. Health 13:673-687

BERNERT JT, GROCE DF, KIMBROUGH RD. (1983) Long-term effects of a single oral dose of polybrominated biphenyls on serum and liver lipids in rats. Tox. Appl. Pharm. 68:424-433

BEYER C. (1976) Neuroendocrine mechanisms in sexual behavior. Subcellular Mechanisms in Reproductive Neuroendocrinology 1:471-485

BIRNBAUM LS, DARCEY DJ, MCKINNEY JD. (1983) Hexabromonaphthalene contaminants of polybrominated biphenyls: chemical composition and disposition in the rat. J. Appl. Envir. Hlth. 12:555-573

BLACKWELL RE, AMES MS. (1971) A sex difference in the rate of rise of plasma LH in rats following gonadectomy. Proc. Soc. Exp. Biol. Med. 136:11-14

BONHAUS DW, MCCORMACK KM, BRASELTON WE, HOOK JB. (1981) Effect of polybrominated biphenyls on hepatic microsomal metabolism of estrogens and uterotropic action of administered estrogen in rats. J. Appl. Envir. Hlth. 8:141-150

BRASELTON WE, ORR JC, ENGEL LL. (1974) Identification by gas chromatography-mass spectrometry of intermediates in the aromatization of modified C19 steroids by human placental microsomes. Steroids 24:411-433

BRILLIANT LB, VAN AMBURG G, ISBISTER J. (1978) Breast-milk monitoring to measure Michigan's contamination with

polybrominated biphenyls. Lancet 2:643-647

BROWN JB. (1955) A chemical method for the determination of oestriol, oestrone, and oestradiol in human urine. Biochem. J. 60:185-195

BROWN-GRANT K. (1976) Control of gonadotropin secretion. Subcell. Mech. Reprod. Endocrinol. 1:485-500

BROWN GC, NIXON R. (1979) Exposure to polybrominated biphenyls: some effects on personality and cognitive function. JAMA 242:523-527

BURSIAN SJ, POLIN D, OLSON BA, SHULL LR, MARKS HL, SIEGEL HS. (1983) Microsomal enzyme induction , egg production, and reproduction in three lines of japanese quail fed polybrominated biphenyls. J. Appl. Envir. Hlth. 12:291-307

CAGEN SZ, PREACHE MM, GIBSON JE. (1977) Enhanced disappearance of drugs from plasma following polybrominated biphenyls. Tox. Appl. Pharm. 40:317-325

CALLARD GV, HOFFMAN RA, PETRO Z, RYAN KJ. (1979) In vitro aromatization and other androgen transformations in the brain of the hamster (Mesocricetus auratus). Biol. Reprod. 21:33-38

CALLARD GV, KUNZ TH, PETRO Z. (1983) Identification of androgen metabolic pathways in the brain of little brown bats (Myotis iucifugus): sex and seasonal differences. Biol. Reprod. 28:1155-1161

CALLARD GV, PETRO Z, RYAN KJ. (1978) Phylogenetic distribution of aromatase and other androgen-converting enzymes in the central nervous system. Endocrinology 103:2283-2290

CALLARD GV, PETRO Z, TYNDALE-BISCOE CH. (1982) Aromatase activity in marsupial brain, ovaries, and adrenals. Gen. Comp. Endocrin. 46:541-546

CARTER LJ. (1976) Michigans PBB incident: chemical mix-up leads to disaster. Science 192:240-242

CASTRACANE VD, ALLEN-ROWLANDS CF, HAMILTON MG, SEIFTER J. (1982) The effect of polybrominated biphenyl (PBB) on testes, adrenal, and pituitary function in the rat. Proc. Soc. Exp. Biol. Med. 169:343-347

CECIL HC, BITMAN J. (1977) Toxicity of polybrominated biphenyl and its effects on reproduction of white leghorn hens. J. Poultry Sci. 57:1027-1036

CELOTTI F, FARINA JMS, SANTANIELLO E, MARTINI L, MOTTA M. (1979) Effect of testosterone, its 5a-reduced metabolites and the corresponding propionate on testosterone metabolism in the ventral prostate and seminal vesicles. J. Steroid Biochem. 11:221-225

CHANDA JJ, ANDERSON HA, GLAMB RW, LOMATCH DL, WOLFE MS, VOORHEES JJ, SELIKOFF IJ. (1982) Cutaneous effects of exposure to polybrominated biphenyls (PBBs): the Michigan PBB incident. Envir. Res. 29:97-109

CHRISTENSEN LW, CLEMENS LG. (1975) Blockade of testosterone-induced mounting behavior in the male rat with intracranial application of the aromatization inhibitor, androst-1,4,6-triene-3,17-dione. Endocrinology 97:1545-1551

CHUNG LWK, MACFADDEN DK. (1980) Sex steroids imprinting and prostatic growth. Invest. Urol. 17:337

CLEMENS LG, GLADUE BA. (1978) Feminine sexual behavior in rats enhanced by prenatal inhibition of androgen aromatization. Horm. Behav. 11:190-201

CLEMENS LG, GLADUE BA, CONIGLIO LP. (1978) Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Horm. Behav. 10:40-53

CLEMENS LG, POMERANTZ SM. (1982) Testosterone acts as a prohormone to stimulate male copulatory behavior in male deer mice (Peromyscus maniculatus bairdi). J. Comp. Physiol. Psycol. 96:114-122

CONNEY AH, KLUTCH A. (1963) Increased activity of androgen hydroxylases in liver microsomes of rats pretreated with phenobarbital and other drugs. J. Biol. Chem. 238:1611-17

COOK H, HELLAND DR, VANDERWEELE BH, DEJONG RJ. (1978) Histotoxic effects of polybrominated biphenyls in Michigan dairy cattle. Envir. Res. 15:82-89

CORBETT TH, BEAUDOIN AR, CORNELL RG, ANVER MR, (1975) Toxicity of polybrominated biphenyls (fireMaster BP-6) in rodents. Envir. Res. 10:390-396

CORBIER P. (1985) Sexual differentiation of positive feedback: Effect of hour of castration at birth on estradiol-induced luteinizing hormone secretion in immature male rats. Endocrinology 116:142-147

CORBIER P, ROFFI J, RHODA J. (1983) Female sexual behavior in male rats: effect of hour of castration at birth. Physiol. Behav. 30:613-616

CORDLE F, KOLBYE AC. (1979) Food safety and public health. Cancer 43:2143-2150

DAHLOF LG, HARD E, LARSSON K. (1978) Influence of maternal stress on the development of the fetal genital system. Physiol. Behav. 20:193-195

DAMASSA DA, KOBSHIGAWA D, SMITH ER, DAVIDSON JM. (1976) Negative feedback control of LH by testosterone: A quantitative study in male rats. Endocrinology 99:736-747

DAMASSA DA, RABII J, SAWYER CH. (1983) Responses of serum LH and FSH to the removal of steroid feedback inhibition Neuroendocrinol. 37:122-130

DAMSTRA T, JURGELSKI W, POSNER HS, VOUK VB, BERNHEIM NJ, GUTHRIE J, LUSTER M, FALK HL. (1982) Toxicity of polybrominated biphenyls (PBBs) in domestic and laboratory animals. Envir. Hlth. Perspec. 44:175-188

DANNAN GA, GUENGERICH FP, KAMINSKY LS, AUST SD. (1983) Regulation of cytochrome P-450. J. Biol. Chem. 258:1282-1288

DANNAN GA, MILESKI GJ, AUST SD. (1982) Purification of polybrominated biphenyl congeners. J. Appl. Envir. Hlth. 9:423-438

DANNAN GA, SLEIGHT SD, AUST SD. (1982) Toxicity and microsomal enzyme induction effects of several polybrominated biphenyls of firemaster. Fund. Appl. Toxicol. 2:313-321

DARJONO, SLEIGHT SD, STOWE HD, AUST SD. (1983) Vitamin A status, polybrominated biphenyl (PBB) toxicosis, and common bile duct hyperplasia in rats. Tox. Appl. Pharm. 71:184-193

DENEF C. (1983) 5a-Dihydrotestosterone formation and its functional significance in rat anterior pituitary. J. Steroid Biochem. 19:235-239

DENT JG. (1978) Characteristics of cytochrome P-450 induced mixed function oxidase enzymes following treatment with PBBs. Envir. Hlth. Perspec. 23:301-308

DENT JG, CAGEN SZ, MCCORMACK KM, RICKERT DE, GIBSON JE. (1977) Liver and mammary arylhydrocarbon hydroxylase and epoxide hydrase in lactating rats fed polybrominated biphenyls. Life Sci. 20:2075-2080

DENT JG, ELCOMBE CR, NETTER KJ, GIBSON JE. (1978) Rat hepatic microsomal cytochrome(s) P-450 induced by polybrominated biphenyls. Drug Metab. Dispos. 6:96-101

DENT JG, MCCORMACK KM, RICKERT DE, CAGEN SZ, MELROSE P, GIBSON JE. (1978) Mixed function oxidase activities in lactating rats and their offspring following dietary expose to polybrominated biphenyls. Tox. Appl. Pharm. 46:727-735

DENT JG, NETTER KJ, GIBSON JE. (1976) Effects of chronic administration of polybrominated biphenyls on parameters associated with hepatic drug metabolism. Res. Comm. Chem. Path. Pharm. 13:75-82

DENT JG, NETTER KJ, GIBSON JE. (1976) The induction of hepatic microsomal metabolism in rats following acute administration of a mixture of polybrominated biphenyls. Tox. Appl. Pharm. 38:237-249

DENT JG, ROES U, NETTER KJ, GIBSON JE. (1977) Stimulation of hepatic microsomal metabolism in mice by a mixture of polybrominated biphenyls. J. Appl. Envir. Hlth. 3:651-661

DERR SK. (1978) In vivo metabolism of exogenous progesterone by PCB-treated female rats. Bull. Envir. Contam. Toxicol. 19:729-732

DESSI-FULGHERI F, LUPO C. (1982) Odour of male and female rats changes hypothalamic aromatase and 5a-reductase activity and plasma sex steroid levels in unisexually reared male rats. Physiol. Behav. 28:231-235

DICARLO FJ, SEIFTER J, DECARLO VJ. (1978) Assessment of the hazards of polybrominated biphenyls. Envir. Hlth. Perspec. 23:351-366

DIERINGER CS, LAMARTINIERE CA, LUCIER GW. (1979) Sex differences in pituitary-gonadal regulation of hepatic 5a-reductase and 16a-hydroxylase. J. Steroid Biochem. 11:1519-1525

DIERINGER CS, LAMARTINIERE CA, SCHILLER CM, LUDIER GW. (1979) Altered ontogeny of hepatic steroid metabolizing enzymes by pure PCB congeners. Biochem. Pharmacol. 28:2511-2514

DOHLER KD, HINES M, COQUELIN A, DAVIS F, SHRYNE JE. (1982) Pre- and postnatal influence of diethylstilboestrol on differentiation of the sexually dimorphic nucleus of the preoptic area of the female rat brain. Neuroendocinrol. Lett. 4:361

DOHLER KD, SRIVASTAVA SS, SHRYNE JE, JARZAB B, SIPOS A, GORSKI. (1984) Differentiation of the sexually dimorphic nucleus in the preoptic area of the rat brain is inhibited by postnatal treatment with an estrogen antagonist. Neuroendocrinol. 38:297-301

DOMINO LE, DOMINO SE, DOMINO EF. (1982) Toxicokinetics of 2,2',4,4',5,5'-hexabromobiphenyl in the rat. J. Tox. Envir. Hlth. 9:815-833

DORNER G, GOTZ F, ROHDE W. (1975) On the evocability of a positive oestrogen feedback action on LH secretion in female and male rats. Endokrinologie 66:369-372

DUNCKEL AE. (1975) An updating on the polybrominated biphenyl disaster in Michigan. JAVMA 167:838-841

DURST HI, WILLETT LB, BRUMM CJ, MERCER HD. (1977) Effects of polybrominated biphenyls on health and performance of pregnant holstein heifers. J. Dairy Sci. 60:1294-1300

EINARSSON K, GUSTAFSSON JA, STENBERG A. (1973) Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. J. Biol. Chem. 248:4987-4997

EL-DEFRAWY, EL-MASRY S, COHEN GM, MANNERING GJ. (1974) Sex-dependant differences in drug metabolism in the rat i. temporal changes in the microsomal drug-metabolizing system of the liver during sexual maturation. Drug Metab. Dispos. 2:267-278

ELLINWOOD WE, HESS DL, ROSELLI CE, SPIES HG, RESKO JA. (1984) Inhibition of aromatization stimulates luteinizing hormone and testosterone secretion in adult male rhesus monkeys. J. Clin. Endorinol. 59:1088-1096

ERSKINE MS. (1983) Effects of an anti-androgen and 5-reductase inhibitors on estrus duration in the cycling female rat. Physiol. Behav. 30:519-524

EYSTER JT, HUMPHREY EB, KIMBROUGH RD. (1983) Partitioning of polybrominated biphenyls (PBBs) in serum, adipose tissue, breast milk, placenta, cord blood, biliary fluid, and feces. Arch. Envir. Hlth. 38:47-53

FARABOLLINI F, LUPO DI PRISCO C, DESSI-FULGHERI F. (1981) Modifications of brain steroidogenesis and plasma steroids after p-chlorophenylalanine-methyl-ester treatment in male rats and rabbits. Pharm. Biochem. Behav. 14:911-914

FARBER T, KASZA L, GIOVETTI A, CARTER C, EARL F, BALAZA T. (1978) Effect of polybrominated biphenyls (firemaster BP-6) on the immunologic system of the beagle dog. Tox. Appl. Pharm. 38:abst.

FEDER H. (1982) Hormonal actions on the sexual differentiation of the genitalia and the gonadotropin regulating systems. in. Neuroendocrinology of Reproduction ed. by N.T. Adler, Plenum, New York

FLORES F, NAFTOLIN F, RYAN KJ, WHITE RJ. (1973) Estrogen formation by the isolated perfused rhesus monkey brain. Science 180:1074-1075

FRAKER PJ. (1980) The antibody-mediated and delayed type hypersensitivity response of mice exposed to polybrominated biphenyls. Tox. Appl. Pharm. 53:1-7

GAY VL, HAUGER RL. (1977) A sex-related pattern of gonadotropin secretion in the castrated rat: Effects of changing the inhibitory steroid or pituitary LH content. Biol. Reprod. 16:527-35

GEORGE FW, OJEDA SR. (1982) Changes in aromatase activity in the rat brain during embryonic, neonatal and infantile development. Endocrinology 111:522-529

GLADUE BA, CLEMENS LG. (1980) Flutamide inhibits testosterone- induced masculine sexual behavior in male and female rats. Endocrinology 106:1917

GLADUE BA, CLEMENS LG. (1980) Masculinization diminished by disruption of prenatal estrogen biosynthesis in male rats. Physiol. Behav. 25:589-593

GOGAN F, BEATTIE IA, HENRY M. (1980) Effect of neonatal administration of steroids or gonadectomy upon estradiol induced luteinizing hormone release in rats of both sexes J. Endocrinol. 85:69-74

GOGAN F, SLAMA A, BIZZINI-KOUTZNETZOVZ B, DRAY F, KORDON C. (1981) Importance of perinatal testosterone in sexual differentiation in the male rat. J. Endocrinol. 91:75-79

GOLDSTEIN JA, LINKO PC, LEVY LA, MCKINNEY JD, GUPTA BN, MOORE JA. (1979) A comparison of a commercial polybrominated biphenyl mixture and 2,3,6,7-tetrabromonaphthalene as inducers of liver microsomal drug-metabolizing enzymes. Biochem. Pharm. 28:2947-2956

GORSKI RA. (1978) Sexual differentiation of the brain. Hospital Practice OCT:55-62

GORSKI RA, GORDON JH, SHRYNE JE, SOUTHAM AM. (1978) Evidence for a morphological sex difference within the medial preoptic area of the rat brain. Brain Res. 148:333-346

GORSKI RA, HARLAN RE, CHRISTENSEN LW. (1977) Perinatal hormonal exposure and tthe development of neuroendocrine regulatory processes. J. Tox. Envir. Hlth. 3:97-121

GORSKI RA, HARLAN RE, JACOBSON CD, SHRYNE JE, SOUTHAM AM. (1980) Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. J. Comp. Neurol. 193:529-539

GORSKI RA, JACOBSON CD. (1981) Sexual differentiation of the brain. Pediat. Androl. ?:109-134

GOTO J, FISHMAN J. (1977) Participation of a nonenzymatic transformation in the biosynthesis of estrogens from androgens. Science 195:80-81

GRAY LE. (1982) Neonatal chlordecone exposure alters behavioral sex differentiation in female hamsters. Neurotoxicol. 3:67-80

GUPTA BN, MCCONNELL EE, GOLDSTEIN JA, HARRIS MW, MOORE JA. (1983) Effects of polybrominated biphenyl mixture in the rat and mouse. Tox. Appl. Pharm. 68:1-18

GUPTA BN, MCCONNELL EE, HARRIS MW, MOORE JA. (1981) Polybrominated biphenyls toxicosis in the rat and mouse. Tox. Appl. Pharm. 57:99-118

GUPTA BN, MCCONNELL EE, MOORE JA, HASEMAN JK. (1983b) Effects of a polybrominated biphenyl mixture in the rat and mouse (ii. lifetime study). Tox. Appl. Pharm.

68:19-35

GUPTA BN, MOORE JA. (1979) Toxicologic assessments of a commercial polybrominated biphenyl mixture in the rat. Amer. J. Vet. Res. 40:1458-1468

GUPTA C, SHAPIRO BH, YAFFE SJ. (1980) Reproductive dysfunction in male rats following prenatal exposure to phenobarbital. Pediat. Pharm. 1:55-62

GUPTA C, YAFFE SJ, SHAPIRO BH. (1982) Prenatal exposure to phenobarbital permanently decreases testosterone and causes reproductive dysfunction. Science 216:640-642

GUPTA D. (1978) Effects of sex hormone antisera on sexual differentiation of the brain. Horm. Brain. Devel. 1:87-97

GUSTAFSSON JA, EDEN S, ENEROTH P,HOKFELT T, ISAKSSON O, JANSSON JO, MODE A, NORSTEDT G. (1983) Regulation of sexually dimorphic hepatic steroid metabolism by the somatostatin- growth hormone axis. J. Steroid Biochem. 19:691-698

GUSTAFSSON JA, ENEROTH P, HOKFELT T, MODE A, NORSTEDT G, SKETT P. (1981) Role of the hypothalamic-pituitary-liver axis in sex differences in susceptibility of the liver to toxic agents. Envir. Hlth. Perspec. 38:129-141

GUSTAFSSON JA, INGELMAN-SUNDBERG M. (1979) Changes in steroid hormonal metabolism in rat liver microsomes following administration of 2,3,7,8 TCDD. Biochem. Pharmacol. 28:497-499

GUSTAFSSON JA, MODE A, NORSTEDT G, ENEROTH P, HOKFELT T. (1983) Central control of prolactin and estrogen receptors in rat liver-expression of a novel endocrine system, the hypothalmo-pituitary-liver axis. Ann. Res. Pharmacol. Toxicol. 23:259-278

GUSTAFSSON JA, SKETT P. (1979) Pituitary control of hepatic steroid metabolism Acta. Biol. Med. Germ. 38:307-20

HAAPARANTA T, GLAUMANN H, GUSTAFSSON JA (1983) Induction of cytochrome P-450 dependant reactions in the rat ventral prostate by ß-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin. Toxicology 29:61-75

HAAPARANTA T, GLAUMANN H, GUSTAFSSON JA (1984) Characterization and endocrine regulation of the cytochrome P-450 dependant microsomal hydroxylation of

5a-androstane-3ß,17ß-diol in the rat ventral prostate. Endocrinology 114:2293

HAHN EF, FISHMAN J. (1984) Immunological probe of estrogen biosynthesis: evidence for the 2b-hydroxylative pathway in aromatizaton of androgens. J. Biol. Chem. 259:1689-1694

HARRIS SJ, CECIL HC, BITMAN J. (1978) Effects of feeding of polybrominated biphenyl flame retardant (firemaster BP-6) to male rats. Bull. Envir. Contam. Toxicol. 19:692-693

HAUSER H, GANDELMAN R. (1983) Contiguity to males in utero affects avoidance responding in adult female mice. Science 220:437-438

HAYASHI S. (1979) Influence of intrahypothalamic implants of antioestrogen or aromatase inhibitor on development of sterility following neonatal androgenation in female rats. J. Ster. Biochem. 11:537-541

HESS JL, POWERS RA. (1978) Polybrominated biphenyl (PBB) contamination of the Pine River, Gratiot and Midland Counties, Michigan. Envir. Hlth. Perspec. 23:19-26

HERSEY RM, GUNSALUS P, LLOYD T, WEISZ J. (1981) Catechol estrogen formation by brain tissue: A comparison of the release of tritium from [2-3H]-estradiol with [6,7-3H]estradiol by rabbit hypothalami in vitro. Endocrinology 109:1902-1911

HILL RH, PATTERSON DG, ORTI DL, HOLLER JS, NEEDHAM LL, SIRMANS SL, LIDDLE JA. (1982) Evidence of degredation of polybrominated biphenyls in soil samples from Michigan. J. Envir. Hlth. Sci. B17(1):19-33

HUDSON RW, MOFFITT PM, OWENS WA. (1983) Studies of the nuclear 5a-reductase of human prostatic tissue: comparison of enzyme activities in hyperplastic, malignant and normal tissues. Can. J. Biochem. Cell Biol. 61:750-755

HUSS JR, MCCONNELL EE, HARVAN DJ. (1976) Chemical and toxicologic evaluation of firemaster BP-6. J. Agric. Food Chem. 26:94-99

IMPERATO-MCGINLEY J, BINIENDA Z, ARTHUR A, MININBERG DT, VAUGHAN ED, QUIMBY FW. (1985) The development of a male pseudohermaphroditic rat using an inhibitor of the enzyme 5-reductase. Endocrinology 116:807

JACKSON TF, HALBERT FL. (1974) A toxic syndrome associated with the feeding of polybrominated biphenyl-contaminated protein concentrate to dairy cattle. JAVMA 165:437-439

JACOBSON CD, CSERNUS VJ, SHRYNE JE, GORSKI RA. (1981) The influence of gonadectomy, androgen exposure, or a gonadal graft in the neonatal rat on the volume of the sexually dimorphic nucleus of the preoptic area. J. Neurosci. 1:1142-1147

JACOBSON CD, SHRYNE JE, SHAPIRO F, GORSKI RA. (1980) Ontogeny of the sexually dimorphic nucleus of the preoptic area. J. Comp. Neurol. 193:541-548

JACOBSON JL, FEIN GG, JACOBSON SW, SCHWARTZ PM, DOWLER JK. (1984) The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. AJPH 74:378-379

JENSEN RK, SLEIGHT SD, AUST SD, GOODMAN JI, TROSKO JE. (1983) Hepatic tumor-promoting ability of 3,3'4,4'5,5'-hexabromobiphenyl: the interelationship between toxicity, induction of hepatic microsomal drug metabolizing enzymes, and tumor-promoting ability. Tox. Appl. Pharm. 71:163-176

JOHNSON EF, SCHWAB GE, MULLER-EBERHARD V. (1979) Multiple forms of cytochrome P-450: Catalytic differences exhibited by two homogeneous forms of rabbit cytochrome P-450. Mol. Pharmacol. 15:708-718

JOHNSON LM, GAY VL. (1983) Influence of perinatal androgenization on the castration response of adult rats. Biol. Reprod. 29:819-823

JOHNSTON CA, DEMAREST KT, MCCORMACK KM, HOOK JB, MOORE KE. (1980) Endocrinological, neurochemical and anabolic effects of polybrominated biphenyls in male and female rats. Tox. Appl. Pharm. 56:240-247

JONDORF WR, WYSS PA, MUHLEBACH S, BICKEL MH. (1983) Disposition of 2,2',4,4',5,5'-hexachlorobiphenyl (6-cb) in rats with decreasing adipose tissue mass. Drug. Metab. Dispos. 11:597-601

JUSTO SN, NEGRO-VILAR A. (1979) A female-like rise in luteinizing hormone and follicle-stimulating hormone after gonadectomy in male rats induced by oestradiol pretreatment. J. Endocrinol. 80:111-116

KAHL R, BUECKER M, NETTER KJ. (1976) Sex difference in the pattern of cytochromes P-450 in rat liver microsomes. in Microsomes and Drug Oxidations. ed. by Ullrich Pergamon Press, New York

KALRA SP, KALRA PS. (1983) Neural regulation of luteinizing hormone secretion in the rat. Endocrine Rev. 4:311-351

KAPPAS A, ANDERSON KE, CONNEY AH. (1983) Nutrition-endocrine interactions: Induction of reciprocal changes in the 5-reduction of testosterone and the cytochrome P-450 dependant oxidation of estradiol. Proc. Nat. Acad. Sci. 80:7646-49

KARSCH FJ, KNOBIL E, DIERSCHKE DJ. (1973) Sexual differentiation of pituitary function: apparent difference between primates and rodents. Science 179:484-486

KASZA L, COLLINS WT, DAPEN CC, GARTHOFF LH, FRIEDMAN L. (1978) Comparative toxicity of polychlorinated biphenyl and polybrominated biphenyl in the rat thyroid gland: light and electron microscopic alterations after subacute dietary exposure. J. Envir. Path. Toxicol. 1:587-599

KATO R. (1974) Sex-related differences in drug metabolism. Drug Metab. Rev. 3:1-32

KAY K. (1977) Polybrominated biphenyls (PBB) environmental contamination in Michigan 1973-1976. Envir. Res. 13:74-93

KIMBROUGH R, BUCKLEY J, FISHBEIN L, FLAMM G, KASZA L, MARCUS W, SHIBKO S, TESKE R. (1978) Animal Toxicology. Envir. Hlth. Perspec. 23:173-184

KIMBROUGH RD, KORVER MP, BURSE VW, GROCE DF. (1980) The effect of different diets or mineral oil on liver pathology and polybrominated biphenyl concentration in tissues. Tox. Appl. Pharm. 52:442-453

KITADA M, OMORI S, IGARASHI T, KANAKUBO Y, KITAGAWA H. (1980) Sex difference in n-demethylation activity of ethlymorphine in rat liver microsomes. Biochem. Biophys. Res. Comm. 97:1527-1534

KLUWE WM, HERRMAN, HOOK JB. (1979) Effects of dietary polychlorinated biphenyls and polybrominated biphenyls on the renal and hepatic toxicities of several chlorinated hydrocarbon solvents in mice. J. Tox.

Envir. Hlth. 5:605-615

KLUWE WM, HOOK JB. (1981) Comparitive induction of xenobiotic metabolism in rodent kidney, testis and liver by commercial mixtures of polybrominated biphenyls and polychlorinated biphenyls, phenobarbital and 3-methylcholanthrene. Toxicology 20:259-273

KLUWE WM, HOOK JB. (1978) Polybrominated biphenyl-induced potentiation of chloroform toxicity. Tox. Appl. Pharm. 45:861-869

KLUWE WM, MCCORMACK KM, BERNSTEIN J, HOOK JB. (1979) Toxicological evaluation of polybrominated biphenyls in developing male rats. Tox. Appl. Pharm. 48:A314

KLUWE WM, MCCORMACK KM, HOOK JB. (1978) Potentiation of hepatic and renal toxicity of various compounds by prior exposure to polybrominated biphenyls. Envir. Hlth. Perspec. 23:241-246

KOBAYASHI RM, REED KC. (1977) Conversion of androgens to estrogens (aromatization) in discrete regions of the rat brain: sexual differences and effects of castration. Soc. Neurosci. Abstracts 3:348

KOSHIKA S, NAKAYAMA Y, TARUI M, TAKEMURA S, HOSAKA M (1984) Properties of 5a-reductase for testosterone, the ability of androgen receptor and testosterone and 5a-dihydrotestosterone concentration in rat submandibular gland 7th International Congress of Endocrinology, Quebec

KREISS K, ROBERTS C. (1982) Serial PBB levels, PCB levels, and clinical chemistries in Michigan's PBB cohort. Arch. Envir. Hlth. 37:141-147

KUBLI-GORFIAS . (1984) Physiological role of 5a- and 5ß-progesterone metabolites on the CNS. TIPS 5:439-42

LANDRIGAN PJ, WILCOX KR, SILVA J. (1979) Cohort study of Michigan residents exposed to polybrominated biphenyls: epidemiologic and immunologic findings. Ann. NY Acad. Sci. 320:284-294

LARSSON K, DEWSSI-FULGHERI F, LUPO C. (1982) Social stimuli modify the neuroendocrine activity through olfactory cues in the male rat. Olfaction and Endocrine Regulation pp:51-62

LEE IP, SUZUKI K, NAGAYAMA J. (1981) Metabolism of benzo[a]pyrene in rat prostate glands following

2,3,7,8-tetrachlorodibenzo-p-dioxin exposure. Carcinogensis 2:823-831

LEVIN W, RYAN D. (1975) Age and sex differences in the turnover of rat liver cytochrome P-450. in Basic and Therapeutic Aspects of Perinatal Pharmacology, ed. by PL Morselli, Raven Press, New York

LIANG T, BRADY EF, CHEUNG A, SAPERSTEIN. (1984) Inhibition of luteinizing hormone-releasing hormone induced secretion of LH in rat anterior pituitary cell culture by testosterone without conversion of 5a-dihydro-testosterone. Endocrinology 115:2311-2317

LOOSE LD, MUDZINSKI SP, SILKWORTH JB. (1981) Influence of dietary polybrominated biphenyl on antibody and host defence responses in mice. Tox. Appl. Pharm. 59:25-39

LUCIER GW, LUI EMK, LAMARTINIERE CA. (1979) Metabolic activation/deactivation reactions during perinatal development. Envir. Hlth. Perspec. 29:7-16

MACLUSKY NJ, NAFTOLIN F. (1981) Sexual differentiation of the central nervous system. Science 211:1294-1302

MACLUSKY NJ, RISKALLA M, KREY L, PARVIZI N, NAFTOLIN F. (1983) Anovulation in female rats induced by neonatal administration of the catechol estrogens, 2-hydroxy-estradiol and 4-hydroxy-estradiol. Neuroendocrin. 37:321-327

MARTINI L. (1982) The 5a-reduction of testosterone in the neuroendocrine structures. Biochemical and physiological implications. Endocrin. Rev. 3:1-25

MARTINI L. (1978) Role of the metabolism of steroid hormones in the brain in sex differentiation and sexual maturation in "Hormones and Brain Development", ed. G. Dorner and M Kawakami, Elsevier North Holland, New York

MATSUMOTO A, ARAI Y. (1983) Sex difference in volume of the ventromedial nucleus of the hypothalamus in the rat. Endocrin. Jpn. 30:277-280

MATTHEWS HB, KATO S, MORALES NM, TUEY DB. (1977) Distribution and excretion of 2,4,5,2',4',5'-hexabromobiphenyl, the major component of firemaster BP-6. J. Tox. Envir. Hlth. 3:599-605

MCCORMACK KM, ARNERIC SP, HOOK JB. (1979) Action of exogenously administered steroid hormones following perinatal exposure to polybrominated biphenyls. J. Tox. Envir. Hlth. 5:1085-1094

MCCORMACK KM, BRASELTON WE, SANGER VL, HOOK JB. (1980) Residual effects of polybrominated biphenyls following perinatal exposure in rats. Tox. Appl. Pharm. 53:108-115

MCCORMACK KM, CAGEN SZ, RICKERT DE, GIBSON JE, DENT JG. (1979) Stimulation of hepatic and renal mixed-function oxidase in developing rats by polybrominated biphenyls. Drug Metab. Dispos. 7:252-259

MCCORMACK KM, HOOK JB. (1982) Effects of lactation and nursing on tissue concentrations of polybrominated biphenyls and on microsomal enzyme activity in mammary gland and liver in maternal rats. J. Envir. Res. 27:110-117

MCCORMACK KM, HOOK JB. (1979) Effects of polybrominated biphenyls on microsomal enzyme activity and action of exogenous steroid hormones during development. Tox. Appl. Pharm. 48:A23

MCCORMACK KM, KLUWE WM, RICKERT DE, SANGER VL, HOOK JB. (1978) Renal and hepatic microsomal enzyme stimulation and renal function following three months of dietary expopsure to polybrominated biphenyls. Tox. Appl. Pharm. 44:539-543

MCCORMACK KM, KLUWE WM, SANGER VL, HOOK JB. (1978) Effects of polybrominated biphenyls on kidney function and activity of renal microsomal enzymes. Envir. Hlth. Perspec. 23:153-157

MCCORMACK KM, LEPPER LF, WILSON DM, HOOK JB. (1981) Biochemical and physiological sequelae to perinatal exposure to polybrominated biphenyls: a multigeneration study in rats. Tox. Appl. Pharm. 59:300-313

MCCORMACK KM, ROTH RA, WALACE KB, ROSS LM, HOOK JB. (1982) Nonrespiratory metabolic function and morphology of lung following exposure to polybrominated biphenyls in rats. J. Tox. Envir. Hlth. 9:27-39

MCCORMACK KM, STICKNEY JL, BONHAUS DW, HOOK JB. (1982) Cardiac and hepatic effects of pre-and postnatal exposure to polybrominated biphenyls in rats. J. Tox. Envir. Hlth. 9:13-26

MCEWEN BS. (1980) Gonadal steroids and brain development. Biol. Reprod. 22:43-48

MCEWEN BS. (1981) Neural gonadal steroid actions. Science 211:1303-1311

MCEWEN BS, LIEVERBURG I, CHAPITAL C, DAVIS PG, KREY LC, MACLUSKY NJ, ROY EJ. (1979) Attenuating the defeminization of the neonatal rat brain: mechanisms of action of cyproterone acetate, 1,4,6-androstatriene- 3,17- dione and a synthetic progestin, R5020. Horm. Behav. 13:269-281

MERCER HD, WILLETT LB, SCHANBACHER FL MOORHEAD PD, POWERS TE. (1978) Use of double-isotope, single-injection method for estimating renal function in normal and polybrominated biphenyl-exposed dairy cows. Amer. J. Vet. Res. 39:1262-1268

MEYER-BAHLBURG FL, EHRHARDT AA. (1980) Neurobehavioral effects of prenatal origin: sex hormones. Rec. Prog. Clin. Biol. Res. 237:93-107

MEYER-BAHLBURG HFL. (1982) Hormones and psychosexual differentiation: implications for the management of intersexuality, homosexuality, and transsexuality. Clin. Endocrinol. Metab. 11:681-701

MILLER AE, RIEGLE GD. (1978) Serum testosterone and testicular response to hCG in young and aged male rats. J. Gerontol. 33:197-203

MIYAIRI S, FISHMAN J. (1983) Novel method of evaluating biological 19-hydroxylation and aromatization of androgens. Biochem. Biophys. Res. Comm. 117:392-8

MIYAIRI S, FISHMAN J. (1985) Radiometric analysis of oxidative reactions in aromatization by placental microsomes. J. Biol. Chem. 260:320-325

MODE A, NORSTEDT G, ENEROTH P, GUSTAFSSON JA. (1983) Purification of liver feminizing factor from rat pituitaries and demonstration of its identity with growth hormone. Endocrinology 113:1250

MOORE CL (1984) Maternal contributions to the development of masculine sexual behavior in laboratory rats. Devel. Psychobiol. 17:347-356

MOORE RW, AUST SD. (1978) Purification and structural characterization of polybrominated biphenyl congeners. Biochem. Biophys. Res. Comm. 84:939-942

MOORE RW, PETERSON RE. (1983) Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on the male reproductive system in rats. FASEB [abst]. Chicago, Ill

MOORE RW, SLEIGHT SD, AUST SD. (1978) Induction of liver microsomal drug-metabolizing enzymes by 2,2',4,4',5,5'-hexabromobiphenyl. Tox. Appl. Pharm. 44:309-321

MOORE RW, SLEIGHT SD, AUST SD. (1979) Effects of 2,2'-dibromobiphenyl and 2,2',3,4,4',5,5'-heptabromobiphenyl on liver microsomal drug metabolizing enzymes. Tox. Appl. Pharm. 48:73-86

NAFTOLIN F, RYAN KJ, DAVIES IJ. (1975) The formation of estrogens by central neuroendocrine tissues. Rec. Prog. Horm. Res. 31:295-319

NAFTOLIN F, RYAN KJ, PETRO Z. (1971) Aromatization of androstenedione by limbic system tissue from human fetuses. J. Endocrinol. 51:795-796

NAFTOLIN F, RYAN KJ, PETRO Z. (1972) Aromatization of androstenedione by the anterior hypothalamus of adult male and female rats. Endocrinology 90:295-298

NASH T. (1953) The colorimetric estimation of formaldehyde by means of the Hantzsch reaction. Biochem. J. 55:416

NASS TE, MATT DW, JUDD HL, LU JKH. (1984) Prepubertal treatment with estrogen or testosterone precipitates the loss of regular estrous cyclicity and normal gonadotropin secretion in adult female rats. Biol. Reprod. 31:723-731

NEBERT DW, ELASHOFF JD, WILCOX KR. (1983) Possible effects of neonatal polybrominated biphenyl exposure on the developmental abilities of children. AJPH 73:286-288

NEWTON JF, BRASELTON WE, LEPPER LF, MCCORMACK KM, HOOK JB. (1982) Effects of polybrominated biphenyls on metabolism of testosterone by rat hepatic microsomes. Tox. Appl. Pharm. 63:142-149

NORDEEN EJ, YAHR P. (1982) Hemispheric asymmetries in the behavioral and hormonal effects of sexually differentiating mammalian brain. Science 218:391-394

NOWICKI HG, NORMAN AW. (1972) Enhanced hepatic metabolism of testosterone, 4-androstene-3,17-dione, and estradiol in chickens pretreated with DDT or PCB.

Steroids 19:85-99

OFNER P, VENA R, LEAV I, HAMILTON DW. (1979) Metabolism of C-19 radiosteroids by explants of canine prostate and epididymus with disposition as hydroxylated products: A possible mechanism for androgen inactivation. J. Steroid Biochem. 11:1367-1379

OFNER P, VENA R, TERRACIO L, DOUGLAS WHJ. (1982) Comparative C19-radiosteroid metabolism in primary monolayer cultures of epithelial cells and fibroblasts from rat ventral prostate, canine prostate, and rat lung. J. Androl. 3:269

O'KEEFE PW. (1978) Formation of brominated dibenzofurans from the pyrolysis of the polybrominated biphenyl fire retardant fireMaster FF-1. Envir. Hlth. Perspec. 23:347-350

O'KEEFE PW (1979) Trace contaminants in a polybrominated biphenyl fire retardant and a search for these compounds in environmental samples. Bull. Envir. Contam. Toxicol. 22:420-425

OLSEN KL. (1983) Effects of 17b-hydroxy-17-methyl-estra-4,9,11-triene-3-one (R-1881): evidence for direct involvement of androgens in the defeminization of behaviour in rats. J. Endocrinol. 98:431-438

ORBERG J, KIHLSTROM JE. (1973) Effects of long-term feeding on polychlorinated biphenyls (PCB, Clophen A60) on the length of the oestrus cycle and on the frequency of implanted ova in the mouse. Envir. Res. 6:176-179

ORBERG J, LUNDBERG C. (1974) Some effects of DDT and PCB on the hormonal system in the male mouse. Envir. Physiol. Biochem. 4:116-120

ORTH J, BADWAY D, WEISZ J. (1981) Testicular function in rat fetuses of normal and stressed mothers: implications for sexual differentiation of the nervous system. Excerpta Med. 559:208-215

ORTI DL, HILL RH, PATTERSON DG, NEEDHAM LL, KIMBROUGH RD, ALLEY CC, LEE HCJ. (1983) Structure elucidation of some minor components of the polybromobiphenyl mixture, firemaster. Arch. Envir. Contam. Toxicol. 12:603-614

PARKINSON A, SAFE SH, ROBERTSON LW, THOMAS PE, REIK LM, LEVIN W. (1983) Immunochemical quantitation of

cytochrome p-450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. J. Biol. Chem. 258:5967-5976

PARROTT RF. (1974) Effects of 17b-hydroxy-4-androsten-19-ol-3-one (19-hydroxytestosterone) and 5-androstan-17b-ol-3-one (dihydrotestosterone) on aspects of sexual behavior in castrated male rats. J. Endocrinol. 61:105-115

PARSONS B, RAINBOW TC, MCEWEN BS. (1984) Organizational effects of testosterone via aromatization on feminine reproductive behavior and neural progestin receptors in rat brain. Endocrinology 115:1412-1417

PASLEAU F, KOLODZICI C, KREMES P, GIELEN. (1981) Ontogenic development of steroid 16-hydroxylase as a tool for the study of the multiplicity of cytochrome P-450. Eur. J. Biochem. 120:213-220

POLAND RL, COHEN SN. (1980) The contamination of the food chain in Michigan with PBB: the breast-feeding question. Rec. Prog. Clin. Biol. Res. 237:129-137

POLIN D, LEAVITT RA. (1984) Colestipol and energy restriction as an approach to hasten removal of PBBs from chickens. J. Tox. Envir. Hlth. 13:659-671

POPOLOW HB, WARD IL. (1978) Effects of perinatal androstenedione on sexual differentiation in female rats. J. Comp. Physiol. Psychol. 92:13-21

PREACHE MM, CAGEN SZ, GIBSON JE. (1976) Perinatal toxicity in mice following maternal dietary exposure to polybrominated biphenyls. Tox. Appl. Pharm. 37:171

PROUGHE RA, ZIEGLER DM. (1977) The relative participation of liver microsomal amine oxidase and cytochrome P-450 in N-demethylation reactions. Arch. Biochem. Biophys. 180:363-373

RAJFER J, COFFEY DS. (1979) Effects of neonatal steroids on male sex tissues. Invest. Urol. 17:3-8

RAUM WJ, MARCANO M, SWERDLOFF RS. (1984) Nuclear accumulation of estradiol derived from the aromatization of testosterone is inhibited by hypothalmic beta-recptor stimulation in the neonatal female rat. Biol. Reprod. 30:388-396

REDDY VV, NAFTOLIN F, RYAN KJ. (1973) Aromatization in the central nervous system of rabbits: effects of castration and hormone treatment. Endocrinology 92:589-594

RENDER JA, AUST SD, SLEIGHT SD. (1982) Acute pathologic effects of 3,3',4,4',5,5'-hexabromobiphenyl in rats: comparison of its effects with firemaster BP-6 and 2,2',4,4',5,5'-hexabromobiphenyl. Tox. Appl. Pharm. 62:428-444

RHEES RW, FLEMING DE. (1981) Effects of malnutrition, maternal stress, or ATCH injections during pregnancy on sexual behavior of male offspring. Physiol. Behav. 27:879-882

RHODA J, CORBIER P, ROFFI J. (1984) Gonadal steroid concentrations in serum and hypothalamus of the rat at birth: aromatization of testosterone to 17ß-estradiol. Endocrinology 14:1754

RICKERT DE, DENT JG, CAGEN SZ, MCCORMACK KM, MELROSE P, GIBSON JE. (1978) Distribution of polybrominated biphenyls after dietary exposure in pregnant and lactating rats and their offspring. Envir. Hlth. Perspec. 23:63-66

RIFKIND AB, TROEGER M, PETSHKE T. (1979) Equality of the rates of mixed function oxidation in livers of male and female chick embryos. Biochem. Pharmacol. 28:1681-1683

ROBERTSON LW, ANDRES JL, SAFE SH. (1983) Toxicity of 3,3',4,4'- and 2,2',5,5'-tetrabromobiphenyl: correlation of activity with aryl hydrocarbon hydoxylase induction and lack of protection by antioxidants. J. Tox. Envir. Hlth. 11:81-91

ROBERTSON LW, PARKINSON A, BANDIERA S, SAFE S. (1981) Potent induction of rat liver microsomal drug metabolizing enzymes by 2,3,3',4,4',5-hexabromobiphenyl a component of firemaster. Chem. Biol. Interact. 35:13-24

ROBERTSON LW, PARKINSON A, CAMPBELL MA, SAFE S. (1982) Polybrominated biphenyls as aryl hydrocarbon hydroxylase inducers: structure-activity correlations. Chem. Biol. Interact. 42:53-66

ROBERTSON LW, PARKINSON A, SAFE S. (1980) Induction of both cytochromes p-450 and p-448 by 2,3',4,4',5-pentabromobiphenyl, a component of

firemaster. Biochem. Biophy. Res. Comm. 92:175-182

ROBERTSON LW, THOMPSON K, PARKINSON A. (1984) Synthesis, characterization and biologic effects of polybrominated naphthalenes. Arch. Toxicol. 55:127-131

ROBERTSON L, PARKINSON A, SAFE S. (1981) Induction of drug-metabolizing enzymes by fractionated commercial polybrominated biphenyls (PBBs). Tox. Appl. Pharm. 57:254-262

ROES U, DENT JG, NETTER KJ, GIBSON JE. (1977) Effect of polybrominated biphenyls on bromobenzene lethality in mice. J. Tox. Envir. Hlth. 3:663-671

ROSELLI CE, ELLINWOOD WE, RESKO JA. (1984) Regulation of brain aromatase activity in rats. Endocrinology 114:192-200

ROSELLI CE, RESKO JA. (1984) Androgens regulate brain aromatase activity in adult male rats through a receptor mechanism. Endocrinology 114:2183-2189

ROSENFELD JM, DALEY JD, OHNO S, YOUNG EV. (1977) Central aromatization of testosterone in testicular feminized mice. Experientia 33:1392-1393

ROSENMAN KD, ANDERSON HA, SELIKOFF IJ, WOLFF MS, HOLSTEIN E. (1979) Spermatogenesis in men exposed to polybrominated biphenyl (PBB). Fertil. Steril. 32:209-213

ROZMAN KK, ROZMAN TA, WILLIAMS J, GREIM HJ. (1982) Effect of mineral oil and/or cholestyramine in the diet on biliary and intestinal elimination of 2,4,5,2',4',5'-hexabromobiphenyl in the rhesus monkey. J. Tox. Envir. Hlth. 9:611-618

RUBIN RT, REINISCH JM, HASKETT RF. (1981) Postnatal gonadal steroid effects on human behavior. Science 211:1318-1324

RUMBAUGH RC, COLBY HD. (1980) Is growth hormone the pituitary feminizing factor mediating the actions of estradiol on hepatic drug and steroid metabolism. Endocrinology 107:719-724

RUPPERT P, CLEMENS LG. (1981) The role of aromatization in the development of sexual behavior of the female hamster (Mesocricetus auratus). Horm. Behav. 15:68-76

RUSH ME, BLAKE CA. (1982) Serum testosterone concentrations during the 4-day estrous cycle in normal and adrenalectomized rats. Proc. Soc. Exp. Biol. Med. 169:216

RYAN KJ. (1959) Biological aromatization of steroids. J. Biol. Chem. 234:268-272

SAMPEREZ S, JOUAN P. (1979) Androgen metabolism, androgen and estrogen receptors in the male rat anterior pituitary. J. Steroid Biochem. 11:819

SANDERS OT, ZEPP RL, KIRKPATRICK RL. (1974) Effect of PCB ingestion on sleeping times, organ weights, food consumption, serum corticosterone and survival of albino mice. Bull. Envir. Contam. Toxicol. 12:394-9

SCHRIEFERS H, GHRAF R, LAX ER. (1972) Sex-specific aglucone patterns of testosterone metabolism in rat liver and their alteration following interference with the sexual differentiation. Hoppe-Seylers Z. Phsyiol. Chem. 353:371-377

SCHWARTZ EL, KLUWE WM, SLEIGHT SD, HOOK JB, GOODMAN JI. (1980) Inhibition of n-2-fluorenylacetamide-induced mammary tumorigenesis in rats by dietary polybrominated biphenyls. JNCI 64:63-67

SCHWARTZ EM, RAE WA. (1983) Effect of polybrominated biphenyl (PBB) on developmental abilities in young children. AJPH 73:277-281

SCHWARTZEL WC, KRUGGEL WG, BRODIE HJ. (1973) Studies on the mechanism of estrogen biosynthesis. (viii. the development of inhibitors of the enzyme system in human placenta. Endocrinology 92:866

SEAGULL EAW. (1983) Developmental abilities of children exposed to polybrominated biphenyls (PBB). AJPH 73:281-285

SELIKOFF IJ. (1979) Polybrominated biphenyls in Michigan [abstract only]. Proc. R. Soc. London Biochem. 205:153-156

SELMANOFF MK, BRODKIN LD, WEINER RI, SIITERI PK. (1977) Aromatization and 5a-reduction of androgens in discrete hypothalamic and limbic regions of the male and female rat. Endocrinology 101:841-848

SHIMAZAKI J, KATO N, NAGAI H, YAMANAAKA H, SHIDA K (1972) 3a-Reduction of 5a-DHT by rat ventral prostate.

Endocrinol. Jpn. 19:97-106

SHIVERICK KT. (1981) In vivo and in vitro effects of ß-naphthoflavone on cytochrome P-450 dependant testosterone hydroxylase activities in liver microsomes. Drug Metab. Dispos. 9:545

SILVA J, KAUFFMAN CA, SIMON DG, LANDRIGAN PJ. (1979) Lymphocyte function in humans exposed to polybrominated biphenyls. J. Reticuloendothelial Soc. 26:341-347

SIMMONS MS, KOTZ KT. (1982) Association studies of polybrominated biphenyls in aquatic systems. Bull. Envir. Contam. Toxicol. 29:58-63

SIMPKINS JW, KALRA PS, KALRA SP. (1981) Alterations in daily rhythmns of testosterone and progesterone in old male rats. Exp. Aging Res. 7:25-31

SLEIGHT SD, SANGER VL. (1976) Pathologic features of polybrominated biphenyl toxicosis in the rat and guinea pig. JAVMA 169:1231-1235

SODERKVIST P, TOFTGARD R, GUSTAFSSON JA. (1982) Induction of cytochrome P-450 related metabolic activities in the rat ventral prostate. Toxicol. Lett. 10:61-69

SODERSTEN P, HANSEN S. (1978) Effects of castration and testosterone, dihydrotestosterone or oestradiol replacement treatment in neonatal rats on mounting behavior in the adult. J. Endocrinol. 76:251-260

SODERSTEN P, HANSEN S, ENEROTH P, WILSON CA, GUSTAFSSON JA. (1980) Testosterone in the control of rat sexual behavior. J. Steroid Biochem. 12:337-346

STEIMER T, HUTCHISON JB. (1980) Aromatization of testosterone within a discrete hypothalamic area associated with the behavioral action of androgen in the dove. Brain Res. 192:586-591

STEWART J, VALLENTYNE S, MEANEY MJ. (1979) Differential effects of testosterone metabolites in the neonatal period on open-field behavior and lordosis in the rat. Horm. Behav. 13:282-292

STROSS JK, SMOKLER IA, ISBISTER J, WILCOX KR. (1981) The human health effects of exposure to polybrominated biphenyls. Tox. Appl. Pharm. 58:145-150

TENNISWOOD M, BIRD CE, CLARK AF. (1982) The role of androgen metabolism in the control of androgen action in the rat prostate. Mol. Cell. Endocrin. 27:89-96

THERON CN, NEETHLING AC, TALJAARD JJF. (1981) Sex-dependant differences in phenobarbitone-induced oestradiol-2-hydroxylase activity in rat liver. S. Afr. Med. J. 60:279-282

THOMPSON EA, SIITERI PK. (1974) The involvement of human placental microsomoal cytochrome P-450 in aromatization. J. BIOL. CHEM. 249:5373-5378

TSUSHIMOTO G, TROSKO JE, CHANG C, AUST SD. (1982) Inhibition of metabolic cooperation in chinese hamster v79 cells in culture by various polybrominated biphenyl (PBB) congeners. Carcinogenesis 3(2):181-185

TUEY DB, MATTHEWS HB. (1980) Distribution and excretion of 2,2',4,4',5,5'-hexabromobiphenyl in rats and man: pharmacokinetic model predictions. Tox. Appl. Pharm. 53:420-431

VALCIUKAS JA, LILIS R, ANDERSON HA, WOLFF MS, PETROCCI M. (1979) The neurotoxicity of polybrominated biphenyls: results of a medical field survey. Ann. NY. Acad. Sci. 320:337-367

VAN-KREY HP, SIEGEL PB, BALANDER RJ, BENOFF FH (1983) Testosterone aromatization in high and low mating lines of gallinaceous birds. Physiol. Behav. 31:153-157

VITO CC, FOX TO. (1982) Androgen and estrogen receptors in embryonic and neonatal rat brain. Devel. Brain Res. 2:97-110

VOM-SAAL FS, BRONSON FH. (1980) Sexual characteristics of adult female mice are correlated with their blood testosterone levels during prenatal development. Science 208:597-599

WALLACH DP. (1978) Pathology of experimentally induced polybrominated biphenyl toxicosis in pregnant heifers. JAVMA 173:150-151

WARD IL. (1972) Prenatal stress feminizes and demasculinizes the behavior of males. Science 175:82-84

WARD IL. (1972) Female sexual behavior in male rats treated prenatally with an anti-androgen. Physiol. Behav. 8:53-56

WARD IL, RENZ FJ. (1972) Consequences of perinatal hormone manipulationo on the adult sexual behavior of female rats. J. Comp. Physiol. Psychol. 78:349-355

WARD IL, WEISZ J. (1980) Maternal stress alters plasma testosterone in fetal males. Science 207:328-329

WEIDENFELD J, SIEGEL RA, YANAI J. (1983) Effect of phenobarbital on in vitro aromatization of testosterone to estradiol by adult male mice brain. J. Steroid Biochem. 18:201-203

WEIL WB, SPENCER M, BENJAMIN D, SEAGULL E. (1981) The effect of polybrominated biphenyl on infants and young children. J. Pediatrics 98:47-51

WEISZ J, BROWN BL, WARD IL. (1982) Maternal stress decreases steroid aromatase activity in brains of male and female rat fetuses. Neuroendocrinology 35:374-379

WEISZ J, GIBBS C. (1974) Conversion of testosterone and androstenedione to estrogens in vitro by the brain of female rats. Endocrinology 94:616-620

WEISZ J, WARD I. (1980) Plasma testoserone and progesterone titers of pregnant rats, their male and female fetuses and neonatal offspring. Endocrinology 106:306

WHALEN RE, DEBOLD JF. (1974) Comparative effectiveness of testosterone, androstenedione and dihydrotestosterone in maintaining mating behavior in the catrated male hamster. Endocrinology. 95:1674-1679

WHALEN RE, OLSEN KL. (1981) Role of aromatization in sexual differentiation: effects of prenatal ATD treatment and neonatal castration. Horm. Behav. 15:107-122

WHALEN RE, REZEK DL. (1974) Inhibition of lordosis in female rats by subcutaneous implants of testosterone, androstenedione or dihydrotestosterone in infancy. Horm. Behav. 5:125-128

WILKE DL, BRASELTON WE JR.. (1983) Rapid assay of hypothalamic aromatase using high performance liquid chromatography. J. Liquid Chromatog. 6(5):861-870

WILLET LB, LIU TTY, DURST HI, SCHANBACHER FL. (1983) Effects of polybrominated biphenyls on the concentration and clearance of steroids from blood. J. Anim. Sci. 56:1135-1144

WILLET LB, SCHANBACHER FL, MOOREHEAD PD. (1983) Effects of polybrominated biphenyls on the excretion of steroids. J. Anim. Sci. 56:1145-1152

WILLETT LB, SCHANBACHER FL, DURST HI, MOORHEAD PD. (1980) Long-term performance and health of cows experimentally exposed to polybrominated biphenyls. J. Dairy Sci. 63:2090-2102

WILSON JD, GRIFFIN JE, GEORGE FW, LESHIN M. (1981) The role of gonadal steroids in sexual differentiation. Rec. Prog. Horm. Res. 37:1-39

WOLFF MS, ANDERSON HA, CAMPER F, NIKAIDO MN, DAUM SM, HAYMES N, SELIKOFFIJ. (1979) Analysis of adipose tissue and serum from PBB (polybrominated biphenyl)-exposed workers. J. Envir. Path. Toxicol. 2:1397-1411

WOOD AW, RYAN DE, THOMAS PE, LEVIN W. (1983) Regio- and stereoselective metabolism of two C19 steroids by five highly purified and reconstituted rat hepatic cytochrome P-450 isozymes. J. Biol. Chem. 258:8839-8847

WORGUL TJ, SANTEN RJ, SMOJLIK E, IRWIN G, FALVO RE. (1981) Evidence that brain aromatization regulates LH secretion in the male dog. Amer. J. Physiol. 241:E246-E250

YAHR P. (1979) Data and hypotheses in tales of dihydrotestosterone. Horm. Behav. 13:92-96