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A TIME-COURSE STUDY OF INTERACTIONS BETWEEN VITAMIN A AND POLYBROMINATED BIPHENYLS IN RATS

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A TIME-COURSE STUDY OF INTERACTIONS BETWEEN VITAMIN A AND POLYBROMINATED BIPHENYLS IN RATS

By

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ABSTRACT

A TIME-COURSE STUDY OF INTERACTIONS BETWEEN VITAMIN A AND POLYBROMINATED BIPHENYLS IN RATS

By

R. Wasito

Seventy-two young male rats were allotted to 4 groups of 18 each. Diets, either vitamin A-deficient or supplemented were fed for 42 days and contained 0 or 100 mg of a commercial mixture of polybrominated biphenyls (PBB) for the last 28 days of the experiment. Adverse clinical signs were not observed. PBB significantly depressed weight gain in rats fed a vitamin A-deficient diet. Hepatic lesions due to PBB toxicosis were not diminished by vitamin A supplementation. The vitamin A-deficient diet containing PBB caused depletion of retinol concentrations in the serum and produced abnormal vitamin A metabolism in the liver as manifested by the appearance of significant amounts of retinyl acetate. Hyperplasia of the bile ducts and squamous metaplasia of the thyroid and salivary glands occurred in vitamin A-deficient rats given PBB.

Dedicated With Love To

Rr. Hastari Wuryastuti, my wife

My mother

My brother and his wife, my sisters and their husbands,
and their children:
Ria, Bagus, Nila, Windri, Galih, Imok, Denta, Uuk and Bayu

You

And in Memory of my Father: R. Mohammad Ichram

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INTRODUCTION

The realization that nutritional imbalances and deficiencies are still major problems in human and animal health has resulted in numerous studies on the role of diet on effects of environmental contaminants (xenobiotics) and carcinogens. Polyhalogenated aromatic hydrocarbons, such as polybrominated biphenyls (PBB), polychlorinated biphenyls (PCB), dibenzofurans, dibenzo-p-dioxin and many others, are cause for concern because their widespread distribution in the environment can result in human and animal exposure. The interaction of nutritional factors, such as vitamin A, and environmental contaminants, such as PBB, is of current research interest and may have important ramifications for human and animal health.

Vitamin A deficiency has a global distribution and is still a serious public health problem in some countries (WHO, 1967; Srikantia, 1982). A number of experiments suggest that vitamin A deficiency is related to the adverse effects of certain xenobiotics such as PCB (Innami et al., 1974) and tetrachlorodibenzo-p-dioxin (TCDD) (Thunberg et al., 1983). Previous work in this laboratory by Darjono (1982) demonstrated that the vitamin A nutritional status has important effects upon the toxicity of PBB. He

evaluated the interaction between vitamin A status and PBB toxicity in two experiments. He found that clinical signs of vitamin A deficiency and mortality occurred earlier in rats fed vitamin A-deficient diets containing 100 mg of PBB/kg than in those fed a vitamin A-deficient diet without PBB. Vitamin A supplementation prevented decreased weight gain and decreased thymic weight associated with PBB toxicosis (Darjono, 1982). Massive enlargement of the common bile duct occurred in rats fed vitamin A-deficient diets containing 100 mg of PBB/kg. Histologically, this lesion consisted of extensive hyperplasia. Serum vitamin A concentrations in rats fed vitamin A-deficient diets were also lowered by PBB as compared to rats fed a diet containing PBB and supplemented with vitamin A. The interaction between vitamin A deficiency and PBB toxicosis also affected vitamin A metabolism in the liver as manifested by the appearance of significant amounts of retinyl acetate in the vitamin A profile. Darjono's experiments were not designed to study the pathogenesis of the lesions. The interaction of vitamin A and PBB toxicosis was also evaluated in a pilot experiment by Wasito and Galbraith (1983, unpublished data). Decreased weight gain, low serum vitamin A concentration, enlargement of the liver, swollen and vacuolated hepatocytes, hyperplasia of the bile duct, keratinization of the ultimobranchial follicles in the thyroid gland and squamous metaplasia of the salivary gland

were observed only after prolonged feeding of a vitamin A-deficient diet containing 100 ppm PBB.

We therefore decided to do a time-course study over a 4-week period so as to better assess the interaction between vitamin A status and PBB. The first objective of this study was to evaluate and compare serum and hepatic vitamin A contents in young rats as affected by the interaction between dietary levels of vitamin A and PBB. The second objective was to evaluate the pathogenesis of the previously described histologic lesions which were due to the combined effects of vitamin A deficiency and PBB with emphasis on those suggestive of vitamin A deficiency. The third objective was to further characterize whether or not the vitamin A nutritional status affected the toxicity of PBB.

LITERATURE REVIEW

Vitamin A

Introduction

Vitamin A is present in the diet either as preformed vitamin A (retinol and its esters) or as its precursor (β-carotene) (Davis, 1978; Gopalan and Rao, 1979). Structurally, the vitamin A molecule consists of a hydrophobic head (β-ionone ring), a conjugated isoprenoid side chain and a polar terminal group. The conjugated isoprenoid chain is a double bond system which is isomerized by light, enzymes or heat to change the shape of the molecule. The polar terminal group can be enzymatically or chemically converted to retinyl palmitate, retinal and retinoic acid (Zile and Cullum, 1983).

Vitamin A is necessary for normal growth and differentiation of epithelial cells and bone (Wolbach, 1954). Retinol, retinyl esters, retinal, and retinoic acid are also capable of supporting these functions (Zile and Cullum, 1983).

Vitamin A is also important in reproduction because it is known to affect spermatogenesis, oogenesis and placental, fetal and embryonal development. Retinol, retinyl esters or retinal are necessary for these functions. Lotthammer

(1979) reported that cattle fed β-carotene deficient rations had delayed ovulation and increased in the incidence of follicular and luteal cysts. In another study, however, these effects were not demonstrated (Folman et al., 1979). Retinoic acid is considered as biochemically inactive insofar as the reproductive functions of vitamin A are concerned (Thompson et al., 1964). However, an in vitro study indicated that retinoic acid activated cell cleavage in the early stage of spermatogenesis and stimulated cellular differentiation in mouse cryptorchid testes (Haneji et al., 1983).

Vitamin A is also responsible for vision and color perception. The active form of vitamin A for this function is retinal derived from retinyl esters or retinol (Wald, 1960). Vitamin A-deficient animals often have xerophthalmia because of keratinization of corneal and conjunctival epithelial cells, and squamous metaplasia and atrophy of lacrimal glands (Wolbach, 1954).

Vitamin A values are given in International Units (IU) of vitamin A. The IU values can be converted to micrograms by applying the following factors: 1 IU of vitamin A = 0.3 μg retinol; 0.6 μg β-carotene; and 1.2 μg other provitamin A carotenoids with vitamin A activity (WHO, 1967). In rats, the minimal requirement for maintaining weight gains, optimal longevity and some hepatic vitamin A storage is about 2,000 IU per kg of diet (Anonymous, National Academy Sciences, 1972).

Metabolism

As already mentioned, the major sources of vitamin A in the diet are β-carotene and retinyl esters. β-carotene is converted to vitamin A primarily in the intestinal mucosa. The biosynthetic process involves two soluble enzymes. β-carotene is cleaved at the central double bond by β-carotene 15-15'-dioxygenase to yield two molecules of retinaldehyde. Retinaldehyde is then reduced to retinol by retinaldehyde reductase (Goodman and Olson, 1969). Dietary retinyl esters are hydrolyzed in the intestinal lumen and the resulting retinol is then absorbed into the mucosal cells. Retinol that is absorbed or newly synthesized in the mucosal cells is reesterified with long chain saturated fatty acids. The retinyl esters formed are then absorbed into the body in association with lymph chylomicrons. During chylomicron metabolism, virtually all of the retinyl esters remaining with the chylomicron remnant are removed from the circulation by the liver (Blomhoff et al., 1982). After uptake of the chylomicron-associated retinyl esters, hydrolysis and reesterification occur in the liver. The retinyl esters formed, mainly retinyl palmitate, are stored in association with lipid droplets in the liver (Goodman, 1980).

Transport of vitamin A from the liver to the tissue is in the form of the free alcohol retinol. In the liver, hydrolysis of retinyl esters is responsible for formation of retinol prior to the mobilization of hepatic vitamin A. The retinol formed is bound to a specific plasma binding protein

(retinol binding protein or RBP) (Rask et al., 1980). The concentration of RBP in plasma is regulated by vitamin A status. Thus, in vitamin A deficiency, RBP molecules are not secreted from the liver. In plasma, RBP forms a complex with thyroxin binding prealbumin. The RBP molecules interact with a cell membrane receptor on epithelial cells and transfer vitamin A across the membrane into the cells (Heller, 1975; Rask and Peterson, 1976). The uptake of vitamin A by the cells causes a reduction of the affinity of RBP for thyroxin binding prealbumin. The RBP molecules which are not able to interact with the thyroxin binding prealbumin are then excreted in the urine (Rask et al., 1980).

In the cell, vitamin A is bound to a cellular receptor protein. Retinol, retinal and retinoic acid are specifically bound to cytosolic-binding protein (Liau et al., 1981).

Retinoic acid is transported by serum albumin (Smith et al., 1973). It is rapidly metabolized to more polar compounds which are excreted in the urine and bile. The major biliary metabolite of retinoic acid is identified as retinol-β-glucuronide (Goodman, 1980).

Pathology of Vitamin A Deficiency and Vitamin A Toxicity

Gross lesions and clinical signs: In the rat, manifestations of vitamin A deficiency include xerophthalmia, ataxia, paresis, humped posture, rough coat and slight focal alopecia (Klein-Szanto et al., 1980).

Vitamin A toxicity affects the skin (dryness and scaling), the hair (dryness and loss), the optic disc (papilledema), the liver (cirrhosis), bone (malformation) and arteries, heart and kidney (calcification) (Grey et al., 1965; Strebel et al., 1969; Russell et al., 1974; Davis, 1978).

Histopathology: As previously mentioned, vitamin A is necessary for the normal differentiation of epithelial cells in many tissues (Wolbach, 1954). A deficiency of vitamin A results in squamous metaplasia in which a squamous keratinizing type of epithelium replaces the normal form of epithelium of salivary glands, trachea, bronchi, testes, kidney, urinary bladder, cornea, conjunctiva, meibomian glands, pancreas and skin. Klein-Szanto et al. (1980) noticed that in vitamin A-deficient rats, males developed squamous metaplasia of the trachea and salivary glands earlier than females. Sialoadenitis, especially of the submaxillary gland was always present. On the other hand, squamous metaplasia of the renal pelvis developed earlier in females.

Others have found that edema and atrophy of testes (Wolbach and Howe, 1925), squamous metaplasia of the bile ducts (Beaver, 1961) and keratinization of the ultimobranchial follicles of the thyroid glands (Krupp, 1972) were related to vitamin A deficiency in rats.

Polybrominated Biphenyls

Introduction

Polybrominated biphenyls (PBB) were manufactured for use as flame retardants in industrial processes. The production of PBB by Michigan Chemical Corporation as a flame retardant under the trade name Firemaster (FM) began in 1970 and ceased in 1975.

An analysis of FM indicated that it consisted of 2% tetrabromobiphenyl, 10.6% pentabromobiphenyl, 62.8% hexabromobiphenyl, 13.8% heptabromobiphenyl and 11.4% other biphenyls (Kay, 1977). The mixture causes a mixed-type induction of liver microsomal drug metabolizing enzymes since it induces phenobarbital (PB)-type and 3-methylcholanthrene (MC)-type microsomal enzymes (Dent et al., 1976). Apparently the toxicity of FM is mostly attributed to its congeners with MC-type induction capability. In addition, mono ortho-substituted congeners in FM, such as 2,4,5,3',4',5'-hexabromobiphenyl (HBB) (Dannan et al., 1978), 2,4,5,3',4'-pentabromobiphenyl (Dannan et al., 1982a) and 2,3,4,5,3',4'-HBB (Dannan et al., 1982b) were reported to be MC-type inducers and were considered toxic. Congener 2,2',4,4',5,5'-HBB comprises approximately 50% of the mixture, is a PB-type inducer, and is relatively nontoxic (Akoso et al., 1982b). Another PB-type inducer, 2,4,5,2',5'-pentabromobiphenyl is also not toxic (Dannan et al., 1982b).

The FM mixture containing PBB begins to melt at 72° C and decomposes at 300 to 400° C. It is insoluble in water and highly soluble in organic solvents (fat soluble). It has a low vapor pressure. PBB can be degraded by ultraviolet radiation (Ruzo and Zabik, 1975).

In 1973 and 1974, livestock feed was contaminated by FM at a facility in Michigan. Clinical signs described in dairy cattle fed PBB-contaminated feed included decreased milk production, anorexia, alopecia and abnormal growth (Jackson and Halbert, 1974). Subsequently, other farm animals were also found to be contaminated by ingestion of PBB-contaminated feed (Damstra et al., 1982). Some investigators associated skin rashes, joint swelling, hematologic changes, immunologic defects, increased susceptibility to respiratory infection and neurologic symptoms to exposure of people to PBB (Bekesi and Holland, 1978; Valciukas, 1978; Weil et al., 1981). However, the levels of PBB in serum or adipose tissue were not well correlated with reported signs of PBB toxicity in man (Brown et al., 1981; Weil et al., 1981).

Metabolism

Results of animal studies indicate that PBB concentrate in hepatic and adipose tissues are poorly metabolized and very slowly excreted. Thus, the potential exists for bioaccumulation and long term effects of PBB exposure (Dent et al., 1976; DiCarlo et al., 1978).

As already mentioned, PBB toxicosis is mostly attributed to congeners with MC-type induction capability. The mechanism of toxicity of PBB is unknown. However, it is suggested that an initial event is the stereospecific reversible binding of a congener to a cytosolic polypeptide receptor molecule called the Ah receptor (Poland et al., 1976). The congener-receptor binding is required for aryl hydrocarbon hydroxylase (AHH) induction (Robertson et al., 1983). The PBB receptor complex is then translocated to the nucleus resulting in the synthesis of liver microsomal drug metabolizing enzymes (P-450) (Nebert et al., 1982).

The various forms of P-450 represent a family of hemoproteins which are monooxygenases that possess catalytic activity towards many substrates. Monooxygenases are enzymes that insert one atom of atmospheric oxygen into their substrates. Accordingly, to perform this monooxygenation, the P-450 receives two electrons from cofactors NADPH or NADH. These electrons are received one at a time via reductases (flavoproteins). It is not known whether the P-450 molecules are arranged in rosettes around reductase molecules or the reductase molecules move among P-450 molecules. In most organisms, the electron chains are embedded in the endoplasmic reticulum, inner mitochondrial membrane and the nuclear envelope. Therefore, monooxygenase activities require the integrity of an electron flow between the cofactor NADPH or NADH and the oxygenated form of P-450. After passage of the electrons to P-450, one atom of

atmospheric oxygen is transferred to the substrate at the P-450 enzyme-active site. This process involves activation of iron of heme. The other atom of oxygen is found in cellular water. It is believed that oxygenation occurs at or within the surface of the microsomal (lipoidal) membrane. The resulting more polar metabolites are water soluble and are then excreted in the urine or bile (Nebert et al., 1982).

PBB are lipophilic and have long half-lives in body tissues. In the rat, the half-life of PBB is 23.1 weeks in serum and 69.3 weeks in fat (Miceli and Marks, 1981). Although PBB are poorly metabolized, excretion of PBB is primarily via the bile into the feces. Milk and eggs are the major routes of excretion in lactating mammals and egg-laying birds, respectively (Matthews et al., 1977). Transplacental transfer of PBB was demonstrated in rats and cows and detrimental effects in young suckling on PBB-contaminated mothers have been reported (Rickert et al., 1978; Willett and Durst, 1978; Moore et al., 1978; Detering et al., 1975).

Pathology

Gross lesions: Hepatomegaly, thymic involution and loss of weight were consistent features noted in PBB-treated rats and mice (Sleight and Sanger, 1976; Gupta and Moore, 1979). Kimbrough et al. (1978) and Kimbrough et al. (1981) observed neoplastic nodules in the livers of rats fed FM FF-1 at high doses. Tumor promoting effects of PBB in rats were also reported by Jensen et al. (1982) who found

increased numbers of γ-glutamyl transpeptidase (GGT) positive enzyme-altered foci and neoplastic nodules. Thyroid enlargement was noted in rats (Sleight et al., 1978) and in sows and their pigs (Werner and Sleight, 1981). Darjono et al. (1983) reported that rats fed a vitamin A-deficient diet containing 100 ppm PBB had extensive enlargement of the common bile ducts.

Histopathology: In the liver, severe hypertrophy and vacuolation of hepatocytes in rats fed 100 ppm or 500 ppm of PBB for 30 or 60 days were noticed by Sleight and Sanger (1976). Similar histologic features were also observed in the liver of sows and their pigs (Werner and Sleight, 1981). Hepatic necrosis (McCormack et al., 1980) and intracytoplasmic inclusions within hepatocytes (Akoso, 1981) were occasionally observed in rats as a result of exposure to PBB. Large acidophilic hepatocytes with enlarged nuclei and multiple nucleoli were described by Jensen et al. (1982) in rats in a study of the tumor promoting activity by PBB. Darjono et al. (1983) observed hyperplasia of the extra-parenchymal bile ducts in rats after prolonged feeding of a vitamin A-deficient diet containing 10 or 100 ppm PBB.

In the thyroid gland, Sleight et al. (1978), Mangkoewidjojo (1979) and Akoso et al. (1982a) observed hypertrophy and hyperplasia of the follicular cells as well as depletion of follicular colloid.

In the thymus, thymic cortical atrophy and loss of demarcation between the cortical and the medullary regions

were observed in rats fed FM FF-1 at high doses (Gupta and Moore, 1979) and in rats given 2,3,4,5,3',4'-HBB (Dannan et al., 1982b).

Interaction Between Vitamin A and Xenobiotics/Carcinogens

Influence of Xenobiotics/Carcinogens on Vitamin A

Research by Goerner (1938) with rabbits revealed that intraperitoneal exposure to dibenzanthracene caused a marked decrease of hepatic vitamin A storage. Decrease of hepatic vitamin A also occurred in rats following exposure to methylcholanthrene (MC) (Bauman et al., 1942) and benzo(a)pyrene (BaP) (Carruthers, 1942). These results suggest that the depletion of vitamin A by the carcinogenic polycyclic aromatic hydrocarbons may be an important factor in cancer. Certain xenobiotics such as polychlorinated biphenyls (PCB) (Kato et al., 1978), phenobarbital (Backes et al., 1979), tetrachlorodiphenyldioxine (TCDD) (Thunberg et al., 1980), ethanol (Sato and Lieber, 1982) and polybrominated biphenyls (PBB) (Mangkoewidjojo, 1979; Akoso et al., 1982a; Darjono, 1982) have also been reported to lower hepatic vitamin A storage. Kato et al. (1978) speculated that PCB and DDT caused decreases in vitamin A levels because these substances induce synthesis of microsomal enzymes that may lead to the metabolism of vitamin A. This hypothesis may be supported by observations that liver microsomal enzymes affect the hydroxylation of

lipid-soluble drugs (Brodie et al., 1958), steroids (Talalay, 1965) and fatty acids (Das et al., 1968).

Aflatoxin B_1 was also reported to lower liver and serum vitamin A levels (Newberne and Suphakarn, 1977). Chikaraishi and Suzue (1977) also reported that aflatoxin may inhibit the conversion of β-carotene to vitamin A as well as enhance the depletion of vitamin A.

Influence of Vitamin A on Xenobiotics/Carcinogens

Innami et al. (1974) observed that rats fed a 0.1% PCB diet supplemented with 3,400 IU of vitamin A/100 g of the PCB diet for 6 weeks grew better than those fed only a 0.1% PCB diet. Rats fed a vitamin A-deficient diet with 0.1% PCB had a significant growth retardation compared to those given only a 0.1% PCB diet. In the thymus, vitamin A was reported to have a protective effect against thymic depletion in rats fed 100 ppm PBB (Darjono et al., 1983).

Moon et al (1983) reported that several retinoids: axerophthene, retinyl acetate (RAc), retinyl methyl ether (RME), N-(4-hydroxyphenyl)retinamide, possess mammary anticarcinogenic activity when given chronically in the diet to experimental animals. Sporn et al. (1977) demonstrated that 13-cis-retinoic acid inhibited the development of bladder cancers in rats caused by direct instillation of N-methyl-N-nitrosurea (MNU) into the bladder. Other investigators have indicated a protective effect of vitamin A against 3-methylcholanthrene (MC)-induced lung tumors in rats (Cone and Nettesheim, 1973) and a decrease in

benzo(a)pyrene (BaP)-induced lung tumors in hamsters (Saffiotti et al., 1967).

Wattenberg (1983) reported that retinoids act by suppressing the expression of neoplasia by cells previously exposed to doses of a carcinogenic agent. However, the mechanisms by which retinoids inhibit carcinogenesis are still unclear. Retinoic acid is thought to act on tissues by binding to a specific intracellular protein or retinoic acid receptor (RAR). There are several reports to support this hypothesis. In one study, 3 human breast carcinomas and 1 lung carcinoma were shown to contain RAR whereas the specimens of the adjacent normal tissues did not contain any detectable RAR (Ong et al., 1975). Significantly higher levels of RAR were reported in cervical, endometrial and ovarian cancers (Palan and Seymour, 1980) and in the transitional cell type of bladder tumors in humans (Fagg et al., 1982).

On the other hand, an in vitro study by Jetten (1983) indicated that retinoids stimulated sarcoma growth factor (SGF) and 12-0-tetradecanoylphorbol-13-acetate (TPA)-induced growth of normal rat kidney fibroblast cells NRK 536-3 (SA 6) in soft agar. These results are in contrast to the observation by Todaro et al. (1978) who demonstrated that retinoids block phenotypic cell transformation produced by SGF using a different subclone NRK 536-7. Retinoids were reported to inhibit the cell proliferation of an SV40-transformed line. However, proliferation of two cell lines

that were transformed by a Kirsten and Moloney strain of murine sarcoma virus and produced growth factor into culture medium was remarkably stimulated by retinoids (Hiragun et al., 1983). Hennings et al. (1982) and Chauvenet and Paque (1982) suggested a potential danger of retinoid therapy when performed on growth factor producing neoplasms. This oncogenic effect of retinoids might explain the retinoid-induced tumor enhancement in hamster cheek pouches initiated with dimethylbenzanthracene (Levij and Polliack, 1968) and in the lungs of hamsters initiated with BaP (Smith et al., 1975).

Comparison Between the Effects of Polybrominated Biphenyls and Vitamin A Deficiency

There are several similarities between the effects of polybrominated biphenyls (PBB) and related compounds and vitamin A deficiency. For example, PBB toxicosis and vitamin A deficiency are known to cause a wasting-type syndrome (Wolbach, 1925; Hayes, 1971; Sleight and Sanger, 1976; Gupta and Moore, 1979).

The thymic involution observed in rats fed FM FF-1 at high doses (Gupta and Moore, 1979) is similar to that reported to occur in vitamin A-deficient rats (Wolbach, 1925). A similar loss of cortical thymocytes was also described in tetrachlorodibenzo-p-dioxin (TCDD) toxicosis (Kociba et al., 1979).

McConnell et al. (1979) reported keratinization and squamous metaplasia of the Meibomian glands in rhesus monkeys highly contaminated with polychlorinated biphenyls (PCB). These epithelial changes were similar to those reported for vitamin A-deficient animals (Wolbach, 1954).

There is evidence that both exposure to PBB and vitamin A deficiency may play a role in carcinogenesis. Kimbrough et al. (1978), Kimbrough et al. (1981) and Jensen et al. (1982) reported that PBB are hepatocarcinogenic in rats. Epidemiological studies have suggested that vitamin A deficiency in humans is associated with cancer of the stomach (Abels et al., 1941) and cancer of the respiratory tract (Basu et al., 1976).

MATERIALS AND METHODS

Experimental Design

Seventy-two male Sprague-Dawley rats[a], weighing approximately 60 g at 21 days old were used. During 2 days of acclimation, the rats were fed a regular commercial pelleted diet[b] and tap water ad libitum.

The rats were randomly allotted to 4 groups of 18 each. Rats in these groups were fed diets either deficient in vitamin A (groups A and B) or containing 30,000 IU retinyl acetate/kg (groups C and D) for 14 days. At this time (day 0), rats in their respective groups were given either 0 (groups A and C) or 100 mg of PBB[c]/kg of diet (groups B and D) for 28 days. The experimental design is illustrated in Table 1.

The rats were housed 6 to a cage in stainless wire-top, plastic cages and the bedding was changed once a week. Room temperature was maintained at 21.1° C. Lights were controlled automatically to allow 12 hours of darkness.

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[a]Charles River Breeding Laboratories, Inc., Portage, Michigan.

[b]Teklad, A Harlan Sprague Dawley, Inc. Co., Winfield, Iowa.

[c]Firemaster BP-6, Michigan Chemical Co., St. Louis, Michigan.

Table 1. Experimental design. Rats were fed diets containing 0 or 100 mg of PBB/kg which were either deficient in vitamin A or supplemented with 30,000 IU retinyl acetate/kg.

Group	Diets		Termination (day)		
	Day -14 to day 0	Day 0 - 28	7	14	28
A	Deficient A	Deficient A	6[a]	6	6
B	Deficient A	Deficient A + 100 mg PBB/kg	6	6	6
C	30,000 IU A/kg	30,000 IU A/kg	6	6	6
D	30,000 IU A/kg	30,000 IU A/kg + 100 mg PBB/kg	6	6	6

[a]Six rats in each group were killed on day indicated.

Feeding Practices

The basal diet for the rats was a commercial vitamin A deficient diet[d]. Diets were supplemented with vitamin A by adding 30,000 IU retinyl acetate[e]/kg feed to the basal diet.

Cottonseed oil was used as the vehicle for mixing vitamin A and PBB in the feed. Diets were mixed at the beginning of the experiment and were refrigerated.

Feed was provided in porcelain containers with stainless steel tops. Drinking water was available ad libitum in inverted bottles with rubber stoppers and stainless steel sipper tubes. Fresh water was provided every other day.

Rats were observed daily for clinical signs. Feed consumption was recorded every other day and body weights were recorded weekly.

Laboratory Investigation Procedures

Collection and Processing of Tissues

Final body weights were recorded prior to the necropsy and rats were killed with ether anesthesia. A blood sample was obtained from the heart while the rat was anesthetized. Serum was removed after coagulation and centrifugation and was stored at -25° C until chemical analyses were done.

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[d]Teklad Mills, Madison, Wisconsin.

[e]Roche Chemical Division, Hoffman-La Roche, Inc., Nutley, New Jersey.

A necropsy was performed immediately after the animal was killed. All tissues were examined grossly and the liver and thymus were weighed with a toploading balance[f] at time of necropsy. The thyroid glands were weighed on an analytical balance[g] after being fixed with 10% neutral buffered formalin for 24 hours.

Tissues for histologic examination were preserved in 10% neutral buffered formalin. Tissues collected included eyelid, eye, salivary gland, thyroid, trachea, brain, thymus, heart, lung, liver, spleen, adrenal gland, kidney, stomach, intestine, pancreas, urinary bladder and testes. Liver tissue for vitamin A analysis was wrapped with aluminum foil and stored at -25° C until analyses were done.

Formalin-fixed tissues were trimmed, processed in an Autotechnicon[h] and embedded in paraffin. The tissues were then cut with a microtome at 5 μ and stained with hematoxylin and eosin.

Vitamin A Analysis

The determination of vitamin A in the serum and liver tissue was done in the Clinical Nutrition Laboratory, Department of Large Animal Surgery and Medicine, Michigan

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[f]Mettler Series P, Model 163 (Readability: 0.01 g), Mettler Instrument Corp., Highstown, New Jersey.

[g]Model H-15 (Readability: 0.0001 g), Mettler Instrument Corp., Highstown, New Jersey.

[h]Histomatic, Model 165, Fisher Scientific Co., Pittsburg, Pennsylvania.

State University. Vitamin A was quantitated by the method established by Stowe (1982) as a modification of the high performance liquid chromatography procedure described by Dennison and Kirk (1977).

In preparation for vitamin A quantitation in the serum, 1 ml of serum was mixed with 1 ml absolute ethanol and vortexed for 5 seconds to form a suspension of the denaturated protein. Two milliliters of hexane (68 to 69° C boiling point) were added to the mixture which was then vortexed for 1 minute and centrifuged at 3000 rpm for 10 minutes. The hexane supernatant was removed and passed through a 0.45 micron Millipore filter[i] in a Swinny-type filter holder. One hundred microliters of aliquot were injected into the chromatographic system[j]. Separation was isocratic in a Microporasil column[j] (30 x 3.9 cm long) with a 60:40 mixture of degassed hexane and chloroform and the mixture was pumped through the HPLC unit at 2.5 ml/minute at 1000 psi. Forms of vitamin A were detected with a Spectrofluorometer[k] set at 330 and 470 nm for excitation and emission wavelengths, respectively, and equipped with a 35 µl flow cell.

Liver vitamin A concentrations on a dry weight basis were determined as follows. To determine dry weight of

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[i]Millipore Corp., Bedford, Massachusetts.

[j]Waters Associated, Inc., Milford, Massachusetts.

[k]Aminco Bowman (J4-8961) Spectrofluorometer, American Instrument Company, Silver Spring, Maryland.

liver, 2 grams of liver tissue were placed in an aluminum pan and dried in an oven at 56° C. The dried liver tissues were weighed after 24 hours in the oven. For the quantitation of the vitamin A level, 1 gram of liver tissue was placed in a large tube and distilled water was added up to 5 ml. The mixture was then homogenized[1] and from this homogenate, 0.5 ml was pipetted into a disposable test tube. An equal amount of absolute ethanol was added and then the mixture was vortexed for 5 seconds. For the extraction, 5 ml hexane was used which was then vortexed for 5 minutes and centrifuged at 3000 rpm for 10 minutes. The hexane supernatant was removed and the subsequent steps were as described for serum samples.

Statistical Evaluation

For organ to body weight ratios and weight gain, the data were analyzed using analysis of variance and Student Newman-Keul's test. For vitamin A analysis, the data for groups A and B or groups C and D were compared by the Student's t-test. The differences were considered significant at the level of $p<0.05$.

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[1]Polytron Homogenizer, Kinematica GmBH, Switzerland.

RESULTS

Clinical Signs

There were no adverse clinical signs of either vitamin A deficiency or PBB toxicosis observed in any of the rats during this experiment. Daily feed intake was not altered throughout the study.

Body Weight

Body weights increased steadily from the start of the experiment until the experiment was terminated. At the end of the experiment, the body weights of rats fed a diet deficient in vitamin A and containing 100 mg of PBB/kg (group B) were the lowest (273.3 g) when compared to rats fed a vitamin A deficient diet (group A; 296.6 g) or to rats fed a diet supplemented with 30,000 IU retinyl acetate and containing 0 (group C; 324.7 g) or 100 mg (group D; 314.1 g) of PBB/kg (Fig. 1).

On day 7, the weight gain of rats in group B was significantly different from groups C and D ($p<0.05$), but not from group A. On day 14, 21 or 28, the weight gain of rats in group B was significantly different from those in groups A, C and D ($p<0.05$).

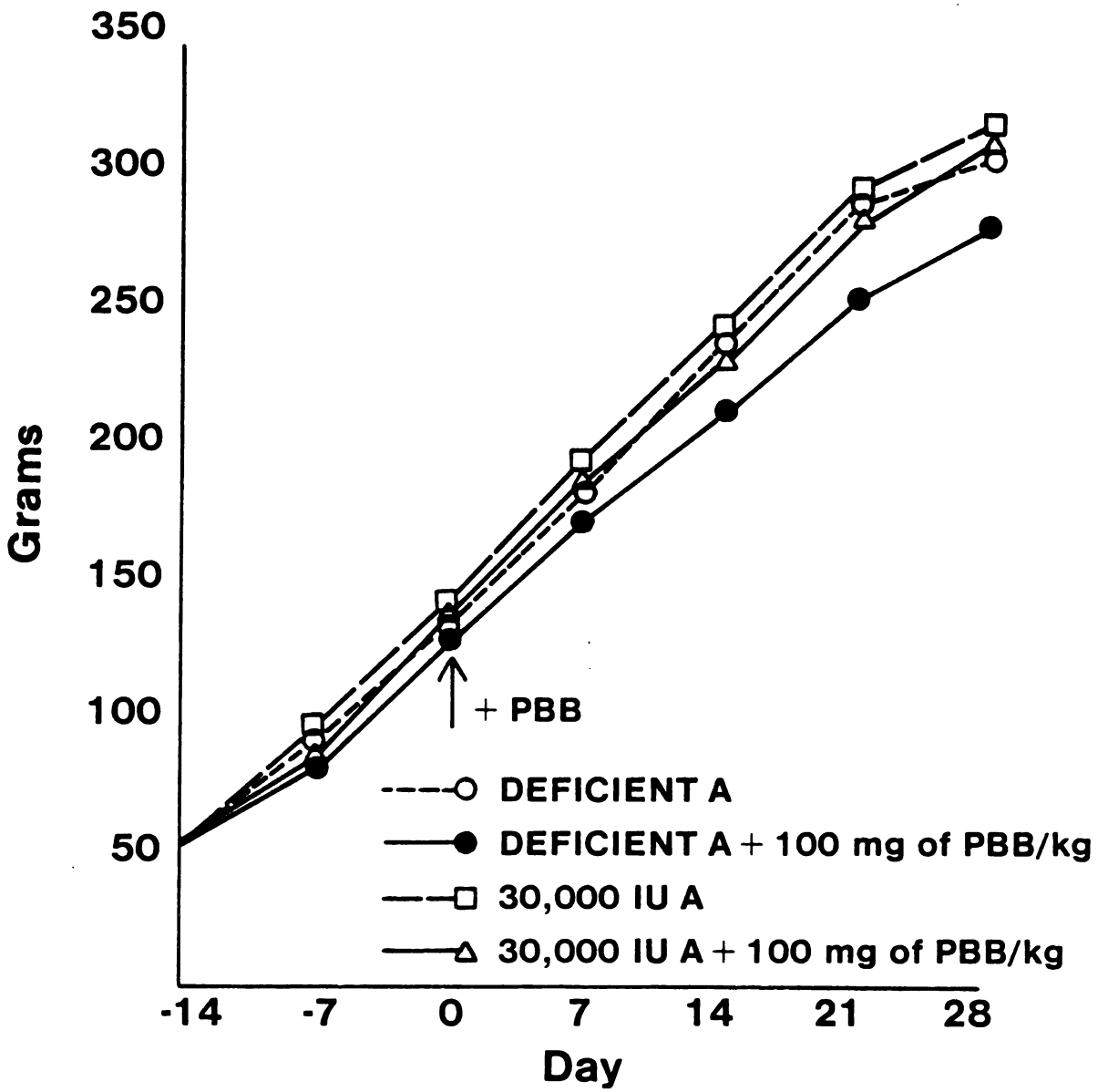

Figure 1. Mean body weights of rats fed diets containing 0 or 100 mg of PBB/kg and either deficient in vitamin A or supplemented with 30,000 IU retinyl acetate/kg.

Organ Weights

Organ to body weight ratios are listed in Tables 2, 3 and 4. Administration of PBB caused significant increases ($p<0.05$) in the liver to body weight ratios at 7, 14 and 28 days (groups B and D). Thymic weight to body weight ratios were not significantly different from each other. Thyroid weight to body weight ratios were significantly increased in groups B and D on day 7 ($p<0.05$) as compared to groups A and C but not in group B on day 14 and not in group D on day 28.

Laboratory Investigation

Serum Vitamin A

Data relating to serum vitamin A concentrations are tabulated (Tables 5, 6 and 7). After 7, 14 or 28 days of dietary treatment with 100 mg of PBB/kg, there were no significant effects on the total vitamin A concentrations in the sera of rats fed a diet supplemented with 30,000 IU retinyl acetate/kg (group D). Total vitamin A concentrations in the sera were still maintained in rats given 100 mg PBB/kg in a vitamin A-deficient diet for 7 or 14 days (group B). By day 28, PBB caused significant decreases of the total vitamin A concentrations in the sera of rats in this group. Decreases were caused mainly by significant lowering of retinol concentrations ($p<0.05$).

Table 2. Organ to body weight ratios at 7 days.

Group	No. of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Organ to body weight ratios: Liver (g/100 g BW)	Organ to body weight ratios: Thymus (g/100 g BW)	Organ to body weight ratios: Thyroid (mg/100 g BW)
A	6	0	0	3.2±0.2[a]	0.39±0.05[c]	9.7±0.8[a]
B	6	0	100	6.0±0.8[b]	0.37±0.09[c]	12.4±2.0[b]
C	5	30,000	0	3.4±0.1[a]	0.40±0.05[c]	9.2±1.3[a]
D	6	30,000	100	5.5±0.5[b]	0.32±0.08[c]	12.7±1.7[b]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Significantly different ($p<0.05$) from groups B and D.

[b]Significantly different ($p<0.05$) from groups A and C.

[c]Not significantly different from each other.

Table 3. Organ to body weight ratios at 14 days.

Group	No. of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Organ to body weight ratios: Liver (g/100 g BW)	Organ to body weight ratios: Thymus (g/100 g BW)	Organ to body weight ratios: Thyroid (mg/100 g BW)
A	6	0	0	3.2 ± 0.1^{a}	0.34 ± 0.05^{c}	7.5 ± 1.3^{d}
B	6	0	100	6.5 ± 0.7^{b}	0.09 ± 0.02^{c}	9.4 ± 1.6^{e}
C	6	30,000	0	3.3 ± 0.2^{a}	0.38 ± 0.06^{c}	8.5 ± 1.1^{d}
D	6	30,000	100	6.0 ± 0.8^{b}	0.30 ± 0.06^{c}	11.2 ± 1.1^{f}

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Significantly different ($p<0.05$) from groups B and D.

[b]Significantly different ($p<0.05$) from groups A and C.

[c]Not significantly different from each other.

[d]Significantly different ($p<0.05$) from group D.

[e]Not significantly different from groups A, C and D.

[f]Significantly different ($p<0.05$) from groups A and C.

Table 4. Organ to body weight ratios at 28 days.

Table 4. Organ to body weight ratios at 28 days.

Group	No. of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Organ to body weight ratios: Liver (g/100 g BW)	Organ to body weight ratios: Thymus (g/100 g BW)	Organ to body weight ratios: Thyroid (mg/100 g BW)
A	6	0	0	3.2±0.3[b]	0.22±0.02[d]	6.6±0.6[e]
B	6	0	100	6.0±0.6[c]	0.18±0.03[d]	8.8±1.4[f]
C	6	30,000	0	2.8±0.1[b]	0.23±0.04[d]	6.8±1.1[e]
D	6	30,000	100	5.5±0.6[c]	0.23±0.06[d]	8.4±1.1[a,g]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]N=5.

[b]Significantly different ($p<0.05$) from groups B and D.

[c]Significantly different ($p<0.05$) from groups A and C.

[d]Not significantly different from each other.

[e]Significantly different ($p<0.05$) from group B.

[f]Significantly different ($p<0.05$) from groups A and C.

[g]Not significantly different from groups A, B and C.

Table 5. Sera vitamin A concentrations at 7 days.

Group	No. of rats	Diet modification: Suppl. vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Serum vitamin A (ng/ml): Retinyl Palmitate	Retinyl Acetate	Retinol	Total
A	6	0	0	28.3±11.0	25.1±28.0	354.6±74.7	408.1±71.3
B	6	0	100	19.6±11.1[a]	3.1±5.3	368.5±135.6[a]	391.3±144.0[a]
C	5	30,000	0	67.6±41.3	0	293.4±109.7	361.0±136.7
D	6	30,000	100	58.5±40.2[b]	0	370.3±112.0[b]	428.8±130.5[b]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Not significantly different from group A.

[b]Not significantly different from group C.

Table 6. Sera vitamin A concentrations at 14 days.

Group	No. of rats	Diet modification: Suppl. vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Serum vitamin A (ng/ml): Retinyl Palmitate	Retinyl Acetate	Retinol	Total
A	6	0	0	38.8±37.9	7.1±12.4	482.0±51.3	528.0±67.4
B	6	0	100	24.3±32.7[a]	9.5±21.2	493.0±55.4[a]	526.0±77.0[a]
C	6	30,000	0	65.6±25.5	4.3±9.6	766.1±89.5	836.1±105.0
D	6	30,000	100	38.6±19.7[b]	12.6±14.9	607.0±146.1[b]	658.3±154.8[b]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Not significantly different from group A.

[b]Not significantly different from group C.

Table 7. Sera vitamin A concentrations at 28 days.

Group	No. of rats	Diet modification: Suppl. vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Serum vitamin A (ng/ml): Retinyl Palmitate	Retinyl Acetate	Retinol	Total
A	6	0	0	46.3±15.7	0	185.0±80.4	231.3±84.0
B	6	0	100	30.3±14.2[a]	0	47.5±24.3[c]	77.8±36.1[c]
C	6	30,000	0	42.5±13.3	29.5±29.6	542.3±43.4	614.3±35.2
D	6	30,000	100	15.8±14.8[b]	59.6±11.7	662.0±130.4[d]	737.5±145.5[d]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Not significantly different from group A.

[b]Significantly different ($p<0.05$) from group C.

[c]Significantly different ($p<0.05$) from group A.

[d]Not significantly different from group C.

Liver Vitamin A

Data for liver vitamin A concentrations are listed in Tables 8, 9 and 10. PBB decreased hepatic vitamin A concentrations in rats fed diets either deficient in vitamin A (group B) or supplemented with retinyl acetate (group D). In rats in group B at 7 or 14 days and in rats in group D at 7, 14 or 28 days, there were significant decreases of the vitamin A concentrations in the livers ($p<0.05$). By day 28, rats in group B had a significant increase in the vitamin A concentration in the liver ($p<0.05$). Although the hepatic vitamin A concentration (μg/g) in rats in groups B and D were decreased at 7, 14 or 28 days, calculation of the data on a mg/whole liver wet weight basis indicated that the decrease was only significant in rats in group D at 14 or 28 days ($p<0.05$). By day 28, the hepatic vitamin A (mg/whole liver) in group B was significantly higher ($p<0.05$) than in group A.

Pathology

Gross Lesions

Gross lesions in rats given PBB were observed at all time points and were mainly in the liver and thyroid glands. Vitamin A content of the diet did not influence the appearance of gross lesions.

Liver: At 7, 14 or 28 days, livers of rats treated with 100 mg of PBB/kg of diet (groups B and D) were

Table 8. Liver vitamin A concentrations at 7 days.

Group	Number of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Liver vitamin A: Retinyl Palmitate (µg/g)	Liver vitamin A: Retinyl Acetate (µg/g)	Liver vitamin A: Retinol (µg/g)	Liver vitamin A: Concentration (µg/g)	Liver vitamin A: Total (mg/liver)
A	6	0	0	34.2±7.3	0	3.1±2.2	37.2±6.8	0.199±0.060
B	6	0	100	13.4±9.7[a]	3.1±4.4	0.7±0.4[a]	17.2±8.7[a]	0.163±0.117[c]
C	5	30,000	0	1684.6±397.2	0	40.2±12.4	1724.9±388.3	9.7±3.4
D	6	30,000	100	1012.4±266.7[b]	0	9.7±3.4[b]	1021.5±227.4[b]	9.5±2.4[d]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Significantly different ($p<0.05$) from group A.

[b]Significantly different ($p<0.05$) from group C.

[c]Not significantly different from group A.

[d]Not significantly different from group C.

Table 9. Liver vitamin A concentrations at 14 days.

Group	Number of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Liver vitamin A: Retinyl Palmitate (µg/g)	Liver vitamin A: Retinyl Acetate (µg/g)	Liver vitamin A: Retinol (µg/g)	Liver vitamin A: Concentration (µg/g)	Liver vitamin A: Total (mg/liver)
A	6	0	0	19.2±5.7	0	0.6±0.3	19.8±5.7	0.145±0.054
B	6	0	100	1.0±0.6[a]	7.5±3.3	0.2±0.3[c]	8.7±2.8[a]	0.112±0.043[c]
C	6	30,000	0	1485.9±504.8	0	17.2±1.1	1503.0±504.9	11.8±4.0
D	6	30,000	100	470.2±139.9[b]	0	15.7±3.7[d]	485.9±141.1[b]	5.9±1.8[b]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Significantly different ($p<0.05$) from group A.

[b]Significantly different ($p<0.05$) from group C.

[c]Not significantly different from group A.

[d]Not significantly different from group C.

Table 10. Liver vitamin A concentrations at 28 days.

Group	Number of rats	Diet modification: Supplemented vitamin A* (IU/kg)	Diet modification: PBB** (mg/kg)	Liver vitamin A: Retinyl Palmitate (μg/g)	Liver vitamin A: Retinyl Acetate (μg/g)	Liver vitamin A: Retinol (μg/g)	Liver vitamin A: Concentration (μg/g)	Liver vitamin A: Total (mg/liver)
A	6	0	0	1.2±1.1	1.1±0.7	0	2.3±1.0	0.020±0.009
B	6	0	100	0.3±0.4[a]	7.8±3.1[c]	0	8.1±3.0[c]	0.118±0.036[c]
C	6	30,000	0	2840.0±457.5	0	13.0±2.9	2853±456.8	24.8±5.8
D	6	30,000	100	734.2±222.8[b]	0	4.7±2.3[b]	738.9±223.7[b]	11.7±2.9[b]

*Vitamin A added to diets of rats in groups C and D 14 days prior to experimental day 0.

**PBB added to diets of rats in groups B and D on day 0.

Data are expressed as mean ± SD.

[a]Not significantly different from group A.

[b]Significantly different ($p<0.05$) from group C.

[c]Significantly different ($p<0.05$) from group A.

enlarged, had a whitish mottling and a slight yellow discoloration.

Thyroid: At 7, 14 or 28 days, dietary treatment with 100 mg of PBB/kg caused the thyroid glands to appear slightly larger than those that had dietary treatments without PBB and have a deep brown discoloration.

Histopathology

Liver: Livers of rats fed diets either deficient in vitamin A (group A) or supplemented with 30,000 IU retinyl acetate/kg (group C) were normal (Fig. 2). By days 7, 14 or 28, the addition of 100 mg of PBB/kg to groups B and D caused enlarged hepatocytes and cytoplasmic vacuolation (Fig. 3). Multifocal areas of necrosis with lymphocyte accumulation occurred in 1 rat in group B at 7 days and in 1 rat in group D at 28 days (Fig. 4).

Bile duct: PBB did not cause any changes in the bile ducts of rats in group D (Fig. 5). However, at 14 and 28 days, the bile ducts of rats fed a vitamin A-deficient diet with 100 mg of PBB/kg (group B) had hyperplasia of the epithelial surfaces and pyknotic nuclei. At 14 days, 3 of 6 rats in group B had slight hyperplasia of the bile ducts (Fig. 6), while at 28 days, there was mild (4 of 6 rats), moderate (1 of 6 rats) or marked (1 of 6 rats) hyperplasia of the bile ducts (Fig. 7, 8 and 9). There were no bile duct lesions observed in this group at 7 days. Only 1 of 6 rats in group A at 28 days had mild hyperplasia of the bile

duct, while no histologic lesions of the bile ducts were observed in any rats before this time.

Thyroid: Lesions in the thyroid gland were observed only in rats in group B at 28 days. The lesion was characterized by early evidence of squamous metaplasia of the ultimobranchial follicles (Fig. 10). Rats from the other groups did not have any changes in the thyroid.

Salivary gland: As with the thyroid, histologic lesions of the salivary gland were observed only in rats in group B at 28 days. There was early evidence of squamous metaplasia of larger ducts (Fig. 11). There were no histologic lesions observed in any of the rats in the other groups.

Lesions were not seen in histologic sections from eyelid, eye, trachea, brain, thymus, heart, lung, spleen, adrenal gland, kidney, stomach, intestine, pancreas, urinary bladder and testes.

Figure 2. Photomicrograph of a liver from a rat fed a vitamin A-deficient diet. Notice the normal lobular pattern with hepatic cords radiating from the central vein toward the portal triads, compact cytoplasm of hepatocytes and prominent sinusoids. (H & E stain, 450X.)

Figure 3. Photomicrograph of a liver from a rat fed a vitamin A-deficient diet and necropsied after 7 days of dietary treatment with 100 mg of PBB/kg. Notice swollen hepatocytes and numerous cytoplasmic vacuoles of various sizes in the central and midzonal areas. (H & E stain, 450X.)

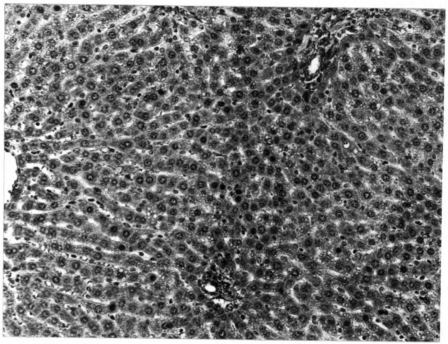

Figure 2

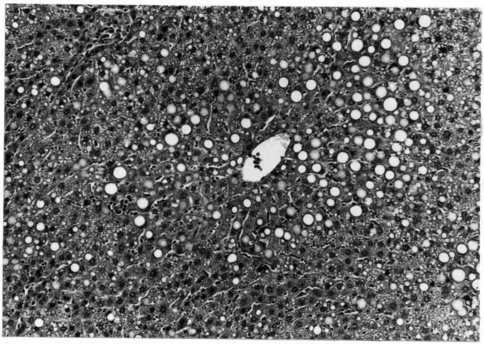

Figure 3

Figure 4. Photomicrograph of a liver from a rat fed a diet supplemented with 30,000 IU retinyl acetate/kg after 28 days of dietary treatment with 100 mg of PBB/kg. Notice an area of necrosis with accumulation of lymphocytes. (H & E stain, 450X.)

Figure 5. Photomicrograph of an extraparenchymal bile duct from a rat fed a diet supplemented with 30,000 IU retinyl acetate/kg after 28 days of dietary treatment with 100 mg of PBB/kg. Notice the normal columnar epithelial cells lining the duct. (H & E stain, 450X.)

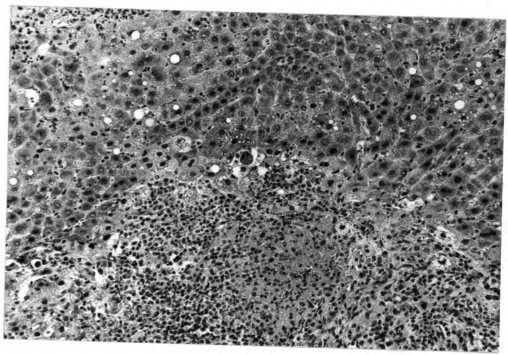

Figure 4

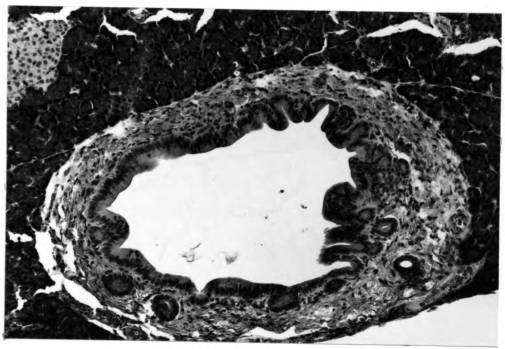

Figure 5

Figure 6. Photomicrograph of an extraparenchymal bile duct from a rat fed a vitamin A-deficient diet after 14 days of dietary treatment with 100 mg of PBB/kg. Notice the slight epithelial hyperplasia with pyknotic nuclei. (H & E stain, 450X.)

Figure 7. Photomicrograph of an extraparenchymal bile duct from a rat fed a vitamin A-deficient diet after 28 days of dietary treatment with 100 mg of PBB/kg to illustrate mild epithelial hyperplasia with pyknotic nuclei. Notice hyperplasia of the epithelial cells as compared to Figure 6. (H & E stain, 450X.)

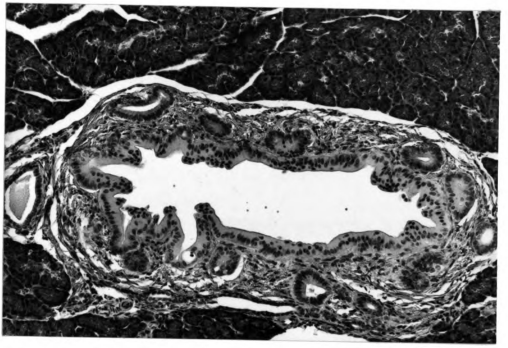

Figure 6

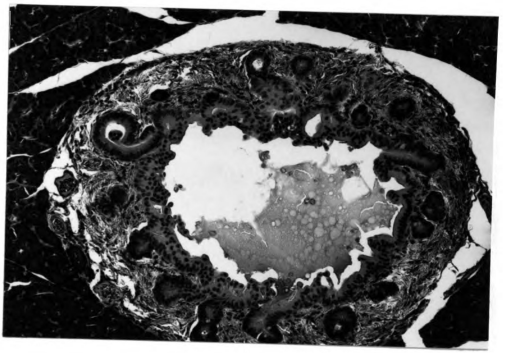

Figure 7

Figure 8. Photomicrograph of an extraparenchymal bile duct from a rat fed a vitamin A-deficient diet after 28 days of dietary treatment with 100 mg of PBB/kg to illustrate moderate epithelial hyperplasia with pyknotic nuclei. Notice hyperplasia of the epithelial cells as compared to Figures 6 and 7. (H & E stain, 450X.)

Figure 9. Photomicrograph of an extraparenchymal bile duct from a rat fed a vitamin A-deficient diet after 28 days of dietary treatment with 100 mg of PBB/kg to illustrate marked epithelial hyperplasia with pyknotic nuclei. Notice the papillary projections of the hyperplastic epithelial cells into the lumen of the duct. (H & E stain, 450X.)

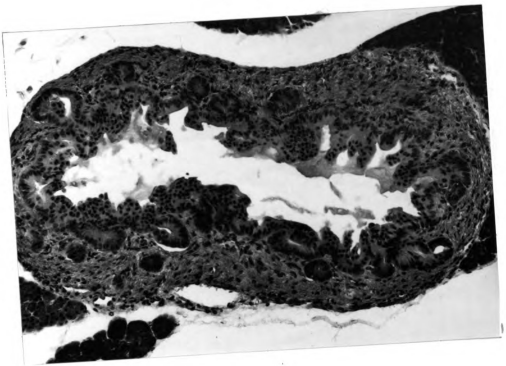

Figure 8

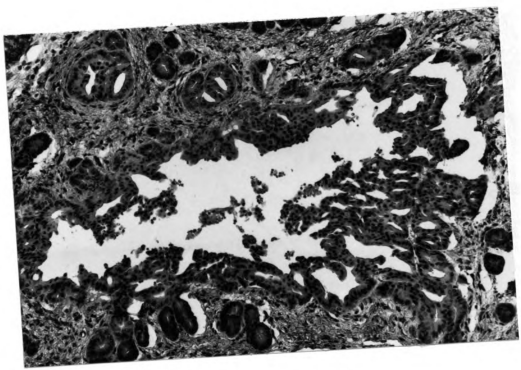

Figure 9

Figure 10. Photomicrograph of a thyroid gland from a rat fed a vitamin A-deficient diet after 28 days of dietary treatment with 100 mg of PBB/kg to illustrate early evidence of squamous metaplasia. Notice keratinization of the ultimobranchial follicles. (H & E stain, 576X.)

Figure 11. Photomicrograph of a salivary gland from a rat fed a vitamin A-deficient diet after 28 days of dietary treatment with 100 mg of PBB/kg to illustrate early evidence of squamous metaplasia. Notice squamous type of epithelial cells lining portions of the duct instead of the usual cuboidal or columnar type of epithelial cells. (H & E stain, 576X.)

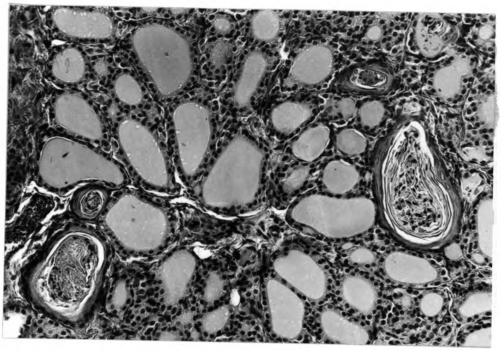

Figure 10

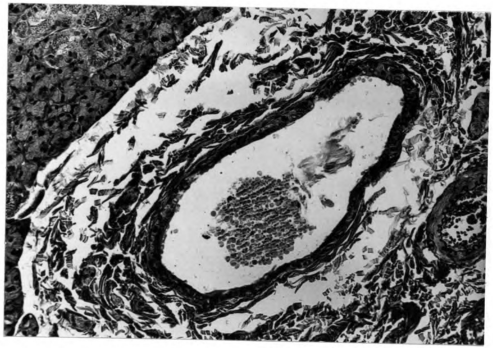

Figure 11

DISCUSSION

In most research on the effects of toxic chemicals in laboratory animals, the animals are fed a diet adequate in vitamin A. The results of many experiments have shown that vitamin A status has an important influence on the toxic effects of environmental contaminants (Innami et al., 1974; Darjono, 1982; Thunberg et al., 1983). In the studies noted above, dietary modifications were made when the experiments were started. Vitamin A deficiency is still a serious veterinary and public health problem (WHO, 1967; Srikantia, 1982). Therefore, people or animals are often depleted in vitamin A when exposed to chemicals such as PBB or related compounds. In the present study, an attempt was made to simulate natural exposure of animals to chemicals such as PBB when they are marginally deficient in vitamin A. This was done by feeding a vitamin A-deficient diet for 14 days prior to the administration of PBB.

Although administration of PBB accelerated the depletion of total vitamin A in the sera in rats fed a vitamin A-deficient diet (group B), neither weight loss nor clinical manifestations of vitamin A deficiency was observed during the time frame of the study. The lowest body weight was observed in rats in group B as compared to the other groups. By day 14 and thereafter, the weight gain of rats

in group B was significantly lower than for the other groups ($p<0.05$). It is possible that the combined effect of a vitamin A-deficient diet and PBB adversely affected the weight gain. Vitamin A deficiency impairs animals' ability to utilize food, especially protein (Hayes, 1971). PBB could cause defective absorption of nutrients from the gut based on the fact that tetrachlorodibenzo-p-dioxin (TCDD) was reported to impair active intestinal absorption of glucose and leucine in male Sprague-Dawley rats (Ball and Chhabra, 1981).

The gross and histologic effects of PBB on the liver were similar to those previously reported (Sleight and Sanger, 1976) and were not diminished by vitamin A supplementation. PBB increased the liver weight to body weight ratios ($p<0.05$) at 7, 14 or 28 days. Histologically, there were enlarged hepatocytes and cytoplasmic vacuoles. In general, a similar manifestation was seen in the thyroid weight to body weight ratios. Thyroid weight was increased in rats in groups B and D. Decreases in thymic weight to body weight ratios and histopathological changes in the thymus were not observed in this study. Darjono (1982) demonstrated that rats fed a vitamin A-deficient diet with 100 ppm PBB had the lowest thymic weight when compared to those fed diets containing either adequate or supplemented vitamin A and 100 ppm PBB. In Darjono's study, however, diets were fed until clinical signs of vitamin A deficiency and death losses occurred in rats fed vitamin A-deficient

diets containing 100 ppm PBB. It is likely that in his experiments, thymic involution was due mainly to vitamin A deficiency and PBB simply accelerated depletion in vitamin A.

In the present study, rats were fed diets either vitamin A-deficient or supplemented 14 days prior to experimental day 0. In rats fed a vitamin A-deficient diet (group A), the hepatic vitamin A concentrations decreased with time (Tables 8, 9 and 10). By day 28, when the hepatic vitamin A concentration was markedly depleted (2.3 µg/g), the vitamin A concentration in the serum was 231.3 ng/ml as compared to the serum vitamin A concentrations of 408.1 ng/ml on day 7 or 528.0 ng/ml on day 14. Therefore, serum vitamin A was still maintained during the period that liver vitamin A stores became severely depleted. Although hepatic vitamin A stores were nearly depleted, only 1 rat in group A had histologic lesions suggestive of vitamin A deficiency at 28 days.

In rats, a vitamin A-deficient diet should be supplemented with approximately 2000 IU vitamin A/kg to attain satisfactory weight gain, optimal longevity and hepatic vitamin A storage (Anonymous, National Academy of Sciences, 1972). Supplementation of the vitamin A-deficient diet with 30,000 IU retinyl acetate/kg (group C) markedly increased the hepatic vitamin A concentrations (from 37.2 µg/g to 1724.9 µg/g at 7 days, 19.8 µg/g to 1503.0 µg/g at 14 days and 2.3 µg/g to 2853.0 µg/g at 28 days). Hepatic

vitamin A concentrations were much higher than those reported by Darjono (1982). He reported a hepatic vitamin A concentration of 97.9 µg/g in rats fed a diet supplemented with 30,000 IU retinyl palmitate/kg for 66 days. The total serum vitamin A concentrations varied between the specified times: 7, 14 or 28 days. By day 7, the concentration of the serum vitamin A in group C (361.0 ng/ml) was nearly the same as in group A (408.1 ng/ml). However, by days 14 and 28, group C had higher serum vitamin A concentrations (836.1 ng/ml and 614.3 ng/ml, respectively) than group A (528.0 ng/ml and 231.3 ng/ml, respectively). Serum vitamin A concentrations in group C at 14 or 28 days were also higher than those reported by Darjono (1982). He reported a serum vitamin A concentration of 456.0 ng/ml in rats fed a diet supplemented with 30,000 IU retinyl palmitate/kg for 66 days. Supplementation of the vitamin A-deficient diet with retinyl acetate was apparently more effective in maintaining vitamin A concentrations when compared to dietary supplementation with retinyl palmitate. It is possible that the retinyl acetate used in this experiment was more stable than the retinyl palmitate used by Darjono. It is also possible that retinyl acetate is more easily absorbed when compared to retinyl palmitate. Darjono's rats were somewhat older but this would still not account for any major differences in vitamin A storage or utilization.

PBB caused significant decreases in total liver vitamin A (mg/whole liver) only in the vitamin A-supplemented rats

at 14 and 28 days. Chemicals such as PBB commonly increase liver size. Values calculated on a μg/g basis may not be an accurate indication of total liver vitamin A. For example, rats in certain groups had a decreased concentration of hepatic vitamin A, but their total hepatic vitamin A was no lower than in the control livers. Therefore, in evaluating hepatic vitamin A associated with exposure to PBB, the total hepatic vitamin A (mg/whole liver) must be considered in addition to the concentration per unit of weight.

In the present study, the decrease of the hepatic vitamin A concentrations was due to the decrease of retinyl palmitate concentrations. The retinyl palmitate concentrations were nearly totally depleted in rats fed a vitamin A-deficient diet (group A). Addition of PBB to diets of rats fed a vitamin A-deficient diet (group B) resulted in the appearance of significant amounts of retinyl acetate in the livers (from 0.0 to 3.1 μg/g at 7 days, 0.0 to 7.5 μg/g at 14 days and 1.1 to 7.8 μg/g at 28 days). Similar effects were observed by Darjono (1982). As a result, by day 28, the total hepatic vitamin A (mg/whole liver) in group B was significantly higher ($p<0.05$) than in group A. Retinyl acetate was not detected in rats fed a diet supplemented with vitamin A and containing PBB (group D). In the normal rat liver, there is primarily retinol and retinyl palmitate which concentrates mainly in the fat storing cells. Ninety-five per cent of the total vitamin A is stored in the liver, predominantly in the form of retinyl

palmitate (De Leeuw et al., 1983). Since the source of retinol for peripheral target tissues is from the liver via hydrolysis of retinyl palmitate, it is possible that an increased turnover of hepatic retinyl palmitate may explain the mechanism of PBB-induced vitamin A deficiency. PBB may alter reesterification of retinyl esters in the liver and lead to an accumulation of retinyl acetate. The effects of PBB on vitamin A metabolite concentrations could occur at either the intestinal or hepatic levels. More likely, the action of PBB on hepatocytes causes an increased production of the esterification enzyme which favors retinyl acetate production. Simultaneously, there could also be a depressing effect upon hepatocytes which favor the enzyme associated with formation of retinyl palmitate. Whether or not the accumulation of retinyl acetate in the liver is harmful to hepatocytes is unknown. Additional research, however, is needed to elucidate the importance of retinyl acetate in PBB exposure.

PBB accelerated depletion of serum vitamin A concentrations in rats in group B to the extent that histologic lesions of vitamin A deficiency developed before noticeable clinical signs of vitamin A deficiency occurred. Rats in group B (3 of 6 rats at 14 days and all rats at 28 days) had histologic lesions suggestive or typical for vitamin A deficiency. The histologic lesions consisted of early hyperplasia of the bile ducts and early evidence of squamous metaplasia of the ultimobranchial follicles in the

thyroid gland and of larger ducts of the salivary gland. Since rats from the other groups had no lesions in the bile ducts, thyroid or salivary glands, the lesions in rats in group B are likely related to the interaction between PBB toxicosis and vitamin A deficiency.

The results are very important to those in human and animal health because they provide further evidence of the ability of PBB or related compounds to accelerate the depletion of vitamin A. Therefore, such chemicals can induce histologic lesions and clinical signs suggestive of vitamin A deficiency. These effects could be more rapid and severe in vitamin A-deficient animals or people.

SUMMARY

Seventy-two male Sprague-Dawley rats, weighing approximately 60 g at 21 days old were allotted to 4 groups of 18 each. Two weeks prior to the beginning of the experiment, rats in 2 groups (A and B) were fed a vitamin A-deficient diet whereas the remaining 2 groups (C and D) were fed a diet supplemented with 30,000 IU retinyl acetate/kg. Starting on day 0, rats were given either 0 (groups A and C) or 100 mg of PBB/kg of diet (groups B and D). Six rats in each group were killed at days 7, 14 and 28, respectively.

There were no clinical signs of either PBB toxicosis or vitamin A deficiency observed during the experiment. Feed intake was relatively normal. However, rats fed a vitamin A-deficient diet containing 100 mg of PBB/kg had the lowest body weight when the experiment was terminated. Weight gain was significantly depressed in this group.

Liver weights were greatly increased in rats fed a diet containing 100 mg of PBB/kg (groups B and D). Histologically, there were enlarged hepatocytes and cytoplasmic vacuolation. The gross and histologic effects of PBB on the liver were not diminished by vitamin A supplementation. Thyroid weight was increased in rats in groups B and D as compared to rats in groups A and C.

Thymic weight was not affected by PBB toxicosis or by vitamin A deficiency in the present study.

Three of six rats fed a vitamin A-deficient diet containing PBB for 14 days had slight hyperplasia of the extraparenchymal bile ducts. By 28 days, the vitamin A-deficient diet containing PBB induced mild to marked hyperplasia of the epithelial cells lining the extraparenchymal bile ducts. Evidence of early squamous metaplasia was seen in the ultimobranchial follicles of the thyroid gland and the larger ducts of the salivary gland.

Analysis of the serum and hepatic vitamin A indicated that rats fed a vitamin A-deficient diet containing PBB (group B) had marked depletion in the retinol concentration in the serum on day 28 and in the retinyl palmitate concentration in the liver on days 14 and 28. Group B also had an abnormal vitamin A profile in the liver manifested by the appearance of significant amounts of retinyl acetate on days 7, 14 or 28 days. This disturbance of vitamin A metabolism in the liver may be related to an alteration in the reesterification of retinyl palmitate.

This experiment has shown that dietary levels of vitamin A have an important influence on effects of exposure to PBB. The combined effect of a high dose of PBB and low vitamin A in the diet greatly accelerated the depletion of serum and hepatic vitamin A concentrations and therefore induced squamous metaplasia of the thyroid and salivary glands and hyperplasia of the bile ducts.

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VITA

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