This is to certify that the

thesis entitled
I. Pathologic Effects of Polybrominated Biphenyls in Rats Fed a Diet Containing Excessive Iodine.
II. Pathologic Changes in Calves after Oral Administration of Excessive Iodine for Six Months.

presented by

Soesanto Mangkoewidjojo

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Pathology

Stuart D Sleight
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Date February 22, 1979

I. PATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS IN RATS FED A DIET CONTAINING EXCESSIVE IODINE

II. PATHOLOGIC CHANGES IN CALVES AFTER ORAL ADMINISTRATION OF EXCESSIVE IODIDE FOR SIX MONTHS

By

Soerakso Nakhasathien

A DISSERTATION

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

DOCTOR OF PHILOSOPHY

Department of Pathology

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ABSTRACT

I. PATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS IN RATS FED A DIET CONTAINING EXCESSIVE IODINE

II. PATHOLOGIC CHANGES IN CALVES AFTER ORAL ADMINISTRATION OF EXCESSIVE IODINE FOR SIX MONTHS

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The pathologic effects of excessive iodine in calves and excessive iodine with or without polybrominated biphenyls (PBB) in rats were investigated.

Ninety-six weanling male Sprague-Dawley rats were used. Four groups of 12 each were fed an iodine adequate diet (IAD) containing 0.2 ppm iodine and 0, 1, 10, or 100 ppm PBB. The remaining 48 rats were similarly divided into 4 groups and fed an iodine excess diet (IED) containing 1,000 ppm iodine and 0, 1, 10, or 100 ppm PBB. Six rats in each group were killed at 30 days and the remainder at 60 days.

Rats fed IAD containing 100 ppm PBB had a marked decrease in rate of weight gain after 45 days. Otherwise, no significant clinical signs were observed.

Liver weight and hepatic cytochrome P_{450} increased at 10 or 100 ppm PBB. There was no significant effect of IED on liver weight or cytochrome P_{450}. Hepatic lesions were especially prominent in rats given 10 or 100 ppm PBB and IED for 60 days. The hepatocytes were swollen and vacuolated. Reticuloendothelial cell hyperplasia occurred

in the liver of rats fed IAD or IED containing 100 ppm PBB. Ultrastructural changes occurred in hepatocytes of rats fed 10 ppm PBB regardless of the level of iodine. Smooth endoplasmic reticulum (SER) was proliferated and rough endoplasmic reticulum (RER) was decreased and dispersed. Mitochondria were reduced in number, and many vacuoles were present. The PBB at 100 ppm produced similar but more pronounced changes. Multilaminated myelin figures were frequently seen.

Feeding IED containing 100 ppm PBB for 60 days produced the most prominent lesions in the thyroid glands. Epithelium was hyperplastic and columnar and colloid was depleted. Ultrastructural changes consisted of an increased number of dense granules in many follicular cells, fewer and shorter microvilli, and dilatation of cisternae.

Rats fed IED containing 100 ppm PBB had squamous metaplasia of respiratory bronchioles and alveoli. Rats fed IED without PBB had squamous metaplasia in the salivary glands, but the liver and thyroid were essentially normal.

The PBB significantly affected serum protein fractions and increased serum cholesterol and liver vitamin A. Generally, concentrations of PBB in tissues were dose related and were highest in fat tissues.

Forty Holstein heifer calves each weighing 250 kg were divided into 4 groups of 10 each and dosed orally with ethylenediamine dihydriodide to provide 0, 50, 250 or 1250 mg iodine/head/day. Five calves of each group were given an intravenous injection of thyrotropin releasing hormone (TRH) at 4-week intervals. Two calves given 1250 mg iodine died by 10 weeks and had severe bronchopneumonia, keratitis, and dermatitis. At 6 months the remaining 38 calves were killed. Bronchopneumonia was evident in calves given iodine with severity

apparently dose related. *Pasteurella multocida* was isolated from pneumonic lungs. Squamous metaplasia of tracheal epithelium occurred in calves given 1250 mg iodine/day. Similar changes were seen in the interlobular ducts of the parotid glands of several calves. Scanning electron microscopy revealed a dose-related disruption of tracheal epithelium.

Thyroid glands of calves given 1250 mg iodine had enlarged follicles and flattened epithelium. Colloid was abundant. Thyroid glands were essentially normal in calves given 1250 mg iodine and TRH. Serum vitamin A but not carotene was depressed in calves given high doses of iodine.

Results indicated that rats and cattle can compensate for excessive iodine. Excessive iodine increased severity of respiratory lesions in cattle and lesions associated with PBB in rats. Reduction of vitamin A associated with excessive iodine or PBB and goitrogenic effects of PBB may have important clinical implications.

DEDICATION

Dedicated with love to my parents

Mr. and Mrs. Mangkoewidjojo

ACKNOWLEDGEMENTS

The author has special pleasure in acknowledging with sincere gratitude Dr. S. D. Sleight, a truly inspiring major professor, whose attitude, understanding, friendship, and indispensable help made this investigation a very rewarding and delightful experience.

Thanks and appreciation are due to the guidance committee of Drs. G. L. Waxler, V. L. Sanger, and E. M. Convey for their impressive cooperation and especially for their advice and help with the dissertation.

The unending assistance and personal interest of Dr. C. K. Whitehair, and the generous consultation of Dr. R. F. Langham, helped the author maintain his enthusiasm during his graduate program.

Full appreciation goes to Drs. Budi Tri Akoso, Pedro Werner, Araquem Telles, Howard Stowe, and Manley Pratt for their valuable assistance during the course of the experiment.

The author is also indebted to Dr. Dale Haggard, Kwan-Yee Leung, M.S., Frank Vicini and Alice Marczewski for their cooperation in this investigation, and to Drs. R. R. Neitzel and J. L. Gill for their help in statistical evaluation.

Shirley Howard, Esther Roege and Betty L. Schoepke are very special people who helped with the laboratory work. Without their skillful and untiring assistance, this investigation would not have

been completed. Valuable assistance was also received from personnel in the histopathology laboratory.

The author is indebted to the administrative staff in the Department of Pathology for their kind cooperation and help in innumerable ways, and to Janice Fuller for the typing of this dissertation.

Thanks go to Dr. R. W. Leader, Chairman of the Department of Pathology, for granting me the opportunity to have graduate training in this department.

The author wishes to express his appreciation to the personnel of Midwest Universities Consortium for International Activities - Agency for International Development - Indonesian Higher Agriculture Education Project, for sponsoring his graduate program.

Finally, the author wishes to acknowledge his deep appreciation to his parents, wife, sons and daughter for their understanding, encouragement, patience and many sacrifices during his overseas training.

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INTRODUCTION

Iodine is one of the trace elements required for normal biological processes in man and animals. Consequently, only a small amount of iodine is needed for supplementation in iodine deficient areas such as the State of Michigan. In veterinary medicine, ethylenediamine dihydriodide (EDDI) has been recommended for prevention and treatment of foot rot and lumpy jaw in cattle, as an expectorant, and as a feed supplement. The apparent beneficial effects of iodine have stimulated feed manufacturers to add iodine to feed supplements. Farmers have in some instances fed excessive iodine to their cattle (McCauley et al., 1972; Wallace, 1975).

Results of a survey in Michigan (Hillman et al., 1976) suggested that health problems were associated with high iodine intake. Other observations indicated that excessive iodine in milk may pose a public health hazard (Lengemann and Comar, 1964; Hillman et al., 1976). Different opinions have been expressed as to whether excessive iodine causes any significant adverse effects in cattle (Herrick, 1972; McCauley and Johnson, 1972). Research on the pathologic effects of excessive iodine intake in calves was especially appropriate because of the current interest in this problem and because of relatively little work in this area.

Contamination with polybrominated biphenyls (PBB) occurred in Michigan in 1973 and involved thousands of cattle and other farm

animals (Carter, 1976). The PBB tragedy has become the largest agricultural disaster in the United States history (Riegle, 1977; Ball, 1977). Further observations indicated that approximately 9 million people in the State of Michigan have PBB in their body.

Since PBB are newly introduced toxic substances into the biological system, there are considerable unanswered questions related to PBB toxicosis in man and animals. Obviously, further research on PBB toxicosis is essential.

Poor growth, decreased milk production, reproductive problems, dermatitis, and abnormal growth of hooves were described in the initial report of PBB contaminated cattle (Jackson and Halbert, 1974). Because signs of PBB toxicosis appeared to be non-specific, there were questions as to whether other toxic chemicals were involved. The combined effects of PBB and iodine toxicosis in cattle appeared to be a possibility.

It was decided to conduct two separate experiments. Experiment I emphasized the effects of feeding rats different levels of PBB added to either an iodine adequate diet (IAD) or an iodine excess diet (IED). Clinical signs, chemical, hematologic, gross, microscopic and electron microscopic changes were evaluated. Experiment II was a portion of a cooperative research project on the effects of chronic feeding of excessive iodine to calves. The gross, microscopic and electron microscopic features were described.

Objectives of the research were:

1. To further evaluate the pathologic effects of excessive iodine in rats.
2. To further evaluate the pathologic effects of PBB in rats.

3. To assess the combined effects of PBB and iodine in rats.
4. To further characterize the pathologic effects of excessive iodine in calves.

LITERATURE REVIEW

A. Nutritional Aspects of Iodine in Nonruminants

Elmer (1938) reviewed the fate of iodine after oral administration. Iodine was found to be absorbed by mucous membranes of the mouth, stomach, [illegible] administered orally to dogs [illegible] Quick [illegible] cited another study that indicated that free iodine was absorbed by the stomach after conversion to KI. Absorption of iodine in the intestine depended upon the form of iodine, but in general its absorption from the large intestine was [illegible] small intestine. Free iodine was more easily [illegible] following absorption, iodine was distributed [illegible] and the thyroid gland had the highest concentration. [illegible] in rats (Leblond, 1954) had the second highest iodine content.

Studies revealed that 24 hours after parenteral administration of physiological amounts of radioiodine to rats, 75.8% of ^{131}I was found in the urine and less than 3% was found in the feces (Johnson and Albert, 1951). The thyroid and gastrointestinal tract contained the greatest amount of ^{131}I. Another experiment in lactating rats indicated that 24 hours after injection of ^{131}I, 15 to 49% of the radioiodine was found in the mammary gland and the thyroid gland had no more than 2% (Potter et al., 1959). In nonlactating rats, 6 to 8% of ^{131}I was found in the thyroid gland. They concluded that the

PART I

PATHOLOGIC EFFECTS OF POLYBROMINATED BIPHENYLS IN RATS FED A DIET CONTAINING EXCESSIVE IODINE

LITERATURE REVIEW

A. Nutritional Aspects of Iodine in Nonruminants

Elmer (1938) reviewed the fate of iodine after oral administration. Iodine was found to be absorbed by mucous membranes of the mouth, stomach and intestine. Nearly 31% of potassium iodide administered orally to dogs was absorbed within 2 hours. He cited another study that indicated that free iodine was absorbed by the stomach after conversion to KI. Absorption of iodine by the intestine depended upon the form of iodine, but in general the absorption from the large intestine was less than from the small intestine. Free iodine was more easily absorbed than KI. Following absorption, iodine was distributed throughout the body, and the thyroid gland had the highest concentration. Skin (Elmer, 1938) or hair (Leblond, 1954) had the second highest iodine content.

Studies revealed that 48 hours after parenteral administration of physiological amounts of radioiodine to rats, 75.8% of ^{131}I was found in the urine and less than 3% was found in the feces (Johnson and Albert, 1951). The thyroid and gastrointestinal tract contained the greatest amount of ^{131}I. Another experiment in lactating rats indicated that 24 hours after injection of ^{131}I, 15 to 49% of the radioiodine was found in the mammary gland and the thyroid gland had no more than 2% (Potter et al., 1959). In nonlactating rats, 6 to 8% of ^{131}I was found in the thyroid gland. They concluded that the

mammary gland had a great ability to concentrate circulating iodine.

Besides urine, feces and milk, elimination of iodine occurred through skin, lung, placenta and salivary gland (Elmer, 1938).

A sufficient amount of iodine in the thyroid gland is necessary for normal production of thyroid hormones, and active transport of iodine is an important action preceding the synthesis of thyroid hormones. The active transport of iodine from a low serum concentration to a high intrathyroidal concentration occurs by an energy-dependent mechanism (Selenkow et al., 1965). The mechanism can be inhibited by perchlorate and stimulated by a thyroid-pituitary feedback mechanism (Degroot and Niepomniszcze, 1977). The iodination of thyroglobulin requires H_2O_2 supplied by microsomal NADPH-cytochrome c reductase or NADH-cytochrome b_5. Electron microscopic observations indicated that iodination of thyroglobulin occurs in the apical surface of the follicular cells (Ekholm and Wollman, 1975). In the normal condition, iodine stimulates synthesis of thyroid hormones when levels of intrathyroidal iodine are low and inhibits synthesis when thyroidal iodine exceeds the maximal level (Selenkow et al., 1965).

Thyroid hormones influence growth and development and regulate metabolism of lipid, carbohydrate, protein and vitamins, as well as calorigenesis (Bernal and Refetoff, 1977). Thyroid affects bone by stimulating all phases of development. This effect is more pronounced in the presence of endogenous parathyroid hormone (Bomme et al., 1975). Administration of thyroid hormones increases oxygen consumption and heat production and accelerates carbohydrate, protein and lipid metabolism (Bernal and Refetoff, 1977).

B. Iodine Toxicosis in Rats

1. Pathophysiology

Most, if not all, of the reports on the effects of excessive iodine in rats were experimental. The effects appeared to be related to the length of the exposure.

Acute antithyroid effects of excessive iodine were shown by Wolff and Chaikoff (1948a) and were then known as the Wolff and Chaikoff phenomena. A single injection of 200 γ of ^{131}I as potassium iodide to normal rats caused inhibition of inorganic binding of iodine by the thyroid for approximately 12 hours. The temporary inhibition was assumed to be related to the concentration of iodine in the plasma. When the plasma iodine fell to a concentration of 15 to 20 γ% the organic binding was resumed. The temporary nature of inhibition could be maintained up to 26 hours if the administration of iodine were continued (Wolff et al., 1949). The period of inhibition could be prolonged if the excretion of iodine through the kidney were impaired (Wolff and Chaikoff, 1948b). A recent report indicated that high iodine levels occurred in rats with renal failure (Robertson et al., 1977). These workers also described enlargement of the thyroid glands of their rats, and they suggested that iodine retention was responsible. However, they pointed out that these findings may not be directly applicable to man because of species differences and because the degree as well as the duration of renal failure is shorter in rats than is commonly seen in man.

Other workers indicated that the adaptation to large doses of iodine was associated with an intrinsic thyroidal mechanism rather than the concentration of iodine in the plasma per se (Braverman and

Ingbar, 1963). *In vitro* studies (Van Sande et al., 1975) suggested that high doses of iodine inhibited cyclic AMP accumulation in the presence of TSH in large animals.

Adaptation to the effects of long-term administration of excessive amounts of iodine to rats was studied by Galton and Pitt-Rivers (1959). Treatment of rats with excessive iodine resulted in suppression of ^{131}I binding in the thyroid gland within 3 to 4 days. Further observations indicated that the rats had little or no evidence of hypothyroidism. An intrathyroidal mechanism related to the reduced organic binding of iodine was proposed by Braverman and Ingbar (1963). Thyroid iodide capacity was reduced in rats fed excessive iodine despite increasing doses of iodine.

The possible role of TSH in the mechanism of escape from temporary inhibition of iodine in the thyroid gland was studied by Liewendahl et al. (1972). They suggested that TSH concentration might be important in the adaptation process since they observed that excessive iodine increased TSH in the pituitary gland. However, Sinadinovic (1976) reported that significant increases of proteolytic activity were found in the thyroid gland of rats exposed to long-term excessive iodine. These rats were able to adapt to excessive iodine without altered TSH secretion. This observation was in line with increased proteolytic activity in the colloid droplets-lysosome fraction found in the iodide-treated rats (Itikawa and Kawada, 1974).

Ingbar (1972) stated that the mechanisms for temporary effects of acute administration of iodine or the adaptive mechanism to prolonged large doses of iodine are still obscure. However, he stressed the importance of the dehalogenation of iodotyrosine and

the renal excretion of iodine. If those mechanisms failed, iodide depressed iodination in the thyroid and myxedema sometimes developed (Degroot and Niepomniszcze, 1977).

2. Pathologic Changes

Most reports on iodine toxicosis in rats were related to thyroid changes. Slight increases in follicular size with minimal changes in thyroid cell height were reported in rats fed excessive iodine for 7 weeks (Galton and Pitt-Rivers, 1959). They claimed that the thyroid of rats responded differently than the thyroid of the chicken. Another study using rats fed 0.119 g iodide/head/day for 9 months resulted in goiter but did not produce hypothyroidism or myxedema (Correa and Welsh, 1960). Histologic examination revealed that most of the follicles were enlarged with excessive colloid accumulation. The epithelial lining cells were flattened. The larger follicles appeared to result from fusion of the adjacent follicles.

The effects of excessive iodine on the structure and function of the thyroid gland were studied by Liewendahl et al. (1972). Male rats fed 20 to 30 mg iodine/head/day for 4 months had statistically significant increases in thyroid weight to body weight ratios. The treated rats gained weight similarly to the controls, but 3 rats died in the course of the experiment without having gross pathologic changes except for gastroenteritis. Histologically, the enlarged thyroid glands did not differ appreciably from the controls. There was a slight tendency towards increased serum thyroxine and decreased free thyroxine formation (Liewendahl et al., 1972).

Excessive iodine was reported to increase mortality of neonatal pups (Ammerman et al., 1964). Milk secretion was decreased or absent.

However, there were no effects on the reproductive performance in male rats. Eklund et al. (1973) reported nephrocalcinosis in rats fed casein containing high amounts of iodide. Gestation time for rats was not affected by iodide but prolonged parturition occurred (Arrington et al., 1965).

C. General Characteristics of Polybrominated Biphenyls

Firemaster BP-6 was manufactured for commercial purposes as a fire retardant in thermoplastic industries (Hoffman, 1977). The PBB were produced by direct bromination of biphenyls resulting in a mixture of at least 18 different compounds because of differences in number and position of bromine atoms on the molecules (Sundstrom et al., 1976; Jacobs et al., 1976). The isomers consisted of tetrabromo- (2.0%), pentabromo- (10.6%), hexabromo- (62.8%) and heptabromobiphenyls (13.2%). The remaining isomers were unidentified (Gutenmann and Lisk, 1975). An unconfirmed report indicated that Firemaster BP-6 contained methylpolybrominated furans (Kay, 1977). A chemically related compound, chlorinated dibenzofuran, which is teratogenic (Corbett et al., 1975), was isolated from polychlorinated biphenyls (PCB) manufactured in Japan (Roach and Pomerantz, 1974). Analyses indicated that Firemaster BP-6 contained approximately 150 ppm pentabromonaphthalene and 70 ppm hexabromonaphthalene (Hass et al., 1977). A similar compound, chlorinated naphthalene, has been well recognized as a cause of hyperkeratosis in cattle (McEntee and Olafson, 1963).

Unlike PCB, the chemical characteristics and stability of PBB are not well known. The PBB are recognized as solid substances (Kolbye, 1977), not soluble in water (11 ppb), but highly soluble in

organic solvents, and nonvolatile (Dunckel, 1975; Jacobs et al., 1976). Additionally, PBB will melt at about 72 C.

In 2% KOH in ethanol, PBB undergo degradation to hexabromobiphenyls, a major component of PBB (Kolbye, 1977). Sunlight was thought to play an important role in nonbiologic degradation of PBB (Ruzo et al., 1976). Under ultraviolet irradiation, PBB degraded 7 times faster than PCB (Ruzo and Zabic, 1975), and heating at 300 to 700 C caused PBB to disintegrate (Kay, 1977). In the ground, PBB eventually undergo oxidative or biological degradation to carbon dioxide, water and bromine ions (Kolbye, 1977).

Laboratory experiments on the fate of PBB in the soil provided evidence that PBB leached very slowly (Filinow et al., 1976). Additionally these workers stated that soil from farms contaminated with PBB in Michigan had PBB concentrations of much less than 0.1 ppm. No PBB were detected in orchard grass grown in soil treated with 100 ppm of PBB. From these studies Jacobs et al. (1976) concluded that plants will not cause serious problems on PBB contaminated farms. However, the persistence of PBB in the soil was considered a source of PBB contamination in the environment (Dunckel, 1975; Kolbye, 1977; Fine, 1976).

D. The Fate of Polybrominated Biphenyls in the Body

Following ingestion, PBB are absorbed from the intestinal tract and carried by the lymphatic system throughout the body (Kolbye, 1977). Since PBB are fat soluble, they tend to accumulate more in the body fat than in other tissues. This simplified pathway was confirmed by a study using radiolabeled 2,4,5,2',2',5' hexabromobiphenyls-^{14}C in rats (Matthews et al., 1977). The PBB were readily absorbed

from the gut since only 7.9% of the dose was excreted within the first 24 hours after a single oral dose of 1 mg/kg. When the same dose was injected intravenously, 0.96% was excreted. From the data they calculated that 93% of PBB were absorbed in the gut, and excretion was almost exclusively through the feces. No radioactivity was detected in the urine. Small amounts of PBB were excreted through the bile, but they were eventually reabsorbed from the gut. Further analyses from extracted tissues convinced these workers that no radioactive compound other than the initial PBB was found, indicating that metabolism of PBB was unlikely.

In cattle, PBB were also easily absorbed from the gastrointestinal tract. After a single intraruminal dose of 6 mg/kg, PBB appeared in the plasma, milk and feces at 6, 13, and 19 hours, respectively. In nonlactating cows, PBB appeared in the plasma and feces at 2 and 13 hours (Willett and Irving, 1975). When cows were fed 10 mg/day of PBB, milk attained a constant concentration of PBB within 30 days (Fries and Marrow, 1975). When the treatment was stopped, the concentration of PBB in the milk decreased 71% in the first 15 days. In addition to the feces as the major route of excretion and urine as the minor one, considerable amounts of PBB were excreted through the milk (Willett and Irving, 1975; Gutenmann and Lisk, 1975).

Results of studies using 20 ppm of PBB fed to White Leghorn hens provided evidence of retention of PBB in the body fat. Elimination of PBB through the eggs was more important than through excreta (Fries et al., 1976). A similar excretion pattern was found in chickens fed PCB (Combs and Scott, 1977).

Analyses of tissues from chronically contaminated cows revealed that PBB were in almost all the body organs. Fat had the highest

concentration, blood had the least, and the brain had the second least concentration of PBB (Fries et al., 1975). Calves which were born dead from PBB-contaminated cows contained 120 to 204 ppm of PBB in their body fat (Detering et al., 1975).

E. Polybrominated Biphenyl Toxicosis

1. History

In 1970, the Michigan Chemical Company started to synthesize polybrominated biphenyls for use as a flame retardant in thermoplastic industries. The product was trade-named "Firemaster BP-6" and was a mixture of polybrominated biphenyls. Approximately 63% were hexabromobiphenyls and the remainder was a mixture of lower or higher brominated biphenyls. From November 1971 to July 1972, the Michigan Chemical Company sent some polybrominated biphenyls to the Cincinnati Chemical Company for pulverization under the name "Firemaster FF-1." The product was packaged in 50-pound bags with "Firemaster FF-1" stenciled on each bag. The Michigan Chemical Company also manufactured and distributed magnesium oxide for use as a feed additive in dairy herds. The magnesium oxide had the trade name "Nutrimaster" (Hoffman, 1977). This product was granulated and similar in appearance to Firemaster FF-1 (Bernstein, 1976).

Early in 1973, a paper shortage led to the Michigan Chemical Company's marketing Firemaster FF-1 in plain brown bags similar to those used for Nutrimaster except for the trade name Firemaster. Ordinarily, PBB had been packaged in preprinted red bags, whereas the brown bags were used to package magnesium oxide (Dunckel, 1975).

In May 1973, 500 to 1000 pounds of Firemaster were erroneously included in a shipment of magnesium oxide (Nutrimaster) to Farm

Bureau Services in Battle Creek, Michigan, and to other Farm Bureau mills (Carter, 1976). In late summer of 1973 a dairy operator near Battle Creek complained of severe problems in his herd that probably were related to feed contamination (Jackson and Halbert, 1974). Samples of feed were sent to the National Animal Disease Center in Ames, Iowa, and showed unusual peaks on the chromatograph (Jackson and Halbert, 1974). On April 26, 1974, a United States Department of Agriculture investigator identified PBB in the feed from the contaminated farm based on mass spectrophotometry and chromatography, and on April 30, 1974, Food and Drug Administration personnel from the Detroit District discovered Firemaster FF-1 packaged in a brown stenciled bag in a feed mill (Kolbye, 1977).

Legal actions were taken to minimize human exposure to PBB. In May 1974, the FDA established a guideline of 1 ppm of PBB on a fat basis in milk and milk products, 0.3 ppm of PBB in animal feed, and 1 ppm in meat. Based on the available data on the toxicology of PBB and PCB, as well as on the improvement of the capability of chemical analyses, the PBB action levels were lowered from 1 ppm in milk and meat, on a fat basis, to 0.3 ppm, from 0.3 ppm to 0.005 ppm in animal feed, and from 0.1 ppm to 0.005 ppm in whole eggs. These actions were taken in November 1974 (Kolbye, 1977). In 1977 the State of Michigan set a guideline of 0.02 ppm on a fat basis for meat and 0.005 ppm for milk.

2. Polybrominated Biphenyl Toxicosis in Rats

a. Signs. Polybrominated biphenyls were potent inducers of microsomal drug metabolizing enzymes, even at very low levels in the diet (Troisi, 1975). The effects lasted for a considerable time

after the PBB were withdrawn. The toxic effects of PBB appeared to depend primarily on their ability to stimulate or inhibit various enzyme systems in the animal body (Kolbye, 1977). The PBB are considered of low acute toxicity, with an LD_{50} of 21.5 g/kg for Firemaster BP-6 in the rat (Kolbye, 1977). However, in some respects PBB are 5 times more active than PCB (Carter, 1976).

The congeners of PBB that are responsible for toxicity are not known. Single oral doses of octabromobiphenyls (OBB) of 1,000 mg/kg did not cause any changes in food consumption or weight gain (Lee et al., 1975a). Similar results related to weight gain and feed efficiency were observed when 25 or 150 mg/kg of PBB were given as a single intraperitoneal injection (Dent et al., 1976a). Food consumption and growth rate were also not affected by feeding PBB for 2 weeks at 5 to 300 ppm (Dent et al., 1976b). There were also no clinical abnormalities in rats fed 1,000 ppm or lower of OBB for 4 weeks (Lee et al., 1975b). On the other hand, Sleight and Sanger (1976) reported a decrease in weight gain and feed efficiency in rats fed 500 ppm of PBB for 30 days. The same effect was also reported in rats fed 500 ppm of hexabromobiphenyls (HBB), the major component of PBB (Farber and Baker, 1974). There were no effects on the survival of the rats reported in these experiments.

Studies in pregnant rats fed 100 µg of PBB/g body weight beginning on the 6th day of pregnancy provided evidence that PBB were nonteratogenic (Ficsor and Wertz, 1976). However, when pregnant rats were given 1,000 or 10,000 ppm of OBB from days 6 through 15 of pregnancy, anasarca and gastroschisis were found in some of the fetuses (Aftosmis et al., 1972a), suggesting that OBB were weak teratogens.

b. Pathologic changes. Morphologic and enzymatic changes in the liver were most commonly reported in PBB toxicosis in rats. Liver weight to body weight ratios were significantly increased in rats chronically fed 1 ppm of PBB or more (Sleight and Sanger, 1976; Dent et al., 1976a,b). Dose-dependent decreases in fetal weight were reported in pregnant rats fed 100 or 1,000 ppm of PBB (Corbett et al., 1975). In addition to hepatic changes, enlargement and petechial hemorrhages of the kidney and thyroid hyperplasia were reported in rats fed OBB (Norris et al., 1975).

Hepatomegaly could also be produced in rats that inhaled fumes caused by heating OBB to 290 C (Aftosmis et al., 1972b). Thyroid hyperplasia was also reported in rats fed 8 mg/kg/day of OBB for 30 days (Norris et al., 1975) or in rats administered 1.0 g/kg of 2,2-bis (2-chlorophenyl,4-chlorophenyl)-1,1-dichloroethane (o,p'-DDD) (Fregly et al., 1968).

Microscopically, hepatocytes were enlarged and vacuolated (Sleight and Sanger, 1976; Aftosmis et al., 1972b). These changes had been reported in rats fed PCB (Kimbrough, 1972). In addition to hepatocellular hypertrophy, cytoplasmic inclusions and margination of cytoplasm were observed under light microscopy in the hepatic cells of rats fed OBB (Lee et al., 1975a). Additionally, hyaline degeneration was found in the kidney of rats fed OBB (Norris et al., 1975).

Electron microscopic observations of livers of rats or mice given PBB disclosed proliferation of smooth endoplasmic reticulum, decreased rough endoplasmic reticulum, detached ribosomes and reduced mitochondrial cristae (Sleight and Sanger, 1976; Kimbrough et al., 1978; Corbett et al., 1978). Comparable changes also developed in the liver after administration of phenobarbital (Burger and Herdson,

1966; Herdson et al., 1964b), thiohydantoin compounds (Herdson et al., 1964a), dichlorodiphenyltrichloroethane (DDT) (Ortega, 1966), PCB (Kimbrough et al., 1972), N-2-fluoroenyl-diacetamide (Mikata and Luse, 1964), and dimethylnitrosamine (Emmelot and Benedetti, 1960).

c. Microsomal enzymes. The PBB have been well accepted as potent inducers of hepatic microsomal enzymes. In general, PBB increased microsomal protein, cytochrome P_{450}, aminopyrine demethylase, UDP-glucuronyl transferase, and NADPH-cytochrome c reductase (Troisi, 1975; Sleight and Sanger, 1976). Other enzymes that had a dose response relationship for exposure to PBB and enzyme induction included epoxide hydratase, aniline hydroxylase, ethoxycoumarin-o-deethylase and benzo(a)pyrene hydroxylase (Dent et al., 1976b). In Dent's 2-week study in rats, doses of 300 ppm of PBB increased the activities of all 8 enzymes measured, and at 4.69 ppm only epoxide hydratase and aniline hydroxylase were elevated. In another study, Moore et al. (1976) provided further evidence that PBB were potent inducers of drug metabolizing enzymes. Rat pups whose mothers were fed 10 ppm of PBB for 18 days had approximately a 40% increase in microsomal protein, and there were dramatic increases in NADPH-cytochrome c reductase, in total cytochrome P_{450}, aminopyrene demethylase, benzpyrene hydroxylase and UDP-glucuronyl transferase. They reported that 10 ppm of PBB in the diet produced toxic effects in the dams, but only 1 ppm in the dam's diet was necessary to produce similar effects in their offspring. A substantial increase of microsomal enzymes occurred as early as 3 days after giving 10 ppm of PBB in

the diet (Troisi, 1975). Similar results could be obtained by a single injection of 90 mg/kg body weight.

Dose-related increases in the amount of microsomal protein and cytochrome P_{450} were also seen in rats fed HBB for 30 days, but the degree of microsomal activity was greater at 50 ppm than at 500 ppm (Farber and Baker, 1974).

Comparative studies between PBB and PCB on their ability to induce 3 enzymes indicated that PBB were more effective inducers than PCB in induction of aminopyrene demethylase, paranitrobenzoate reductase and pentobarbital hydroxylase (Garthoff et al., 1977). The maximum induction occurred at 50 ppm of PBB or 50 ppm PCB, except for pentobarbital hydroxylase. In this instance, the maximum induction for PCB occurred at 500 ppm. It was concluded that PBB produced a greater response at lower levels than did PCB.

Polybrominated biphenyls had mixed function properties in inducing drug metabolizing enzymes because in certain respects PBB induced drug metabolizing enzymes in a similar way to phenobarbital and 3-methylcholanthrene (3MC) (Dent et al., 1976b; Dent, 1978). Similar properties had been suggested for PCB (Dent et al., 1976a).

3. Polybrominated Biphenyl Toxicosis in Other Species

a. Signs. Jackson and Halbert (1974) described the clinical signs seen in a herd of 400 cows exposed to PBB-contaminated feed. Initial clinical signs consisted of anorexia, reduced milk production and increased frequency of urination and lacrimation. Lameness, shrinking of the udder but normal rectal temperature were reported. In more advanced stages, they described abnormal growth of hooves and loss of hair. A marked decrease in feed consumption and an

increase in reproductive disorders were reported in field observations of dairy cattle contaminated with PBB (Prewitt et al., 1975).

Studies in pregnant cows that were contaminated at least 7 to 9 months prior to the end of gestation revealed that PBB was transferred to the fetus. The PBB were thought to be embryotoxic (Detering et al., 1975).

A field study compared 16 herds of dairy cattle exposed to low levels of PBB to 15 control herds (Mercer et al., 1976). They concluded that milk composition and production, serum chemistry profiles and calf mortality were not significantly different between the two groups.

Moorhead et al. (1977) reported excessive lacrimation, salivation, diarrhea, dehydration, depression and abortion in cows fed 25 g of PBB/day. Although the signs of PBB toxicosis in cows appeared to be non-specific, cows experimentally given 5,000 ppm of PBB had the same signs as cows naturally intoxicated by PBB (Fine, 1976).

b. Pathologic changes. Gross lesions reported in PBB contaminated cows included suppurative bronchopneumonia, hematomas, and peritoneal and thoracic abscesses (Jackson and Halbert, 1974). Testicular lesions were also reported in young bulls fed a diet containing PBB.

Experimental studies in which pregnant cows were fed 25 g of PBB/day resulted in atrophy of thymus, enlarged kidneys, a thickened gallbladder, metritis and enteritis (Moorhead et al., 1977). Reduced weight of the spleen and bursa of Fabricius (Ringer and Polin, 1977) and general edema (Heineman and Ringer, 1976) were reported in PBB-fed chickens.

Microscopic changes in tissues of cattle (Moorhead et al., 1977) included tubular dilatation and degeneration of the proximal convoluted tubules and marked hyperplasia and dilatation of the mucous glands of the gallbladder. Hyperkeratosis was described in the epithelium of the eyelids. Pneumonia was reported in cows experimentally and naturally exposed to PBB (Jackson and Halbert, 1974; Moorhead et al., 1977).

Clinical pathologic data were not consistent in PBB-intoxicated cows (Trapp et al., 1975). However, Moorhead et al. (1977) reported significant increases in serum glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), blood urea nitrogen (BUN) and bilirubin in the serum of cows fed as much as 25 g of PBB/day. Hematologic values were significantly altered in White Leghorn cockerels fed 75 ppm of PBB for 5 weeks (Heineman and Ringer, 1976). On the other hand, when chickens were fed 20 ppm of PBB or less, there was no appreciable alteration in egg production, egg weight, eggshell thickness and weight gain (Lillie et al., 1975).

Experiments in guinea pigs resulted in no signs of toxicosis when they were fed diets containing 1 or 10 ppm of PBB. Significant mortality occurred at 100 ppm of PBB or higher (Sleight and Sanger, 1976), and there was marked enlargement of the liver. Despite no observable microscopic lesions at 1 or 10 ppm of PBB, electron microscopic examination disclosed numerous vacuoles and myelin bodies. Liver enlargement was also produced by a single dose of 1 g/kg HBB in guinea pigs (Aftosmis et al., 1972b). The PBB at a dosage of 1,000 ppm given for 7 to 18 days to pregnant mice caused exencephaly or cleft palate (Corbett et al., 1975). From these results they concluded that PBB are weak teratogenic compounds. When 100 ppm of

PBB were fed to mice from day 4 of gestation, the percentage of dead and resorbed fetuses was increased (Preache et al., 1976). Decreases in fetal weight occurred when PBB were fed from day 8 of gestation. At 100 ppm, PBB reduced the number of offspring. Results of studies of dermal toxicity indicated that OBB were relatively nontoxic. The daily administration of 0.1 g/kg for 10 days to rabbits did not produce liver enlargement (Aftosmis et al., 1972b). Japanese quail were relatively resistant to OBB, since acute toxicity occurred only at doses higher than 12.5 g/kg.

MATERIALS AND METHODS

A. Experimental Animals

Ninety-six male rats of the Sprague-Dawley strain[a] purchased at weaning age were used in this experiment. The initial body weight varied from 120 to 150 g. The factorial design of the experiment is outlined in Table 1. Six rats from each dietary subgroup were killed at 30 days and the rest were killed at 60 days of the experiment.

Table 1. The basic experimental design

Levels of PBB (ppm)	Number of Rats	
	Iodine Adequate Diet[a]	Iodine Excessive Diet[b]
0	12	12
1	12	12
10	12	12
100	12	12

[a] 0.2 ppm supplied as ethylenediamine dihydriodide (EDDI).

[b] 1,000 ppm supplied as EDDI.

Six rats of each subgroup were killed on the 30th day and the remaining rats were killed on the 60th day.

[a] Life Science Division of Mogul Corporation, Madison, WI.

The rats were kept in wire-topped cages allowing 3 rats per cage. All rats were housed in the same room and maintained at 23±1 C with a relative humidity of 55±5%. Lights were controlled automatically to allow light from 8 a.m. to 8 p.m.

During a 2-day initial acclimatization, the rats were fed a commercial pelleted diet[a] and tap water *ad libitum*. The rats were then adapted to a finely ground diet containing adequate iodine and distilled water for another 2 days before the experiment was started. The basic experimental feed was a finely ground diet deficient in iodine.[b] Diets representing either iodine adequate diet (IAD) or iodine excessive diet (IED) were made by mixing the basic feed with a calculated amount of ethylenediamine dihydriodide[c] (EDDI) to get concentrations of iodine of 0.2 or 1,000 ppm, respectively. Each subgroup of 12 rats was fed either IAD or IED supplemented with either 0, 1, 10, or 100 ppm of PBB.[d] The feed was placed in porcelain containers with stainless steel tops. Distilled water was supplied *ad libitum* in inverted bottles with stainless steel sipper tubes.

Clinical signs and feed consumption were recorded every day, and body weight was measured twice weekly. Appropriate precautions were employed to protect the people working with these rats. Gloves, coats and masks were changed appropriately to avoid any contamination

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[a] Purina Rat Chow, Ralston Purina Co., Checkerboard Square, St. Louis, MO.

[b] Remington iodine deficient diet, United Biochemical Corporation, Cleveland, OH.

[c] Hi-Amine, Pitman-Moore, Inc., Washington Crossing, NY.

[d] Firemaster, Michigan Chemical Co., St. Louis, MI.

of feed from one group to the other. Bags used for each batch of feed were clearly labeled.

B. Collection of Samples

Feed but not water was removed 24 hours prior to necropsy. Final body weights were measured within a few hours of the time of necropsy. The rats were killed with ether anesthesia and blood samples were obtained by cardiac puncture using a 21-gauge needle. Blood samples for hematologic examination were collected in tubes containing ethylenediamine tetraacetic acid, and direct blood smears were made immediately after drawing the blood. Blood without anticoagulant was collected in tubes and, after coagulation and centrifugation, the serum was removed, placed in different tubes, and stored at -4 C for further chemical analyses.

A complete necropsy was performed. To attain satisfactory fixation of lungs, 1 ml of buffered formalin was injected intratracheally and the trachea was ligated. The kidney and liver were weighed with a top-loading balance.[a] The thyroid glands were weighed with an analytical balance.[b] Urine was drawn from the bladder by direct puncture at the time of necropsy.

Samples of brain, pituitary gland, eye, liver, kidney, spleen, thyroid gland, adrenal gland, salivary gland, pancreas, thymus, trachea, lung, heart, esophagus, stomach, small and large intestine, testes, urinary bladder, skeletal muscle, and lymph node were fixed

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[a]Mettler Series P, Model 163 (readability 0.001 g), Mettler Instrument Corporation, Hightstown, NY.

[b]Model H-15 (readability 0.0001 g), Mettler Instrument Corporation, Hightstown, NY.

in 10% neutral buffered formalin for histologic examination. Bone was decalcified[a] prior to histologic processing.

Pieces of liver and thyroid gland were sliced into approximately 2 mm blocks and fixed in cold 3% glutaraldehyde for electron microscopic examination.

Tissues for PBB and vitamin A analyses were wrapped in aluminum foil and kept frozen at -70 C until the time of analyses.

C. Examination of Samples

1. Hematology

Blood smears were stained with Wright's stain,[b] and differential leukocyte counts were made by counting and classifying 200 white blood cells. Noncoagulated blood samples were used for red blood cell counts and white blood cell counts, packed cell volume and hemoglobin determinations. Red blood cells and white blood cells were counted with an electronic counter.[c] Hemoglobin concentration was determined by using a cyanmethemoglobin standard[d] and readings were made with a spectrophotometer.[e] Packed cell volume was measured by drawing the blood into microhematocrit tubes,[f] centrifuging for 5 minutes at 3,000 g and reading with a microhematocrit reader.[g]

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[a] RDO, Du Page Kinetic Laboratory, Inc., Downers Grove, IL.

[b] Hema-Tek slide stainer, Ames Co., IN.

[c] Coulter Electronic, Inc., Hialeah, FL.

[d] Hycel, Inc., Houston, TX.

[e] Perkin Elmer Coleman 4, Coleman Instruments Div., Oak Brook, IL.

[f] Capillary tubes, Scientific Products, Evanston, IL.

[g] International Micro-Capillary Reader, International Equipment Co., Boston, MA.

2. Blood Chemistry

Spectrophotometric measurement of BUN, SAP and SGOT was done by using Eni-Gemsaec reagent.[a] The values were expressed in mg/dl for BUN and International Units/liter (IU/l) for SAP and SGOT.

3. Urinalysis

Protein content and specific gravity were determined by a total solid (TS) refractometer.[b] Urobilinogen, bilirubin, blood, ketone bodies, glucose, pH and protein were estimated by using a dipstick.[c]

4. Serum Electrophoresis

Serum protein, lipid and LDH isoenzymes were determined by electrophoretic analysis.[d] Serum for protein determination was electrophoresed in a buffer[d] (pH 8.6-9.0) for 15 minutes at 180 volts and stained with a special stain.[e] After dehydration in methanol for 2 minutes, the plates were cleared in a 25% acetic acid in methanol solution for 5 to 10 minutes and were then dried at 50 to 60 C for 5 to 10 minutes. The strips were then quantitated by densitometry.[f]

Serum for lipid analyses was applied to a Titan III XW plate[g] for 20 minutes at 165 volts. The strips were then stained in oil red O

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[a]Smith Kline Instruments, Inc., Sunnyvale, CA.

[b]Golden Refractometer, American Optical Co., Buffalo, NY.

[c]Multistick, Ames Co., Division Miles Laboratory, Inc., Elkhart, IN.

[d]Helena Laboratory Corporation, Beaumont, TX.

[e]Ponceau S, Helena Laboratory Corporation, Beaumont, TX.

[f]Quick Quant II, Helena Laboratory Corporation, Beaumont, TX.

[g]Helena Laboratory Corporation, Beaumont, TX.

for 1 hour in a covered rotator and then washed in water to remove excess stain and precipitates. Following a dip in methanol for 10 seconds, the plates were rinsed with water for 5 seconds. The plates were dipped in a mixture of 4 parts of glycerin and 1 part of methanol for 5 to 10 minutes and then placed in a sealed plastic bag. The plates were scanned with a densitometer.

Serum for LDH isoenzyme determination was electrophoresed in a buffer for 10 minutes at 300 volts. Meanwhile, a substrate plate was prepared by pipetting 1 ml of the LDH substrate onto a wetted acetate plate. After electrophoresis, the sample plate was blotted firmly and carefully "sandwiched" to the substrate plate. After incubation for 15 minutes, the plates were scanned at 540 nm.

5. Polybrominated Biphenyl Analysis

Two grams of fat tissues were homogenized with 1 ml distilled water in tubes. The tubes were silanized by using dimethyl dichlorophenol silane. The homogenates were sequentially extracted with 10 ml redistilled petroleum ether (PE) and incubated in a 40 C shaker-water bath to allow evaporation. Approximately 5 ml of the remaining solution was added to 20 ml saturated acetonitril (CH_3CN) and mixed thoroughly by a shaker. The mixture was centrifuged at 1,000 rpm for 5 minutes. The separated CH_3CN was removed carefully using a Pasteur pipet and a small amount of NaCl was added. An additional 10 ml of PE was added, mixed and centrifuged again. The PE layer was transferred to 15 ml centrifuge tubes. This extraction step was repeated 3 times to maximize the results. The PE was evaporated to approximately 2 ml.

The florisil columns were prepared by filling a 5 3/4 inch Pasteur pipet with activated florisil[a] up to approximately 2 cm from the top of the pipet. A small amount of glass wool was placed at the tapered end of the pipet to hold the florisil. A small amount of anhydrous $NaSO_3$ was added to the top of the florisil. The column was washed with 1 to 2 ml PE and the washing was discharged. The sample was transferred into the column and eluted with 3 ml of 6% anhydrous ether in PE. The eluate was collected into a labeled centrifuge tube and was then evaporated. The dried sample was redissolved with 100 µl of PE just prior to injection into the chromatograph.[b] The redissolved samples were kept stable in ice. Results were compared with standards of known concentrations of PBB ranging from 1, 0.5, 0.1, 0.05, and 0.001 µg/µl that had been processed by the same procedures. Similar procedures were employed for nonfat tissues, except additional saturated CH_3CN was not used.

6. Vitamin A Analysis

The procedure for determination of vitamin A in the liver was adapted from the methods described by Garry et al. (1970) and Harris and Navia (1977).

One and one-half grams of liver tissue were homogenized with 6 ml demineralized water. One milliliter of homogenate was saponified with 2 ml 1% ethanolic pyrogallic acid and 1 ml 50% KOH. The tissue samples were heated at 60 C for 30 minutes. After cooling at room temperature for 10 minutes, 4 drops of ethanol were added to the

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[a]Florisil PR, 2-0280, 60/100 mesh, Supelco, Inc., Bellefonte, PA.

[b]GC model 3200, Finnigan, Inc., Sunnyvale, CA.

samples. The samples were then extracted with 4 ml PE. After centrifugation for 5 minutes at 1,000 rpm, the PE layer containing free retinol was removed and was transferred into a 10 ml volumetric flask. The extraction was repeated once. The extract was made up to 10 ml by adding PE.

Microcolumns were made from 5 3/4 inch disposable glass pipets. Glass wool was used to plug the tapered end of the pipets. Approximately 100 mg of salicilic acid crystals were added to the column.

A 0.2 ml sample of extract was transferred to the prepared salicilic acid column and the column was then washed with 0.5 ml PE. The washing was discarded. The residue in the column was eluted with 0.4 ml isopropanol. The eluate was collected in the disposable tubes and was then made up to 1 ml by adding a sufficient amount of isopropanol.

The samples were read in a fluorometer[a] against a blank. The results were calculated using a standard curve that was prepared from known amounts of purified vitamin A.[b]

7. Histologic Preparation

Formalin-fixed tissues were processed in an Autotechnicon and embedded with paraffin.[c] The tissues were sectioned at 5 to 6 microns and stained with hematoxylin-eosin for light microscopic examination. Frozen sections of selected tissues were stained with oil red O for

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[a]Turner Fluorometer, Arthur H. Thomas Co., Philadelphia, PA.

[b]Retinol, Crystalline Synthetic Type X, Sigma Chemical Co., St. Louis, MO.

[c]Tissue Prep, Fisher Scientific Co., Chemical Manufacturing Division, Fair Lawn, NJ.

lipid identification. Pituitary gland and adrenal gland were stained with periodic acid-Schiff orange G (Sheehan and Hrapchak, 1973). The von Kossa stain was used for calcium.

8. Transmission Electron Microscopy

Karnovsky's-fixed tissues were minced into 0.5 to 1.0 mm^3 blocks and were then washed with Zetterqvist solution (Pease, 1964) at pH 7.4. The washed tissues were then postfixed in 1% osmium in Zetterqvist solution. After dehydration with graded alcohols, the tissues were transferred to propylene oxide and embedded in a mixture of Epon and Araldite.

Semithin sections were made and stained with toluidine blue for orientation of the object. Thin sections were cut with a glass knife on an ultramicrotome[a] and were stained with uranyl acetate and lead citrate before examination with an electron microscope.[b]

G. Statistical Evaluation

The data were analyzed using analysis of variance, and the degrees of significance were determined by the Student's t-test.

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[a] LKB Ultratome III^R, Instrument Group 8800, Sweden.

[b] EM 9S2, Carl Zeiss, Germany.

RESULTS

A. Clinical Signs

During the entire course of the experiment, there were no remarkable clinical signs observed in any of the rats. All rats survived until the end of the experiment. However, food consumption was decreased in rats fed IED and 100 ppm of PBB for 60 days, especially in the last 2 weeks.

B. Body Weight

The average body weights of the rats fed IAD and 0, 1, 10, and 100 ppm of PBB for 60 days are presented in Figure 1. Growth appeared to be unaffected by 0, 1, and 10 ppm of PBB, but feeding of IAD containing 100 ppm of PBB decreased rate of gain after about 15 days and a marked decrease was noticed after about 45 days of treatment.

Figure 2 depicts the average body weight of rats fed IED containing 0, 1, 10, or 100 ppm PBB for 60 days. The growth was not affected by 1 ppm of PBB, but a tendency towards a decrease in rate of gain was observed in rats fed IED and 10 ppm of PBB. The greatest effect on body weight occurred after about 45 days in rats fed IED containing 100 ppm of PBB.

The main factor affecting body weight was PBB (Table 2). The only observable effect was caused by the interaction between PBB and duration of the treatment.

Figure 1. Means of body weight of rats fed an iodine adequate diet (IAD) containing different levels of polybrominated biphenyls (PBB) for 60 days.

Figure 2. Means of body weight of rats fed an iodine excess diet (IED) containing different levels of PBB for 60 days.

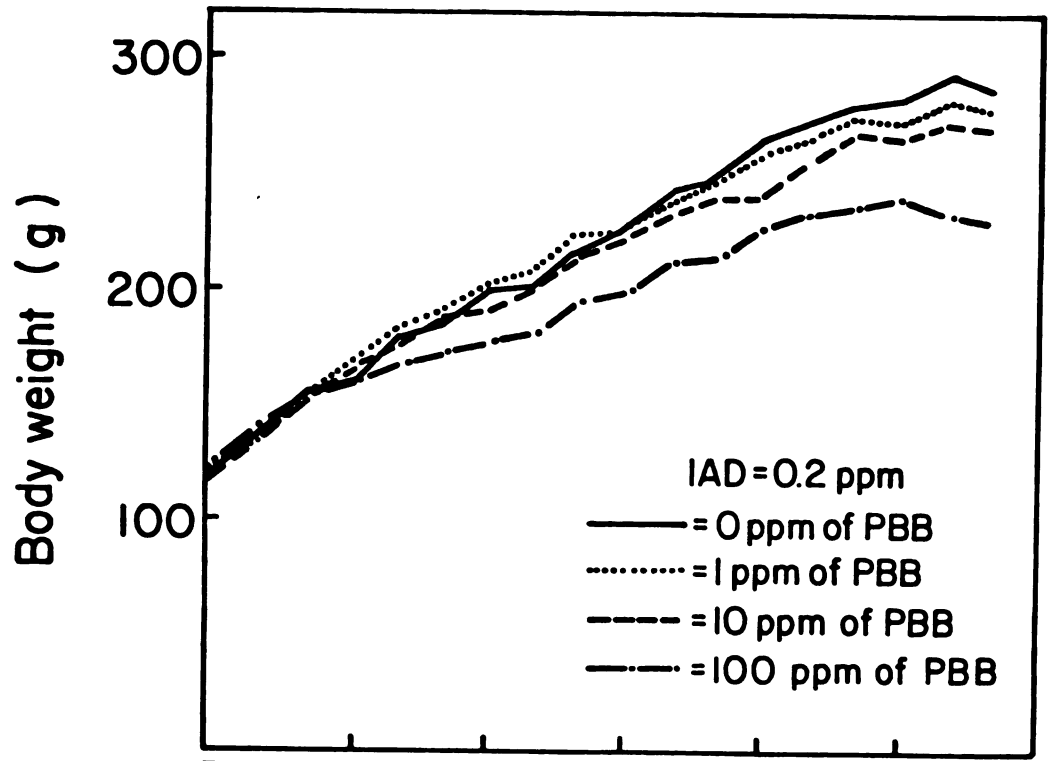


Figure 1

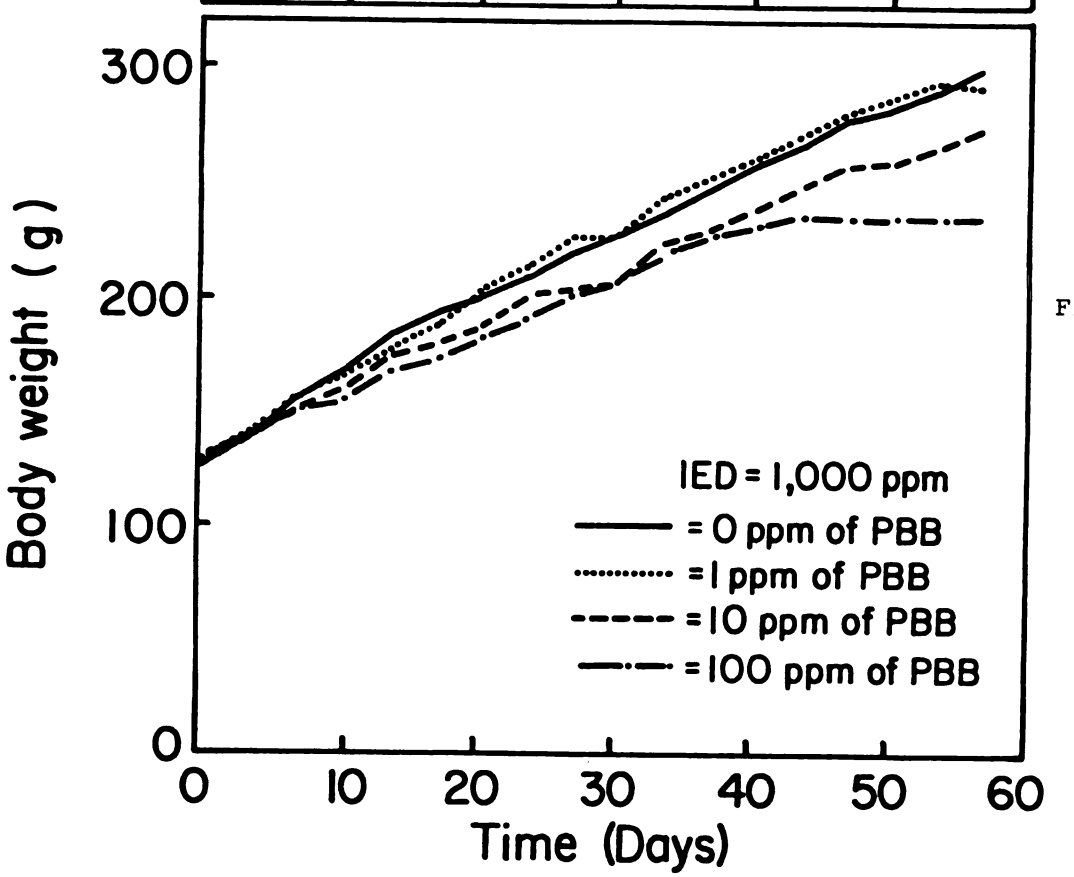


Figure 2

Table 2. The effects of polybrominated biphenyls (PBB), duration of treatment, and their interaction on the final body weight of rats fed different levels of PBB for 30 and 60 days

Levels of PBB in the Diets (ppm)	Final Body Weight (g)	
	30 days	60 days
0	214.08±3.90	288.83±5.23
1	210.17±5.47	285.92±6.34
10	206.33±4.04	265.00±4.35[b]
100	191.17±4.70[a]	229.58±4.35[b]

The values were expressed in means±SE of 6 rats.

[a]Different ($P<0.05$) compared to the control of the same group at 30 days.

[b]Different ($P<0.01$) compared to the control of the same group at 60 days.

The final body weight of rats fed 100 ppm of PBB for 30 days was significantly less ($P<0.05$) than that of rats fed 0 ppm of PBB. At 60 days the rats given 100 ppm of PBB or 10 ppm of PBB had final body weights significantly lower ($P<0.01$) than the rats fed 0 ppm of PBB. There were no differences in average body weight among the groups that could be attributed to the combination of PBB and iodine.

C. Organ Weights

1. Kidney

The IED caused a decrease ($P<0.01$) in kidney weight to body weight ratios from an average of 0.847±0.16 to 0.809±0.08 g/100 g body weight regardless of the levels of PBB and duration of the treatment. There was no effect of PBB on the weight of the kidney.

2. Liver

The effects of interaction between iodine and PBB on liver weight to body weight ratios are indicated in Figure 3. The interaction was confirmed by statistical analysis ($P<0.05$). The effects of treatment at 30 days were similar to those of 60 days, suggesting no interaction related to duration of the treatment.

Figure 4 shows the group means of liver weight to body weight ratios of rats fed IAD or IED containing 0, 1, 10, or 100 ppm of PBB. In rats fed IAD, an addition of 10 ppm of PBB caused increases ($P<0.05$) in liver weight to body weight ratios, and supplementation of 100 ppm of PBB produced a greater increase ($P<0.01$). Furthermore, increases ($P<0.01$) in liver weight to body weight ratios were evident in rats fed IED containing 10 ppm or 100 ppm of PBB. As indicated in Figure 3, there were no significant differences between liver weight to body weight ratios at 30 days and at 60 days that could be related to the treatment.

3. Thyroid Gland

The analysis of variance of thyroid weight to body weight ratios of rats fed IAD or IED containing 0, 1, 10, or 100 ppm of PBB for 30 or 60 days is shown in Table 3.

Effects of PBB, iodine, duration of the treatment and their interaction on thyroid weight to body weight ratios are shown in Figure 5. In general, at 30 days these ratios were smaller ($P<0.005$) in the rats fed IED containing 0 or 1 ppm of PBB than in those of rats fed IAD at the same levels of PBB. On the other hand, there was no significant effect on the thyroid weight to body weight ratios if the rats were fed IED containing 10 or 100 ppm of PBB.

Figure 3. The effects of PBB, iodine, duration of treatment and their interaction on liver to body weight ratios of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

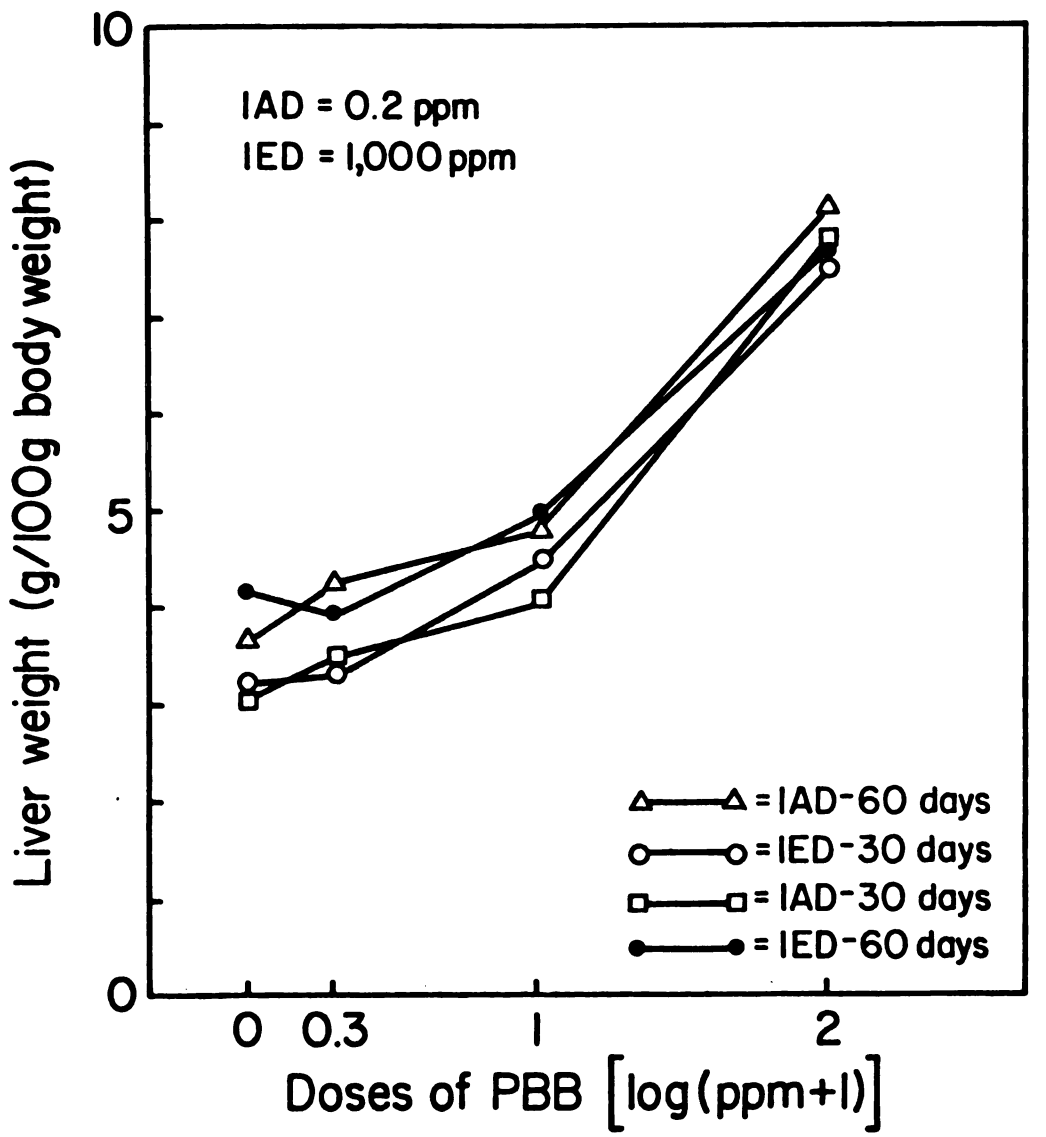
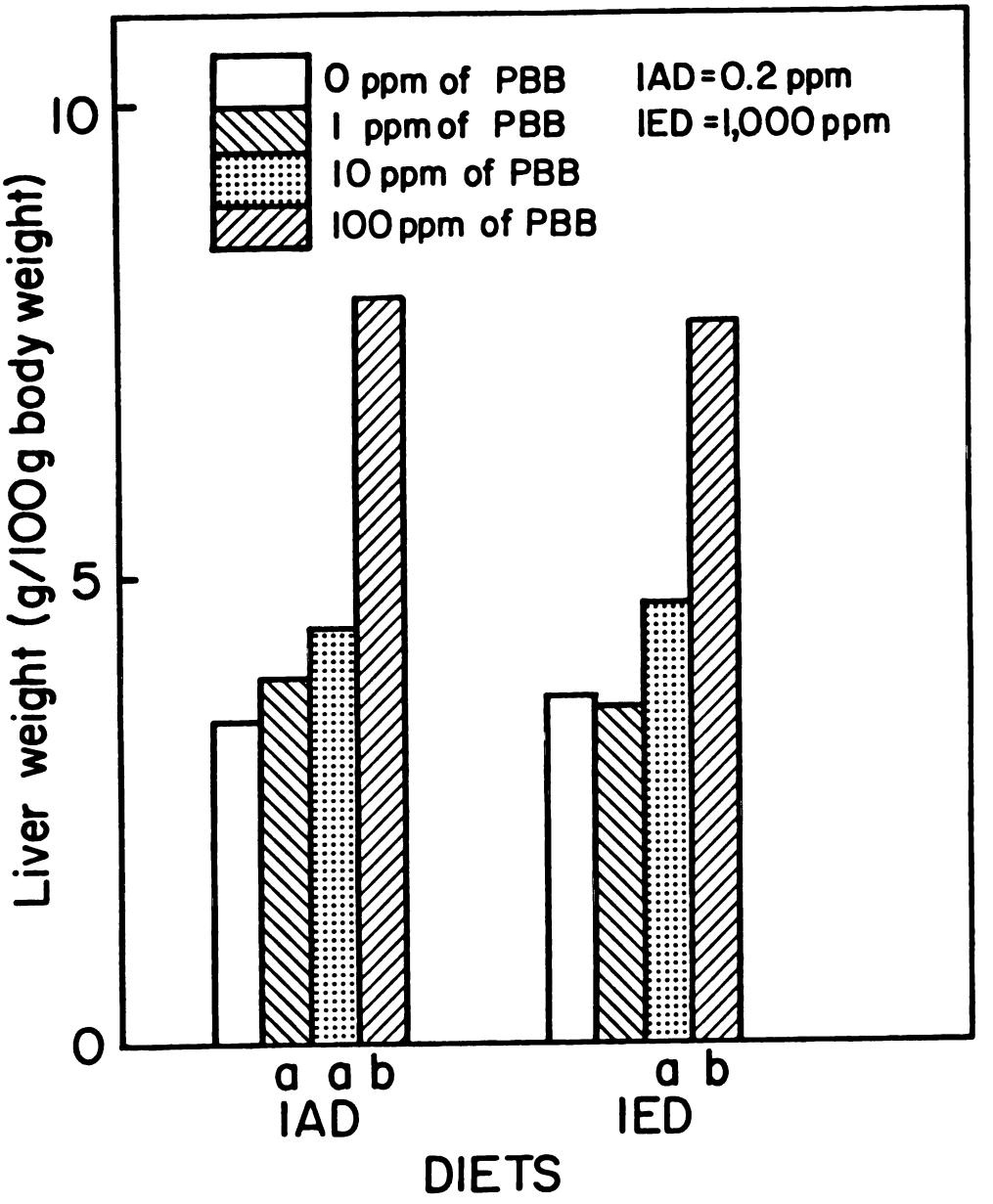


Figure 4. The effects of PBB, iodine and their interaction on liver to body weight ratios of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.



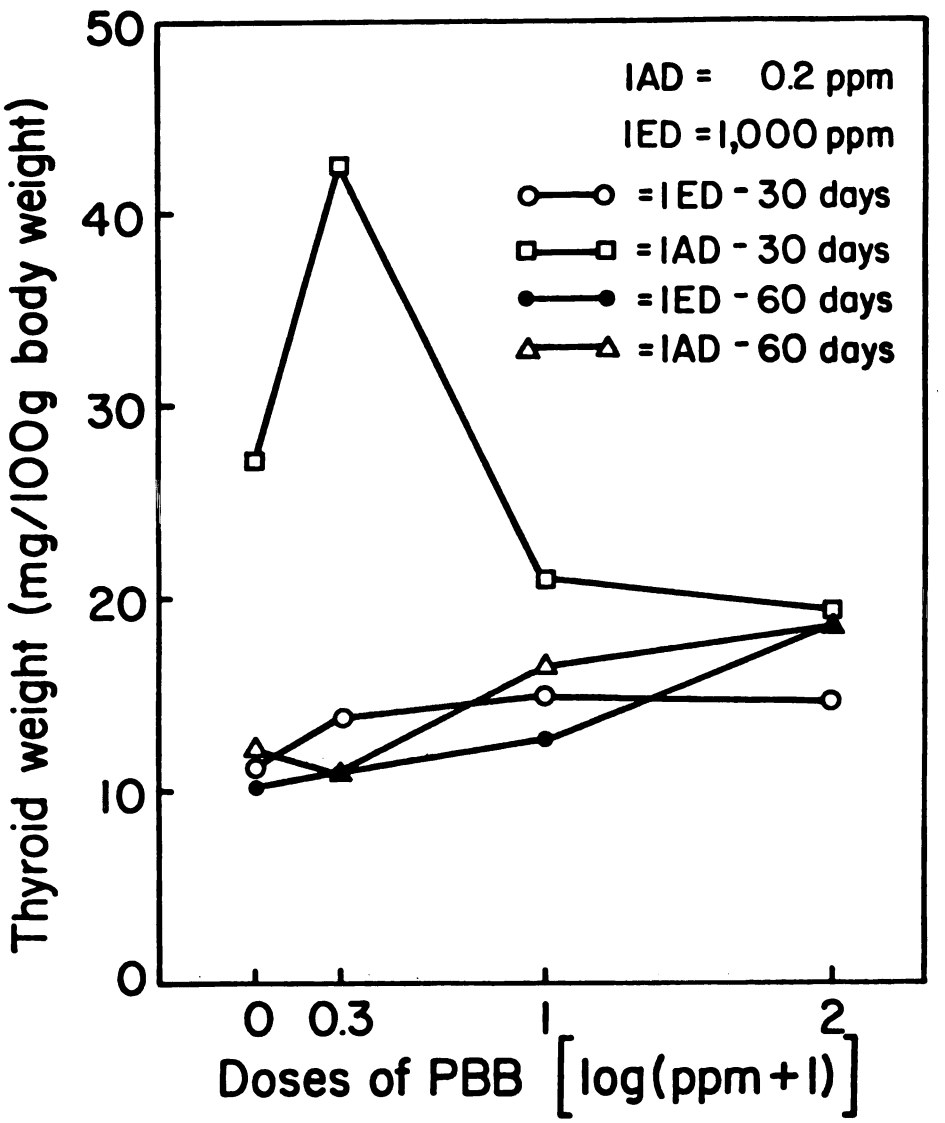
[a]Significantly different ($P<0.05$) from 0 ppm at the same levels of iodine.

[b]Highly significant difference ($P<0.01$) from 0 ppm at the same levels of iodine.

Table 3. Analysis of variance of thyroid weight to body weight ratios of rats fed an iodine adequate diet (IAD) or an iodine excess diet (IED) containing different levels of PBB for 30 and 60 days

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	F Statistic	Approx. Significance Probability of Stat.
PBB	53.135655	3	17.711885	3.607163	.020
Iodine	167.1526	1	167.1526	34.041919	<0.0005
Days	310.0681	1	310.0681	63.147769	<0.0005
PBB Iodine	50.178092	3	16.726031	3.406386	.025
PBB Days	135.5403	3	45.180097	9.201277	<0.0005
Iodine Days	125.4120	1	125.4120	25.541127	0.0005
PBB Iodine Days	62.242292	3	20.747431	4.225375	.010
Residual Error	235.6895	48	4.910198		
Total	1139.4185	63			

Figure 5. The effects of PBB, iodine, duration of treatment and their interaction on thyroid to body weight ratios of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.



At 60 days, addition of 100 ppm of PBB to either IAD or IED caused significant ($P<0.01$ and $P<0.005$, respectively) increases in the thyroid weight to body weight ratios. This effect was not seen in rats fed IED containing 0, 1, or 10 ppm of PBB at 60 days. Table 4 summarizes the organ weight to body weight ratios.

D. Urinalysis

There were no consistent results in the urinalysis. Protein was present in urine of the controls and in the treated rats. However, there was a tendency towards increased protein content associated with PBB treatment.

E. Hematology

There were no significant differences in hematologic profiles that could be related to any of the treatments. Erythrocytes, leukocytes, hemoglobin and packed cell volume values were in the normal range. The morphologic appearance of erythrocytes, leukocytes and platelets was essentially normal.

F. Blood Chemistry

Data obtained from BUN determinations indicated that there was no significant ($P>0.05$) effect of PBB on BUN values. However, IED caused decrease ($P<0.05$) in BUN from 18.02 ± 0.49 to 16.81 ± 0.50 mg/dl regardless of the levels of PBB. Additionally, at 60 days the BUN was significantly higher ($P<0.05$) than at 30 days.

A factor affecting the concentration of SGOT was the interaction between iodine and duration of the treatment. At 60 days but not at 30 days, rats fed IED had a significantly ($P<0.05$) lower concentration

Table 4. Organ weight to body weight (BW) ratios of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Levels in the Diets		Kidney (g/100 g BW)[a]		Liver (g/100 g BW)[a]		Thyroid (mg/100 g BW)[b]	
Iodine	PBB	30 days	60 days	30 days	60 days	30 days	60 days
IAD	0	0.885±0.055	0.823±0.336	3.170±0.141	3.757±0.071	12.497±1.998	4.108±0.234
	1	0.918±0.263	0.805±0.070	3.497±0.130	4.262±0.284	18.378±2.788	4.010±0.355
	10	0.903±0.254	0.805±0.024	4.040±0.135	4.832±0.150	10.440±1.109	6.107±0.487
	100	0.890±0.226	0.748±0.076	7.815±0.146	8.147±0.160	10.125±0.247	8.403±1.068
IED	0	0.868±0.030	0.767±0.291	3.258±0.115	4.165±0.154	5.155±0.467	3.578±0.252
	1	0.868±0.199	0.745±0.024	3.305±0.079	3.912±0.291	6.840±2.788	3.995±0.437
	10	0.887±0.440	0.747±0.361	4.525±0.124	4.957±0.232	7.603±1.300	4.935±0.713
	100	0.835±0.032	0.757±0.039	7.630±0.346	7.713±0.409	7.710±0.626	8.390±1.228

[a]Data represented as means±SE (n=6).

[b]Data represented as means±SE (n=4).

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of SGOT (132.88±6.02 IU/l) than rats fed IAD (216.17±25.07 IU/l). The effect was not related to the levels of PBB.

Table 5 contains the analysis of variance of the effect of factors on SAP. There were effects of PBB ($P<0.0005$), iodine ($P<0.0005$), interaction between PBB and iodine ($P<0.05$), PBB and duration of the treatment ($P<0.05$), as well as iodine and duration of the treatment ($P<0.05$). However, there were no significant effects of the duration of the treatment alone, and there was no three-way interaction between PBB, iodine and duration of the treatment.

The effects of PBB, iodine and their interaction on SAP are presented in Figure 6. Rats fed IED containing 0, 1, or 10 ppm of PBB had a lower concentration ($P<0.05$) of SAP than rats fed IAD at the same levels of PBB. On the other hand, there were no significant differences in SAP of rats fed IAD from those fed IED containing 100 ppm of PBB. In general, SAP had a tendency to decline related to the increase of PBB levels.

G. Serum Electrophoresis

1. Protein Fractions

The effects of treatment factors and their interactions on protein fractions in the serum are summarized in Table 6.

The only factor affecting albumin was PBB. Group means for albumin were significantly reduced from 49.81±4.09% in rats fed 0 ppm of PBB to 40.28±3.47% in rats fed 100 ppm of PBB. There was no significant effect of lower levels of PBB on albumin.

The effects of PBB, duration of the treatment and their interaction on beta globulin (%) are illustrated in Figure 7. At 30 days, beta globulin had a tendency to increase proportionally to the elevated

Table 5. Analysis of variance of the effect of treatment factors on serum alkaline phosphatase of rats fed IAD or IED containing different levels of PBB for 30 days and 60 days

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Squares	F Statistic	Approx. Significance Probability of Stat.
PBB	72139.46	3	24046.49	19.636130	0.0005
Iodine	37604.17	1	37604.17	30.707201	0.0005
Days	975.3750	1	975.3750	0.79648186	0.375
PBB Iodine	10500.83	3	3500.2778	2.858293	0.042
PBB Days	10302.79	3	3434.2639	2.804387	0.045
Iodine Days	12150.00	1	12150.00	9.921573	0.002
PBB Iodine Days	7005.0000	3	2335.0000	1.906739	0.135
Residual Error	97968.33	80	1224.6042		
Total	248645.96	95			

Figure 6. The effects of PBB, iodine and their interaction on serum alkaline phosphatase of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

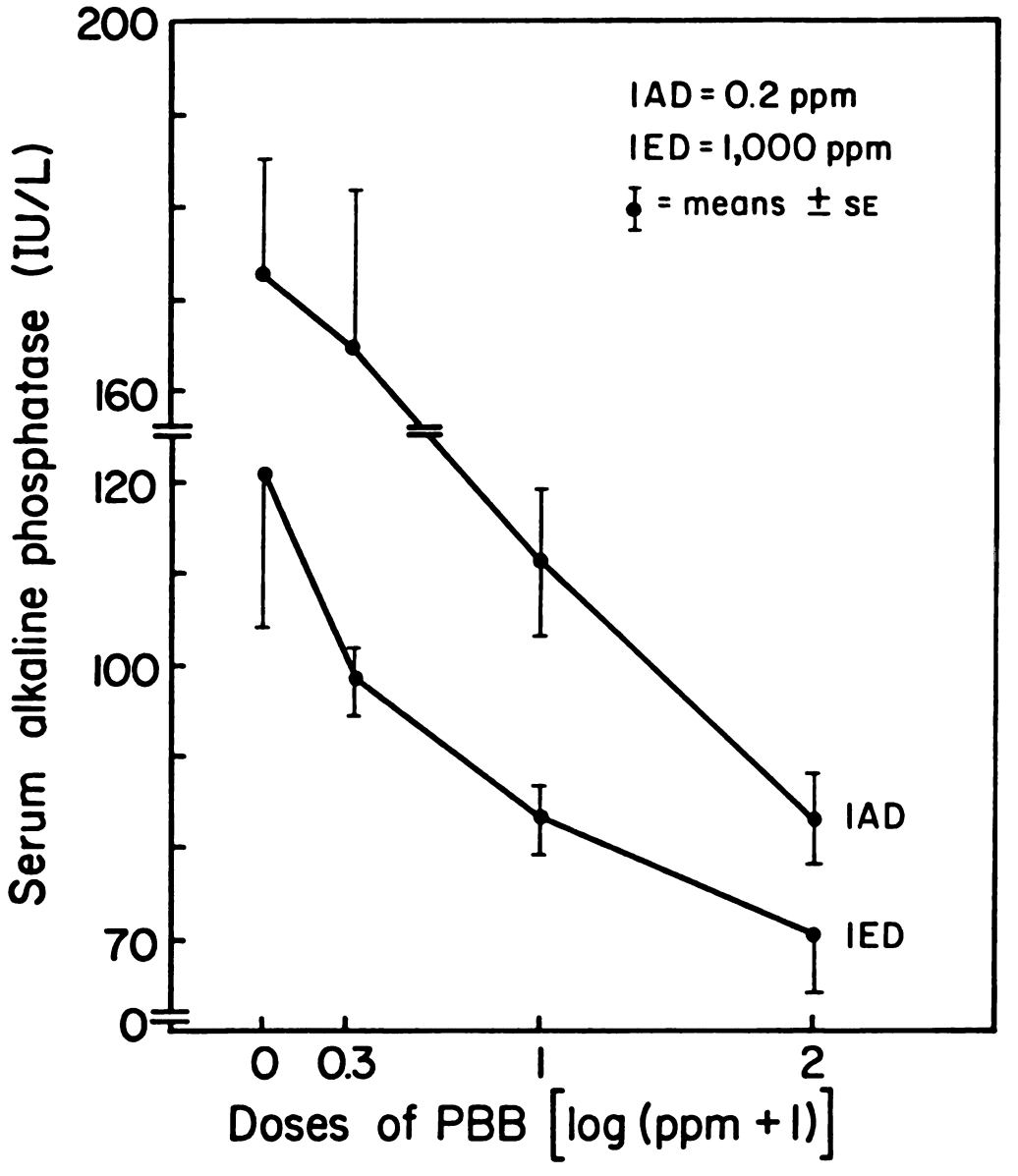


Table 6. The results of statistical evaluation of the effects of treatment factors and their interactions on protein fractions in the serum of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Parameters	Treatment Factors						
	PBB	I	D	PBB X I	PBB X D	I X D	PBB X D
Albumin	b	NS	NS	NS	NS	NS	NS
Globulin							
Alpha 1	NS	NS	NS	NS	NS	NS	NS
Alpha 2	NS	NS	NS	NS	NS	NS	NS
Beta	b	NS	a	NS	b	NS	NS
Gamma	b	b	a	NS	b	a	b
Total	a	NS	NS	NS	NS	NS	NS
A/G ratio	b	NS	NS	NS	NS	NS	NS

I = Iodine, D = Days (duration of treatment), X = Interaction

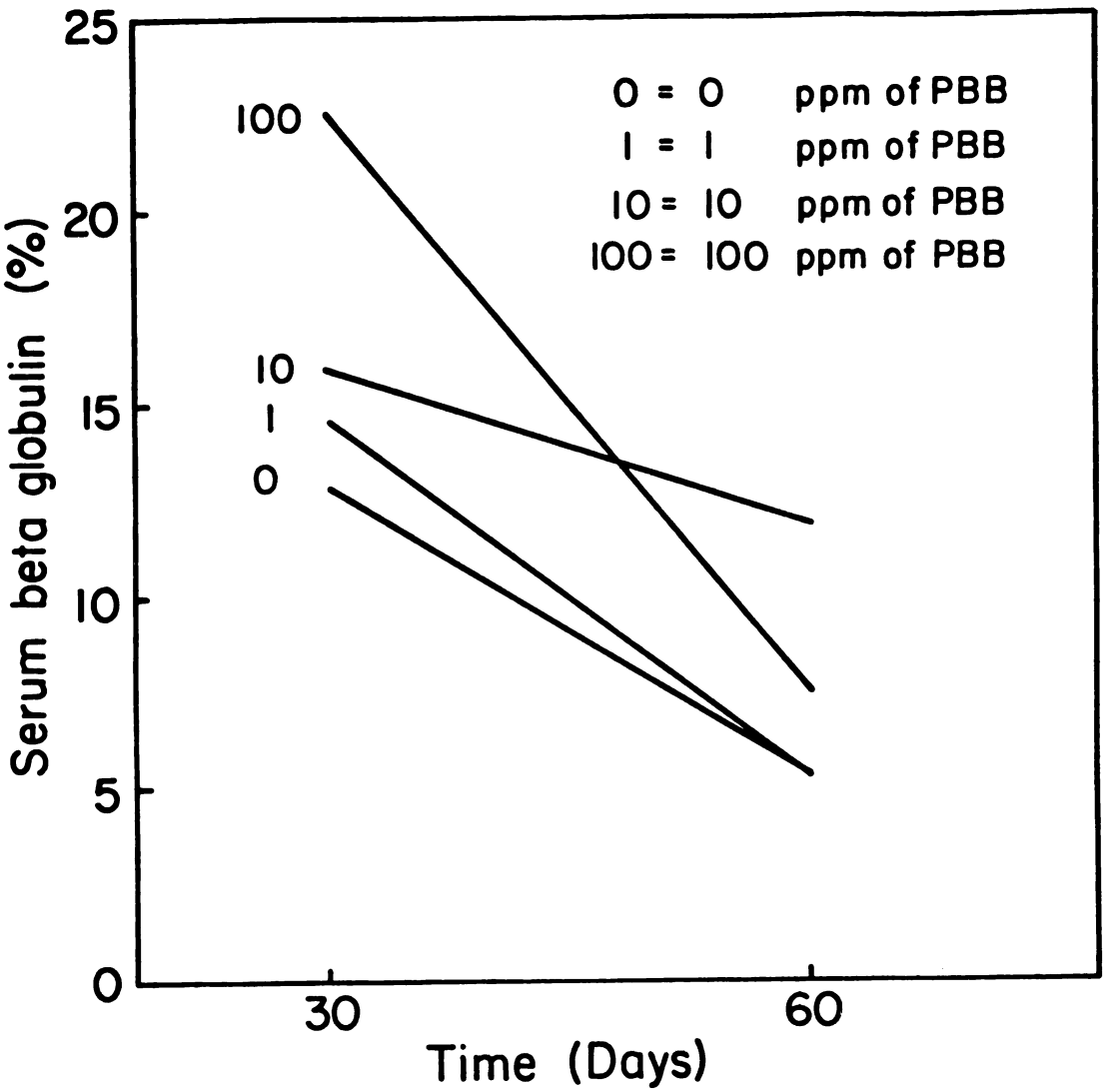
[a]Different ($P<0.05$).

[b]Different ($P<0.01$).

NS = Not significant.

Serum beta globulin (%)

Figure 7. The effects of PBB, duration of treatment and their interaction on serum beta globulin (%) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.



doses of PBB. At 60 days the concentration of beta globulin seemed to be unaffected by the levels of PBB, except for an increase at 10 ppm of PBB. At 60 days, the concentration of beta globulin in the serum was significantly lower ($P<0.05$ to $P<0.001$) as compared to 30 days at the same levels of PBB.

The effects of PBB, iodine, duration of treatment and their interaction on gamma globulin (%) in the serum are presented in Table 7.

In general, the concentration of gamma globulin at 60 days was significantly higher than at 30 days. There seemed to be no dose-related effects of PBB on gamma globulin. At 30 days, rats fed IAD and 100 ppm of PBB had less ($P<0.05$) gamma globulin than rats fed IAD and 0 ppm of PBB. Rats fed IED containing 1 ppm of PBB had more ($P<0.05$) gamma globulin than those of rats fed IED containing 0 ppm of PBB.

At 60 days, rats fed IAD containing 1 ppm or 100 ppm of PBB had more ($P<0.05$ and $P<0.001$, respectively) gamma globulin than rats fed IAD and 0 ppm of PBB. However, at 10 ppm of PBB, gamma globulin was reduced ($P<0.05$). A significant decrease ($P<0.001$) of gamma globulin was also found in rats fed IED containing 10 ppm of PBB as compared to rats fed 0 ppm of PBB and IED.

The PBB were also the only factor affecting total globulin and A/G ratio. Total globulin was increased ($P<0.05$) from 50.18±4.11% in rats fed 0 ppm of PBB to 59.59±3.56% in rats fed 100 ppm of PBB. There were no significant effects of 1 or 10 ppm of PBB on total globulin.

Group means for A/G ratio tended to be reduced with increasing doses of PBB. Rats fed 100 ppm of PBB had a lower ($P<0.001$) A/G ratio

Table 7. The effects of PBB, iodine, duration of treatment and their interaction on gamma globulin in the serum (%) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Levels of Iodine	PBB (ppm)	Gamma Globulin in the Serum (%) 30 days	60 days
IAD	0	5.68±5.42	18.35±2.19
	1	6.23±2.60	23.06±3.69
	10	3.23±2.76	12.33±2.55[a]
	100	0.30±0.10[a]	28.35±1.91[b]
IED	0	0.80±0.78	19.77±2.10
	1	6.75±1.34[a]	20.23±2.06
	10	1.30±0.74	3.73±3.39[b]
	100	2.23±1.00	17.20±3.83

The values were means±SD of 3 rats.

[a]Different ($P<0.05$) from 0 ppm at the same levels of iodine and same days.

[b]Different ($P<0.001$) from 0 ppm at the same levels of iodine and the same days.

(0.68±0.10) as compared to ratios in rats fed 0 ppm of PBB (1.01±0.18). Rats fed diets containing 1 ppm of PBB had a lower ($P<0.01$) A/G ratio (0.84±0.11) as compared to rats fed 0 ppm of PBB (1.01±0.18). There were no significant effects of PBB at 10 ppm on A/G ratio.

The effects of PBB, duration of the treatment and their interaction on serum gamma globulin (expressed in grams) are presented in Figure 8. At 30 days, supplementation of 10 or 100 ppm of PBB in the diets caused a decrease ($P<0.05$) in gamma globulin. On the other hand, at 60 days, although 10 ppm of PBB caused a reduction ($P<0.01$) of gamma globulin, a dosage of 100 ppm of PBB caused a significant increase ($P<0.001$) of gamma globulin. There was no significant effect ($P>0.05$) of 1 ppm of PBB on gamma globulin at 30 or 60 days.

2. Lipids and Lactic Dehydrogenase Isoenzymes

The effects of treatment factors on lipid and LDH isoenzymes are presented in Table 8.

As indicated in Table 8, statistical analysis revealed that excessive iodine caused a significant effect on the level of triglycerides in the serum. Group means for triglyceride values for rats fed excessive iodine were significantly ($P<0.001$) lower (34.17±37.04 mg/dl) than in rats fed an adequate level of iodine (127.50±49.57 mg/dl). Treatment with IED caused a decrease ($P<0.05$) of triglycerides as compared to feeding an IAD regardless of the level of PBB (Figure 9).

The effect of PBB on the concentration of serum cholesterol is presented in Figure 10. Incorporation of 100 ppm of PBB caused an increase ($P<0.05$) of cholesterol values in rats fed IED or IAD.

The crossed lines in Figure 11 suggested that LD-2 values were affected by an interaction between iodine and duration of the treatment.

Figure 8. The effects of PBB, duration of treatment and their interaction on serum gamma globulin (g/dl) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

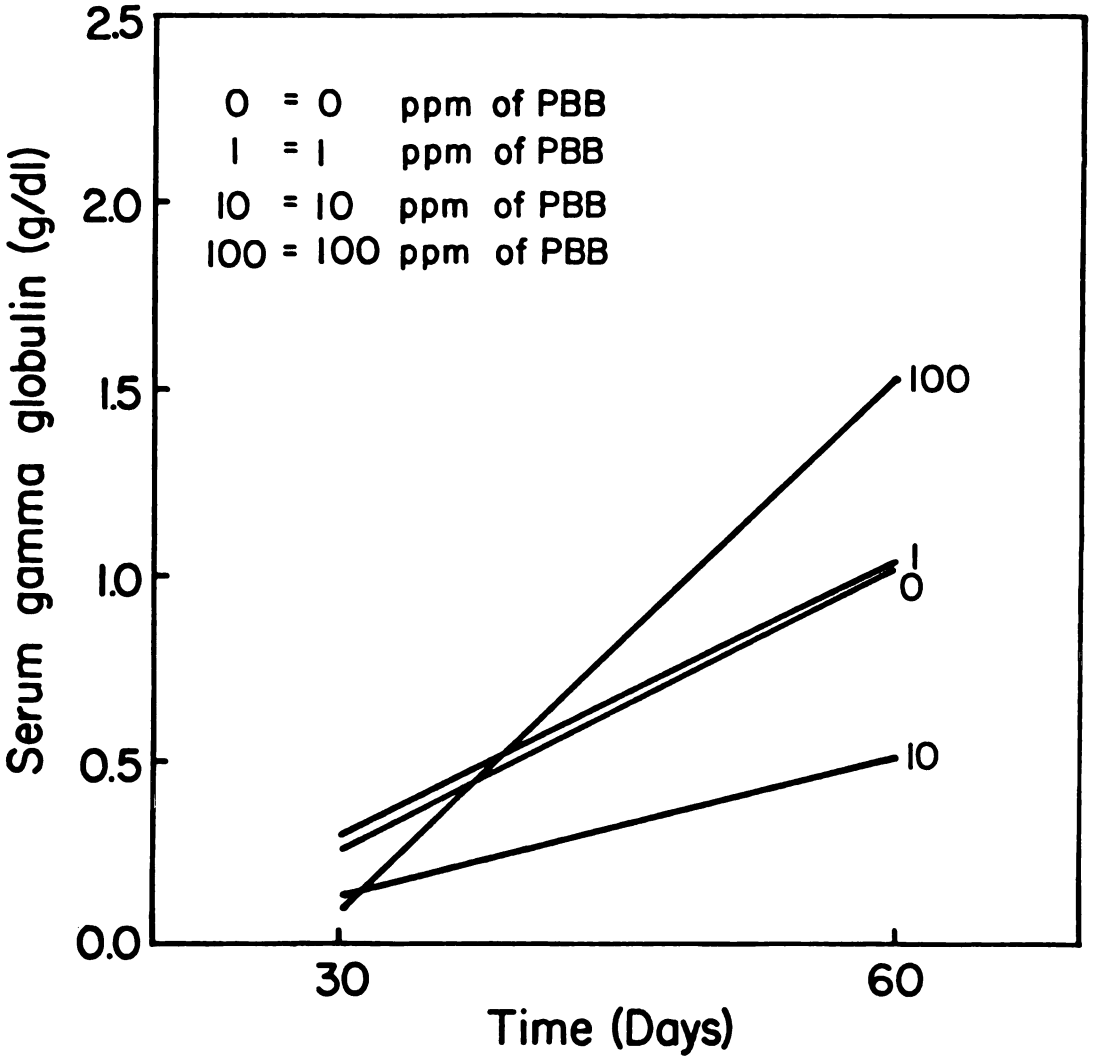


Table 8. The results of statistical evaluation of the effects of treatment factors and their interaction on lipid and lactic dehydrogenase isoenzymes in the serum of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Parameters	Treatment Factors						
	PBB	I	D	PBB X I	PBB X D	I X D	PBB X I X D
Triglyceride	NS	b	NS	NS	NS	NS	NS
Cholesterol	a	NS	NS	NS	NS	NS	NS
LDH isoenzymes:							
LD-1	NS	NS	NS	NS	NS	NS	NS
LD-2	a	NS	NS	NS	NS	a	NS
LD-3	NS	NS	NS	NS	NS	a	NS
LD-4	NS	NS	NS	NS	NS	NS	NS
LD-5	NS	NS	NS	NS	NS	a	NS

I = Iodine, D = Days (duration of treatment), X = Interaction

[a]Different ($P<0.05$).

[b]Different ($P<0.001$).

NS = Not significant.

Figure 9. The effects of PBB and iodine on serum triglycerides (mg/dl) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

Figure 10. The effects of PBB and iodine on serum cholesterol (mg/dl) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

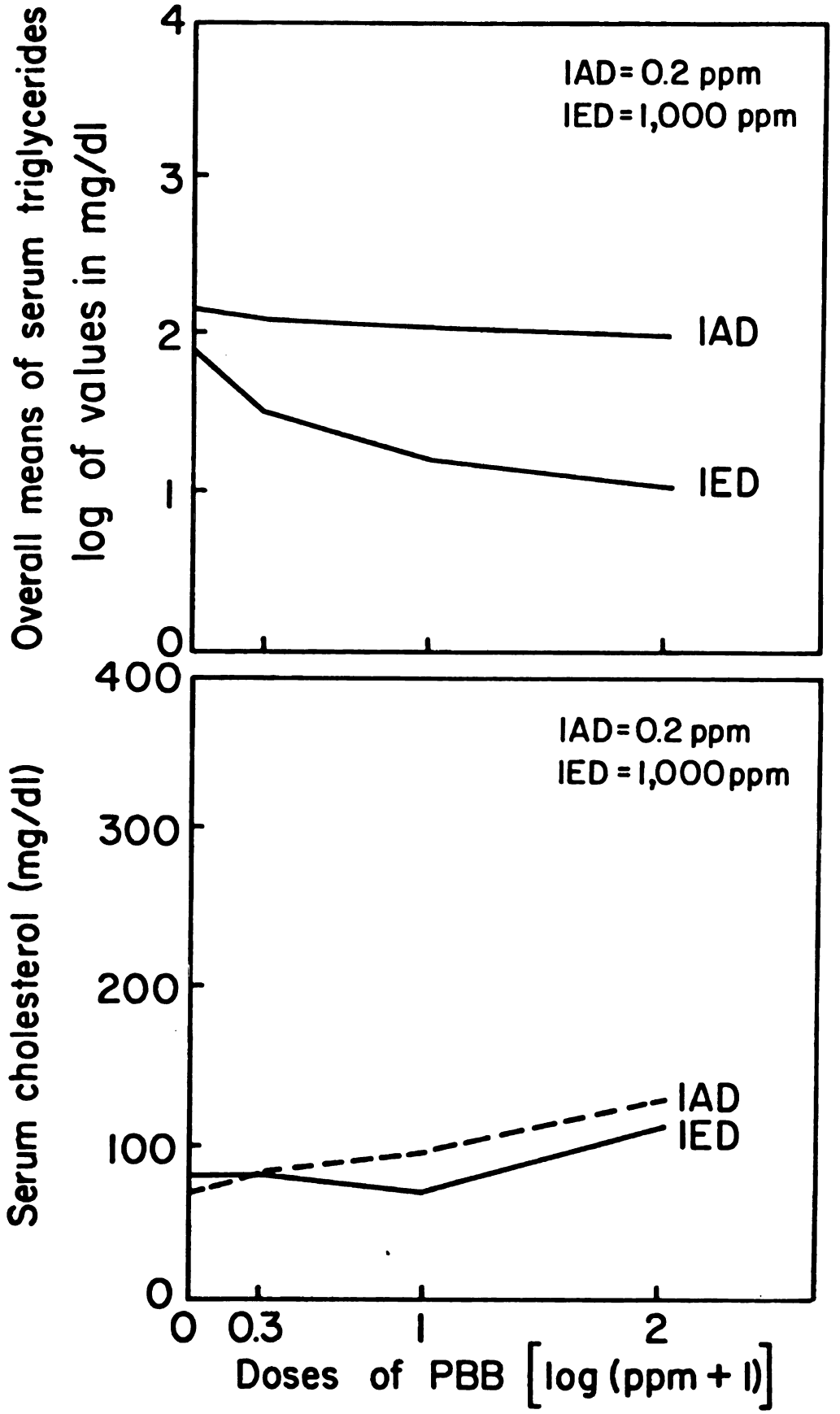


Figure 9

Figure 10

At 60 days, supplementation of excessive iodine in the diets caused a decrease ($P<0.001$) in LD-2 as compared to values in rats fed IAD. The effects of PBB on LD-2 were apparently dose related. The LD-2 values were increased in rats fed IED containing 1 or 10 ppm of PBB. However, LD-2 values were reduced in rats fed IAD and 1 or 10 ppm of PBB.

The effects of iodine and duration of treatment on LD-3 values are indicated in Figure 12 by nonparallel lines. At 60 days rats fed IAD had higher ($P<0.05$) LD-3 values (0.97±0.25%) than at 30 days (0.64±0.36%). At 30 days, rats fed IED had higher ($P<0.05$) LD-3 values (0.91±0.21%) than rats fed IAD (0.64±0.36%).

The LD-5 values were not affected by PBB but were affected by excessive iodine. At 60 days rats fed IED had higher ($P<0.05$) values for LD-5 (6.39±1.17%) than rats fed IAD (5.17±1.17%). There was no effect of excessive iodine at 30 days (Figure 13).

H. Tissue Analysis

1. Cytochrome P_{450}

The results of determination of cytochrome P_{450} in the liver are presented in Table 9.

Statistical analysis indicated significant effects of PBB ($P<0.05$), duration of the treatment ($P<0.05$), interaction between PBB and duration of the treatment ($P<0.05$), and interaction between PBB, iodine and duration of treatment ($P<0.05$) on the concentration of cytochrome P_{450} in the liver. However, there was no significant ($P<0.05$) effect of iodine on the level of cytochrome P_{450} in the liver.

Figure 11. The effects of iodine, duration of treatment and their interaction on serum LD-2 (%) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

Figure 12. The effects of iodine, duration of treatment and their interaction on serum LD-3 (%) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

Figure 13. The effects of iodine, duration of treatment and their interaction on serum LD-5 (%) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

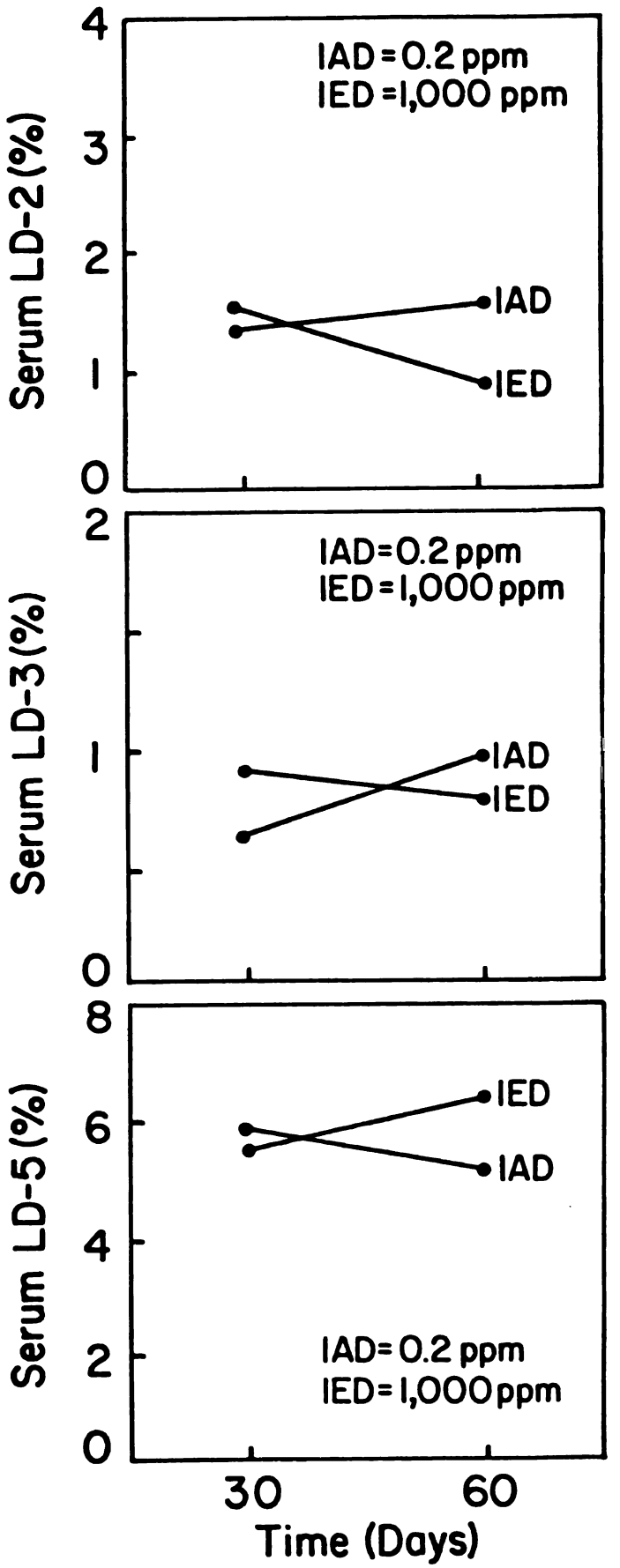


Figure 11

Figure 12

Figure 13

Table 9. The concentration of cytochrome P_{450} in the liver of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Levels of Iodine	PBB (ppm)	Concentration of Cytochrome P_{450} (nmoles/mg protein)	
		30 days	60 days
IAD	0	0.9212±0.1357	0.8231±0.0250
	1	1.0517±0.0430	1.3774±0.1860
	10	1.9683±0.1594	2.3305±0.1665[b]
	100	2.8476±0.1889	3.1166±0.1136[b]
IED	0	0.7862±0.0270	0.9448±0.1590
	1	0.9930±0.0513	1.0952±0.0996
	10	2.0870±0.0303[b]	2.4327±0.3444[b]
	100	2.9441±0.2950[b]	2.6174 0.3214[b]

[a]The values were means±SD of 3 samples.

[b]Different ($P<0.001$) from 0 ppm at the same levels of iodine and the same days.

The effects of PBB, iodine, duration of the treatment and their interaction on cytochrome P_{450} in the liver are illustrated in Figure 14. At 60 days, but not at 30 days, supplementation of 100 ppm of PBB to IAD caused an increase ($P<0.05$) of cytochrome P_{450} in the liver when compared to rats fed IED without 100 ppm of PBB.

By using an overall multiple regression equation to determine the effects of PBB on cytochrome P_{450} [$Y = 1.31 = 0.61$ (Log PBB) + 0.104 (Log PBB^2)], no significant increases of cytochrome P_{450} at 1 ppm of PBB were evident. However, if the dose exceeded 1 ppm of PBB, the levels of cytochrome P_{450} in the liver increased linearly.

2. Vitamin A

The results of the determination of vitamin A in the liver are given in Table 10.

Statistical analysis indicated no significant effects of excessive iodine on the concentration of vitamin A in the liver. On the other hand, there were effects ($P<0.005$) of PBB and duration of treatment, and the interaction between PBB and duration of treatment was significant ($P<0.05$).

Figure 15 illustrates the effects of PBB, duration of treatment and their interaction on the concentration of vitamin A in the liver. Generally, increasing doses of PBB caused decreases in the concentration of vitamin A in the liver.

3. Polybrominated Biphenyls

In general, there were dose related increases in PBB concentrations in the tissues (Table 11).

Figure 14. The effects of PBB, iodine, duration of treatment and their interaction on liver cytochrome P_{450} (nmoles/mg protein) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

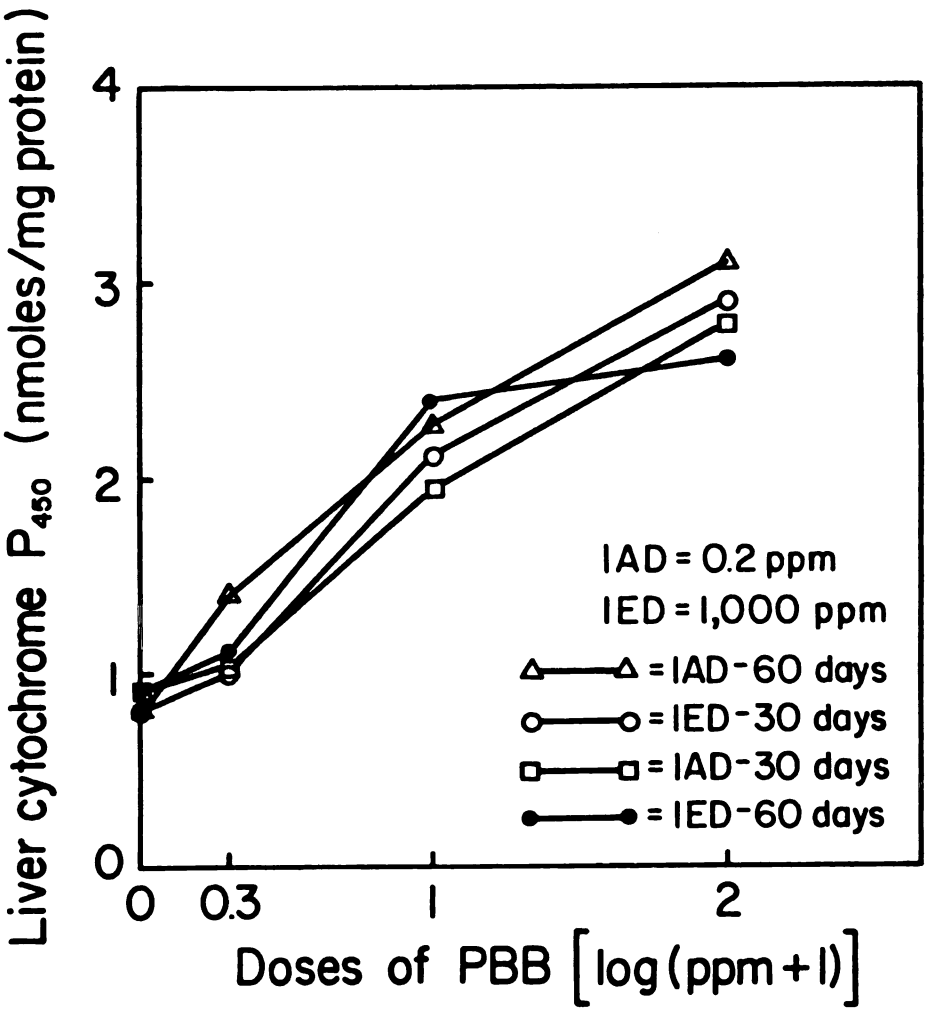


Table 10. The concentration of liver vitamin A (μg/g) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Levels of Iodine	PBB (ppm)	Concentration of Vitamin A in the Liver (μg/g)[a]	
		30 days	60 days
IAD	0	38.77±1.00	22.86±4.97
	1	29.81±1.99	14.91±0.99
	10	17.89±1.99	11.93±1.99
	100	11.93±0.00	12.42±0.99
IED	0	43.73±0.00	24.60±2.73
	1	39.75±7.95	17.89±2.49
	10	23.85±11.93	15.90±0.99
	100	7.95±0.00	9.44±1.49

[a]The values were means±SD of 2 pooled samples of 3 rats each.

Figure 15. The effects of PBB, duration of treatment and their interaction on liver vitamin A (µg/g) of rats fed IAD or IED containing different levels of PBB for 30 and 60 days.

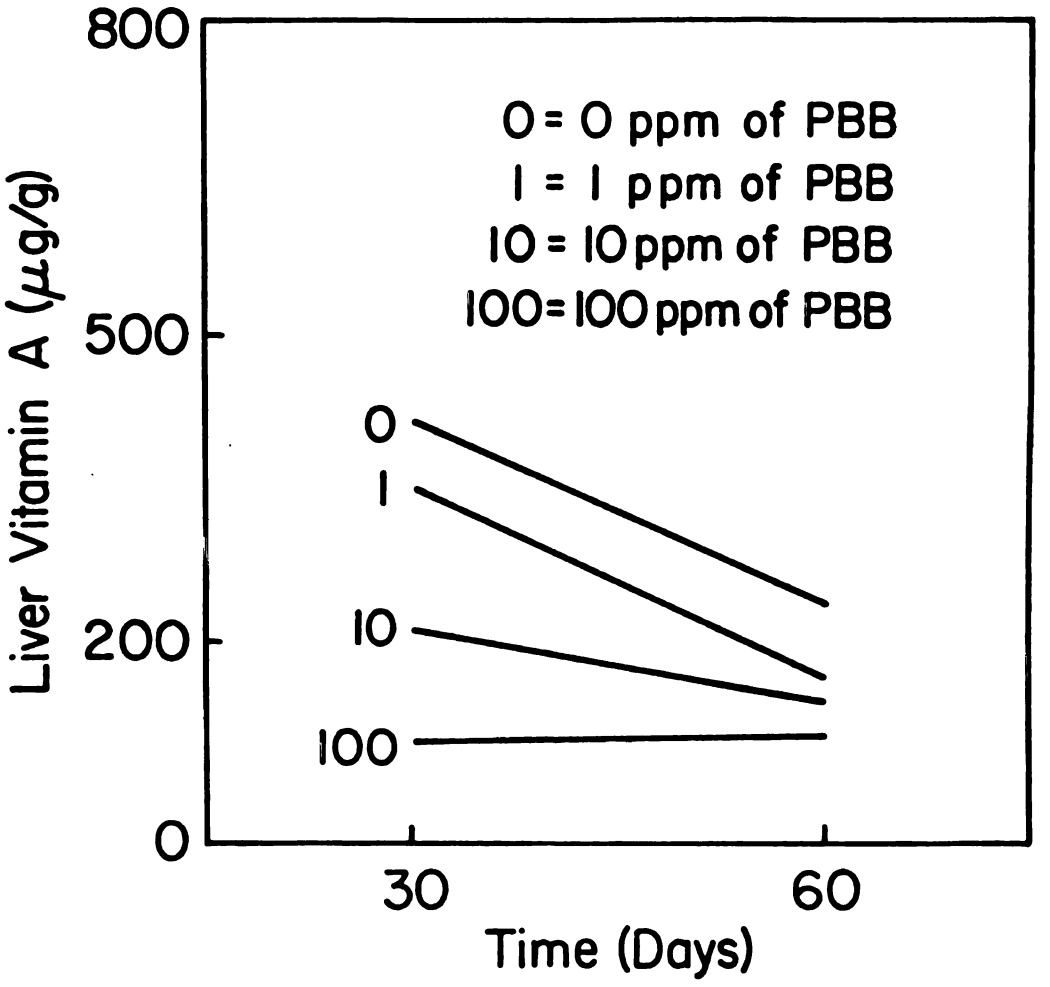


Table 11. The concentrations of PBB in the tissues of rats fed IAD or IED containing different levels of PBB for 30 and 60 days

Diet Supplementation		Concentrations of PBB in the Tissues (ppm)							
		Thyroid		Liver		Kidney		Fat	
Iodine	PBB (ppm)	30 days	60 days	30 days	60 days	30 days	60 days	30 days	60 days
IAD	0	0	0	0	0	0	0	0	a
	1	0.007	0.000	0.001	0.001	0.006	0.007	0.015	a
	10	0.027	0.053	0.026	0.017	0.021	0.017	0.157	a
	100	0.160	0.260	0.497	0.468	0.183	0.156	1.025	a
IED	0	0	0	0	0	0	0	0	0
	1	0	0.003	0.001	0.001	0.007	0.004	0.057	0.045
	10	0.027	0.033	0.056	0.122	0.019	0.020	0.070	0.738
	100	0.320	0.213	0.383	0.748	0.196	0.355	2.026	3.154

[a]Technical problems resulted in inappropriate values.

I. Histopathology

1. Thyroid Gland

The thyroid glands of rats given IAD and 0 ppm of PBB for 30 days were essentially normal. Typically, the peripheral follicles were larger and were lined by a lower epithelium than those in the central areas. Follicles in the central areas were lined by cuboidal epithelium (Figure 16). All follicles contained eosinophilic homogeneous colloid.

The thyroid glands of rats fed IAD and 1 ppm of PBB had similar morphologic features to those given IAD containing 0 ppm of PBB. The first noticeable pathologic changes were found in rats fed IAD and 10 ppm of PBB. A number of follicles had taller epithelium than normally seen. The colloid was pale and absorptive vacuoles were frequently seen on the periphery of the colloid. The most marked changes were found in the rats given IAD containing 100 ppm of PBB (Figure 17). The thyroid glands were hyperplastic and characterized by an increased number of follicles. The majority of the follicles were small and had a small lumen. The follicular cells were columnar and slightly foamy. Several follicles had a slight protrusion of lining cells into the lumen and contained either granular colloid or none at all.

Slight changes were found in the thyroid glands of rats treated with IED containing 0 ppm of PBB. The peripheral follicles were lined by low cuboidal or thin epithelium. Some follicles had a tendency to be confluent with the adjacent follicles. In some instances the lumens contained coarse colloid. The central follicles were maintained as smaller follicles. The epithelial cells in many follicles were larger than the controls and increased in height. The cytoplasm was foamy and most of the nuclei were vesicular. Similar morphologic appearances

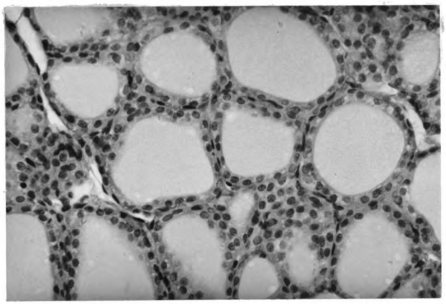


Figure 16. Thyroid follicles of a control rat at 30 days. The follicles were lined by cuboidal or low cuboidal cells and had homogeneous colloid. H&E stain, 400X.

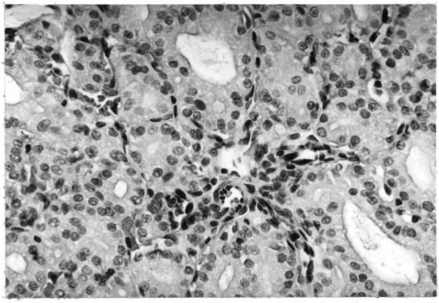


Figure 17. Thyroid follicles of a rat fed IAD containing 100 ppm PBB for 30 days. Hyperplastic follicles were evident. The follicles were small, increased in number and were lined by tall epithelial cells. The colloid was scanty. H&E stain, 400X.

were seen in rats given 1 ppm of PBB at the same levels of iodine. However, distinct changes were found in the thyroid glands of rats fed IED and 10 ppm of PBB. The thyroid glands were hyperplastic with colloid lacking or precipitated. The most marked changes were found in the thyroid gland of rats given IED containing 100 ppm of PBB. The thyroid glands consisted mainly of medium-sized follicles lined by columnar epithelium. The superficial border of the lining cells was irregular and the colloid was pale or scanty.

With a few exceptions, extending feeding to 60 days caused little additional changes. The thyroid glands of rats fed IAD and 0 ppm of PBB for 60 days were within normal limits (Figure 18). Various-sized follicles were lined by regular cuboidal epithelium and filled with homogeneous colloid. There were no pathologic changes detected in the thyroid glands of rats fed IAD and 1 ppm of PBB. However, 100 ppm of PBB produced hyperplastic changes of the thyroid glands that were similar to those described at 30 days with the same levels of dosage. The majority of the follicles were small and contained scanty colloid. The epithelium was columnar instead of cuboidal as normally seen. A few mast cells had infiltrated interfollicular tissues.

Changes observed in rats fed IAD containing 10 ppm of PBB for 60 days were similar but less prominent than seen at 100 ppm of PBB.

Feeding IED without additional PBB produced histologic features that were similar to those described at 30 days with the same level of dosage. However, at 60 days the majority of the follicles contained heterogeneous colloid and had a foamy appearance of epithelial cells (Figure 19). Most of the follicles were of medium size. The cells were slightly increased in height. Occasionally, degenerated cells were seen in the lumen of the follicles.

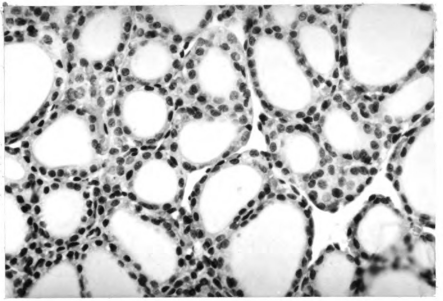


Figure 18. Thyroid follicles of a control rat at 60 days. Notice the similarity to those at 30 days (Figure 16). H&E stain, 400X.

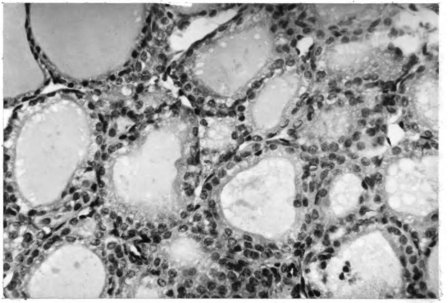


Figure 19. Thyroid follicles of a rat fed IED containing 0 ppm PBB for 60 days. Notice slight changes of the cell height. The cells were foamy in appearance and the colloid was not homogeneous. H&E stain, 400X.

Moderate thyroid hyperplasia was observed in rats fed IED containing 1 ppm of PBB. The follicles were small and had columnar epithelium. The colloid was depleted and granular. More distinct hyperplasia was found in rats fed IED and 10 ppm of PBB. A few follicles had no colloid and others had coarse colloid.

The most pronounced thyroid changes for the entire experiment were found in rats fed IED containing 100 ppm of PBB for 60 days. The sizes of the peripheral and central follicles were about equal and of medium size. The epithelium was tall. The nuclei were stained darker than normal. Prominent papillary projections into the lumen were seen in a number of the follicles (Figure 20). The remnants of colloid were seen as granular material in the lumen. The interfollicular tissues were hypervascular.

2. Liver

The microscopic appearance of the liver of rats fed IAD containing 0 ppm of PBB for 30 days is illustrated in Figure 21. Basically, the histologic picture was within normal limits. The hepatic cords radiated from the central veins and were separated by prominent sinusoidal spaces. The polyhedral hepatocytes had relatively large and centrally located nuclei with prominent nucleoli and little chromatin. Binucleated hepatocytes were occasionally seen. The Kupffer cells were distributed throughout the parenchyma. The intrahepatic ducts were lined by a low simple epithelium.

At 30 days, the livers of rats fed IED were similar to those of rats fed IAD and the same level of PBB. Feeding 1 ppm of PBB produced slight changes consisting of small vacuoles in the cytoplasm, but other basic structures were normal. At 10 ppm of PBB, similar but more

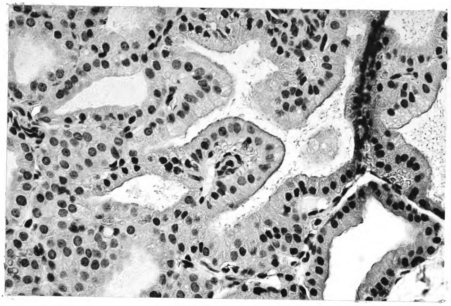


Figure 20. Thyroid follicles of a rat fed IED containing 100 ppm PBB for 60 days. Notice columnar epithelial cells lining the follicles. Short projections of the epithelium were present. The colloid was scanty and granular. H&E stain, 400X.

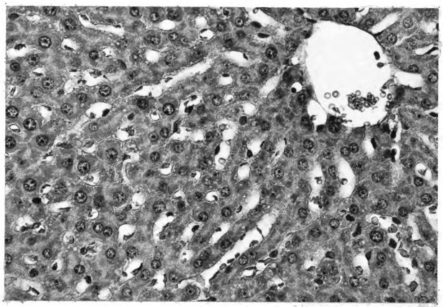


Figure 21. Liver section of a control rat. Notice regular arrangement of hepatic cords and prominent sinusoids. H&E stain, 400X.

severe changes were evident. The hepatocytes were moderately swollen and vacuolated. Small to moderately-sized vacuoles that were positive for the oil red O stain were diffusely distributed in most of the hepatic lobules. A few cells were necrotic and mitoses were occasionally seen among the swollen hepatocytes. More definitive changes were seen in the rats fed 100 ppm of PBB in combination with IAD for 30 days. The hepatocytes were markedly swollen and the fatty metamorphosis was more distinct. The swelling in the liver cells apparently initiated in the portal triads and extended to the surrounding areas, thereby compressing the relatively normal cells (Figure 22). Relatively normal hepatocytes were commonly seen in a narrow area right next to the central veins. In these areas the sinusoids were maintained as open spaces and contained a few red blood cells.

Similar histologic changes were observed in rats fed IED and 100 ppm of PBB for 30 days. Additionally, in this group of rats occasional cytoplasmic inclusions in the form of a "ring" were found in the swollen hepatocytes. The inclusions were homogeneous and eosinophilic and were usually located in the cytoplasm of nonvacuolated hepatocytes or in those that had small vacuoles. Sometimes the "ring" encircled more than one small vacuole. There were many individual hepatocytes which were necrotic. The nuclei were shrunken or pyknotic and the cytoplasm was eosinophilic. Kupffer cells were enlarged and increased in number, and the nuclei were plump and had less distinct chromatin.

Microscopic features of the liver of rats given 0 ppm of PBB in combination with IAD or IED for 60 days were essentially the same as for those at 30 days at the same dose. Generally, addition of PBB to

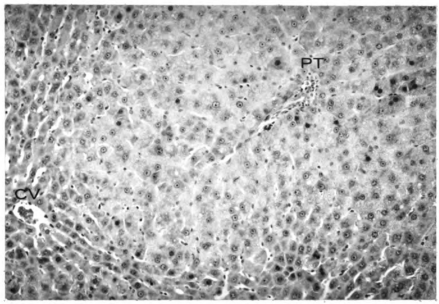


Figure 22. Liver section of a rat fed IAD containing 100 ppm PBB for 30 days. The hepatocytes around portal triads (PT) were markedly swollen, leaving normal-looking hepatocytes around the central vein (CV) and hepatic cords radiating from the central vein. Numerous hepatocytes had pyknotic nuclei. H&E stain, 160X.

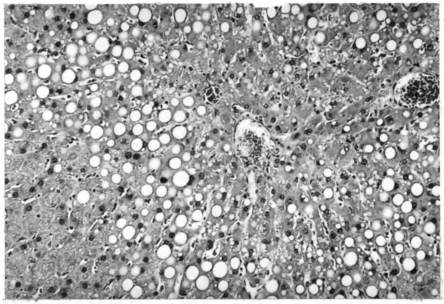


Figure 23. Liver section of a rat fed IAD containing 100 ppm PBB for 60 days. Notice numerous cytoplasmic vacuoles of various sizes in the midzonal area, with relatively few cytoplasmic vacuoles around the central vein. H&E stain, 160X.

either IAD or IED for 60 days caused more severe lesions than at 30 days. The size of hepatocytes and vacuoles was increased in rats given higher doses of PBB.

As with the hepatic lesions at 30 days, the most severe changes were seen in the liver of rats fed IAD containing 100 ppm of PBB. The hepatocytes were swollen greatly, and the cytoplasmic vacuoles were larger than at 30 days. Most of the sinusoids were compressed by swollen hepatocytes, especially in the areas close to the portal triads. The hepatocytes adjacent to the central vein were usually almost normal or had less cytoplasmic vacuoles (Figure 23). These changes appeared to be similar to a midzonal type of fatty metamorphosis. Cells with small cytoplasmic vacuoles had a granular or foamy appearance. In the areas with less vacuoles, ring-like structures and margination of cytoplasm were more commonly seen. Different sizes and shapes of ring structures that apparently were developmental stages of the inclusions were seen in the cytoplasm. The large inclusions seemed to result from fusion of smaller ones. In general, the larger inclusions had thicker "capsules." A number of individual hepatocytes were necrotic, as characterized by shrunken or pyknotic nuclei and eosinophilic cytoplasm. Kupffer cells were increased in number and size. Focal hepatic necrosis was occasionally seen with a cellular infiltrate that consisted mainly of neutrophils and a few lymphocytes.

The liver of rats fed IED containing 100 ppm of PBB had more advanced lesions than those seen with IAD at the same level of PBB. The hepatocytes were greatly enlarged and ring-like structures were thicker. In the hepatic lobules that had numerous ring-like structures, the cytoplasmic vacuoles were mostly small (Figure 24). In some

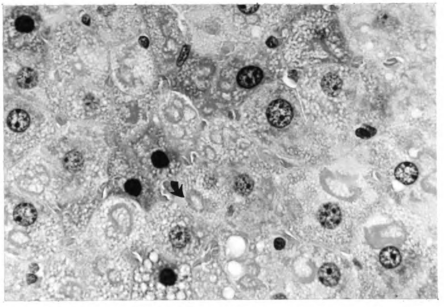


Figure 24. Liver section of a rat fed IED containing 100 ppm PBB for 60 days. The hepatocytes were markedly swollen and contained mostly small vacuoles. The sinusoids were inapparent. Ring-like structures of various sizes were present in the swollen cells (arrow). Several hepatocytes had pyknotic nuclei. H&E stain, 640X.

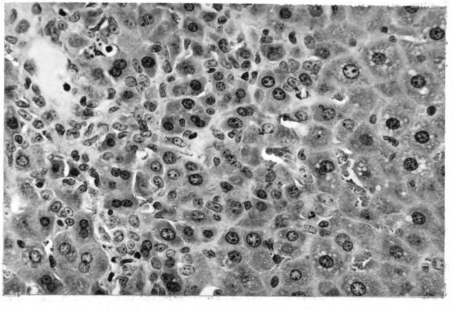


Figure 25. Liver section of a rat from the same group as Figure 24. Reticuloendothelial cell hyperplasia was present. These cells had relatively large nuclei with pale staining chromatin and had scanty cytoplasm. Some hepatocytes seemed to be compressed by the hyperplastic cells. H&E stain, 320X.

sections the type of fatty change was either centrolobular or more midzonal with larger vacuoles. Additionally, prominent reticulo-endothelial (RE) cell hyperplasia was found in the liver of rats fed diets containing 100 ppm of PBB for 60 days. The RE cells infiltrated among the hepatocytes. The cytoplasm was scanty, and the nuclei were large, plump or bean-shaped with less prominent chromatin (Figure 25).

3. Lung

In general, all of the rats had slight to moderate changes in the lungs. However, the most significant lesions appeared in rats fed the higher levels of PBB, especially after 60 days. Aggregations of lymphoid cells especially around the bronchioles were a common finding. In some areas, foamy macrophages were found in the affected alveoli.

The most prominent lung lesions were found in the rats fed IED containing 100 ppm of PBB for 60 days. The lung had patchy areas of inflammation with an infiltration of lymphocytes, macrophages and a few neutrophils. Alveolar septa were thickened. Squamous metaplasia was observed in the respiratory bronchioles (Figure 26). The metaplastic cells had prominent keratin granules in the cytoplasm and keratin materials were on the surface. Cellular debris was found in the lumen of the respiratory airways. The basal cells had darker nuclei than those on the superficially located cells. Squamous metaplasia was also seen in the alveoli resulting in narrowing of the lumen (Figure 27). The superficial cells had either elongated or pyknotic nuclei. Degenerated cells were frequently seen in the lumen. Keratinization was less prominent than was seen in the metaplastic respiratory bronchioles.

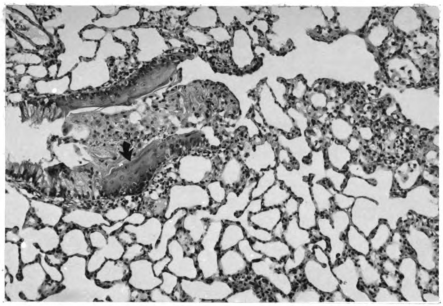


Figure 26. Lung section of a rat fed IED containing 100 ppm PBB for 60 days. Prominent squamous metaplasia was seen in the terminal bronchioles (arrow). Many alveoli had inflammatory cells in the lumen. H&E stain, 300X.

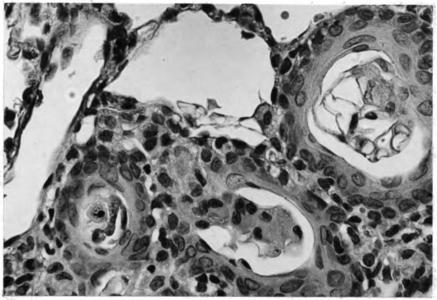


Figure 27. Lung section of a rat from the same group as in Figure 26. Notice squamous metaplasia of the alveoli and narrowing of the lumen. The alveolar cells were enlarged. H&E stain, 640X.

4. Salivary Gland

The most consistent pathologic change in the salivary glands was squamous metaplasia in the interlobular ducts (Figure 28). These changes were consistently observed in the salivary glands of rats treated with IED with or without supplementation of PBB. The ducts tended to be narrower and lined by 2 or more layers of metaplastic cells instead of 1 simple layer of cells as normally seen.

Several ducts were almost completely occluded by the metaplastic epithelium (Figure 29). The metaplastic cells had dark-staining nuclei. Frequently the upper layer of the metaplastic cells had pyknotic nuclei and eosinophilic cytoplasm. Degenerated cells were sometimes found in the lumen of the ducts. Mitotic figures were frequently seen in the basal layer of the stratified cells.

The acini of the salivary glands were essentially normal, and no infiltrative cells were observed.

5. Pituitary Gland

Pathologic changes were found in the pituitary glands of rats fed IED. These changes appeared not to be strictly dependent on the duration of the treatment nor the levels of PBB. The changes were found in several rats at 30 or 60 days regardless of the level of PBB. However, the most pronounced pituitary cell changes were found in the rats fed IED containing 100 ppm of PBB. The pituitary cells that supposedly were chromophobes were swollen and foamy. A number of these cells had pyknotic nuclei (Figure 30). Some rats fed IED at 0 ppm of PBB or higher for 30 or 60 days had cysts containing eosinophilic material and lined by flat cells (Figure 31). The cysts appeared to have formed in spaces left by the degenerated cells.

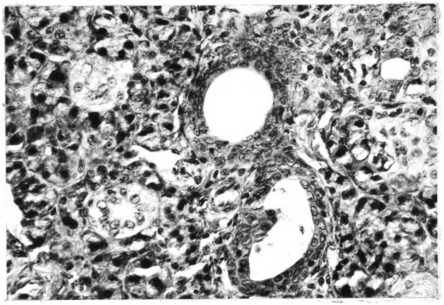


Figure 28. Salivary gland of a rat fed IED and 0 ppm of PBB at 30 days. Notice squamous metaplasia of interlobular duct. The metaplastic cells had dark-staining nuclei. H&E stain, 400X.

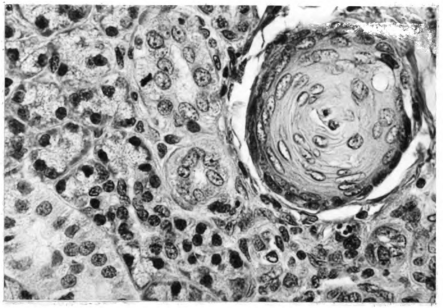


Figure 29. Salivary gland of a rat fed IED and 100 ppm of PBB for 60 days. Advanced metaplastic changes were seen in the interlobular ducts. The interlobular duct was occluded by the metaplastic cells. H&E stain, 640X.

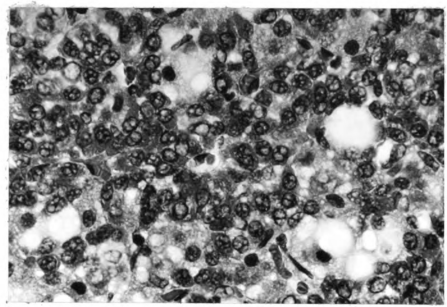


Figure 30. Pituitary gland of a rat fed IED containing 100 ppm of PBB for 30 days. Notice many chromophobe cells were swollen and foamy and had pyknotic nuclei. H&E stain, 400X.

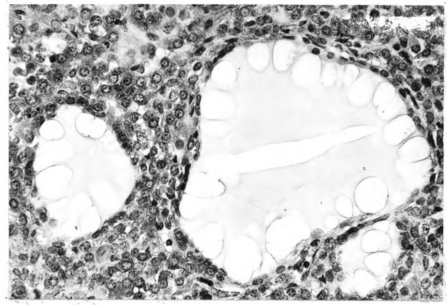


Figure 31. Pituitary gland of a rat fed IAD and 100 ppm of PBB for 60 days. Notice cysts with flat lining cells and containing proteinaceous material. H&E stain, 350X.

J. Electron Microscopy

1. Thyroid Gland

a. Thyroid gland of control rats. Examination of the fine structure of thyroid follicular cells of rats fed IAD containing 0 ppm of PBB revealed that the epithelial lining cells were low cuboidal and the apical plasma membrane bordering the lumen of the follicles had many microvilli. The fingerlike microvilli had a circular cross section and contained material that had a density similar to the apical cytoplasm. Rough endoplasmic reticulum (RER) was the most prominent of the cytoplasmic organelles (Figure 32). The characteristic parallel arrangement of RER sometimes diverged and formed sac-like structures. In most instances, these vesicles contained less dense material. The Golgi complex was usually located at the apical pole of the nucleus. The nuclei had several invaginations and had abundant heterochromatin. Large colloid droplets were not commonly seen. Membrane-bound cytoplasmic dense bodies that probably were primary or secondary lysosomes were encountered occasionally. Mitochondria were characterized by their elongated or ovoid shape and moderate electron density. These were distributed randomly throughout the cytoplasm except in the basal portion of the cells.

b. Thyroid gland of treated rats. Submicroscopic changes in follicular cells were noticed in rats fed PBB in the diet. At 10 ppm of PBB the changes were less prominent than those at 100 ppm of PBB. Additionally, the changes at 60 days were more pronounced than those at 30 days. The combination of PBB and IED produced the most pronounced changes.

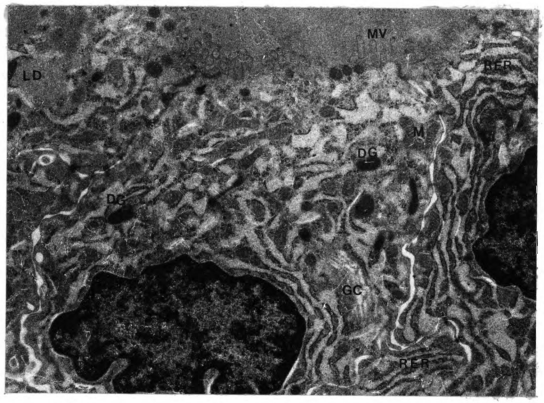


Figure 32. Electron micrograph of control thyroid follicular cells. Notice parallel arrangement of well developed rough endoplasmic reticulum (RER). A number of microvilli (MV) protruded into the lumen. Mitochondria (M) had maintained their integrity. Some dense granules (DG), less dense granules (LD) and Golgi complex (GC) were also seen. Lead citrate and uranyl acetate stain, 14,000X.

Rats fed IAD containing 10 ppm of PBB had larger and taller follicular cells than those in rats fed IAD and 0 ppm of PBB. The RER became dilated and tended to form wider cisternae. The cisternae contained fine granular materials. The electron dense bodies were increased in number and occasionally were fused with colloid droplets.

The follicular cells of rats given 100 ppm of PBB were definitely columnar (Figures 33 and 34). Most of the cells had a bulging surface with dome-shaped formations. Many of the cells had fewer and shorter microvilli. Most of the cisternae were irregularly dilated and only a portion of the RER had maintained their parallel arrangement. Dense granules were more prominent and were significantly increased in number. Sometimes the granules were located in the apical areas or right beneath the surface membrane. In a number of cells several mitochondria were swollen and had less prominent cristae. The Golgi complex became less prominent. Large vacuoles that probably were dissolved lipids were occasionally seen in the cytoplasm.

The most prominent follicular changes were found in rats fed IED containing 100 ppm of PBB. The apical borders were markedly irregular with the apical surface bulging into the lumen. Most of the cells had numerous dense granules that were located between the nucleus and the surface border. The granules were slightly less dense than those in rats fed IAD and 100 ppm of PBB but were more irregular in shape. Many large colloid droplets were present among the scattered dense granules. Frequently, the large colloid droplets were surrounded by smaller dense granules (Figures 35 and 36). The RER were moderately dilated, resulting in a lacy appearance of the cytoplasm. The Golgi complex was less prominent than in the controls. Most of the follicular cells had fewer or shorter microvilli. The colloid was not homogeneous.

Figure 33. Electron micrograph of thyroid follicular cells of a rat fed IAD containing 100 ppm of PBB for 30 days. Notice increased cell height, dilated rough endoplasmic reticular cisternae (CT), and the increased number of dense granules (DG). Lead citrate and uranyl acetate stain, 4,000X.

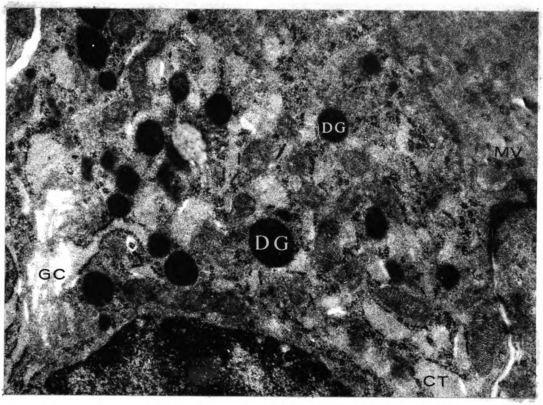


Figure 34. Higher magnification of a thyroid follicular cell of a rat from the same group as Figure 33. Notice numerous prominent dense granules (DG), dilated cisternae (CT), a reduced number of microvilli (MV) and indistinct Golgi complex (GC). Lead citrate and uranyl acetate stain, 22,270X.

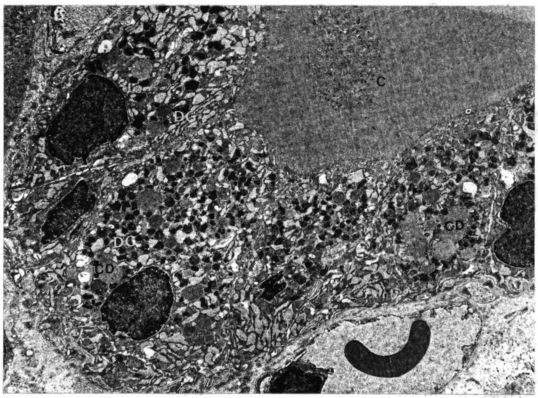


Figure 35. Follicular cells of a rat fed IED containing 100 ppm of PBB for 60 days. Notice marked increase in number of dense granules (DG) and colloid droplets (CD). The cells were relatively tall. The colloid (C) was granular. Lead citrate and uranyl acetate stain, 4,000X.

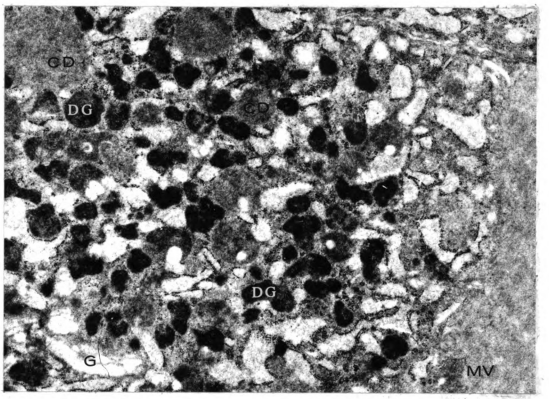


Figure 36. Higher magnification of Figure 35. Notice less distinct Golgi apparatus (G), the marked increase in number and irregularly shaped dense granules (DG), and colloid droplets (CD). The microvilli (MV) are reduced in number. Lead citrate and uranyl acetate stain, 15,700X.

Noticeable changes were found in rats given IED with 0 ppm of PBB. Follicular cells were either cuboidal or columnar. Numerous colloid droplets were located mostly in the middle parts of the cells (Figures 37 and 38). The RER was reduced in number and was located mainly in the apical regions. The RER beneath the surface border had maintained its parallelism, and microvilli were slightly increased in number. The nuclei were basally located. The intercellular spaces were sometimes dilated. Mitochondria appeared to be decreased in number and less distinct. There were more dense granules than in the controls, but fewer than were seen in rats given a combination of IED and 100 ppm of PBB.

2. Liver

a. Liver of control rats. Electron micrographs of liver cells of rats given IAD and 0 ppm of PBB for 30 days were essentially normal. The hepatocytes had normal size and shape, and the internal structures of the cells were well oriented.

The border of the hepatocytes was smooth with lateral interdigitations. Short microvilli protruded into the bile canaliculi and space of Disse. Bile canaliculi were located between neighboring hepatocytes. Nuclei were relatively large, round and centrally located. Light euchromatin and dense heterochromatin were scattered in the central and peripheral areas of the nuclei, respectively. Nuclear membranes were well developed with many nuclear pores.

One of the most striking features in the cytoplasm was a large number of relatively small, round or elongated mitochondria (Figure 39) that were randomly distributed throughout the cytoplasm. The mitochondrial matrix was dense and contained indistinct tubular cristae.

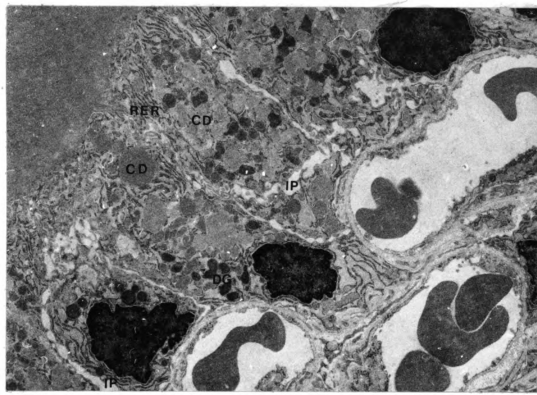


Figure 37. Thyroid follicular cells of a rat given IED containing 0 ppm of PBB for 60 days. The cells were relatively tall. Numerous colloid droplets (CD) and a moderate number of dense granules (DG) were seen in the cytoplasm. Parallel arrangement of rough endoplasmic reticulum (RER) is mostly maintained. Intercellular spaces (IP) were dilated. Lead citrate and uranyl acetate stain, 5,000X.

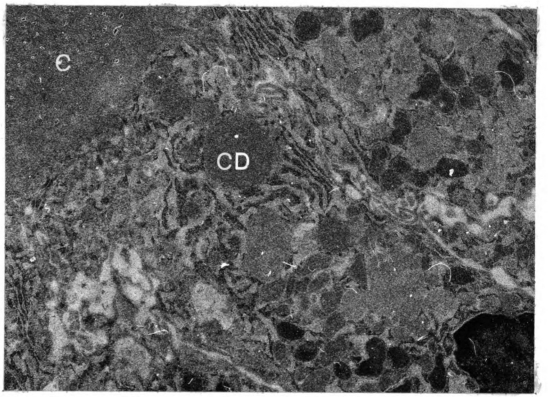


Figure 38. Higher magnification of Figure 37. Notice the colloid droplets (CD) had the same electron density as the follicular colloid (C). Lead citrate and uranyl acetate stain, 9,700X.

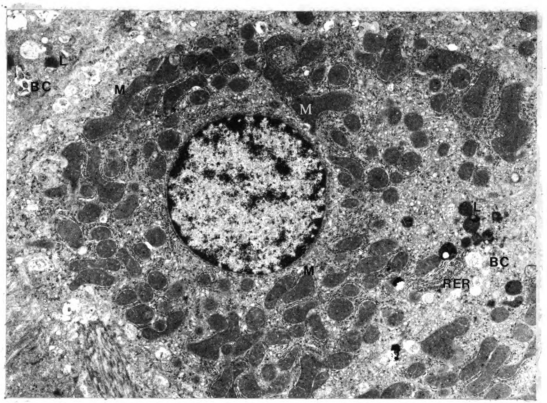


Figure 39. Electron micrograph of a control hepatocyte. Numerous mitochondria (M) were distributed randomly throughout the cytoplasm. Rough endoplasmic reticulum (RER) is well developed and has prominent ribosomes. Lysosomes (L) were located in the cytoplasm around bile canaliculi (BC). Lead citrate and uranyl acetate stain, 8,000X.

Rough endoplasmic reticulum (RER) had numerous ribosomes arranged in groups of parallel cisternae. The RER was distributed mostly in the areas among the mitochondria. There were also free ribosomes present in the same areas. Smooth endoplasmic reticulum (SER) generally occupied the areas that had few mitochondria. Glycogen particles were frequently closely associated with SER. Lysosomes were mainly located in the cytoplasm around the bile canaliculi.

b. Liver of the treated rats. Electron microscopic examination of the hepatocytes of rats fed IED did not reveal marked changes that could be related to the treatment. The architecture of the hepatocytes was normal, as was the distribution of the organelles. However, there was a slightly increased number of mitochondria and SER in a number of hepatocytes.

More changes occurred as PBB were added to the diet. In general, the changes at 60 days were more pronounced than those at 30 days. Furthermore, a combination of IED and 100 ppm of PBB resulted in more severe lesions than those in rats fed IAD and 100 ppm of PBB. The severity of electron microscopic changes was generally proportional to the doses of PBB rather than to the amount of iodine in the diets.

One of the most prominent changes was proliferation of SER at 10 or 100 ppm of PBB. Definite changes were found in the hepatocytes of rats fed 10 ppm of PBB regardless of the level of iodine. The proliferated SER was tortuous and vesiculated and contained electron dense materials (Figures 40 and 41). In the areas of proliferated SER, RER was decreased or dispersed and mitochondria were also depleted. Many vacuoles of different sizes were seen throughout the cytoplasm.

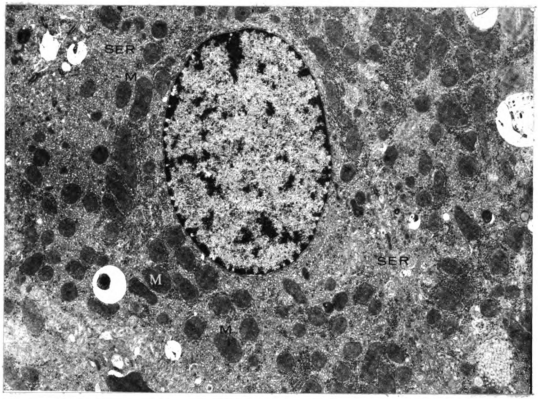


Figure 40. Electron micrograph of liver cells of a rat fed IAD and 10 ppm PBB for 30 days. Notice increased amount of smooth endoplasmic reticulum (SER) and dispersion of mitochondria (M). Lead citrate and uranyl acetate stain, 7,500X.

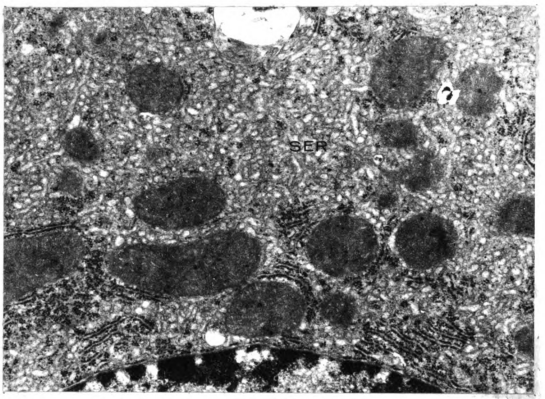


Figure 41. Higher magnification of Figure 40. Smooth endoplasmic reticulum (SER) was proliferated and vesiculated. Lead citrate and uranyl acetate stain, 22,000X.

In rats fed 100 ppm of PBB the changes were more striking. The RER was markedly decreased or dispersed. Many of the ribosomes were free in the cytoplasm among the altered RER. The RER was mostly displaced to near the nuclear periphery or to the periphery of the cells. This feature seemed to correspond to the margination of cytoplasm as seen under light microscopy. The cisternae were frequently enlarged or vesiculated.

Another remarkable change was the proliferation or vesiculation of SER. The hyperplastic SER seemed to have displaced other organelles (Figure 42). Glycogen particles were reduced in number and several mitochondria were swollen. Some other mitochondria were enclosed by membranous tubes that appeared to be denuded RER. Numerous fat droplets of different sizes were seen throughout the cytoplasm.

Another signifciant alteration was the formation of inclusion bodies. These bodies were seen at 30 days and had a characteristic fine structure. At the end of the experiment, more extensive myelin bodies were observed. These figures were identified in the liver of rats fed 100 ppm of PBB in IAD or IED. The myelin figures were more numerous, larger and thicker than seen at 30 days. Frequently, in one cell there were 2 or more smaller myelin figures. The size of the bodies appeared to increase by the apposition of the layers or by the confluence of smaller bodies.

In what appeared to be early stages of the formation of myelin bodies, several closely laminated membranes sometimes enclosed lipid droplets. The more developed bodies were larger and multilaminated. They consisted of closely paired smooth membranes that mostly were denuded RER. The paired laminated membranes were formed concentrically. The peripheral layer of the myelin bodies was frequently seen

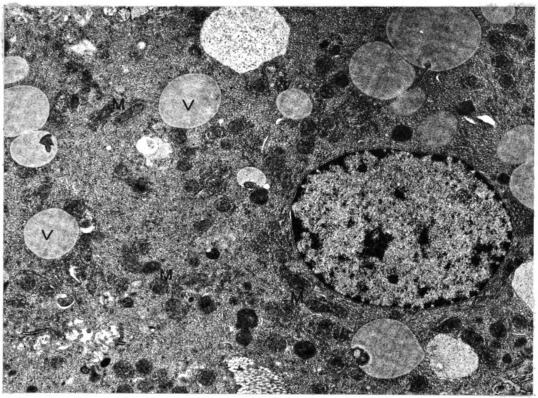


Figure 42. Electron micrograph of a liver cell of a rat fed IAD and 100 ppm PBB for 30 days. Smooth endoplasmic reticulum (SER) was markedly hyperplastic and displaced other organelles. Many vacuoles (V) were present. Mitochondria (M) were relatively reduced in number. Lead citrate and uranyl acetate stain, 6,800X.

as the continuation of RER (Figures 43 and 44). The concentric laminated membranes sometimes encircled mitochondria.

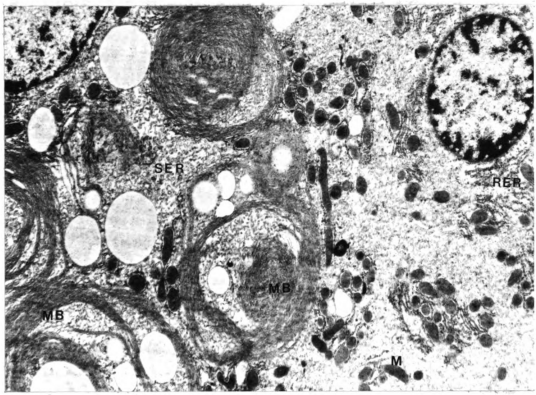


Figure 43. Electron micrograph of a liver cell of a rat fed IED containing 100 ppm of PBB for 60 days. Notice moth-eaten feature of hyperplastic smooth endoplasmic reticulum (SER), reduction in number of mitochondria (M), dispersion of rough endoplasmic reticulum (RER), and myelin bodies (MB). Lead citrate and uranyl acetate stain, 7,500X.

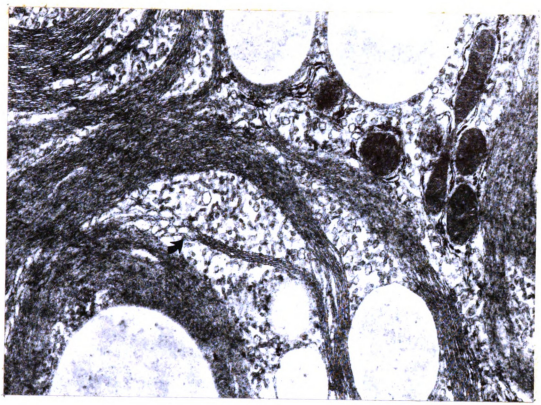


Figure 44. Higher magnification of Figure 43. Notice the transition between myelin bodies and denuded rough endoplasmic reticulum (arrow). Lead citrate and uranyl acetate stain, 7,500X.

DISCUSSION

A. General

There were no apparent clinical signs of disease except for a decreased rate of weight gain in rats given the highest doses of PBB. Similar observations were reported by Lee et al. (1975a) and apparently were related to the relatively low toxicity of PBB. However, with specific methods Tilson et al. (1978) observed behavioral and neurologic changes in rats given PBB, and the changes were still present after the termination of the treatment.

By 30 days in the present experiment, diets containing 100 ppm of PBB suppressed weight gains. At 60 days, doses as low as 10 ppm of PBB lowered the rate of weight gains. Sleight and Sanger (1976) reported decreases in body weight in rats fed 500 ppm of PBB for 30 days. Similar effects were also seen in rats given 100 to 150 ppm of PBB for 30 days (Tilson et al., 1978). The cause of the retarded growth was not known, but probably it was related to changes in organs such as the thyroid gland and liver.

As indicated in Figures 1 and 2, there was no significant effect of excessive iodine on the body weight. Liewendahl et al. (1972) also reported no effects on the body weight of rats given 20 to 30 mg iodine/head/day for 4 months.

B. Laboratory Results

Hematologic evaluations indicated that there were no significant differences related to the treatment. Slight changes in packed cell volume and in the number of red blood cells of rats fed PCB were reported by Bruckner et al. (1974), and these findings were supported by Oishi et al. (1978). Trapp et al. (1975) reported nonconsistent clinical pathologic values in Michigan dairy cows exposed to PBB. In contrast, in a controlled experiment significant alterations in hematologic values were reported in chickens given PBB (Heineman and Ringer, 1976).

In regard to the effects of iodine, Webster et al. (1966) reported mild anemia in dogs dosed with excessive iodine. A tendency towards hypoplastic anemia was described by Hillman et al. (1976) in cows naturally exposed to high levels of iodine. In the present experiment, as much as 1,000 ppm iodine did not produce significant effects on the hematologic values. There was no effect of PBB on BUN. Similarly, administration of 100 ppm of PBB for 30 days and 90 days to rats had no effect on BUN (McCormack et al., 1978). In contrast, feeding a diet containing an excessive amount of iodine resulted in depression of BUN values. Whether the excessive iodine interfered with the synthesis or excretion of BUN was not determined. Blaxter (1948a) reported an increased urea excretion in sheep fed iodinated casein.

Excessive iodine in the diet evidently also had a significant effect on SGOT at 60 days. The decreased level of SGOT was considered to be clinically insignificant when compared to an increase of SGOT. Increased BUN and SGOT was reported in cows given 25 g PBB/head/day (Durst et al., 1978). In another study (Oishi et al., 1978), BUN values were decreased in rats fed 1 ppm of polychlorinated

dibenzofurans (PCDF), and SGOT was increased at 10 ppm of PCDF. Significantly increased SGOT was reported in mice fed 100 ppm of PBB for 14 days, but SGOT was not increased at 28 days (Kluwe et al., 1978). Similarly, in the present study there were no significant effects of PBB on SGOT at 30 or 60 days.

As indicated in Figure 6, SAP was affected by PBB or iodine in the diet. The SAP had a tendency to decline as the doses of PBB were increased. A similar pattern for a decrease in SAP was seen in rats fed a combination of IED and PBB. Oishi et al. (1978) reported that there were no changes in SAP values in rats fed 100 ppm of PCB for 4 weeks. Ku et al. (1978) stated that SGOT and SAP in growing pigs were not affected by feeding PBB in the diet. In the present experiment, IED containing PBB caused a greater reduction of SAP when compared to the IAD containing the same level of PBB. Whether the reduction of SAP was related to decreased production or increased excretion needs further study.

The PBB had significant effects on serum albumin, beta globulin, gamma globulin, total protein, and A/G ratios (Table 6). Oishi (1977) reported increases in beta and gamma globulin and decreases in alpha-1 globulin in rats fed 100 ppm of PCB with no effects on the values for albumin and alpha-2 globulin. He explained that increased globulin was attributed to liver injuries. In the present experiment, inflammation was found occasionally in the liver, but marked inflammatory processes were most pronounced in the lung of rats fed 100 ppm of PBB. Based on electrophoretic pattern of serum protein, Sleight et al. (1978) reported apparent functional alterations in the liver of rats fed PBB. The evaluation of gamma globulin concentration expressed in grams appeared to be more informative (Figure 8). At

30 days, feeding 10 or 100 ppm of PBB caused decreases in the serum gamma globulin. Luster et al. (1978) reported that giving 30 mg/kg of PBB depressed immune response in rats. A significant decrease of gamma globulin was found also in rats fed 10 ppm of PCB and the changes were not dose related (Vos and DeRoij, 1972). These workers speculated that the nondose-related response of gamma globulin was perhaps due to antigenic stimulation. A nondose-related response for gamma globulin was also seen at 60 days in this experiment. Gamma globulin was significantly increased in rats fed 100 ppm of PBB but was decreased at 10 ppm of PBB. Schanbacher et al. (1978) found a decrease in serum albumin in heifers given 25 g PBB for about 35 days. They further stated that the alteration of serum protein was associated with interference of protein synthesis in the liver. The effects of polyhalogenated hydrocarbons on serum protein appeared to be dependent on the species and dosages. Ku et al. (1978) reported no consistent effect of 200 ppm of PBB on the serum electrophoretic profile in growing pigs. Similar results were reported in guinea pigs given 50 ppm of PCB (Vos and DeRoij, 1972).

Dietary administration of PBB (Sleight and Sanger, 1976) or PCB (Litterst and Loon, 1974) had been shown previously to increase the cytochrome P_{450} microsomal drug metabolizing enzymes. Troisi (1975) reported that PBB was a stronger inducer than was PCB. Dent et al. (1976b) found no elevation of cytochrome P_{450} in rats given 4.69 ppm of PBB for 2 weeks but an increase was seen at 18.75 ppm of PBB. In the present experiment, there was no increase in cytochrome P_{450} in rats fed 1 ppm of PBB for 60 days, but feeding diets containing 10 ppm of PBB produced a significant increase of cytochrome P_{450}. This effect coincided with liver weight changes in that no significant

differences of liver weight were seen in rats fed 1 ppm of PBB but were seen at 10 ppm or 100 ppm of PBB.

Doses up to 100 ppm of PBB did not cause a significant effect on serum triglycerides in this experiment, but excessive iodine reduced the amounts of triglycerides. Oishi et al. (1978) reported a decrease in serum triglycerides in rats given 100 ppm of PCB or 10 ppm of PCDF. In the same experiment they also found an increase in serum cholesterol. In a preliminary study, Garthoff et al. (1977) discovered that cholesterol values were increased in rats fed 5 ppm or more of PBB or 500 ppm of PCB. These workers stated further that the change in serum cholesterol was the most noticeable effect seen in the blood chemistry profile.

The increase of LD-3 and LD-5 in rats given IED but not in those given PBB was not anticipated. The elevation of LD-5 is usually associated with liver or skeletal muscle injuries. Histologically, there were no detectable lesions in the liver or skeletal muscle of rats given IED without PBB. The elevation of LD-3 is commonly associated with lung lesions. The lung lesions were more pronounced in rats fed a combination of excessive iodine and PBB than in those fed IED alone. The increase of LD-2 was probably due to hemolysis of the samples.

Generally, PBB concentrations in the tissues were dose related and were highest in the fat. At 30 days, addition of 100 ppm of PBB to IAD resulted in PBB levels in fat 2 times higher than in the liver. Lee et al. (1975b) reported a similar comparison in the calculated bromine residues in rats fed 100 ppm of OBP for 4 weeks. The length of treatment apparently affected the levels of PBB in the tissues. In the present experiment, feeding IED containing 100 ppm of PBB for

60 days resulted in concentrations of PBB in the fat 4 times greater than in the liver. Harris et al. (1978) found that rats fed 100 ppm of PBB for 10 weeks had concentrations of PBB in the fat more than 30 times as great as in the liver.

C. The Effect of Polybrominated Biphenyls on Vitamin A

Treatment with diets containing PBB reduced vitamin A in the liver but there were no clinical signs of vitamin A deficiency observed. A marginal vitamin A deficiency was reported to cause a decrease in rate of weight gain but did not stop growth (Phillips and Nockels, 1977). This statement seemed to apply to the condition of rats in this experiment.

There was a lack of information on an association between PBB and vitamin A storage in the liver. However, similar compounds have been studied extensively and possible mechanisms of the reduction of vitamin A in the liver by these compounds were elucidated.

The toxic effects of PCB were claimed to be due in part to the occurrence of vitamin A deficiency since PCB accelerated vitamin A deficiency (Innami et al., 1975). In a recent experiment Innami et al. (1976) suggested that PCB caused a decrease in serum vitamin A levels. This statement was based on the finding that retinol binding protein decreased significantly in the serum of rats fed a diet containing 0.1% PCB. Liver vitamin A was also reduced. Cecil et al. (1973) postulated that PCB or DDT caused destruction of vitamin A and alteration of lipid metabolism. However, Phillips (1963) stated that DDT decreased utilization of carotene and reduced vitamin A concentration in the liver. He further speculated that DDT reduced vitamin

A primarily by altering lipid metabolism rather than by damaging the liver or by affecting the conversion of carotene to vitamin A.

The PCB and DDT have been well accepted as being able to stimulate drug metabolizing enzymes including cytochrome P_{450}. When drug metabolizing enzymes were induced, a reduction of vitamin A occurred (Innami et al., 1976). Furthermore, they speculated that powerful oxygen was produced in the process of a hydroxylation reaction catalyzed by cytochrome P_{450}. This active oxygen then coupled with a superoxide generating system that probably was responsible for the reduction of vitamin A. A similar explanation is suggested for PBB, since PBB has similar effects on drug metabolizing enzymes in the liver.

Another possibility for the action of PBB on vitamin A would be through thyroid impairment. Histologic and electron microscopic observations indicated that PBB may have caused some degree of thyroid impairment leading to hypothyroidism.

All rats given IED alone or in combination with PBB had prominent squamous metaplasia in the interlobular ducts of the salivary glands. This consistent finding was observed at 30 or 60 days. It was stated that the concentration of iodine in the saliva was very high (Elmer, 1938). Possibly an even higher concentration of iodine occurred in the saliva of rats given excessive iodine. The iodine-concentrated saliva may have irritated epithelial cells of the interlobular ducts leading to metaplastic changes.

Slight changes in the thyroid glands were found in the rats given excessive iodine in the diets. More marked changes were found in the rats fed IED containing 100 ppm of PBB for 60 days. In these rats, squamous metaplasia was observed in the lung and salivary glands. Keratinization in the salivary glands and respiratory tract had been

described in rats with vitamin A deficiency (Moore, 1957). It seemed likely that the treatment combination of IED and 100 ppm of PBB interfered with vitamin A more severely than did excessive iodine or PBB alone.

D. Histopathology

1. Thyroid Gland

By 30 days, the addition of PBB to IAD tended to reduce the thyroid weight. At 60 days, the same treatment caused increases in thyroid weight with a significant effect occurring in rats fed IAD containing 100 ppm of PBB. The reason for this pattern of changes is not known. Studies in chickens (Hurst et al., 1974) indicated that PCB depressed thyroid size at low levels or early in the experiment but stimulated growth at higher doses or later in the experiment.

Histologic examination revealed that at 60 days more significant changes were seen than at 30 days. The earliest observable changes in the thyroid glands were found in the rats fed 10 ppm of PBB added to either IAD or IED. The thyroid glands were hyperplastic with decreased colloid. These changes suggested that the glands were in a hypothyroid state. The degree of hyperplasia did not always coincide with an increase in thyroid weight. Perhaps the decreased colloid influenced the lack of increase in thyroid weight.

The mechanism of the effect of PBB on the thyroid glands is not clearly understood. Norris et al. (1975) stated that thyroid hyperplasia in rats fed OBB may have been associated with the competition between iodine and bromine. Hyperplasia of the thyroid glands was also reported for PCB in rats (Yamane et al., 1975), for DDT or p,p'-DDT in avian species (Richert and Prahland, 1972; Jefferies and

French, 1969), for DDD in rats (Fregly et al., 1968), and for 4,4'-oxydianiline in rats and mice (Hayden et al., 1978). Yamane et al. (1975) provided evidence that PCB enhanced the biliary excretion of thyroxine. Fregly et al. (1968) hypothesized that DDD might enhance thyroid metabolism or increase clearance of thyroxine by the liver. Secondary hypothyroidism was proposed by Jefferies and Parslow (1976). They stated that PCB had a direct effect on the pituitary gland. Apparently the effects of PBB on the thyroid glands are similar to the other polyhalogenated hydrocarbons.

Histologically, the thyroid glands of rats fed IED containing PBB had more marked changes than those seen in rats given IED or PBB alone. This suggested an interaction of the effects of IED and PBB. This supposition was enhanced by statistical analysis as shown in Table 3.

Rats fed IED had slight changes in the thyroid glands. The follicles were lined by slightly thickened cells and contained pale colloid. Slight increases in follicular size and minimal changes of the cell height were reported in rats fed excessive amounts of iodine for 7 months (Galton and Pitt-Rivers, 1959). Apparently, the duration of treatment caused different effects. Four months of feeding high doses of iodine to rats produced enlarged thyroid glands without significant histologic changes (Liewendahl et al., 1972). However, administration of high doses of iodine for 9 months produced definite enlargement of follicles with excessive amounts of colloid (Correa and Welsh, 1960).

Rats given IED were apparently able to adapt to high levels of iodine by 60 days. An inhibiting effect of high doses of iodine on

thyroid glands occurred for a short period of time (Wolff and Chaikoff, 1948a; Galton and Pitt-Rivers, 1959), and then disappeared spontaneously. Prolonged feeding of excessive iodine produced thyroid changes (Correa and Welsh, 1960), but the rats had little or no evidence of hypothyroidism (Galton and Pitt-Rivers, 1959). If the adaptive mechanism failed, hypothyroidism could develop (Degroot and Niepomniszcze, 1977). In the present experiment, whether or not the slight changes in the thyroid glands caused by IED had any effect on any metabolic process was not determined.

The overall features of the thyroid glands indicated that PBB was goitrogenic but the effects were not manifested clinically, indicating that the rats were in a state of compensated hypothyroidism. Instead of hyperplastic thyroids, a compensatory type of colloid goiter was reported in Japanese quail fed DDT (Richert and Prahland, 1972).

2. Liver

In general, the effects of PBB on liver weight were dose related with the heaviest livers seen at 100 ppm of PBB. Hepatomegaly was commonly reported in rats given PBB. The increases in liver weight appeared to be related to the increases in lipid and endoplasmic reticulum as seen by light and by electron microscopy.

Basically, the histologic features of the liver of rats fed PBB were similar to those described previously (Sleight and Sanger, 1976; Kimbrough et al., 1978). However, at 30 days in this experiment, treatment with 100 ppm of PBB produced hypertrophy of the hepatocytes that appeared to initiate from the periportal area and left narrow areas around the central vein with almost normal hepatocytes. At

60 days a different pattern of fatty metamorphosis was found in that the changes were either midzonal or more centrolobular. These features were different from what was generally described as centrolobular fatty metamorphosis in laboratory animals exposed to PBB. Probably the type of fatty changes depended upon the degree of toxicity of the chemicals and the time of exposure to specific areas of the hepatic lobules. Herdson et al. (1964a) reported that after 5 days of treatment of rats with thiohydantoin, the abnormal cytoplasmic vacuolation was predominantly in the midzones of the lobules. By the seventh day the vacuolation was generalized.

Rats given IED without PBB did not have detectable lesions. However, rats fed IED containing PBB had more pronounced lesions in the liver than those rats fed PBB and IAD. The most remarkable lesions were found in rats fed IED containing 100 ppm of PBB for 60 days, indicating an interaction between excessive iodine and PBB in producing lesions. Many cytoplasmic inclusions were found. The ring-like inclusions seemed to represent condensation of cytoplasmic materials. Large cytoplasmic inclusions that could be seen by light microscopy were observed in rats fed OBB (Lee et al., 1975a). The incidence of cytoplasmic inclusions produced by PCB at 100 ppm was higher than at 500 ppm of PCB (Kimbrough et al., 1972). In the present experiment, the highest frequency of inclusions was found in the rats given 100 ppm of PBB, the maximum dose used.

Rats given 100 ppm of PBB had proliferation of reticuloendothelial cells in the liver. This lesion was seen especially in rats fed IED containing 100 ppm of PBB for 60 days. Kimbrough et al. (1972) reported that rats fed PCB had Kupffer cells and perivascular macrophages which contained hemosiderin. They did not specifically

mention hyperplasia of these cells. On the other hand, Allen et al. (1976) described reticuloendothelial cell hyperplasia in the liver of rats fed PCB. Kupffer cell proliferation and hyperplasia of the reticuloendothelial system were reported in vitamin A deficient rats (Moore, 1957). Whether the reticuloendothelial cell hyperplasia observed in the present experiment was caused by a direct effect of PBB or by vitamin A deficiency could not be judged.

3. Other Organs

Histopathologic changes in the lung included focal inflammatory processes and squamous metaplasia in the respiratory bronchioles and alveoli. The most severe inflammatory changes were seen at the higher doses of PBB. There were suggestions that PBB (Luster et al., 1978) and PCB (Vos and DeRoij, 1972) exposure could produce suppression of the immune response in rodents. If such is the case, the lung would be one of the most vulnerable organs to infection.

Squamous metaplasia developed in the interlobular ducts of the salivary glands and respiratory airways of rats treated with IED or with IED in combination with 100 ppm of PBB. Similar changes were commonly reported in animals with vitamin A deficiency. This suggests a relationship between the treatment and the vitamin A in the body. The reduction of vitamin A may have been clinically important in individuals exposed to PBB.

The reasons for the formation of pituitary cysts and for the cellular changes in the pituitary gland seen in this experiment are obscure. Microcysts and vacuolation of chromophobe cells were seen in the pituitary glands of rats given IED alone or in combination with PBB. Hayden et al. (1978) described thyroid changes and pituitary

cell alterations in rats and mice given 4,4'-oxydianiline. The basophils were enlarged, foamy or vacuolated. They suggested that pituitary changes were initiated by drug-altered thyroid function. Large pituitary cysts in cattle (Madsen et al., 1942) and proliferation of beta cells in rats (Sutton and Brief, 1939) caused by vitamin A deficiency were described.

E. Electron Microscopy

1. Thyroid Gland

Significant differences from the controls were observed in electron micrographs of the thyroid gland of rats fed 10 ppm of PBB added to either IAD or IED. There were changes in the number and size of colloid droplets, the number and shape of dense granules, microvilli and some organelles. Significant changes were also found in size and height of follicular cells. Basically the changes were dose and time related. However, in rats fed IED without PBB the changes were apparently not time related.

The thyroid follicular cells of rats fed diets containing 10 or 100 ppm of PBB had indications of increased activity as shown by increases in height, dilatation of cisternae and an increased number of colloid droplets. The RER became less parallel. Similar changes were described in thyroid glands after exposure to cold (Dempsey and Peterson, 1955). However, in rats fed 100 ppm of PBB, the alterations were more extreme. The cisternae were more dilated, and the contents were less dense. Many mitochondria were indistinct, and some of them were swollen. Probably these features are comparable to the changes in follicular cells after administration of propylthiouracil as described by Dempsey and Peterson (1955). The increase in activity

may have served to compensate for the declining function of the thyroid glands caused by PBB.

Rats given PBB also had more lysosomes and the microvilli were decreased and shorter. All of these changes had much similarity to those seen in rats fed PCB. Based on histologic, histochemical and ultrastructural observations, Collins et al. (1977) reported that rats fed 50 ppm of PCB for 4 weeks had columnar follicular cells with an increased number of lysosomal bodies and colloid droplets, abnormal microvilli and dilated cisternae. More severe changes were found in rats fed 500 ppm of PCB. Although the reason for the light and electron microscopic changes in the follicular cells caused by PBB have not been explained, Collins et al. (1977) found that the ultrastructural alterations of the thyroid follicles in rats fed PCB were accompanied by a significant reduction of thyroxine in the serum. It is quite possible that a reduction of thyroxine also occurred in rats fed PBB. Studies indicated that PCB enhanced biliary thyroxine excretion in rats (Yamane et al., 1975), and DDT increased metabolism of thyroxine in Japanese quails (Richert and Prahland, 1972). Hormonal analyses and determination of the basal metabolic rates of rats given PBB might help explain the thyroid changes.

The IED containing PBB produced more striking ultrastructural changes. It is suggested that the addition of PBB to an IED caused more dramatic effects on the follicular cells.

2. Liver

Administration of PBB produced marked alterations in the membrane system of the liver. Smooth endoplasmic reticulum proliferated at 10 ppm of PBB or higher. The hypertrophic SER appeared to be related to the pale appearance of the perinuclear zones and to the foamy cytoplasm of the hepatocytes. Increased SER and vesiculation have been induced by different toxic chemicals (Herdson et al., 1964a,b; Ortega, 1966; Kimbrough et al., 1972; Lee et al., 1975a; Sleight and Sanger, 1976). These changes probably represented the natural response of the hepatocytes to toxic agents. The metabolism of drugs in the liver has often been related to the hyperplasia of SER (Remmer and Merker, 1963). It was speculated that drug metabolizing enzymes liberated by SER had easy access to fat soluble toxic materials (Lee et al., 1975b). Based on morphological and biochemical considerations, Remmer and Merker (1963) concluded that increases in amount and activity of SER was a non-specific adaptation to the administration of drugs. In contrast, there were no appreciable morphologic changes and increases in SER or increases in drug metabolizing enzymes in the liver of rats given IED alone.

In rats fed diets containing 100 ppm of PBB, the hepatic RER lacked normal organization. The membranes were mostly decreased in number, displaced and denuded of ribosomes. Increase in SER accompanied by disorganization of SER was reported after treatment in rats with 3'-methyl-4-dimethylaminobenzene, a carcinogenic substance (Porter and Bruni, 1959). Although similar changes in SER and RER were observed in the liver of rats fed PBB, it does not necessarily mean that PBB are carcinogenic. The changes in the mitochondria and reduction in

glycogen particles were considered as other non-specific alterations produced by toxic chemicals such as PBB.

A striking feature observed in the liver of rats fed 100 ppm of PBB was the formation of myelin bodies. The different sizes of these bodies likely represented different stages of development. Myelin bodies developed in the liver of animals after treatment with different chemical agents (Herdson et al., 1964a,b; Ortega, 1966; Kimbrough et al., 1972; Lee et al., 1975a,b; Sleight and Sanger, 1976). Electron microscopically, Lee et al. (1975b) classified myelin bodies as smooth, ribosome-studded or glycogen-studded. The myelin figures seen in the rats fed PBB seemed to be of the fingerprint type, as Steiner et al. (1964) mentioned in their review. However, one chemical may produce several types of whorled membrane complexes (Steiner et al., 1964).

The morphologic and functional significance of the myelin bodies is still unclear. Many of the inclusion bodies found in this experiment were exceptionally large and could be detected by light microscopy. Large myelin bodies were also reported in rats treated with DDT (Ortega, 1966). These structures appeared to have developed from either RER or SER as seen in rats fed 100 ppm of PBB (Figure 44). The same consideration had been stated by Hruban et al. (1965). Since myelin bodies have been found in livers from animals given polyhalogenated hydrocarbons and in other chemical poisonings, they must be considered as non-specific evidence of cell injury.

Lee et al. (1975b) postulated that the whorled figures might represent modified lysosomes formed by focal degradation of the cytoplasm. However, previous cytochemical studies indicated that these figures could not be considered as "typical" lysosomes since they were not positive for acid phosphatase (Hruban et al., 1965). Herdson et

al. (1964a) stressed that an association between the cytoplasmic inclusions and the detoxication of the compound could not be ruled out. They observed that as long as the treatment was continued, the myelin bodies persisted. When treatment was discontinued, the myelin bodies degenerated and RER was restored. Steiner et al. (1964) postulated that "fingerprint" formation was associated with increased metabolic activities of the hepatocytes, especially anabolic processes, since they found glycogen particles in relation to free ribosomes. Further studies are necessary to evaluate the role of myelin bodies in response to different chemical substances.

SUMMARY

Ninety-six male Sprague-Dawley rats weighing approximately 125 g each were randomly allotted into 8 groups of 12 each. Four groups were fed an iodine adequate diet (0.2 ppm) and the other 4 a diet containing 1,000 ppm of iodine. Polybrominated biphenyls (PBB) were added at concentrations of 0, 1, 10, or 100 ppm. Six rats in each group were killed at 30 and the remainder at 60 days.

In general, there were no clinical signs of disease except for a decrease in rate of weight gain in rats given the highest doses of PBB. The hematologic values were essentially normal. Electrophoretic profiles of serum proteins suggested that hepatic function was altered by PBB. Concentration of vitamin A in the liver was significantly depressed by PBB, especially at the highest doses. There were no alterations of serum vitamin A levels that could be related to the excessive iodine treatment.

Diets containing 100 ppm of PBB and 1,000 ppm of iodine induced squamous metaplasia in the lung and the most pronounced histologic and electron microscopic changes in the thyroid glands and liver. The iodine excess diet caused squamous metaplasia in the ducts of salivary glands but did not cause prominent pathologic and weight changes in the liver and thyroid. Cysts and cellular changes were seen in the pituitary gland of rats given excessive iodine or PBB. Lesions in the liver produced by PBB were dose related. Liver to

body weight ratios were significantly increased at 10 or 100 ppm of PBB at 30 and 60 days. These changes were well correlated with the levels of cytochrome P_{450} in the liver.

The results of this investigation indicate that PBB are goitrogenic. High levels of iodine in the diet are not highly toxic to rats, but may increase the toxicity of PBB. The reduction of serum vitamin A may have clinical implications important for individuals exposed to PBB.

PART II

PATHOLOGIC CHANGES IN CALVES AFTER ORAL ADMINISTRATION OF EXCESSIVE IODINE FOR SIX MONTHS

LITERATURE REVIEW

A. Nutritional Aspects of Iodine in Ruminants

Iodine is an essential trace element for mammals including ruminants, and lack of iodine is the principal cause of goiter. Based on iodine requirements for man, a cow of 500 kg requires about 1 mg iodine/day (Hemken, 1970). In iodine deficient areas, incorporation of 0.0076% stabilized iodine salt at 1% of the grain ration provides the needed iodine in dairy cows (National Research Council, 1971). In high producing cows fed rations containing iodine-binding agents such as soybean meal, additional iodine may be required (Hemken, 1970).

Iodine is easily absorbed from the gastrointestinal tract of the ruminant. Barua et al. (1964) reported that approximately 70 to 80% of orally administered radioiodide was absorbed from the rumen. The absorption occurred also throughout the other parts of the gastrointestinal tract. Iodine was found in almost all of the body tissues. Nonthyroid tissues contained about 0.006% to 0.4% as much as thyroid gland on a unit weight basis. The muscle had the smallest concentration of iodine (Miller et al., 1975b).

One experiment indicated that approximately 79% of ^{131}I was found in the thyroid gland of cows given an oral dose of 100 μc of ^{131}KI (Lengemann and Comar, 1964). The thyroid uptake of ^{131}I was affected by iodine intake, stage of lactation, season (Swanson et al., 1957) and pregnancy (Brown-Grant, 1961).

The absorption of iodine from the rumen may be influenced by the solubility of the compound (Miller et al., 1975b) or by the presence of iodine-binding agents in the ration (Miller et al., 1975a). A single intravenous or oral dose of EDDI was metabolized in a similar way to NaI when both were dissolved in water (Miller and Swanson, 1973). However, EDDI was retained in the tissue longer than iodine from NaI.

When radioiodine was given to cattle, sufficient iodide was resecreted into the abomasum to substantially increase the concentration of the administered radioiodine. The rate of secretion of iodine was about 18 times the rate of absorption (Miller et al., 1975b).

Under normal conditions, organic iodine is conjugated in the liver and secreted in the bile (Hemken, 1970). Iodine is secreted into the small intestine and the concentration of iodine in the intestine increases substantially (Barua et al., 1964).

The main excretory pathways of iodine in lactating cows are through the feces, urine and milk. Total daily excretion in feces during 6 days of oral dosing of EDDI was about 39% and for NaI was 41% (Miller and Swanson, 1973). When NaI was administered intravenously, the average excretion was 32% (Miller et al., 1975b). Radioactive assays provided evidence that cows excreted 30 to 35% of a daily dose of ^{131}I in urine and feces (Lengemann and Comar, 1964).

Cows have an effective mechanism for recycling iodine. Even if the iodine supply is limited, the amount lost through the mammary gland can be effectively reduced and available iodine can be recycled (Swanson, 1972). A homeostatic mechanism had been assumed by Long et al. (1956) in cows given high doses of iodine. These workers reported that when KI feeding was increased to 44 mg/day, the quantities of

inorganic iodine in the serum rose proportionally to the amount of KI fed. On the other hand, when the dose reached 2.816 gm/day, the concentration of inorganic iodine in the serum did not increase proportionally.

An excess amount of iodine in the milk occurred in cows fed large doses of iodine (Lengemann and Comar, 1964). The presence of iodine in milk either nutritionally associated or as a result of dipping teats may cause public health consequences (Lengemann and Comar, 1964; Iwarsson and Ekman, 1973). Studies in sheep indicated that the level of iodine in ewe milk was a reliable indicator of daily iodine intake, and a concentration of iodine below 8 µg/100 ml of milk indicated iodine deficiency in the ewe (Mason, 1976).

Other pathways of iodine excretion include the salivary and sweat glands, but the amount in these fluids was not significant (Brown-Grant, 1961).

B. Regulation of Activities of the Thyroid Follicular Cells

Thyrotropin releasing hormone (TRH) is produced by the hypothalamus and has an indirect effect on the production and secretion of thyroid hormones. The TRH stimulates the anterior pituitary gland to synthesize and release thyroid stimulating hormone (TSH). The TSH is known as a prime regulator of follicular cells and stimulates the thyroid gland to synthesize and secrete thyroid hormones (Pantic, 1974).

The mechanism of action of TSH on the thyroid gland has been proposed by Quint (1974). The TSH binds a receptor on the outer surface of the follicular cells. This binding activates adenylcyclase which converts ATP to cAMP on the inside of the follicular cells. The

cAMP appears to increase iodine trapping, incorporate iodine into thyroglobulin, hydrolyze thyroglobulin, and release T_3 and T_4 (Quint, 1974). By a feedback mechanism the TRH stimulates the anterior pituitary gland to synthesize and release TSH. Production of T_3 and T_4 due to the action of TSH on follicular cells will block the stimulating effect of TRH upon the pituitary gland (Quint, 1974).

The effect of TSH on intact and altered thyroid gland has been studied by electron microscopy and by cytochemistry (Seljelid, 1967a,b,c). In a thyroid gland suppressed by administration of thyroxine, injection of TSH caused the appearance of colloid droplets in the apical cytoplasm (Seljelid, 1967b). The colloid droplets were usually larger in TSH-stimulated follicular cells than in nonstimulated follicles in untrated animals. Sixty to seventy-five minutes after TSH administration, most colloid droplets had acid phosphatase activity. Seljelid (1967b) further noticed that there was fusion between colloid droplets and lysosomes. In another study Seljelid (1967c) found that in suppressed thyroid glands, the lysosomes were dispersed throughout the cytoplasm. After stimulation with TSH the lysosomes tended to be located more superficially. The lysosomes appeared to move toward the apex before the colloid droplets were found. Seljelid (1967c) interpreted the apical movement of lysosomes as a direct effect of TSH and not secondary to the appearance of colloid droplets. The appearance of intracellular droplets has been observed in TSH-stimulated thyroids of pigs (Ekholm and Smeds, 1966).

Another feature found in thyroxine-suppressed follicular cells included the absence of colloid droplets with a predominance of lysosomes in the cytoplasm (Seljelid, 1967a). The cells appeared to be somewhat shorter than in normal glands. The microvilli were shorter

than in normal glands. Cytoplasmic protrusions other than the microvilli were not seen. On the other hand, in TSH-stimulated follicles, the microvilli appeared longer with varying length. Cytoplasmic protrusions (pseudopods) appeared on the luminal surface. The shape and size of the pseudopods were highly variable (Seljelid, 1967a). Similar features were only rarely encountered in normal thyroid gland. The pseudopods were consistently observed in stimulated thyroid follicles, even in follicles with markedly depleted colloid (Zeligs and Wollman, 1977). In the normal follicles these pseudopods would be expected to extend laterally and then to roll up within the follicular lumen and form colloid droplets. In the TSH-stimulated follicles, the pseudopods lost their ability to roll up (Zeligs and Wollman, 1977).

C. Therapeutic Aspects of Iodine

Iodine compounds have long been used to treat goiter and inflammatory processes or as an expectorant. In human medicine iodine compounds have also been used to manage bronchial asthma (Gutknecht, 1977).

Doses of 200 to 400 mg of EDDI/day/head were effective for both prevention and treatment of severe foot rot in feeder cattle (Burch, 1957). Burch stated that EDDI is more potent against foot rot than penicillin. Ten grams of EDDI at each feeding for 10 days was effective as a treatment for actinomycosis in cattle (Key and Loffer, 1956). Long et al. (1956) suggested that 3 g/day of KI at 2-day intervals was necessary to treat systemic mycotic diseases. This dosage was required to maintain adequate levels of the serum inorganic iodine.

Ethylenediamine dihydriodide has also been recommended as an expectorant in the treatment of respiratory diseases in cattle

(Herrick, 1972). Baker (1953) was convinced that EDDI was highly effective for the correction of breeding failure in cows.

The effect of iodine appeared to be related to its involvement in the inflamed areas or its effect on the invasive agent itself. Miller et al. (1973) presented evidence that the concentration of iodine was significantly higher in inflamed tissues than in the normal tissues. Another study indicated that administration of KI caused inhibition of granuloma formation by its "antifibrotic" action (Mielens et al., 1968). Iodine was also believed to reduce vascularity in the thyroid gland. This action of iodine would be beneficial during thyroid surgery.

The anti-inflammatory properties of iodide have been postulated as related to the uncoupling of oxidative phosphorylation (Middlebrook and Szent-Györgyi, 1955). This effect is similar to the biochemical properties of anti-inflammatory drugs such as salicylates (Whitehouse, 1964). In the presence of H_2O_2 and myeloperoxidase, human and guinea pig polymorphonuclear cells were able to fix iodine and thereby promoted the killing of microorganisms (Simmons and Karnovsky, 1973; Klebanoff, 1967). Results of another study suggested that when phagocytosis was induced iodination was enhanced. The iodination reaction within leukocytes was thought to have a bactericidal function (Woeber et al., 1972). The degree of iodination was well correlated with the bactericidal activity of the cell (Pincus et al., 1971). In contrast, Stone and Willis (1967) reported that iodide enhanced the inflammatory response by causing the inflamed tissues to become more suppurative.

D. Iodine Toxicosis in Cows

1. Signs

Toxicosis has been associated with either dietary or therapeutic use of iodine. Prolonged ingestion of excessive iodine in cows was thought by Hillman et al. (1976) to have caused thyroid impairment resulting in hypo- or hyperthyroidism. The clinical signs commonly observed were lacrimation, nasal discharge, hypersalivation, loss of or rough hair, decreased milk production, loss of weight, or poor growth.

The different clinical signs and sequelae appeared to be related to the condition of the animals. Coughing and hyperthermia with high mortality were reported in cows subjected to various stress factors or which had respiratory disease and received 312 mg of EDDI/day (McCauley et al., 1972). They concluded that EDDI aggravated the disease process. In unstressed feedlot cattle given 500 mg EDDI/day, death or growth retardation were not observed and the signs of iodinism disappeared after removal of EDDI from the feed (McCauley et al., 1973). The frequency of coughing was increased in calves fed 50 mg EDDI and 45 g urea/day (Rosiles et al., 1975).

Another field study was conducted by Wallace (1975), in which dairy cattle received more than 10 times the daily requirement of iodine. In addition to the common clinical signs of iodinism, he observed lameness and overgrown hooves. Furthermore, he pointed out that iodine toxicosis should be taken into consideration in cattle with the signs mentioned.

Differences in tolerance were recognized in calves fed excessive iodine. Calves could tolerate daily oral doses of 10 mg/100 pounds

live weight, but at 30 mg/100 pounds several calves developed signs of toxicity, including skin changes and digestive disturbances characterized by reddish-brown feces (Forbes et al., 1932).

In another experiment, some lactating cows produced cream with an objectionable odor caused by feeding of excessive iodine (Forbes et al., 1932). McCauley et al. (1972) reported metritis and diarrhea in several dairy cows with a history of being fed 680 to 1700 mg EDDI. These cows were anorectic and were unresponsive to therapy.

Fetal death or complications at parturition were documented in pregnant cows irradiated with excessive amounts of ^{131}Iodine (Clarke and Clarke, 1975). ^{131}Iodine caused thyroid damage in dairy heifers (Miller and Swanson, 1969) and resulted in lowered production and altered composition of milk.

Individual variability was observed in Holstein bull calves fed 10 to 200 ppm iodine in the form of calcium iodide. Significant decreases in feed intake and weight gain were noticed in calves given 50, 100, and 200 ppm (Newton et al., 1974). They claimed that the minimum toxic dose of iodine for calves was approximately 50 ppm and that 25 ppm might cause problems. In the earlier study, Newton et al. (1972) gave 0 to 200 ppm of iodine as calcium iodate. Feed intake was suppressed in all groups supplemented with iodine, but feed efficiency was depressed only at 200 ppm. Serum analysis indicated that all calves fed supplemental iodine had a much higher concentration of serum iodine. In contrast, Long et al. (1956) reported no clinical signs in mature Jersey cows given increasing doses of KI up to 2.816 gm (=2.15 gm I). Herrick (1972) described recovery in 2 steers within 2 weeks after the steers had been given as much as 20,000 mg EDDI/day. He concluded that EDDI was not detrimental to cattle.

No signs of toxicity were reported in calves fed 1.15 gm of iodine in the form of cuprous iodate or KI despite the fact that the serum iodine concentration reached 1,400 µg/100 ml (Kuebler, 1957). However, Rosiles et al. (1975) found about the same levels of iodine in the serum of calves fed 500 mg EDDI/day for 2 weeks, and the calves had signs of iodinism. Iodinism with occasional signs of hyperthyroidism might also develop after prolonged use of iodine preparations such as used in the treatment of actinobacillosis (Clarke and Clarke, 1975).

2. Pathologic Changes

There was a scarcity of information on the pathologic features of iodine toxicosis in cows. Different responses as to the thyroid weight were found in calves given excessive iodine (Newton et al., 1974). Iodine when given at 200 ppm in the diet caused an increase in size of the thyroid gland in some cows. In contrast, in another trial in which 0 to 50 ppm of iodine was used, the thyroid weights tended to be smaller than normal. In addition, the mean adrenal gland weight of the calves fed 25 or 50 ppm of iodine was significantly heavier than in the controls.

McCauley et al. (1972) observed 4 herds of beef or dairy cattle with a history of receiving an excessive amount of EDDI. The postmortem examination revealed tracheitis, bronchopneumonia with excessive fluid in the bronchial trees, and fibrinopleuritis. Bacteriologic and virologic examination of some of the cows did not reveal pathogens normally related to bovine respiratory disease. They stated that EDDI affected the host response mechanism.

Protruding eyes and loss of hair around the neck, eyes or over the back were found in some herds in Michigan (Hillman et al., 1976). They suggested the possibility of exophthalmic goiter. Laboratory analysis indicated that some of the cows had hypoplastic anemia, but the total white blood cell counts were normal. In one herd they found amounts of iodine in milk that could be potentially hazardous to human health.

Feeding studies done by Newton et al. (1974) resulted in all calves fed supplemental iodine having high concentrations of iodine in the serum throughout the trial. Those fed 200 ppm of iodine in the diet had significantly lower hemoglobin and serum calcium concentrations. At necropsy, thyroid hypertrophy and adrenocortical hyperplasia were found. The calculated iodine intake averaged 107 mg/day, and that was about 10 times the normal daily requirement.

E. Iodine Toxicosis in Man and Other Species

High doses of iodine have been known to cause goiter in some individuals. On the other hand, iodine is used to treat goiter and hypothyroidism (Selenkow, 1965). Administration of expectorants containing excessive iodine had been reported to produce hyperthyroidism (Gutknecht, 1977). Treatment of nontoxic goiter with elevated doses of iodine tended to increase the severity of goiter (Vagenakis et al., 1972). Recurrence of myxedema with symptoms of hypothyroidism had been reported in human patients due to excessive intake of KI and the adverse effects ceased when KI administration was stopped.

In animals other than cows, toxic effects of excessive iodine application have been documented. Excessive iodine intake in pregnant mares produced mortality in foals (Drew et al., 1975). The mares had

an iodine intake of approximately 83 mg/day in the feed. An enlarged thyroid was observed in one mare, and the dead foals had hyperplastic thyroids. The foals did not respond to treatment and had increased amounts of protein-bound iodine in the serum.

Lambs fed EDDI or KI ranging from 94 to 785 mg/head/day for 22 days had signs of toxicosis (McCauley et al., 1973). The lambs given higher doses were more lethargic, consumed less feed, and grew more slowly than did lambs given smaller doses. Excessive nasal discharge and skin changes were not found, but all lambs had hyperthermia. Marked lesions of bronchopneumonia were found in 4 lambs given large doses. Histologically, the thyroid glands of the treated lambs were essentially normal. It was suggested that iodine may affect the defense mechanism of animals with acute or chronic infection.

Blaxter (1968b) produced hyperthyroidism by feeding excessive iodinated casein to 6 sheep. The sheep had hyperthermia, increased pulse rate and respiratory discomfort, and they were reluctant to move. Two sheep died during the experiment. The weights of liver, heart, kidney and adrenal gland were increased. Histologic observations included normal to regressed thyroid glands, loss of most of the body fat, deficiency of medullary tissues of the adrenal gland or lipid depletion of the zona reticularis. The lung was emphysematous and pneumonic. Hobbs and Hansard (1952) mentioned that high doses of iodine may cause abortion and may also intensify signs of phosphorus deficiency in sheep.

Feed containing 400 ppm $Ca(IO_3)_2$, when fed to pigs for 97 days, produced no signs of toxicosis. However, the thyroid weight and serum iodine levels increased, whereas iron concentration in the liver was reduced (Newton and Clawson, 1977). Doses of either 800

ppm or 1,600 ppm of iodine caused the pigs to grow slowly. Feed consumption and hemoglobin concentration were reduced. Swine were not affected by dietary levels of iodine that were toxic to rabbits and rats (Arrington et al., 1965). However, young pigs under 4 weeks were adversely affected by feeding 4 ppm of KI (Frape et al., 1969).

Mortality of dogs started within a week after oral administration of 200 mg KIO_3/kg and all dogs died when given 250 mg KIO_3 (Webster, 1966). Clinical signs were emesis, anorexia, prostration, hemoglobinuria and coma. Necrosis was found in the liver, kidney, mucosa of the gastrointestinal tract, and urinary bladder. Retinal pigmentation was sometimes present. At the lower level of treatment, occasional vomiting and listlessness were noticed. Hematologic changes included mild anemia with a reduced M:E ratio. Histopathologic lesions were mainly hemosiderosis in the spleen, liver and kidney. There was also gastroenteritis.

Hamsters were given 2,500 ppm of iodine in feed without significant effects except for a slightly reduced feed intake and weaning weights (Arrington et al., 1965). On the other hand, Wolff (1969) described acute and chronic thyroiditis with desquamation of follicular cells in hamsters chronically fed iodide at 10 times the daily requirement. These workers hypothesized that some predisposing factors might have contributed to the development of goiter associated with excessive iodine intake.

Pigmentary degeneration was reported in rabbits given intravenous injections of 5 ml of 2% sodium iodate (Sorby et al., 1941). These changes were absent in albino rabbits. Oral administration of 500 to 1,000 ppm iodate for 2 or 5 days caused increased mortality in newborn rabbits (Arrington, 1965).

Administration by stomach tube of 500 mg/kg of 3 to 6% KIO_3 caused extensive degeneration of gastric parietal cells in mice (Highman et al., 1955), whereas retinal degeneration was found in mice and guinea pigs given intraperitoneal injections of 115 to 142 mg/kg KIO_3. However, retinal changes were not seen after prolonged administration of 0.25 to 0.5% KIO_3 in drinking water.

The toxic effects of excessive iodine in chickens have been studied. Fertility was not affected but embryonic death was greater in hens fed excessive iodine. Adverse effects of iodine upon egg production were transitory, since all hens resumed laying within 7 days after removal of excessive iodine. Young chicks were more susceptible to excessive iodine (Mayberry, 1968). Day-old chicks treated with 0.5% KI or NaI in drinking water gained weight slowly, had delayed comb development, and had a lack of feather growth. Paralysis occurred on the seventh day of treatment, and serum levels of GOT, LDH and creatine phosphokinase (CPK) were elevated. It was postulated that iodide altered the muscle enzyme systems.

Incidental iodine toxicosis was reported in captured penguins (Russell, 1977). The penguins were kept in a place that was disinfected daily with an improper dilution of an organic iodine. All penguins that died had prior signs of lethargy and lameness. The thyroid glands had varying degrees of follicular hyperplasia, and distended follicles were lined by columnar or flattened epithelium.

MATERIALS AND METHODS

A. Experimental Animals

Forty Holstein heifer calves were used to investigate the pathologic effects of daily feeding of excessive iodine for 6 months. The calves were purchased from 2 farms near Indianapolis, Indiana, and were housed in Barn A of the Michigan State University Veterinary Research Farm. The calves were kept in pens which accommodate 3 to 4 animals each. The average initial body weight was approximately 250 pounds.

Before the experiment was started, all calves were given an injectable anthelmintic[a] (2.0 ml/100 pounds body weight) and were vaccinated with *Brucella abortus* strain 19 and a mixed leptospiral bacterin.[b]

The feed consisted of 2 to 3 pounds of commercial calf pellets[c] daily and freely available second-cutting alfalfa hay. The basic experimental design is outlined in Table 1. The calves were divided into 4 groups of 10 each. Five calves of each group were given an intravenous injection of thyrotropin releasing hormone (TRH) at a dose of 15 µg/100 kg body weight every 28 days. Ethylenediamine

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[a]Levasole, Pitman-Moore Company, Washington Crossing, NJ.

[b]Affiliated Laboratories, Division of Whitmoyer Labs, Inc., Horshman, PA.

[c]Ralston Purina Company, St. Louis, MO.

Table 1. The basic experimental design

Levels of Iodine (mg/day)	Number of Calves	
	TRH	No TRH
0	5	5
50	5	5
250	5	5
1250	5	5

Iodine was in the form of ethylendiamine dihydriodide.

The calves were killed after 6 months of treatment.

Thyrotropin releasing hormone (15 µg/kg of body weight) was given intravenously every 28 days.

dihydriodide (EDDI) was added to water and given by drenching to supply the daily oral dosage of either 0, 50, 250, or 1250 mg of iodine/head/day. The calves were killed 6 months after the experiment was started, and complete necropsies were performed.

B. Collection of Samples

Within a few minutes after the calves were killed, the thyroid gland and a portion of the trachea were removed. Some of the thyroid gland was sliced into pieces approximately 2 x 2 mm and fixed in Karnovsky fixative (Karnovsky, 1965) for electron microscopy. The tracheal mucosa was covered with Karnovsky fixative and excess exudate was removed by washing with the fixative. A piece of tracheal mucosa (approximately 1 x 2 cm) was removed with a sharp razor blade and was attached with needles to a small rectangular piece of plastic foam. The mounted tissues were floated in a plastic container filled with

Karnovsky fixative and kept in a refrigerator until further processing.

Samples of skin, brain, pituitary gland, eye, lacrimal gland, salivary gland, thyroid gland, thymus, trachea, lung, heart, liver, spleen, urinary bladder, gallbladder, pancreas, kidney, adrenal gland, uterus, ovary, rumen, reticulum, omasum, abomasum, small and large intestine, and lymph node were taken and fixed in 10% buffered formalin for light microscopic examination. Pieces of liver were fixed in Carnoy's fluid.

C. Examination of Samples

1. Histologic Preparation

Carnoy's-fixed tissues were stained with Best's carmine for glycogen identification. Frozen sections were made and stained with oil red O for lipid identification. Pituitary glands were stained with Masson's trichrome, and the adrenal glands were stained with periodic acid-Schiff orange G. Other tissues for histologic examination were processed and evaluated as described in Part I of this dissertation.

2. Transmission Electron Microscopy

Samples of thyroid gland were processed and evaluated as described in Part I of this dissertation.

3. Scanning Electron Microscopy

The procedure for scanning electron microscopy was adapted from Malik and Wilson (1975). The Karnovsky-fixed tissues were mounted on the bottom metal holder by using 10% gelatin. The tissues were then postfixed in buffered osmium tetroxide. After postfixation the tissues

were rinsed 5 times in distilled water and immersed in a fresh, filtered, saturated solution of thiocarbohydrazide for 20 to 30 minutes. After rinsing 5 times with distilled water, the tissues were incubated in 1% osmium tetroxide for 2 to 3 hours. The procedure starting from fixation with buffered osmium tetroxide was done twice. The tissues were then dehydrated in graded ethanol for 4 hours. The tissues were critical point dried by using CO_2 and were examined with a scanning electron microscope[a] with accelerating voltage of 20 to 30 KV.

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[a]ISI, II, Mini-Scan, Japan.

RESULTS

A. General

Signs of respiratory disease were seen in most calves during the early period of the experiment. The signs were especially severe in calves given 250 and 1250 mg of iodine/day and consisted of cough, mucopurulent nasal discharge, seromucous ocular discharge and hypersalivation. Rough and falling hair and scaly skin were observed around the eyes, neck, and over the back and flank. The signs were diminishing in the last couple of months of the experiment.

One calf given 1250 mg of iodine/day died at 4 weeks and another in the same group was moribund and was killed at 10 weeks. These calves had severe respiratory discomfort and were severely depressed, lethargic, and weak. The other calves survived and were killed at 6 months.

B. Gross Lesions

1. External Lesions

Occasional areas of scaly skin were observed in the calves given 1250 mg of iodine/day and killed at 6 months, but the skin and hair were essentially normal. Congested conjunctiva and excessive nasal discharge were also apparent in these calves.

The 2 calves that died were emaciated and had roughened hair. Areas of alopecia and dry scaly skin were found on the neck, back, flank, and around the eyes. The conjunctiva was markedly congested.

2. Respiratory Tract

The principal gross lesions in the calves killed at 6 months were in the lungs. Consolidation of portions of the apical and cardiac lobes was consistently seen in calves given 1250 mg of iodine/day. Consolidation of the diaphragmatic lobes was seen occasionally. The affected areas comprised approximately 25% of the lung tissue. The mediastinal lymph nodes were swollen and congested. The mucosa of the larynx and trachea was thickened and congested, and exudate was seen on the tracheal mucosa of most of these calves.

Five of ten calves given 250 mg iodine/day had slight to moderate lung lesions. The other 5 calves did not have significant lesions. A partial consolidation of the apical and cardiac lobes and the anterior tip of diaphragmatic lobes was seen in the affected lungs. The consolidated areas comprised approximately 10% of the pulmonary tissues. Pleuritis with fibrous adhesions of the pleura to the thoracic wall was also seen in some calves. The mucosa of the trachea of the calves was somewhat thickened and had a moderate amount of exudate on the surface. Otherwise, there were no gross lesions.

Three of ten calves given 50 mg iodine/day had slight gross lesions in portions of the apical lobes. The affected areas comprised 5 to 10% of the lung tissue, and the tracheal mucosa appeared to be slightly thickened, but no exudate was seen. The mediastinal lymph nodes appeared to be normal. Gross lesions were not seen in the calves

given no additional iodine. The gross lung lesions are summarized in Table 2.

Table 2. Gross estimation of consolidated lung tissue of calves given different levels of iodine for 6 months

Levels of Iodine (mg/day)	Affected Lung Tissue (%)	Number of Calves
0	0	10
50	0	7
	5	3
250	1-2	5
	10	5
1250	25	8
	60	2[a]

[a]Two calves died in the first 10 weeks of the experiment.

The 2 calves given 1250 mg iodine and that died early in the experiment had the most severe lesions in the respiratory tract. The tracheal mucosa had petechial and ecchymotic hemorrhages and had mucopurulent exudate on the surface. Additionally, the mucous membranes were thickened and velvety with longitudinal and cross ripples. The lungs had dark red to grayish areas of consolidation in approximately 60% of all lobes. The consistency was firm but more friable than normal and the cut surface was dry. Necrotic lobules with distinct lines of demarcation were frequently present. Emphysematous lobules and interstitial emphysema were commonly observed. Fibrinous pleuritis was characterized by rough and thickened pleura and by adhesions to the thoracic wall and was seen primarily in the anterior part of the

lungs. The bronchi were filled with seromucous or mucopurulent exudate. Bronchial and mediastinal lymph nodes were swollen, brownish and edematous. Cultures from the lungs revealed an extremely heavy growth of *Pasteurella multocida* (*P. multocida*).

3. Other Organs

Gross lesions in the other organs were found only in the calves given 1250 mg iodine and which died during the early period of the experiment. The liver of these calves was enlarged with rounded edges and was mottled, friable and yellowish. The cut surface was greasy and bulging, and a piece of the liver floated when put into water. The gallbladder was thickened and edematous. In 1 calf an adhesion of the liver to the serosa of the reticulum was found, and a small abscess (3 cm in diameter) was present in this area of the liver.

C. Histopathology

1. Respiratory Tract

a. Trachea. Lesions were seen mainly in calves given 1250 mg iodine/day. In some areas the mucosal surface had become disrupted and epithelial cells were necrotic. In other areas there was squamous metaplasia and loss of cilia (Figures 1 and 2). The metaplastic cells were stratified and had prominent intercellular bridges. The cytoplasm was slightly eosinophilic and the nuclei were mostly centrally located. There was a mild to moderate infiltration of lymphocytes and neutrophils into the lamina propria. Some of the submucosal glands were hyperplastic and had an enlarged lumen. Similar but more severe

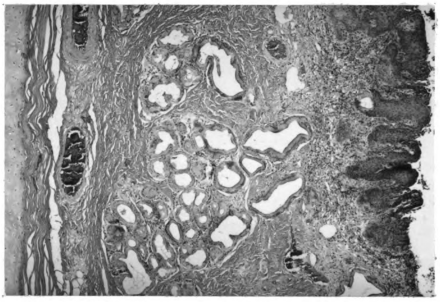

Figure 1. Trachea of a calf given 1250 mg iodine/day and killed at 6 months. Submucosal glands are hyperplastic. Mucosal epithelium had undergone squamous metaplasia with prominent formation of rete pegs. H&E stain, 50X.

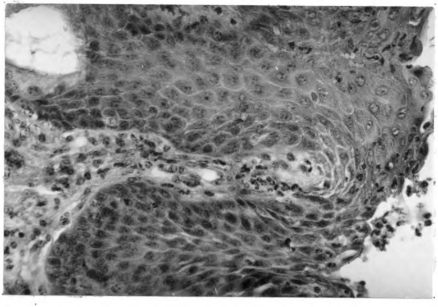

Figure 2. Higher magnification of Figure 1. Notice loss of cilia, squamous metaplasia with prominent intercellular bridges, rete pegs and infiltration of inflammatory cells in the lamina propria. H&E stain, 400X.

lesions were seen in the trachea of the 2 calves that died early in the experiment.

b. Lung. Lesions in the consolidated areas of the lungs were typical for a mild to moderate bronchopneumonia and the degree of involvement appeared to be dose related.

Fibrinous or purulent exudate was often found in the central portion of severely affected areas. Heavy infiltrations of neutrophils and some mononuclear cells into the lumen of bronchi and bronchioles were seen. The large bronchi had hypertrophic mucosal cells projecting into the lumen. In some areas marked alteration of bronchioles was observed. In necrotizing bronchioles, the necrotic tissues and fibrin were replaced by granulation tissue which appeared to have developed from adjacent denuded bronchial mucosa. The granulation tissue frequently occluded the lumen of the bronchioles (Figure 3). Bronchial and bronchiolar walls were infiltrated with mononuclear cells and a number of polymorphonuclear leukocytes. The changes in the bronchioles appeared to resemble bronchiolitis obliterans. Peribronchial lymph follicles were markedly hyperplastic and had frequently compressed the adjacent bronchioles. Among the alveoli which contained fibrin, there were some in which macrophages, considerable polymorphonuclear leukocytes and a few lymphocytes were mixed with the fibrinous exudate. Multinucleated giant cells were observed in more advanced stages of the process (Figure 4). A number of eosinophils were also found in other areas. Lymph vessels were dilated or obstructed. Occasional thromboses were also seen. In more nearly normal areas many alveoli contained serous exudate. The affected

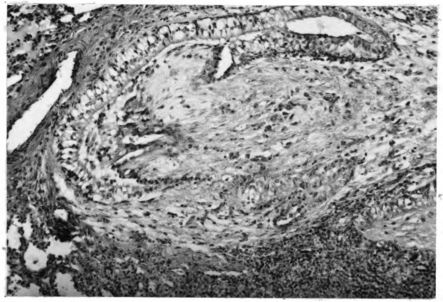

Figure 3. Bronchiole of a calf given 1250 mg iodine/day and killed at 6 months. Notice bronchiectasis and denuded mucosa. Reactive connective tissue is seen in the bronchiole. H&E stain, 120X.

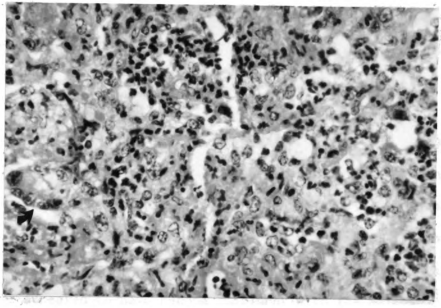

Figure 4. Lung of a calf given 1250 mg iodine/day and killed at 6 months. Notice alveoli are filled with numerous polymorphonuclear cells. Alveolar septa are thickened. Giant cells were present (arrow). H&E stain, 300X.

pleura was thick, irregular and fibrinous. The normal texture was disrupted and inflammatory cells were present.

More extensive lesions were found in the lungs and pleura of the 2 calves given 1250 mg iodine/day that died early in the experiment.

2. Thyroid Gland

The thyroid glands of the control calves were surrounded by thick capsules of dense irregular connective tissue. Capsular extension into the parenchyma comprised the trabeculae. The trabeculae had extended deeply into interfollicular areas and formed thin and loose connective tissue around the thyroid follicles. The follicles were relatively large and filled with homogeneous colloid. The sizes of the follicles were slightly variable and they were lined mostly by cuboidal epithelium (Figures 5 and 6). The epithelial lining cells had round dark nuclei which were basally located. Absorptive vacuoles were frequently observed on the surface of the cells, and the cells had a foamy appearance.

Figure 7 depicts the follicles of the thyroid gland of calves given 1250 mg iodine/day and which died before the termination of the experiment. The majority of the follicles were enlarged but varied in size. The follicles were filled with relatively pale colloid and were lined by flat or very low cuboidal epithelium. Many of the follicles appeared to have become confluent with the adjacent follicles. Even the small follicles were lined by very thin follicular cells. In some instances, degenerated epithelial cells were found in the lumen of the follicles. In these follicles the colloid was less homogeneous than normally seen.

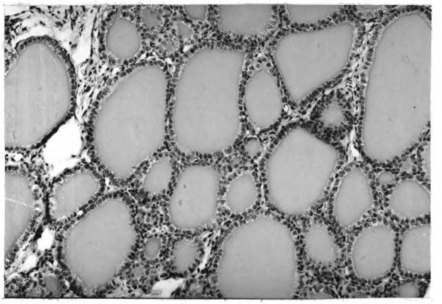

Figure 5. Thyroid follicles of a control calf. The follicles are of small to medium size and filled with homogeneous colloid. H&E stain, 120X.

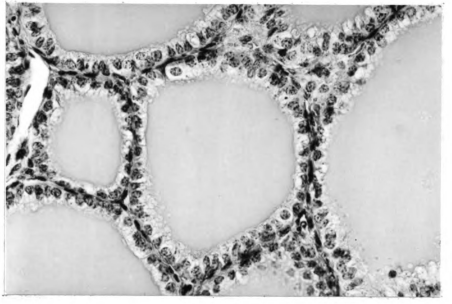

Figure 6. Higher magnification of Figure 5. Follicles are lined with cuboidal epithelium. H&E stain, 340X.

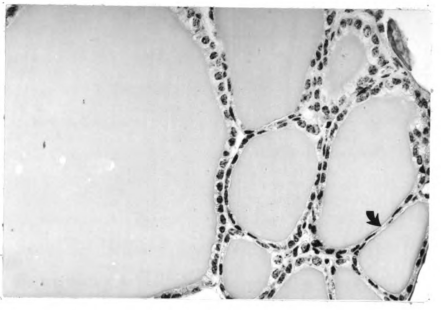

Figure 7. Thyroid follicles of a calf given 1250 mg iodine/day and that died early in the experiment. Notice the large and small follicles are lined by flattened cells. Notice the confluence of adjacent follicles (arrow). H&E stain, 300X.

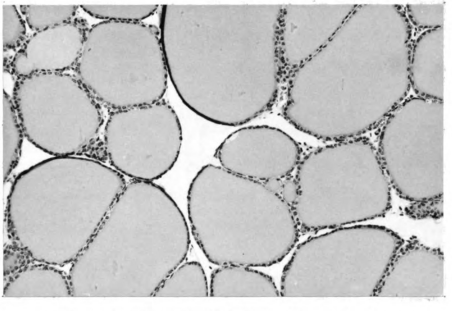

Figure 8. Thyroid follicles of a calf given 1250 mg iodine/day and killed at 6 months. The follicles are enlarged, relatively uniform and contain abundant colloid. Epithelium is flattened. H&E stain, 120X.

Two of three calves given 1250 mg iodine/day and no TRH from the same group had similar changes to those just described. The follicles were more uniform in size and contained abundant pale colloid. Several follicles had granular colloid, and a number of follicles had a tendency to be confluent with each other. The interfollicular septa were thin, and many of the nuclei were pyknotic (Figure 8).

There appeared to be some differences in the thyroid gland related to administration of TRH. The morphologic features of the thyroid follicles of 4 of 5 calves given 1250 mg iodine/day and TRH are presented in Figure 9. Generally, the thyroid gland consisted of follicles that were not much different from the controls. The follicles were of moderate size and contained homogeneous eosinophilic colloid. Low cuboidal or cuboidal epithelium lined the follicles. Resorptive vacuoles were commonly seen on the edge of the colloid. Only one calf from the same group had a thyroid gland with an appearance similar to the changes described for the calves given 1250 mg iodine/day without TRH challenge.

When the thyroid glands of calves given 250 mg iodine/day were compared with the thyroid from calves given the same dosage and TRH, the differences were similar but less prominent than those described for calves given 1250 mg iodine. However, there were no detectable differences or changes in the thyroid glands of the 10 calves given 50 mg iodine/day and the thyroid glands of the controls were essentially normal.

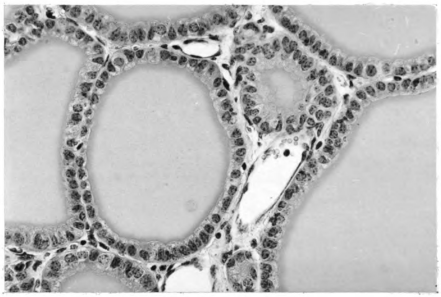

Figure 9. Thyroid follicles of a calf given 1250 mg iodine/day and TRH and killed at 6 months. Follicles are filled with homogeneous colloid and lined by cuboidal epithelium. Notice similarity to section from control calf in Figure 6. H&E stain, 300X.

3. Other Organs

Significant pathologic features were found in the salivary glands of 3 calves. Squamous metaplasia was evident in 2 calves given 1250 mg iodine/day and in another given 250 mg iodine/day. The interlobular ducts of the parotid glands were lined by more than one layer of epithelial cells. The metaplastic cells had dark elongated nuclei and more compact cytoplasm. Detached epithelial cells were frequently seen in the lumen of the ducts. The morphologic features of the acini were essentially normal. Figure 10 illustrates the changes described.

A seborrheic dermatitis was present in the scaly areas of the skin of calves given 1250 mg iodine/day. There were no lesions in the other organs of calves killed at 6 months.

Lesions in the skin were especially severe in the calves that died at 4 and 10 weeks. Hyperkeratosis was observed in many areas. Sloughed necrotic tissue was seen on the surface of the epidermis (Figure 11). A number of hair follicles were degenerated and sweat glands were markedly dilated and lined by very thin epithelium. Moderate infiltration of mononuclear cells and eosinophils was found in the corium. The stratum germinativum consisted of cells with relatively large and dark-staining nuclei.

Examination of the eyes from the same calves that died early in the experiment revealed a keratitis characterized by increased vascularity and an infiltration of polymorphonuclear cells into the lamina propria of the cornea (Figure 12). The blood vessels were dilated and filled with numerous red blood cells. The epithelial cells of the cornea were vacuolated and probably had undergone hydropic degeneration.

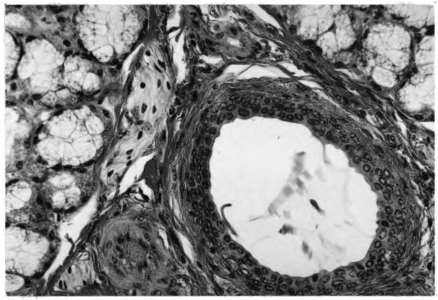

Figure 10. Interlobular duct of the parotid gland of a calf given 1250 mg iodine/day and killed at 6 months. The duct in lined by a multilayer of metaplastic epithelium. H&E stain, 350X.

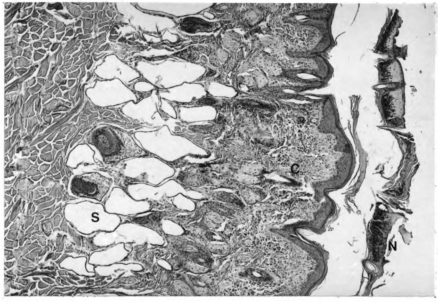

Figure 11. Skin from a calf given 1250 mg iodine/day and that died early in the experiment. Notice sloughing of necrotic tissues on the surface (N), infiltration of inflammatory cells in the corium (C), and marked dilatation of sweat glands (S). H&E stain, 50X.

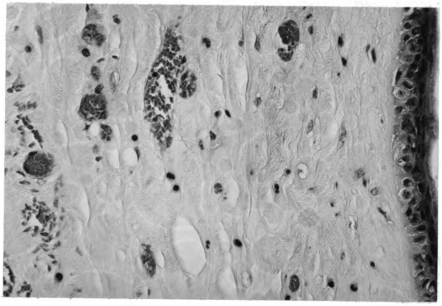

Figure 12. Cornea of a calf given 1250 mg iodine/day and that died early in the experiment. Notice hypervascularity and infiltration of inflammatory cells. H&E stain, 300X.

In the adrenal gland of calves that died early in the experiment the main pathologic changes were found in the cortex. Numerous cells in the zona fasciculata were swollen, and many others had eosinophilic cytoplasm and pyknotic nuclei. A marked decrease in the secretory granules was seen in the zona glomerulosa.

Moderate pathologic changes were also found in the kidneys of the 2 calves that died early in the experiment. Many tubular epithelial cells had undergone hydropic degeneration. The cells had a foamy appearance and had pyknotic nuclei. Small focal aggregations of lymphocytes were found in the cortex. The glomeruli and Bowman's capsules were essentially normal.

Pronounced pathologic changes were seen in the liver from the same calves. The hepatic cells were swollen and had diffusely distributed vacuoles in the cytoplasm. The vacuoles varied from small to moderate in size and were positive for lipid with oil red O. The hepatic sinuses were mostly compressed. Portal fibrosis with moderate proliferation of bile ducts was seen in limited areas. Marked infiltration of mononuclear cells and eosinophils was found in the fibrotic portal triads.

D. Transmission Electron Microscopy

1. Thyroid Gland

a. Thyroid glands of control calves. The continuity of follicular cells of the thyroid glands from control calves was well preserved. The follicular cells were cuboidal and were separated by narrow intercellular spaces. The apical border of the cells was irregular and the subapical cytoplasm appeared to be denser than the rest of the

cytoplasm. Luminal colloid appeared to be homogeneous but granular in texture. A number of blunt-ended microvilli protruded out of the apical cytoplasmic membrane bordering the lumen of the follicles. Dome-shaped structures similar to pseudopods were observed only rarely.

The normal follicular cells had well developed endoplasmic organelles. The Golgi complex was generally located next to the nucleus. Rough endoplasmic reticulum (RER) had intact cisternal membranes rich in ribosomes. Free ribosomes were dispersed or in clusters. The cisternae were of varying width and were filled with finely granular materials (Figure 13).

Colloid droplets were present in the apical portion of all cells but varied considerably in number and size. In some sections several colloid droplets were found in one cell. In other sections the droplets were only occasionally seen. The colloid droplets had a smooth surface and had a similar density to that of the luminal colloid. In general, the control follicular cells did not contain large colloid droplets. A number of bodies that appeared to be lysosomes were found in most cells and were located predominantly in the apical areas. They had a distinct membrane and were very dense. The occurrence of the dense bodies varied from cell to cell. Lipid droplets were occasionally seen. Most of the nuclei had irregular outlines.

b. Thyroid glands of calves given 1250 mg iodine/day with no TRH administration. Figure 14 illustrates the electron microscopic features of the thyroid follicular cells of calves given 1250 mg iodine/day with no TRH challenge. Morphologic features of the follicles varied from follicle to follicle, but the majority of

Figure 13. Electron micrograph of thyroid follicular cells from a control calf. Well developed mitochondria (M) were distributed throughout the cytoplasm. Colloid droplets (CD) were occasionally seen. Ribosomes were distinct. Uranyl acetate and lead citrate stain, 23,800X.

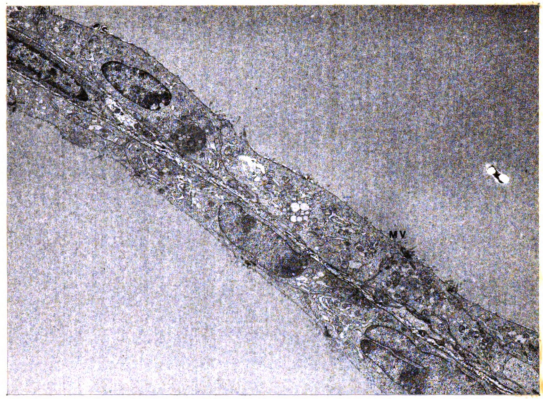

Figure 14. Thyroid follicular cells of a calf given 1250 mg iodine/day and killed at 6 months. The epithelium is flat and microvilli (MV) are decreased in number. Uranyl acetate and lead citrate stain, 3,500X.

these cells were shorter than normally seen. Cytoplasmic pseudopods were rarely seen. There appeared to be a decrease in number of colloid droplets and dense granules and a general overview indicated a reduction in number of mitochondria. The Golgi complex was less distinctive. In some cells the mitochondria were swollen and had disrupted cristae (Figure 15). Disruption of RER was observed, as were free ribosomes. The cisternae of RER were small and narrow, and the RER had less distinct ribosomes. The nuclei had regular nuclear membranes and the chromatin was less condensed.

In areas next to the enlarged and apparently inactive follicles with flat epithelium, there were follicles that appeared to be more active (Figure 16). These follicles had taller epithelium and had dilated cisternae. The RER was more distinctive and had dense ribosomes. The Golgi complex seemed to be hypertrophic and was characterized by numerous vesicles and infoldings. In other cells, less dense bodies were apparently fused with more dense granules and apparently had formed secondary lysosomes.

Similar but much less distinctive changes were found in the follicular cells of calves given 250 mg iodine/day. In the calves given 50 mg iodine/day, no detectable differences could be observed.

c. Thyroid glands of calves given 1250 mg iodine/day and TRH. Variation of follicular cells was observed, but the majority of the cells were cuboidal. The number of large colloid droplets had increased in most of the follicular cells (Figure 17). The colloid droplets were located in the apical areas, had a low electron density, and were homogeneous similar to luminal colloid. In the other cells several dense bodies that probably were lysosomes appeared mainly in

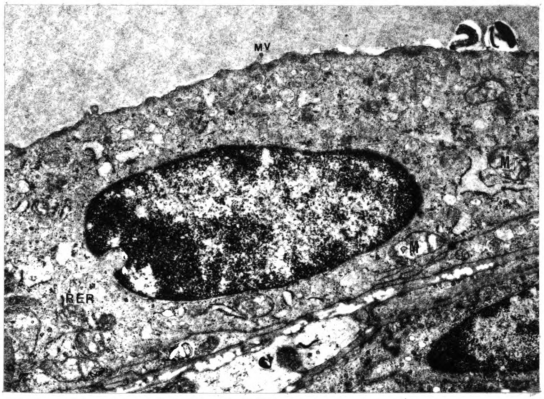

Figure 15. Thyroid follicular cells of a calf given 1250 mg iodine/day and killed at 6 months. Cells had few microvilli (MV), disrupted endoplasmic reticulum (RER), distorted mitochondria (M), and disarrangement of cristae. Uranyl acetate and lead citrate stain, 7,000X.

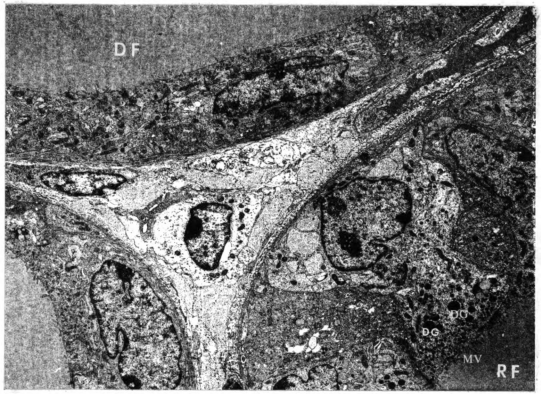

Figure 16. Follicles of a calf given 1250 mg iodine/day and killed at 6 months. Notice reactive follicles (RF) next to depressed follicles (DF). In the reactive follicles the cells are taller and have an increased number of microvilli (MV) and dense granules (DG). Uranyl acetate and lead citrate stain, 3,700X.

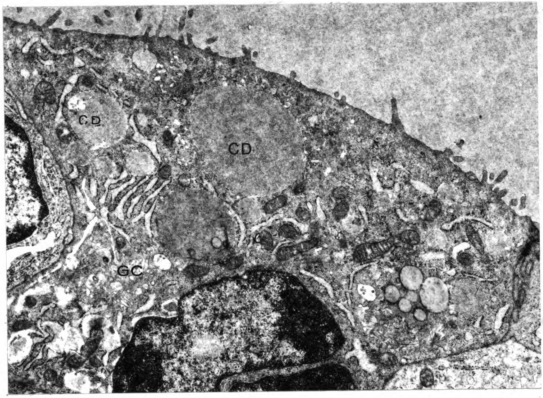

Figure 17. Electron micrograph of thyroid follicular cells of a calf given 1250 mg iodine/day and TRH and killed at 6 months. Notice increased large colloid droplets (CD), distinct rough endoplasmic reticulum (RER) and Golgi complex (GC). Uranyl acetate and lead citrate stain, 12,880X.

the apical cytoplasm. These bodies had a tendency to be near colloid droplets. Cytoplasmic protrusions resembling pseudopods had projected from the surface of the cells into the follicular lumen. The projections had different sizes and shapes. Additionally, RER had undergone moderate expansion and had dilated cisternae. The Golgi complex appeared to be more prominent than in calves given 1250 mg iodine/day without TRH. The nuclei appeared to be more indented.

Changes were much less prominent in follicular cells of the calves given 250 mg or 50 mg iodine/day and TRH.

d. Thyroid glands of calves given no iodine supplementation and TRH. The ultrastructure of follicular cells of calves given no iodine supplementation but which were injected with TRH is presented in Figure 18. Generally, the morphologic features were similar to those in calves given 1250 mg iodine/day and stimulated with TRH. Dome-shaped structures were less commonly seen in this group of calves. The mitochondria, Golgi complex and RER were more distinct. Dilatation of cisternae was less pronounced than in those calves given 1250 mg iodine and TRH. A few colloid droplets and dense granules appeared in the apical cytoplasm.

E. Scanning Electron Microscopy

The sample of trachea taken from control calves had well arranged cilia of uniform length and size. The cilia were erect, had blunt ends, and covered the entire surface of the ciliated epithelial cells (Figure 19). Several smooth globs, apparently mucus, were present on the cilia. The edges of the epithelial cells could be recognized by the space between clusters of the cilia.

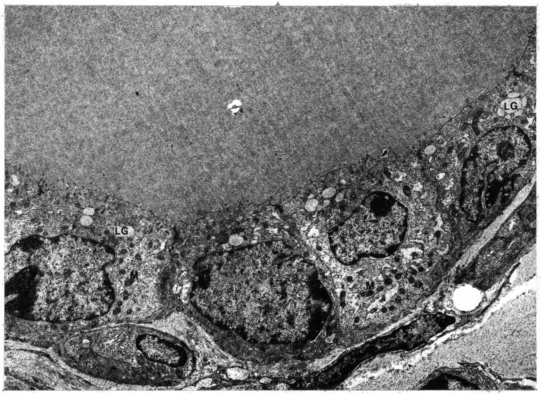

Figure 18. Thyroid follicular cells of a calf given no supplemental iodine but given TRH and killed at 6 months. The cuboidal epithelium is still maintained. Mitochondria (M) are nearly normal or increased in number. Several less dense granules (LG) are seen on the apical region of the cells. Uranyl acetate and lead citrate stain, 4,400X.

Figure 19. Scanning electron micrograph of the trachea of a control calf to illustrate regularity in size, shape and length of cilia covering the ciliated mucosal epithelium. 3,900X

Figure 20. Scanning electron micrograph of the trachea of a calf given 50 mg iodine/day and killed at 6 months. Notice the similarity to the control calf, except for the presence of more mucus droplets on the surface (M). 3,900X

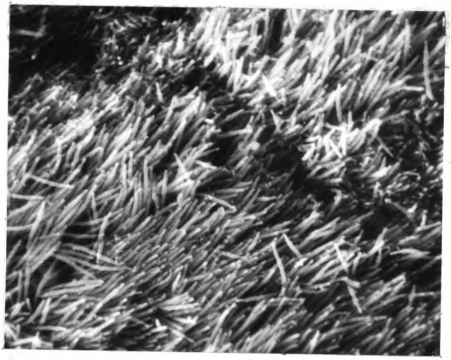

Figure 19

Figure 20

Figure 20 illustrates the appearance of epithelial cells of the trachea of the calves given 50 mg iodine/day. Minimal cilial changes could be detected, but in general the morphological features were not much different from the controls. The levels of the tips of the cilia were uneven, probably due to partially broken tips.

In calves given 250 mg iodine/day, an irregular bulging surface of nonciliated cells was seen. The nonciliated cells had microvilli of varying length and size. The ciliated cells had markedly broken cilia and abundant mucous material was found on the surface (Figure 21). The cilia were disrupted and in many cells were partially or almost completely gone. In another specimen, the integrity of the epithelial cells was disrupted. A number of red blood cells was observed at the broken surface and indicated hemorrhage (Figure 22).

More pronounced changes were found in the trachea of the calves given 1250 mg iodine/day. Numerous ciliated cells were partially or completely denuded of cilia. The nonciliated cells had shorter or disrupted microvilli. Figure 23 was taken in an area that had a more advanced lesion. Almost completely denuded cells were seen. The surface was quite irregular and had necrotic debris on it. A depressed surface frequently was observed. In another sample there was some evidence of squamous metaplasia. The denuded epithelial surface was flat. In other areas, bulging cells, broken surfaces, and hemorrhages were occasionally found.

F. Laboratory Analyses

A tendency towards decreased final body weight and increased thyroid weight was related to the increasing doses of iodine. The calves given 1250 mg iodine/head/day had significantly heavier thyroid

Figure 21. Scanning electron micrograph of the trachea of a calf given 250 mg iodine/day and killed at 6 months. Notice abundant mucus droplets (M) and exudate material (E) on the surface. Numerous cilia were missing which resulted in partially or completely denuded epithelium (DE). 2,400X

Figure 22. Another area from the same calf as shown in Figure 21. Red blood cells (RBC) on and between disrupted epithelial surface (ES) indicating hemorrhage. 2,500X

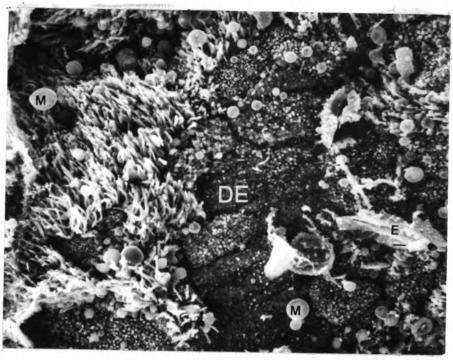

Figure 21

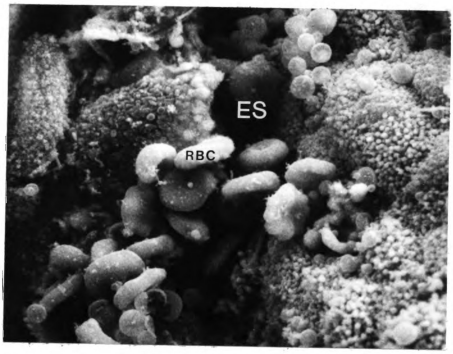

Figure 22

Figure 23. Scanning electron micrograph of the trachea of a calf given 1250 mg iodine/day and killed at 6 months. The bulging mucosa has irregularly-sized epithelial cells. The cilia are practically absent. Notice also some degenerated epithelial cells (arrow). 1,300X

glands as compared to the controls ($P<0.05$). On the other hand, the calves given the same dosage had significantly lower body weight as compared to the controls ($P<0.05$). There were no significant effects ($P>0.05$) of iodine supplementation on the weight of adrenal and pituitary gland.

Table 4 gives the results of serum analysis for vitamin A. The concentration of vitamin A in the sera decreased as the doses of iodine were increased. The concentration of vitamin A in the sera of calves given 1250 mg iodine/day was less than 50% of the controls. On the other hand, the concentration of carotene was about the same in all calves.

Table 3. Body and organ weight of calves given different levels of iodine for 6 months

Levels of Iodine (mg/day)	Final Body Weight[a] (kg)	Organ Weight[a] (g/100 kg bw)		
		Thyroid	Adrenal[b]	Pituitary
0	278± 5.6	7.4±0.4	4.6±0.1	0.49±0.01
50	273± 7.8	7.3±0.5	4.8±0.3	0.43±0.01
250	260± 8.2	9.7±0.9	4.6±0.1	0.43±0.02
1250	225±12.4[c]	10.0±1.1[c]	5.1±0.4	0.46±0.03

[a]Values are means±SE.

[b]Paired weight.

[c]Significantly different from the controls ($P<0.05$).

Table 4. Vitamin A and carotene concentration in the serum of calves given different levels of iodine for 6 months

Levels of Iodine[a] (mg/day)	Vitamin A (µg/100 ml)	Carotene (µg/100 ml)
0	266	676
50	257	668
250	188	666
1250	102	567

[a]Iodine was in the form of ethylenediamine dihydriodide.

DISCUSSION

A. General

Signs of iodine toxicity were apparent during the first 3 months of the experiment, but the signs gradually diminished in the last 3 months. Two calves given the highest doses of iodine died during the first 10 weeks with signs of respiratory disease and with skin changes. Several deaths in cattle with signs of respiratory distress and hyperthermia were reported in cattle given up to 500 mg/day of EDDI (McCauley et al., 1973). They stated that EDDI caused the disease process to be more severe. The 8 remaining calves given 1250 mg iodine/day and calves given lower doses of iodine survived until the end of the experiment. Apparently these calves adapted to the excessive iodine, as indicated by an improved health status during the last 3 months. However, the calves given the highest doses were still suffering from a mild respiratory disease. Whether or not excessive iodine was related to death losses or severe clinical signs apparently depended on the degree of stress or infection and the dosage or duration of treatment (McCauley et al., 1972). Several workers emphasized the biochemical mechanism by which the thyroid gland adapted to higher iodine intake in rats (Galton and Pitt-Rivers, 1959; Braverman and Ingbar, 1963; Liewendahl et al., 1972; Wolff et al., 1949). Other tissues besides the thyroid gland may be important in adaptation to excessive iodine. These may include the gastrointestinal tract, kidney, salivary gland, and mammary glands.

The experiment was not designed to characterize the pathogenesis of lesions associated with iodine toxicosis. Since control calves were not killed at the time that the 2 calves died early in the experiment, it cannot be said with certainty that the lesions in these calves were associated with iodine toxicosis. Therefore, one must be critical in the comparative assessment of lesions in calves that died early in the experiment with lesions seen in calves that were killed at the end of the experiment.

B. Histopathology

Pathologic changes in calves fed excessive iodine were found primarily in the lung and thyroid gland. Calves given 1250 mg iodine/day had the most pronounced lesions, which were especially prominent in the 2 calves that died. Bronchopneumonia and fibrinous pleuritis affecting approximately 60% of the lung tissue were found in these 2 calves. The other 8 calves from the same group had about 25% consolidation of lung tissue. Less severe lesions were observed in calves given smaller doses (Table 2), strongly suggesting an association between the dose of iodine and inflammation. Hamilton and Geever (1952) stated that administration of potassium iodide to tuberculous guinea pigs increased the severity of the inflammation. Marchese et al. (1952) reported that iodized oil bronchography caused the bronchogenic spread of infection or the activation of an unstable progressive tuberculosis in man. In another study (Stone and Willis, 1967), iodine appeared to have enhanced the inflammatory process. The negative effect of iodine may be related to uncoupling of oxidative phosphorylation, thereby making the cells unable to utilize ATP (Middlebrook and Szent-Györgyi, 1955).

Heavy growth of *P. multocida* was observed in the culture of lung tissue taken from calves that died earlier in the experiment. However, light to moderate growth occurred in nasal swabs taken from calves that were clinically normal. A similar result from normal looking cows was reported by Hoerlein et al. (1961). Excessive iodine may have caused the calves to be more susceptible to infection.

Pasteurella organisms were commonly isolated from animals with shipping fever (Carter, 1954). On the other hand, experimental studies indicated that cultures of *P. multocida* and *P. haemolytica* failed to cause illness even after the calves were stressed with cortisol and diethylstilbestrol (Gale and Smith, 1958). These workers concluded that *Pasteurella* organisms apparently were not the primary agent of shipping fever. The organisms were thought to be potentially pathogenic and other synergistic agents were required for disease to develop (Hamdy et al., 1958; Trapp et al., 1966). It was hypothesized that alteration and damage of mucosa allowed *P. multocida* to multiply and cause disease (Hetrick et al., 1963; Hoerlein et al., 1961).

In the present experiment, alteration of tracheal mucosa was observed by light and scanning electron microscopy. Squamous metaplasia was found primarily in the calves given 1250 mg iodine/day.

Vitamin A deficiency is one of the causes of squamous metaplasia. Vitamin A concentration in the serum tended to decrease with the increased doses of iodine. Calves given 1250 mg iodine/day had serum levels of vitamin A which were less than 50% of the controls (Table 4).

Squamous metaplasia was also seen in the duct of the parotid gland of several calves (Figure 10). Jungherr et al. (1950) claimed that squamous metaplasia in the duct of the parotid gland was pathognomonic for vitamin A deficiency in cows.

There was no definitive information on the effect of excessive iodine on vitamin A content in the body. Evidence of a correlation between vitamin A and thyroxine was indicated by an observation that signs of hypovitaminosis A paralleled those of thyroid insufficiency (Elmer, 1938). Thyroxine was reported to have an important role in conversion of carotene to vitamin A (Bernal and Refetoff, 1977). Elmer (1938) also mentioned that thyroidectomized goats had yellow milk as a result of elimination of provitamin A by mammary glands. In another report, Johnson and Bauman (1947) stated that in the hyperthyroid animal vitamin A storage was increased, and in hypothyroid states the stores of vitamin A were markedly low. Whether the hypothyroid state developed first and was followed by hypovitaminosis A or vice versa was not known. Frape et al. (1959) reported that vitamin A had considerable influence on the rate of thyroid secretion and insufficient or excessive intake of vitamin A lowered the rate of thyroid secretion. Jungherr et al. (1950) reported mild hyperplasia of the thyroid gland in calves with vitamin A deficiency. In the present experiment, hyperplasia of the thyroid gland was not found, but calves given 1250 mg iodine/day had significant thyroid alterations consisting of enlarged follicles filled with an excessive amount of colloid. The follicular cells were thin. These changes apparently indicated reduced thyroid function, although hypothyroidism was not manifested clinically.

The weight of thyroid glands tended to increase with the increased iodine dosage, with the heaviest thyroid glands found in calves given 1250 mg iodine/day. A tendency towards heavier thyroid glands was also reported by Newton et al. (1972) in cows given excessive iodine.

Another possibility of adverse effects of iodine would be related to an ability of iodine to produce direct effects on particular tissues. Highman et al. (1955) reported that intraperitoneal injections of potassium iodide in mice and guinea pigs caused retinal degeneration. However, retinal changes were not observed in this experiment.

Portal cirrhosis, fatty metamorphosis of the liver, tubular cell necrosis of the kidney, and cellular changes of the adrenal glands were found in the calves given 1250 mg iodine and which died in the early period of the experiment. These lesions were probably not directly related to the treatment but were related to intercurrent disease. However, degenerative changes of proximal parts of the nephron characterized by hydropic degeneration were reported in cattle with vitamin A deficiency (Langham et al., 1941). Furthermore, Webster et al. (1966) reported marked fatty changes in dogs dosed with excessive iodine. Similar changes were observed in fatal iodine poisoning in man (Finkelstein and Jacobi, 1937). Additionally, Jungherr et al. (1950) found portal cirrhosis in calves with vitamin A deficiency.

The alterations found in the adrenal glands consisted mainly of degenerative changes. These changes were different than the adrenocortical hyperplasia described by Wallace (1975).

Conjunctivitis and keratitis were found in the calves given high doses of iodine, especially in the first half of the experiment. Excessive ocular discharge was commonly reported in iodinism in calves (McCauley et al., 1973; Wallace, 1975; Hillman et al., 1976). Similar features were found in man (Goodman and Gilman, 1975), but no histologic descriptions were included.

Scaly skin was also one of the common signs of iodine toxicosis in cows. In this experiment the seborrheic type of dermatitis was characterized by an infiltration of eosinophils and mononuclear cells. Necrotic remnants were found on the skin surface (Figure 11) and suggested that the healing process was going on. Skin eruptions sometimes occurred in human patients who had been taking iodide as adjunctive therapy for asthma. The acne-type lesion had infiltrations of neutrophils as well as mononuclear cells and plasma cells (Moschella, 1975). Pathologic changes in the eyes and skin were thought to be related to a hypersensitivity-type reaction as described in human medicine (Moschella, 1975).

C. Electron Microscopy

Ultrastructural changes were found primarily in the calves given 1250 mg iodine daily with or without TRH injection.

The thyroid glands of the calves given 1250 mg iodine alone consisted of 2 distinct populations of follicles. The majority of the follicles were characterized by thin cells and had a decreased number of mitochondria. Some mitochondria had distortions of shape and a lack of regular arrangement of the cristae (Figure 15). A minority of the follicles appeared to be in a reactive state. These follicles were frequently located next to the depressed follicles (Figure 16). These reactive cells were thicker and had more microvilli and more secretory granules than the depressed cells. The reactive cells might be interpreted as compensating for the depressed follicular cells in order to maintain homeostasis.

Calves given 1250 mg iodine/day and TRH seemed to have more reactive follicles than those calves given 1250 mg iodine alone.

Most of the follicular cells had an increased number of large colloid droplets in the cytoplasm (Figure 17). It is difficult to judge whether these less dense droplets were secretory substance being discharged from the follicular cells or were reabsorbed colloid. The larger droplets were likely being reabsorbed, as suggested by Kurosumi and Fujita (1974). Evidence of phagocytic capability of follicular cells was indicated by the appearance of cytoplasmic pseudopods frequently seen on the apical surface of the cells. Increased numbers of large droplets and pseudopod formation were reported in follicular cells injected with thyroid stimulating hormone (TSH) (Seljelid, 1967a). Another feature that seemed to be related to increased activity of follicular cells was the more numerous microvilli protruding into the lumen as found in calves fed 1250 mg iodine/day and injected with TRH. Injection of TRH accelerated absorption of luminal colloid and iodination of thyroglobulin and released T_3 and T_4 (Kurosumi and Fujita, 1974). Increased numbers of microvilli that sometimes were branched were reported in stimulated follicular cells (Pantic, 1974).

In the present experiment the exogenous TRH apparently stimulated the pituitary gland to synthesize and release TSH, and subsequently the TSH stimulated the activity of the follicular cells. The effect of exogenous TSH was reported to be more pronounced in depressed thyroid than in the euthyroid gland.

Thyroid glands of calves given 1250 mg iodine seemed to be depressed, whereas administration of TRH apparently either prevented depression or restored thyroid gland activity towards normal. One may question the duration of TRH action, since the last administration of TRH was about 10 days prior to the killing of the calves. In man,

repeated administration of TRH gave a peak response at about 4 days (Hirooka, 1976) and it was not known if the thyroid was still stimulated after 10 days. Reichlin (1975) reported that in hypothyroidism in rats, the turnover rate of TRH was slower and the excretion of TRH through the kidney was also slow. Hypothyroidism might lead to prolonged effects of TRH. Similarly, TRH could have prolonged effects in calves given 1250 mg iodine/day, even though hypothyroidism was not seen clinically.

Results of scanning electron microscopic examination of the surface of the trachea indicated that the degree of severity of the lesions was proportional to the levels of iodine supplementation. The severity of tracheal lesions seemed to be closely related to those in the lungs. The lesions in the lung and trachea could have resulted from different etiologic agents (Nelson, 1974; Smith et al., 1972) and may not have been directly related to excessive iodine.

Several conclusions could be drawn based on the results of the present experiment:

1. Excessive iodine intake seemed to cause the calves to be more susceptible to infections.

2. Oral administration of excessive iodine caused reduction of vitamin A in the sera.

3. Although doses of 1250 mg/head/day appeared to have detrimental effects in some calves in the early period of the experiment, most of the calves were able to compensate for the excessive iodine.

SUMMARY

Forty Holtein heifer calves weighing 250 kg were divided randomly into 4 groups of 10 each and dosed orally with ethylenediamine dihydriodide to provide 0, 50, 250, or 1250 mg of iodine/head/day. Five calves in each group were given an intravenous injection of thyrotropin releasing hormone (TRH) at 4-week intervals. The last injection was given 10 days before the calves were killed.

Two calves given 1250 mg of iodine died by 70 days and had severe bronchopneumonia. After 6 months of treatment, the remaining 38 calves were killed. Pneumonia was evident in calves given excessive iodine with the severity apparently dose related. Squamous metaplasia of tracheal epithelium occurred in all calves given 1250 mg of iodine. Similar changes were seen in the interlobular ducts of the parotid gland of 2 calves given 1250 mg of iodine and 1 given 250 mg. Scanning electron microscopy revealed dose-related changes in the tracheal mucosa. *Pasteurella multocida* was isolated from the pneumonic lungs.

Changes in the thyroid gland of calves given 1250 mg of iodine consisted of enlarged follicles, flattened epithelium and abundant colloid. In calves given 1250 mg of iodine and TRH, the histologic and electron microscopic features of the thyroid were similar to the controls.

Serum vitamin A concentrations were depressed in calves given high doses of iodine, but the concentration of carotene in the serum was not affected.

In general, calves were able to compensate for excessive iodine after an initial period of increased susceptibility to respiratory infection.

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