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INVESTIGATIONS ON THE EFFECTS OF POLYCHLORINATED BIPHENYLS ON THE FUNCTION AND STRUCTURE OF THE THYROID GLAND OF ADULT AND PERINATAL RATS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

William Thomas Collins, Jr., D.V.M.

The Ohio State University

1979

Reading Committee
Dr. Charles C. Capen
Dr. Steven E. Weisbrode
Dr. Larry A. Nagode

Approved By

Advisor
Department of Veterinary Pathobiology
Copyright by
William Thomas Collins, Jr., D.V.M.
1979
VITA

February 12, 1945
Born - Cincinnati, Ohio

1967
B.A., Ohio Wesleyan University, Delaware, Ohio

1968
M.S., University of Kentucky, Lexington, Kentucky

1969 - 1972
Captain, U.S. Army, Medical Service Corps., Medical Research and Development, Middle America Research Unit, Canal Zone, Panama

1976
D.V.M., The Ohio State University, Columbus, Ohio

1976 - 1979
Postdoctoral Research Fellowship, National Institutes of Health, Department of Veterinary Pathobiology, The Ohio State University, Columbus, Ohio

PUBLICATIONS


FIELDS OF STUDY

Major Field: Veterinary Pathology

Studies in Environmental Pathology and Toxicology. Professor Charles C. Capen

Studies in Endocrine Pathology. Professor Charles C. Capen
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CHAPTER I

EFFECT OF POLYCHLORINATED BIPHENYL (PCB)
ON THE THYROID GLAND:
ULTRASTRUCTURAL AND BIOCHEMICAL INVESTIGATIONS

INTRODUCTION

The widespread contamination of the environment with polychlorinated biphenyls (PCB) has been well documented in several recent reports. These compounds, because of their nonflammability, high dielectric constant, and plasticizing abilities, have gained widespread use in industry as dielectric fluids in capacitors and transformers, hydraulic and heat transfer fluids, as well as plasticizers and solvents in adhesives and sealants. The escape of PCB into the environment through sewage outfalls and industrial disposal into waterways plus their long half-life have led to detectable levels in the adipose tissue of a large proportion of the human population. PCB residues have been detected in rivers and oceans plus tissues of fish, wildlife, cattle and poultry. Escape of PCB into the air from plasticized materials, leakage of lubricants, hydraulic and heat transfer fluids, and leaching from waste dumps also may contribute significant amounts of PCB to the environment. The disease-producing
capability of these compounds has been documented in human beings, cattle, and poultry following accidental contamination of foodstuffs with PCB.\textsuperscript{8-10}

Intoxication with PCB results in the production of lesions in several organ systems. In the liver, PCB is a potent inducer of microsomal enzymes, resulting in the proliferation of smooth endoplasmic reticulum in hepatocytes and may cause fatty degeneration and necrosis.\textsuperscript{11-13} Epidermal hyperplasia and hyperkeratosis, porphyria, and degeneration of lymphoid tissues and kidney have been reported following PCB intoxication.\textsuperscript{14,15} PCB also has been shown to have an adverse effect on reproduction and growth in several different animal species.\textsuperscript{16-18}

Recent evidence indicates that PCB may cause alterations in thyroid structure and thyroxine metabolism. In birds administered PCB, there is enlargement of the thyroid gland and alteration of $\text{I}^{131}$ uptake by follicular cells.\textsuperscript{19,20} The administration of PCB to rats resulted in an increased $\text{I}^{131}$ uptake, lowered serum thyroxine concentration, reduced protein-binding of thyroid hormone, and increased conjugation of thyroxine and excretion of thyroxine-glucuronide in the bile.\textsuperscript{21-24} These findings suggest that some of the metabolic alterations produced by intoxication with PCB may be related to alterations in thyroid structure and function. Therefore, the objectives of this investigation were (1) to evaluate the histopathologic, histochemical, and ultrastructural changes in thyroid follicular cells produced by the acute and chronic administration of high and low doses of PCB to rats, (2) to correlate the structural alterations in follicular cells with changes in serum thyroxine concentration, and (3) to investigate the persistence of the effects of PCB on the ultrastructure of thyroid follicular cells and serum thyroxine levels.
MATERIALS AND METHODS

Eight-week-old male Osborne-Mendel rats (FDA colony) were fed Purina rat chow mixed with Aroclor 1254 (Monsanto Co., Inc., St. Louis, Missouri) at concentrations of 50 and 500 parts per million (ppm) for 4 and 12 weeks. The PCB was mixed into the pulverized feed using corn oil as a vehicle. Control rats received a similar diet with 3% corn oil but without PCB and were housed in a separate room. The delayed and long-term effects of PCB on the thyroid were investigated by feeding rats 50 and 500 ppm PCB mixed into rat chow for 12 weeks followed by 12 and 35 week intervals without receiving the compound prior to euthanasia. Rats in the high dose groups received 50 ppm of PCB for the first 6 weeks but were switched to 250 ppm for the last 6 weeks due to anorexia and weight loss. All diets were stored at -25°C in hexane-cleaned metal containers until use. The animals were housed individually in stainless steel cages with wire mesh bottoms. The daily lighting schedule was 12 hours of light followed by 12 hours of darkness. Feed and water were available ad libitum.

Thyroid glands were collected from 5 rats at each time interval (4 weeks, 12 weeks, 12 weeks with 12 week recovery, 12 weeks with 35 week recovery) and dose level (0, 50, 500 ppm PCB) for ultrastructural evaluation. The rats were sacrificed immediately at the end of each experimental interval using carbon dioxide asphyxiation and thyroid glands were collected for electron microscopic, histopathologic, and histochemical evaluation. Tissue for electron microscopy were minced immediately under fixative into 0.5 to 1.0 mm³ blocks, fixed in cold 3% glutaraldehyde with 0.1 M sodium cacodylate and buffered at
pH 7.4, postfixed in 1% osmiumtetroxide in s-collidine, dehydrated in graded ethanols, transferred to propylene oxide, and embedded in Epon (Shell Chemical Company, New York, N.Y.). Thin sections were cut with a diamond knife on an LKB or Reichert OmU2 ultramicrotome and floated on a water bath buffered at pH 7.4. Sections were stained with uranyl acetate and lead citrate and were examined with a Phillips 200 or 300 electron microscope.

Five additional rats from each time interval and dose level were used for histochemical evaluation of the thyroid gland and serum levels of thyroid hormone. Tissues for histochemistry were collected in dry ice at necropsy. Acid phosphatase naphtol-AS-B1 reaction was evaluated on selected thyroid sections from rats in all experimental groups. Thyroid glands for histopathologic evaluation were fixed in phosphate-buffered formalin and stained with hematoxylin and eosin, and the periodic acid-Schiff reaction. Serum thyroxine was determined on 5 rats of each time interval and dose level of PCB by radioimmunoassay (Bio-Science Laboratories, Van Nuys, CA).

RESULTS

Histopathology and Histochemistry

Control Rats. The thyroid glands of control rats in all groups were similar histologically and will be described together. They were composed of prominent follicles lined by a single layer of cuboidal and low columnar epithelial cells (Figure 1). Follicular cells had a lightly eosinophilic cytoplasm and a centrally placed, round, basophilic nucleus. Colloid in the follicular lumens was either homogeneous or slightly vacuolated near the periphery. Thyroid C-cells were present in small clumps of 3 to 4 cells between follicles or
as individual cells within follicular walls. Interfollicular capillaries were separated from follicles by a thin basement membrane.

**Low Dose PCB (50 ppm) for 4 Weeks.** Thyroid glands were slightly enlarged and follicles were small and lined by a single layer of columnar epithelium. The cytoplasmic area of follicular cells was lightly eosinophilic and vacuolated. Acid phosphatase activity in follicular cells was increased compared to control rats.

**High Dose PCB (500 ppm) for 4 weeks.** Thyroid glands were enlarged and composed of small, irregularly shaped follicles lined by single or multiple layers of columnar epithelial cells. Papillary projections of hyperplastic follicular cells extended into the lumens of some follicles (Figure 2). Numerous cytoplasmic processes projected into the colloid from the apical surface of follicular cells. Acid phosphatase activity in the cytoplasm of follicular cells was increased compared to control rats.

**Low Dose PCB (50 ppm) for 12 Weeks.** Thyroid glands were enlarged and composed of small follicles lined by single or multiple layers of columnar cells. Cytoplasmic processes extended from the apical surfaces of follicular cells into the colloid. The cytoplasm of follicular cells was vacuolated and the nucleus was basally situated. Acid phosphatase activity in follicular cells was strong and increased compared to control rats (Figure 3).

**High Dose PCB (500 ppm) for 12 Weeks.** Thyroid glands had similar changes as those described in rats fed 500 ppm PCB for 4 weeks. Papillary
projections of hyperplastic follicular cells and prominent cytoplasmic processes extended into the colloid (Figure 4). The cytoplasmic area of follicular cells appeared vacuolated and had strong acid phosphatase activity.

Delayed Effects of PCB. Thyroid follicles in rats receiving either 50 or 500 ppm PCB for 12 weeks followed by an interval of 12 weeks without PCB prior to euthanasia were lined by a single layer of columnar cells. Multiple layers of follicular cells with papillary and cytoplasmic projections were not present in thyroids of rats from this group. The cytoplasm of follicular cells only had occasional vacuoles and there was peripheral scalloping of colloid.

Long-term Delayed Effects of PCB. Thyroid glands of rats fed either 50 or 500 ppm PCB for 12 weeks and followed by an interval of 35 weeks without PCB prior to euthanasia were similar to those of control rats. Thyroid follicles were large and lined by a single layer of cuboidal to low columnar cells. The cytoplasm of follicular cells was uniformly eosinophilic and the nucleus was located centrally. There was only a mild increase in acid phosphatase activity in follicular cells compared to controls.

Ultrastructural Evaluation of Thyroid Glands

Control Rats. Thyroid glands of control rats from all groups were similar and were composed of follicles lined by a single layer of cuboidal cells situated on a thin basement membrane. The cytoplasm contained long profiles of rough endoplasmic reticulum with numerous cisternae containing a finely granular electron-dense material. The cisternae appeared as long narrow spaces and were not dilated. The Golgi apparatus was of moderate size and composed of
flattened layers of smooth membranes associated with small dense granules. Mitochondria with transverse cristae were scattered throughout the cytoplasmic area. A narrow layer of electron-dense apical vesicles was present near the luminal border immediately beneath the microvilli. Microvillar projections from the luminal surface were uniform in width and length. Occasional membrane-limited colloid droplets and small, round lysosomal bodies were present in the cytoplasm.

**Low Dose PCB (50 ppm) for 4 Weeks.** Follicular cells were larger and more columnar than in controls (Figure 5). Profiles of rough endoplasmic reticulum were numerous and their cisternae frequently were dilated with a finely granular material. The Golgi apparatuses were more prominent than in controls and associated with many small granules. The oval nucleus was basally placed in follicular cells. Microvilli were shortened, irregular in shape, and had abnormal branching (Figure 6). Long projections of follicular cell cytoplasm often extended from the apical surface into the luminal colloid (Figure 7). Electron-dense apical vesicles appeared to accumulate immediately beneath the microvilli. Large abnormally shaped lysosomal bodies with a heterogenous internal structure were present in increased numbers compared to control rats.

**High Dose PCB (500 ppm) for 4 Weeks.** Follicular cells were more columnar than in controls and occasionally were present in multiple layers lining thyroid follicles (Figure 8). The cytoplasmic area contained long profiles of rough endoplasmic reticulum often irregularly dilated by a finely granular material. Mitochondria were more frequently swollen and had disrupted cristae
compared to thyroids in control rats. The basally located nucleus was oval and contained coarsely granular chromatin. Striking changes were detected in the microvilli on the luminal border of follicular cells as described in the previous group. Microvilli were shortened and irregularly branched. Areas of the luminal surface of follicular cells were completely devoid of microvilli. These areas often had large cytoplasmic projections extending into the lumenal colloid. Numerous apical vesicles were present immediately beneath the altered luminal surface. Membrane-limited colloid droplets and abnormally large electron-dense lysosomal bodies were increased in numbers within the cytoplasm.

Low Dose PCB (50 ppm) for 12 Weeks. Follicular cells were more columnar than in control rats and had small profiles of rough endoplasmic reticulum irregularly dilated with finely granular material. The Golgi apparatus was compressed near the nucleus and was less extensive than in controls. Mitochondria were large but frequently were swollen and had disrupted cristae. There was marked reduction in the number and length of microvilli on the luminal surface of follicular cells. The surface of follicular cells was irregular and had large projections of apical cytoplasm that extended into the colloid. There was a marked increase in large, membrane-limited colloid droplets in the cytoplasm. In addition, lysosomal bodies were increased in follicular cells of rats of this group. They were extremely electron-dense, irregular in size and shape, and occasionally appeared to be fused with the colloid droplets.
High Dose PCB (500 ppm) for 12 Weeks. Thyroid follicles were lined by single or occasionally multiple layers of columnar cells. The Golgi apparatus and rough endoplasmic reticulum were less well developed in follicular cells due to the abnormal accumulation of numerous lysosomal bodies and colloid droplets (Figure 9). The cytoplasmic area of some hypertrophied follicular cells appeared to be distended by the large numbers of lysosomal bodies and colloid droplets. Mitochondria were more irregular and swollen with disrupted cristae than in rats from any of the other experimental groups. Microvilli on the lumenal surfaces of most follicular cells appeared to be abnormally short, blunt, and branched (Figure 10). Extensive areas of the lumenal surface of follicular cells were devoid of microvilli and occasionally unique cytoplasmic projections extended into the colloid. These changes were similar but more extensive than in rats receiving 50 ppm PCB for 12 weeks.

Delayed Effects of Low Dose (50 ppp) PCB. Follicular cells were more columnar than in control rats and often had a hypertrophied cytoplasmic area (Figure 11). There was less evidence of multiple layers of follicular cells lining follicles than in the previous experimental groups. Mitochondrial swelling and disruption of cristae were less evident than in rats killed immediately after receiving PCB for 12 weeks. Microvilli on the lumenal surface were fewer in number and shorter than in control rats but were more normal in shape than in rats receiving PCB for 12 weeks and killed immediately. Few apical vesicles were present beneath the microvilli. The numbers of irregularly shaped extremely dense lysosomal bodies remained increased compared to controls.
Delayed Effects of High Dose (500 ppm) PCB. Thyroid follicles were lined either by tall columnar or hypertrophied cuboidal cells but there was less evidence of multiple layers of follicular cells than in rats receiving PCB for 12 weeks and killed immediately. The rough endoplasmic reticulum was less well developed than in controls and the Golgi apparatus was small. Occasional mitochondria were swollen and had disrupted cristae. Microvilli on the luminal surface of some follicular cells were short and blunt; however, many cells had normal appearing microvilli. Large cytoplasmic projections from the luminal surface were observed infrequently. Numerous irregular lysosomal bodies and colloid droplets also were present in follicular cells.

Long-term Delayed Effects of Low and High Dose PCB. Follicular cells of rats receiving either 50 or 500 ppm of PCB for 12 weeks followed by an interval of 35 weeks without PCB ultrastructurally were similar to those of control rats (Figure 12). The endoplasmic reticulum was well developed and consisted of long profiles with narrow cisternae containing a finely granular material. Dilated profiles of endoplasmic reticulum were present but they were considerably reduced compared to previous experimental groups. The Golgi apparatus was prominent and was associated with numerous dense granules. Mitochondria in follicular cells was present in similar numbers as in control rats. Only an occasional mitochondrion was swollen with disruption of cristae. Microvilli on the luminal surface were numerous and had a normal configuration. Many apical vesicles were present immediately beneath the microvilli. Lysosomal bodies and colloid droplets were markedly decreased in most follicular cells compared to previous experimental groups. Lysosomal bodies
present in follicular cells resembled those in controls and were round with a more homogeneous internal structure. Only a few follicular cells contained numerous irregularly shaped lysosomes similar to those in the previous experimental groups. The residual ultrastructural alterations in follicular cells of rats from this group appeared to be minimal by comparison to all other PCB-treated rats.

Ultrastructural alterations were not detected in thyroid C-cells related to PCB administration.

**Serum Thyroxine**

Serum thyroxine concentration of control and experimental rats fed PCB were determined by radioimmunoassay and groups were compared statistically using Students' T-test (Table 1). Thyroxine levels were significantly reduced in rats fed 50 and 500 ppm PCB daily for 4 and 12 weeks. The greatest reduction in serum thyroxine levels occurred at 12 weeks in rats receiving 500 ppm PCB (Table 1). Serum thyroxine had returned toward normal values in rats administered either high or low doses of PCB for 12 weeks followed by a 12 week recovery interval without PCB prior to euthanasia but remained significantly lower than in control rats (Table 1). In rats receiving PCB for 12 weeks followed by an interval of 35 weeks without PCB the serum thyroxine had returned to within normal range (Table 1).

**DISCUSSION**

The daily feeding of polychlorinated biphenyl compounds to rats produced striking ultrastructural alterations in thyroid follicular cells and in the
metabolism of thyroid hormone. There was a progressive accumulation of large abnormally shaped lysosomal bodies with strong acid phosphatase activity and numerous colloid droplets in follicular cells. Mitochondria were swollen and cristae often were disrupted. The lowest dose of polychlorinated biphenyl (50 ppm) administered to rats in this investigation for 4 weeks resulted in the formation of blunt, irregularly branched microvilli with unique cytoplasmic projections extending into the lumenal colloid. These ultrastructural changes were interpreted to be a direct effect of PCB and were associated with a significant decrease in serum thyroxine levels after 4 weeks.

Follicular cells in rats receiving PCB for 4 and 12 weeks were hypertrophied and more columnar than in controls. They often were present in multiple layers lining thyroid follicles and occasionally extended as papillary projections of hyperplastic follicular cells into the lumen. The rough endoplasmic reticulum and Golgi apparatus were well developed in follicular cells and numerous apical vesicles were present near the lumenal surface. These ultrastructural changes suggesting increased secretory activity were interpreted to be a compensatory reaction by follicular cells to the lowered blood thyroxine levels and were similar to those reported following thyroid stimulation by thyrotrophin. These findings are consistent with the report of Bastomsky who detected increased uptake by the thyroid gland following PCB administration in rats. In birds, this effect of PCB appears to be accentuated and results in a goitrous enlargement of the thyroid gland. A similar compensatory hypertrophy and hyperplasia of follicular cells has been produced in animals by other polycyclic hydrocarbon compounds which also are inducers of hepatic microsomal enzymes.
More severe ultrastructural lesions were observed in thyroid follicular cells with the higher dose (500 ppm) of PCB or with chronic administration of the compound. After feeding 500 ppm of PCB for 12 weeks, extensive areas of the luminal surface of follicular cells were devoid of microvilli and abnormal cytoplasmic projections extended into the lumen. Increased numbers of abnormal lysosomes and numerous colloid droplets accumulated in the cytoplasm of follicular cells, resulting in displacement and compression of the rough endoplasmic reticulum and Golgi apparatus. Serum thyroxine levels were markedly decreased in rats that had severe ultrastructural lesions in follicular cells after receiving either 50 or 500 ppm for 12 weeks.

Residual effects of PCB on thyroid structure and function were observed in rats evaluated 12 weeks after the last dose; however, minimal alterations persisted in follicular cells after 35 weeks. Microvilli were fewer in number and shorter than in controls after 12 weeks recovery, large accumulations of lysosomal bodies and colloid droplets remained in the cytoplasm, and serum thyroxine levels had increased compared to rats fed PCB for 12 weeks and killed immediately but were still significantly lower than in controls. These residual effects presumably were related to the long half-life of PCB and storage in adipose tissue. Follicular cells of rats fed PCB for 12 weeks followed by an interval of 35 weeks prior to evaluation were similar ultrastructurally to controls and serum thyroxine levels had returned to within the normal range.

The striking ultrastructural alterations in thyroid follicular cells of rats following PCB administration probably contributed in part to the highly significant decrease serum thyroxine levels in experimental rats. In spite of the
 alterations in microvillar structure, follicular cells appeared capable of taking up colloid droplets by endocytosis. However, the increased lysosomal bodies with strong acid phosphatase activity in experimental rats appeared unable to interact with colloid droplets in a normal manner and hydrolyze the cleavage of active thyroid hormone from the molecular structure of thyroglobulin in colloid droplets. This resulted in a striking accumulation of colloid droplets and lysosomal bodies after chronic administration of PCB, resulting in a displacement of synthetic organelles such as the endoplasmic reticulum and Golgi apparatus.

In addition to the direct effect on follicular cells, PCB compounds are known to significantly enhance the peripheral metabolism of thyroxine and to reduce the binding of thyroid hormones to serum proteins. This results in a lowering of serum thyroxine and protein-bound iodine levels in rats.\textsuperscript{21} The biliary excretion of thyroxine is enhanced (4 to 5 fold) by PCB and there is an increased proportion of biliary\textsuperscript{125}I as thyroxine-glucuronide.\textsuperscript{23} The increased hepatic conjugation of thyroxine to glucuronic acid and excretion in the bile in rats receiving PCB probably are secondary to the induction of hepatic microsomal thyroxine-UDP glucuronyltransferase.\textsuperscript{24}

The results of this investigation and studies reported in the literature demonstrate a highly significant reduction in serum thyroxine by PCB. The lowering of circulating thyroxine levels by PCB is dose- and time-dependent, and appears to be the combined result of a direct effect on thyroid follicular cells with an interference in hormone secretion plus an enhanced peripheral metabolism of thyroxine. Some of the metabolic alterations produced by PCB
intoxication in experimental animals and human beings such as decreased weight gain, reduced feed efficiency, decreased reproductive performance, and skin lesions with hyperpigmentation may be related to an alteration in thyroid function.

**SUMMARY**

Polychlorinated biphenyls (PCB) produced ultrastructural lesions in thyroid follicular cells and reductions in serum thyroxine levels in rats that were time- and dose-dependent. The acute effects (4 week) of PCB (50 and 500 ppm) consisted of an accumulation of lysosomal bodies and colloid droplets in follicular cells with abnormalities of microvilli on the luminal surface. The chronic administration (12 week) of PCB (50 and 500/250 ppm) resulted in a striking distention of many follicular cells with large lysosomal bodies with strong acid phosphatase activity and colloid droplets, blunt and abnormally branched microvilli, and mitochondrial vacuolation. These ultrastructural alterations in follicular cells were associated with a highly significant reduction in serum thyroxine with both the low and the high dose of PCB. Follicular cells remained responsive to the lowered thyroxine level after feeding PCB for 4 and 12 weeks and underwent moderate compensatory hypertrophy and hyperplasia. Thyroid follicles were smaller than in controls and were lined by more columnar cells that occasionally formed papillary projections into the colloid. Residual ultrastructural alterations persisted for 12 weeks following cessation of feeding the compound, and serum thyroxine levels were significantly lower than in control rats. However, 35 weeks after discontinuing PCB, thyroid follicular
cells were similar to those in controls and serum thyroxine levels had returned to normal. The striking ultrastructural lesions in follicular cells produced by feeding PCB to rats appeared to contribute to the lowering of serum thyroxine levels, in combination with the known stimulation of peripheral thyroxine metabolism by these compounds. Certain metabolic alterations produced by PCB intoxication in experimental animals and human beings may be related to an alteration in thyroid function.
Table 1 — Serum Thyroxine Levels (Mean ± SE) of PCB-Treated and Control Rats Determined by Radioimmunoassay.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Acute effects (4 wks.PCB) (µg/dl)</th>
<th>Chronic effects (12 wks.PCB) (µg/dl)</th>
<th>Delayed effects (12 wks.PCB; 12 weeks no PCB) (µg/dl)</th>
<th>Long-term delayed effect (12 wks.PCB; 35 weeks no PCB) (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>6.66 ± 0.3</td>
<td>7.18 ± 0.4</td>
<td>7.86 ± 0.8</td>
<td>6.18 ± 0.9</td>
</tr>
<tr>
<td>50 ppm PCB</td>
<td>4.80 ± 0.3†</td>
<td>1.96 ± 0.2‡</td>
<td>4.90 ± 0.1*</td>
<td>5.86 ± 1.2</td>
</tr>
<tr>
<td>500/250 ppm PCB</td>
<td>2.10 ± 0.2‡</td>
<td>1.78 ± 0.08‡</td>
<td>3.01 ± 0.8†</td>
<td>6.02 ± 1.3</td>
</tr>
</tbody>
</table>

N = 5 rats per dose and interval.

*P < 0.025.

†P < 0.005.

‡P < 0.001.
Figure 1. Follicles lined by cuboidal epithelium and containing partially vacuolated colloid in thyroid gland of rat. (H & E, X 315).
Figure 2. Irregularly sized follicles in thyroid gland of rats fed 500 ppm PCB for 4 weeks. Many follicles (F) were smaller than in control rats, lined by tall columnar epithelium, and contained sparse colloid. Papillary projections of hyperplastic follicular cells (arrow) and apical cytoplasmic processes (arrow head) extend into the follicular lumens. (H & E, X 315).
Figure 3. Papillary projections of hyperplastic follicular cells (arrow) extend into the lumen of a thyroid follicle lined by tall columnar cells.
Rat fed 500 ppm PCB for 12 weeks. (H & E, X 315).
Figure 4. Strong acid phosphatase reaction in apical portions of thyroid follicular cells of rat fed 50 ppm PCB for 12 weeks. (X 315).
Fig 4
Figure 5. Thyroid follicular cell with expanded cytoplasmic area containing numerous dilated profiles of rough endoplasmic reticulum (E), a large Golgi apparatus (G), and prominent lysosomal bodies (L). Microvilli are short and abnormal in shape (arrow), and portions of the apical cytoplasm (P) project into the follicular lumen. Rat fed 50 ppm PCB for 4 weeks. (X 11,000).
Figure 6. Follicular cell with large apical cytoplasmic processes (arrow) extending into follicular lumen. The hypertrophied follicular cells contain dilated profiles of rough endoplasmic reticulum (E), colloid droplets (C), and many large lysosomal bodies (L). Rat fed 50 ppm PCB for 4 weeks. (X 5800).
Figure 7. Apical surface of thyroid follicular cell with abnormally short, branched microvilli (arrow) and cytoplasmic projections (P) extending into the follicular lumen. Rat fed 50 ppm PCB for 4 weeks. (X 26,000).
Figure 8. Multiple layers of hyperplastic follicular cells lining thyroid follicle. The large cytoplasmic area of follicular cells contains numerous dilated profiles of rough endoplasmic reticulum (E) and large Golgi apparatuses (G). Microvilli extending into the follicular lumen (L) are short and abnormally branched (arrows). Rat fed 500 ppm PCB for 4 weeks. (X 5800).
Figure 9. Thyroid follicular cell with an expanded cytoplasmic area filled with closely packed colloid droplets (C) and large lysosomal bodies (L). The rough endoplasmic reticulum (E), Golgi apparatus, and mitochondria are poorly developed and often compressed by the numerous colloid droplets and lysosomes. Short microvilli (arrow) extend into follicular colloid (FC). Rat fed 500 PPM PCB for 12 weeks. (X 4700).
Figure 10. Large membrane-limited colloid droplets (C) and numerous lysosomal bodies (L) in thyroid follicular cell of a rat fed 500 ppm PCB for 12 weeks. Short, abnormally branched microvilli (arrow) project from the apical surface of the follicular cell into the lumen (L). (X23,600).
Figure 11. Tall columnar follicular cells with markedly increased numbers of lysosomal bodies and short abnormally shaped microvilli lining a thyroid follicle. The cytoplasmic area is filled with large irregularly shaped lysosomes (L), colloid droplets (C), and dilated profiles of rough endoplasmic reticulum (E). Follicular lumen (L). Rat fed 50 ppm PCB for 12 weeks followed by a 12 week interval with no PCB prior to euthanasia. (X 5700).
Figure 12. Thyroid follicular cell with similarly developed microvilli (arrow) and cytoplasmic organelles as in control rats, except for a moderate increase in the numbers of round lysosomal bodies (L). Rat fed 50 ppm PCB for 12 weeks followed by a 35 week interval with no PCB prior to euthanasia. Follicular lumen (F), interfollicular capillary (C). (X 10,300).
CHAPTER II

ULTRASTRUCTURAL AND FUNCTIONAL ALTERATIONS OF RATS PRODUCED BY POLYCHLORINATED BIPHENYLS COMPARED WITH IODIDE EXCESS AND DEFICIENCY, AND THYROTROPIN AND THYROXINE ADMINISTRATION

INTRODUCTION

Polychlorinated biphenyls (PCB) are persistent and toxic industrial compounds which have caused widespread contamination of the environment. (Anon. 1972; Gustafson, 1970; Kimbrough, 1974; Kolbye, 1972). Detectable levels of PCB have been found throughout the environment and in tissues of animals and man (Finklea et al., 1972; Hammond, 1972; Price et al., 1972). Exposure to PCB results in the induction of hepatic microsomal enzymes and causes fatty degeneration and necrosis of hepatocytes (Grant et al., 1974; Kasza et al., 1975; Kimbrough et al., 1972). Epidermal hyperplasia and hyperkeratosis, porphyria, and degeneration of lymphoid and renal tissues have been reported following PCB intoxication (Vos and Beems, 1971; Vos and Koeman, 1970). PCB also has an adverse effect on reproduction, growth, and development in both animals and man (Ax and Hansen, 1975; Hansen et al., 1975; Hansen et al., 1976).
Some of these alterations may be related to the recent findings that exposure to PCB causes a significant reduction of serum levels of thyroxine (Bastomsky, 1974; Bastomsky and Murphy, 1976; Bastomsky and Wyse, 1975; Bastomsky et al., 1976; Collins et al., 1977). The lowering of serum thyroxine appeared to be due to alterations in thyroid structure and peripheral thyroxine metabolism. In rats administered PCB thyroid follicular cells accumulated colloid droplets and large abnormally shaped lysosomes, suggesting an interference in the lysosome-colloid droplet interaction necessary for the secretion of thyroid hormones (Collins et al., 1977). Ultrastructurally, colloid droplets did not appear to fuse with lysosomes thereby preventing the cleavage of thyroid hormones from the thyroglobulin molecule by acid hydrolases. PCB also affected microvilli on follicular cells. Microvilli were short, blunt or branching, fewer in number, and large cytoplasmic processes extended into the follicular lumen.

In addition to the alterations in follicular cell structure, PCB is a potent inducer of hepatic microsomal enzymes (Bruckner et al., 1973 and 1974; Litterst et al., 1972; Villeneuve et al., 1971). Exposure to PCB resulted in induction of the UDP-glucuronyltransferase needed for the conjugation of thyroxine and increased excretion of thyroxine-glucuronide in the bile (Bastomsky, 1974; Bastomsky and Murphy, 1976; Bastomsky and Wyse, 1975; Bastomsky et al., 1976). In spite of the increased peripheral metabolism of thyroxine and ultrastructural lesions in thyroid glands produced by PCB, follicular cells appeared to be responsive to chronic thyrotropin stimulation and underwent hypertrophy and hyperplasia with increased uptake of $^{131}\text{I}$, endocytosis of
colloid, and increased synthesis of lysosomes (Bastomsky, 1974; Collins et al., 1977). Therefore, the objectives of this investigation were: 1) to evaluate the histologic and ultrastructural lesions in thyroid follicular cells resulting from feeding low and high levels of PCB compared with alterations resulting from acute stimulation by exogenous thyrotropin, chronic stimulation produced by iodide deficiency and excess, and chronic suppression by exogenous thyroxine, and 2) to correlate these structural alterations with serum levels of thyroxine and triiodothyronine in order to further define the pathogenesis of the thyroid lesions produced by PCB.

MATERIALS AND METHODS

Eight-week-old, male, Osborne-Mendel rats were used in the experiment. Aroclor 1254 (PCB) was administered to 3 groups of rats (6 rats per group) at concentrations of 5, 50, and 500 parts per million for 4 weeks. The PCB was mixed into powdered Purina rat chow using corn oil as a vehicle. Rats receiving PCB were kept isolated in a separate room from other rats used in the experiment. Six rats were fed an iodine deficient (Remington) diet (Teklad Test Diets, Madison, Wisconsin) and distilled water for 4 weeks. Six rats were fed an iodine-excess diet that was prepared by mixing potassium iodide (KI) into powdered Purina rat chow at a concentration of 1%. Rats whose thyroids were acutely stimulated received 0.5 international units (I.U.) thyrotropin (TSH) (Thytropar, Armour Pharmaceutical Co., Phoenix, Arizona) intramuscularly and 3 rats were killed at 30 min, 1, 4, 8, and 24 h post-injection. These control rats were administered only saline. Six rats whose thyroids were chronically
suppressed received 100 ug of sodium levothyroxine subcutaneously each day for 4 weeks. Six control rats were fed Purina rat chow.

One of each pair of thyroid glands was collected for scanning and transmission electron microscopy at the end of the experimental period. Half of one thyroid lobe was fixed whole in 3% glutaraldehyde and the remaining portion was minced into 1 mm$^3$ pieces in cold 3% buffered glutaraldehyde (E.M. Grade; Electron Microscopy Sciences, Fort Washington, PA. 19034) in 0.1M sodium cacodylate. The opposite thyroid lobe was collected for histopathologic evaluation and was stained with hematoxylin and eosin (H & E), periodic acid-Schiff (PAS), or both. Thyroid tissue for electron microscopic evaluation was washed twice in 0.1M cacodylate buffer and post-fixed in 1.33% osmium tetroxide in s-collidine buffer at pH 7.4 for 1 hr. Both minced and whole pieces of thyroid from each rat were dehydrated through ascending concentrations of ethyl alcohol. Thyroids for transmission electron microscopy were transferred to propylene oxide and embedded in Epon 812 (Shell Oil Co., New York, N.Y.). One micron thick sections were cut from each block and stained with toluidine blue for light microscopic evaluation and selection of the most appropriate area for sectioning. Thin sections for electron microscopic evaluation were cut at 600 to 800 Å on a Reichert Om-U2 ultramicrotome and mounted on 300 mesh copper grids. The sections from each rat were stained with uranyl acetate and lead citrate, and examined with a Philips 200 or 300 electron microscope.

Thyroids for scanning electron microscopic evaluation were dried in a Sorvall critical point dryer, coated with gold-palladium, and examined with an AMR 1000S microscope.
Serum thyroxine and triiodothyronine were determined for each rat. Serum collected was frozen (-20°C) until completion of the experiment when all samples were assayed at the same time. Serum thyroxine and triiodothyronine were determined by radioimmunoassay (V.K. GanJam, unpublished data).

RESULTS

Histopathology and Ultrastructure

Control Rats. The thyroid glands of control rats were composed of large follicles lined by a single layer of cuboidal or low columnar epithelial cells. Follicular cells had a lightly eosinophilic cytoplasm and a central basophilic nucleus. Colloid in the follicular lumen was either homogeneous or slightly vacuolated near the periphery. Thyroid C-cells were present in small clumps of 3 or 4 cells between follicles or as individual cells within follicular walls. Interfollicular capillaries were separated from follicles by a thin basement membrane.

Ultrastructurally, the cytoplasm of follicular cells had lamellar arrays of rough endoplasmic reticulum (RER) with narrow cisternae that contained a finely granular material (Fig. 1). The Golgi apparatus was of moderate size and composed of flat layers of smooth membranes associated with small dense granules. Mitochondria with transverse cristae were dispersed throughout the cytoplasm. The nucleus was centrally or basally positioned and had peripherally clumped chromatin. A narrow layer of electron-dense apical vesicles were
present immediately beneath the microvilli along the luminal plasma membrane. Occasional membrane-bound colloid droplets and round electron-dense lysosomes were present in the cytoplasm, particularly in the apical portion of follicular cells.

The luminal surfaces of follicular cells were slightly rounded and contained variable numbers of microvilli of uniform length and width (Fig. 2). Microvilli were fewer in number, short, and located near cell boundaries in the larger, peripheral follicles. Numerous microvilli covering most of the luminal surface of follicular cells were present in the smaller follicles located in the central part of the thyroid. Only an occasional cytoplasmic pseudopodium protruded into the follicular lumen of control rats.

5 ppm PCB. Thyroid glands of rats fed 5 ppm PCB had ultrastructural evidence suggesting increased activity compared to controls. Follicles were lined by columnar follicular cells which had a lightly eosinophilic vacuolated cytoplasm with a basally located nucleus. The follicular lumen was reduced in size and contained a homogeneous colloid. Large membrane-limited colloid droplets and irregular lysosomes were more numerous in rats fed PCB than in controls and occupied much of the cytoplasmic area (Fig. 3). The cytoplasm had numerous lamellar arrays or dilated profiles of RER (Fig. 3). The cisternae contained a finely granular material similar to that found in the control rats. The Golgi apparatus was small and was composed of flat cisternae associated with a few small dense granules. Apical vesicles were reduced in number. The apical surface of follicular cells was rounded and protruded into the follicular
lumen (Fig. 4). Microvilli were shorter and less numerous compared to control rats. Occasional cytoplasmic projections extended from the apical surface of follicular cells into the lumen.

50 ppm PCB. Thyroid glands were moderately enlarged and composed of follicles lined by tall columnar cells that had a vacuolated, lightly eosinophilic cytoplasm (Fig. 5). Follicular lumens were reduced in size and contained homogeneous colloid. PAS-positive colloid droplets were increased in the apical portion of follicular cells (Fig. 5). Occasional papillary projections of apical cytoplasm extended into the lumen of follicles (Fig 6). Numerous large colloid droplets and irregular lysosomes filled the apical cytoplasm. There was limited evidence of colloid droplet-lysosome interaction. The RER was dilated and contained a finely granular, electron-dense material. The Golgi apparatus was small, had few associated granules, and apical vesicles were reduced in number. Mitochondria were vacuolated and were displaced peripherally in some follicular cells by the increased numbers of colloid droplets and lysosomes. The luminal surfaces of follicular cells were rounded and protruded into the follicular lumen. Microvilli were short, blunt or abnormally branched. Luminal surfaces of many follicular cells were devoid of microvilli and cytoplasmic projections extended into the colloid.

500 ppm PCB. Thyroid glands were enlarged and composed of small follicles lined by a single layer of tall columnar follicular cells. Papillary projections of hyperplastic cells and cytoplasmic processes from apical surfaces extended into the luminal colloid. Numerous large colloid droplets and irregular
lysosomes filled the apical cytoplasm. There was little evidence of fusion between membranes of colloid droplets and lysosomes (Fig 7). Both dilated profiles and lamellar arrays of RER were present in follicular cells (Fig. 7). Mitochondria often were vacuolated and were displaced peripherally in many follicular cells by the abnormal accumulation of colloid droplets and lysosomes. The Golgi apparatus was small and apical vesicles were observed infrequently in the narrow zone immediately beneath microvilli. The apical surface of follicular cells was swollen and protruded into the follicular lumen, thereby reducing its size (Fig. 8). Microvilli were short, blunt, and often abnormally branched. Cytoplasmic processes devoid of microvilli extended from the luminal surface into the colloid.

**Iodide-Excess Diet.** Thyroid glands of rats fed a diet containing 1% potassium iodide were enlarged compared to rats fed the control diet. Follicles were lined by a single layer of tall columnar cells with a vacuolated eosinophilic cytoplasm and basal nucleus (Fig. 9). Numerous PAS-positive granules were present in the cytoplasm. Papillary projections of hyperplastic follicular cells often extended into the lumen.

The apical cytoplasm of follicular cells was distended by numerous large colloid droplets and irregular lysosomes. There was little evidence of fusion between membranes of colloid droplets and lysosomes. The RER was present as dilated profiles and occasional lamellar arrays. Mitochondria often were vacuolated and displaced peripherally in some follicular cells by the numerous colloid droplets and lysosomes (Fig. 10). Golgi apparatuses were of moderate
size and associated with many small dense granules. Apical vesicles were numerous and located immediately beneath the microvilli in comparison to PCB-treated rats.

Many follicular cells contained intracellular microfollicles located within the apical portion of the cytoplasm. These microfollicles were lined by many microvilli of uniform length and width (Fig. 11). Numerous apical vesicles were present immediately beneath the microvilli. The material contained within the follicles was of similar density as the colloid within the lumen of larger follicles.

The apical surface of follicular cells was rounded and protruded into the follicular lumen. Microvilli present within the central part of the apical plasma membrane were numerous, whereas those near the cell borders were fewer in number and long (Fig. 12).

**Iodine Deficient Diet.** Thyroid glands of rats fed a diet deficient in iodine were enlarged compared to control rats. Follicular lumens were reduced in size and lined by tall columnar cells with a vacuolated cytoplasm and a basal nucleus. Follicular cells had a marked increase of both dilated profiles and lamellar arrays of RER (Fig. 13). Cisternae often were distended by a finely granular material. There was an increase in round lysosomes and colloid droplets in follicular cells. Membranes of many lysosomes and colloid droplets were observed to be fused. The Golgi apparatus was of normal size and apical vesicles were moderately decreased.

The apical surface of follicular cells was rounded and extended into the follicular lumen. Microvilli were of uniform shape, long, arranged
close together, and more numerous than in controls. Occasional pseudopod-like
processes of apical cytoplasm extended into the follicular lumen (Fig. 14).

Thyrotropin Stimulation. There was hypertrophy of follicular cells 30 min
to 4 h after intramuscular administration of 0.5 I.U. of TSH. Follicular cells
were tall columnar with a basal nucleus and had a vacuolated cytoplasm
containing large PAS-positive granules (Fig. 15). The follicular lumen appeared
as a narrow slit-like opening in many follicles.

The apical cytoplasm was distended with numerous large colloid droplets
(Fig. 16). Lysosomes were increased and were morphologically similar to those
in control rats. Membranes of many lysosomes were fused with colloid droplets.
Individual profiles and lamellar arrays of RER were dilated with a finely
granular material. The vesicles normally associated with the Golgi apparatus
were reduced in number. Apical vesicles were reduced in the zone beneath the
microvilli, reflecting an increased exocytosis of protein in response to acute
thyrotropin stimulation. Mitochondria in some follicular cells were displaced
peripherally by the numerous colloid droplets and lysosomes. Microvilli were
either long and branching or were replaced by pseudopod-like projections of
apical cytoplasm that contained colloid droplets (Fig. 16). Hypertrophied
follicular cells were rounded and had pseudopodia that extended into the
follicular lumen (Fig. 17).

Eight hours after administration of 0.5 I.U. TSH follicular cells were tall
columnar and contained many large colloid droplets which appeared to compress
the numerous dilated profiles and lamellar arrays of RER (Fig. 18). Round
lysosomes were increased and the limiting membrane of many appeared to be
fused with colloid droplets. The Golgi apparatus was associated with many small granules and apical vesicles were present immediately beneath the microvilli. Pseudopod-like projections of apical cytoplasm were not present 8 h post-TSH. Microvilli were numerous and closely grouped near the more central portion of the luminal plasma membrane. Near the junctions of adjacent follicular cells microvilli were longer and not as closely arranged together.

Twenty-four hours post-injection of 0.5 I.U. TSH hypertrophied follicular cells had fewer large colloid droplets than at 8 h and lysosomes were similar to those in controls. The RER consisted of dilated profiles and lamellar arrays. Microvilli were uniform in length and width, and distributed similar to those in control rats.

**Thyroxine Suppression.** Thyroid glands of rats administered 100 µg of sodium levothyroxine daily for 4 weeks were smaller than controls. Follicular cells were low cuboidal with a central nucleus surrounded by a small cytoplasmic area. Follicular lumens appeared to be enlarged due to the decrease in the size of follicular cells. Colloid was uniformly dense and PAS-positive with few peripheral vacuoles. The diminished cytoplasmic area of follicular cells contained only a few small profiles of RER and scattered mitochondria (Fig. 19). Round, homogeneous lysosomes were scattered throughout the cytoplasm. The Golgi apparatus was small, associated with a few granules, and apical vesicles were markedly decreased compared with controls. Microvilli on the lumenal surface were widely separated and short but of uniform length and width (Fig. 20). The remaining microvilli were present primarily near the junctions of adjacent follicular cells.
Radioimmunoassay of Serum Thyroid Hormone Levels

The serum thyroxine and triiodothyronine concentrations in each group of experimental rats were compared to the control group using the Student t test (Table 1). Thyroxine levels were significantly reduced in rats fed 50 and 500 ppm PCB and in rats fed iodide-excess and deficient diets. The greatest reduction in thyroxine levels was in rats fed 500 ppm PCB.

Thyroxine levels were significantly increased in rats administered thyroxine and TSH. There was a significant increase in serum $T_4$ 1 h after the intramuscular injection of TSH which remained elevated up to 8 h post-injection (Table 2). By 24 h after TSH stimulation $T_4$ levels had returned to normal.

Serum $T_3$ concentrations in experimental rats were more variable than $T_4$ levels. Serum $T_3$ was significantly decreased in rats fed 500 ppm PCB. There was no significant difference in serum $T_3$ levels between controls and rats fed 50 ppm PCB or an iodide-excess diet. Serum $T_3$ significantly increased in rats fed an iodide deficient diet, rats administered thyroxine, and following TSH injection (30 min, 1 and 8 h).

**DISCUSSION**

PCB produced dose-dependent alterations in thyroid follicular cells that were distinct from those changes resulting from either acute stimulation by thyrotropin, chronic stimulation by iodide-deficient and -excess diets, or chronic suppression by thyroxine. Thyroid follicular cells in rats fed PCB underwent both hypertrophy and hyperplasia from endogenous TSH stimulation.
in response to the lowered serum thyroxine and triiodothyronine. Colloid droplets and abnormal lysosomes accumulated in follicular cells and there was minimal ultrastructural evidence of lysosome-colloid droplet interaction necessary for the secretion of thyroid hormones. Microvilli were short, blunt or branching and projections of apical cytoplasm devoid of microvilli extended into the lumen.

Thyroid follicular cells of rats fed an iodine deficient diet also underwent hypertrophy and hyperplasia with abundant RER similar to that reported by Lupulescu and Petrovici (1968) but in contrast to the changes produced by PCB there was not an accumulation of large colloid droplets and abnormal lysosomes. In addition, there was ultrastructural evidence of the normal fusion of lysosomes and colloid droplets that is necessary for the secretion of thyroid hormones. This lysosome-colloid droplet interaction was not observed in the rats exposed to PCB. Rats fed the iodine deficient diet had more numerous and longer microvilli on follicular cells in contrast to the abnormal structure and reduction of microvilli in rats fed PCB. The pseudopodia engulfing colloid in iodine-deficient rats also were unlike the abnormal extensions of apical cytoplasm into the follicular lumen observed in PCB-treated rats.

Follicular cells in rats fed the iodide-excess diet accumulated lysosomes and colloid droplets with little evidence of fusion similar to that observed in PCB-exposed rats. Microvilli were long and numerous as opposed to the irregular and bizarre forms of microvilli on the luminal surface of follicular cells following the administration of PCB. The abnormal accumulation of colloid droplets and lysosomes with limited interaction in follicular cells of rats
fed the iodide-excess diet may be related to an inhibition of lysosomal enzymes required to cleave thyroid hormones from thyroglobulin in the colloid droplets. Decreased proteolysis of thyroglobulin has been reported in the thyroid glands of rats fed similar excessive amounts of iodide over a 3 week period (Takeuchi et al., 1970). An inhibition in the degradation of thyroglobulin may explain the accumulation of colloid droplets in rats fed excess iodide and have contributed to the abnormal accumulation of colloid droplets and lysosomes in follicular cells of rats fed PCB. The inhibition of proteolysis of thyroglobulin may be more complete in PCB-treated rats leading to a decreased release of thyroid hormones from follicular cells into the circulation. Serum thyroxine concentrations were dramatically decreased in rats fed 50 and 500 ppm PCB, whereas in rats fed iodide-excess diets the decrease, although significant, was less striking.

The acute stimulation with thyrotropin resulted in hypertrophy of follicular cells containing an increased number and evidence for fusion of colloid droplets with lysosomes as has been observed by other investigators (Lupulescu and Petrovici, 1968; Pantić and Kalušević, 1974). These changes were unlike those observed in rats exposed to PCB or fed a diet containing excess iodide. In addition, the fine structure of lysosomes was different in the thyroids of rats acutely stimulated by thyrotropin. Lysosomes in follicular cells of rats receiving PCB were large, irregular, heterogenous, and not fused with colloid droplets whereas in TSH-stimulated rats they were small, round, uniformly osmiophilic, and often fused with colloid droplets.
Changes in the apical plasma membrane also were different in follicular cells acutely stimulated by TSH compared to PCB-treated rats. The microvilli of TSH-stimulated rats either were long and branching or were replaced by pseudopodia containing colloid droplets. Microvilli were fewer in number and short with abnormal branching in PCB-treated rats. Cytoplasmic processes with few organelles and no colloid droplets extended into the follicular lumen of rats exposed to PCB. These changes in microvilli were associated with elevated circulating levels of thyroid hormones after the administration of TSH but markedly diminished serum thyroxine and triiodothyronine following PCB.

Suppression of the thyroid by exogenous thyroid hormone administration resulted in involution of follicular cells similar to that reported by other investigators (Lupulescu and Petrovici, 1968. A few short microvilli of uniform length and width were present on the apical plasma membrane, especially near the junction with adjacent follicular cells. They were distinctly different from the bizarre, blunt, abnormal microvilli observed in PCB-treated rats.

The ultrastructural lesions produced by PCB in thyroid follicular cells were unique, distinct from changes associated with acute or chronic stimulation or suppression of follicular cells, and contributed to the significant reduction in serum levels of thyroid hormones. Follicular cells remained responsive to stimulation by endogenous thyrotropin after exposure to PCB as evidenced by the hypertrophy and hyperplasia with increased development of RER and an increased uptake of colloid droplets. The principal effect of PCB on follicular cells appeared to be on limiting colloid droplet-lysosome interaction thereby inhibiting the proteolysis of thyroglobulin necessary for the release of thyroid hormones.
SUMMARY

Histologic and ultrastructural lesions in thyroid follicular cells of Osborne-Mendel rats produced by feeding diets containing 5, 50, and 500 ppm polychlorinated biphenyls (PCB) for 4 weeks were compared with alterations produced by low (Remington diet) and high (1% KI) iodide diets, chronic thyroxine administration, and a single injection of thyrotropin.

Exposure to PCB resulted in a dose-dependent hypertrophy and hyperplasia of follicular cells with an abnormal accumulation of large colloid droplets and irregular lysosomes. There was limited evidence of colloid droplet-lysosome interaction necessary for the secretion of thyroid hormones. Microvilli on luminal surfaces were decreased, abnormally shaped, and short. Serum thyroxine and triiodothyronine were decreased significantly after feeding PCB.

Follicular cells in rats fed the iodine-deficient diet underwent hypertrophy and hyperplasia with increased development of rough endoplasmic reticulum. They had increased numbers of round lysosomes and colloid droplets. There were numerous uniformly long microvilli and occasional pseudopodia were present engulfing colloid. Serum thyroxine was significantly decreased but triiodothyronine levels were increased. The iodide-excess diet resulted in hypertrophy and hyperplasia of follicular cells which contained numerous colloid droplets and abnormally shaped lysosomes with little evidence of fusion. Numerous long microvilli extended into the lumen. Many follicular cells contained unique intracytoplasmic microfollicles lined by uniform microvilli. Serum thyroxine was significantly reduced.
Follicular cells of rats chronically administered thyroxine underwent involution and had only a few short microvilli, scattered round lysosomes, and poorly developed organelles. Serum thyroxine and triiodothyronine were elevated significantly. Thyrotropin (TSH) produced hypertrophy of follicular cells by 30 min post-injection with increased large colloid droplets and small round lysosomes. Large pseudopod-like projections of follicular cells were involved in endocytosis of colloid. There was evidence of fusion of lysosomal membranes with colloid droplets. Circulating thyroid hormone levels were significantly elevated from 30 min to 8 h after administration of thyrotropin but normal by 24 h.

The ultrastructural alterations produced by PCB in the structure of microvilli were distinct from changes observed with acute stimulation by TSH, long-term stimulation by iodide excess or deficient diets, and chronic suppression by thyroxine. The changes in lysosomal structure and the accumulation of colloid droplets with little evidence of fusion in follicular cells of rats fed PCB closely resembled the findings in rats fed the iodide-excess diet. The principal effect of PCB on follicular cells appeared to be on limiting colloid droplet-lysosome interaction thereby inhibiting the proteolysis of thyroglobulin necessary for the release of thyroid hormones.
Table 1. Thyroxine ($T_4$) and Triiodothyronine ($T_3$) concentrations of Rats Administered PCB, Iodine Deficient or Excess Diets, or Thyroxine.

<table>
<thead>
<tr>
<th>Group</th>
<th>$T_4$ (µg/dl)</th>
<th>$T_3$ (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>3.71 ± 0.04</td>
<td>86.80 ± 2.0</td>
</tr>
<tr>
<td>5 ppm PCB</td>
<td>3.56 ± 0.10</td>
<td>105.96 ± 3.0†</td>
</tr>
<tr>
<td>50 ppm PCB</td>
<td>2.14 ± 0.10‡</td>
<td>82.13 ± 8.7</td>
</tr>
<tr>
<td>500 ppm PCB</td>
<td>0.78 ± 0.04‡</td>
<td>72.18 ± 3.7*</td>
</tr>
<tr>
<td>1% KI</td>
<td>3.22 ± 0.10*</td>
<td>87.06 ± 5.6</td>
</tr>
<tr>
<td>I-deficiency</td>
<td>2.74 ± 0.40*</td>
<td>125.15 ± 6.8*</td>
</tr>
<tr>
<td>$T_4$-suppression</td>
<td>8.55 ± 0.9‡</td>
<td>384.23 ± 30.9‡</td>
</tr>
</tbody>
</table>

N = 6/group Mean ± Standard error of mean
* P<0.025
† P<0.005
‡ P<0.001
Table 2. Thyroxine (T₄) and Triiodothyronine (T₃) Concentrations After the Administration of 0.5 Units of Thyrotropin.

<table>
<thead>
<tr>
<th>Group</th>
<th>T₄ (µg/dl)</th>
<th>T₃ (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>2.76 ± 0.03</td>
<td>79.2 ± 8.0</td>
</tr>
<tr>
<td>30 min. post-TSH</td>
<td>3.13 ± 0.17</td>
<td>108.7 ± 4.2*</td>
</tr>
<tr>
<td>1 hr. post-TSH</td>
<td>3.70 ± 0.10†</td>
<td>112.7 ± 2.3+</td>
</tr>
<tr>
<td>4 hr. post-TSH</td>
<td>3.86 ± 0.06‡</td>
<td>124.0 ± 3.4‡</td>
</tr>
<tr>
<td>8 hr. post-TSH</td>
<td>3.60 ± 0.30*</td>
<td>90.8 ± 5.5</td>
</tr>
<tr>
<td>24 hr. post-TSH</td>
<td>2.73 ± 0.13</td>
<td>75.1 ± 4.1</td>
</tr>
</tbody>
</table>

N = 3/group  Mean ± Standard error of mean
* P<0.025
† P<0.005
‡ P<0.001
Figure 1. Thyroid follicular cell in a control rat with lamellar arrays and dilated profiles of rough endoplasmic reticulum (E), mitochondria (M), and a well developed Golgi apparatus (G). A narrow layer of apical vesicles (A) is located beneath the microvilli. X 14,100
Figure 2. Scanning electron micrograph of luminal surface of thyroid follicular cells from a control rat with many finger-like microvilli (arrows) of uniform length and width. X 21,300
Figure 3. Columnar follicular cell lining thyroid follicle from a rat fed 5 ppm PCB for 4 weeks. Numerous large membrane-limited colloid droplets (C) and irregular dense lysosomes (L) fill much of the cytoplasmic area. Apical vesicles are reduced in number immediately beneath the microvilli (arrows). Both lamellar arrays and dilated profiles of rough endoplasmic reticulum (E) were present in follicular cells. X 15,100
Figure 4. Rounded luminal surface of follicular cells with short microvilli (arrows) in a rat fed 5 ppm PCB for 4 weeks. X 8,000
Figure 5. Thyroid follicles with narrow lumens (L) lined by columnar follicular cells (arrows) from a rat fed 50 ppm PCB for 4 weeks.
Figure 6. Columnar follicular cells containing dilated profiles of rough endoplasmic reticulum (E), large colloid droplets (C), and numerous lysosomes (L) lined thyroid follicles of rats fed 50 ppm PCB for 4 weeks. Luminal surfaces of many follicular cells had long processes of apical cytoplasm (P) that extended into the follicular lumen. X 10,500
Figure 7. Thyroid follicular cell with an expanded cytoplasmic area filled with closely packed colloid droplets (C) and large lysosomes (L) in a rat fed 500 ppm PCB for 4 weeks. The rough endoplasmic reticulum (E) and Golgi apparatus are poorly developed and compressed by the numerous colloid droplets and lysosomes. Short, blunt microvilli (arrows) extend into the follicular colloid. X 12,300
Figure 8. Scanning electron micrograph of the luminal surfaces of follicular cells of rats fed 500 ppm PCB. Luminal surfaces were rounded and protruded into the follicular lumen. Microvilli (arrows) were short, irregularly shaped or absent from areas of the apical plasma membrane. X 16,800
Figure 9. Thyroid follicles lined by columnar follicular cells from a rat fed an iodide-excess diet. The lumen (L) is narrow and contains little colloid. PAS, X 315
Figure 10. Thyroid from rat fed 1% KI diet for 4 weeks. Large colloid droplets (C) and irregularly shaped lysosomes (L) fill the cytoplasm displacing other organelles peripherally in columnar follicular cells. Scattered apical vesicles (A) are present beneath the uniform finger-like microvilli. X 8,700
Figure 11. Intracellular microfollicles (MF) in the apical cytoplasm of follicular cells. The microfollicles are lined by long, uniform microvilli (arrows) and contain a finely granular electron-dense material similar to colloid (C). Rat fed 1% KI diet for 4 weeks. X 7,000
Figure 12. Scanning electron micrograph illustrating numerous microvilli in the central part of the luminal surface. Microvilli are fewer in number and longer near the border with adjacent follicular cells (arrows). Thyroid from rat fed 1% KI diet for 4 weeks. X 14,000
Figure 13. Columnar follicular cells with dilated profiles of rough endoplasmic reticulum (E), many large colloid droplets (C), and numerous lysosomes in a rat fed an iodine deficient diet for 4 weeks. The Golgi apparatus (G) is displaced peripherally by the colloid droplets and lysosomes. Apical vesicles are reduced in number and uniform microvilli project from the apical plasma membrane into the colloid. X 13,300
Figure 14. Scanning electron micrograph of luminal surface of thyroid follicular cell from a rat fed an iodine deficient diet for 4 weeks. Numerous long, closely packed microvilli and small pseudopod-like processes of apical cytoplasm (arrows) extend into the follicular lumen. X 14,400
Figure 15. Columnar follicular cells lining thyroid follicles from a rat 2 h post-TSH. The lumen (L) is narrow and contains little colloid. PAS, X 315
Fig 15
Figure 16. Columnar follicular cells with large apical pseudopodia (P) extending into the colloid from a rat 30 min after injection of 0.5 I.U. thyrotropin. Microvilli (arrows) are long and branching. Large colloid droplets (C), dilated profiles of rough endoplasmic reticulum (E), and small lysosomes (L) are present in the cytoplasm. Apical vesicles are reduced in the narrow zone beneath the microvilli. X 15,600
Figure 17. Luminal surface of hypertrophied follicular cells 4 h post-TSH illustrating cytoplasmic pseudopodia (P) and numerous long microvilli (arrows). X 5,000
Figure 18. Hypertrophied follicular cell containing numerous colloid droplets (C) and scattered lysosomes (L) in a rat 8 hours post-injection of 0.5 I.U. The rough endoplasmic reticulum (RER) and mitochondria (M) are displaced peripherally by the colloid droplets. Apical vesicles (A) are increased and numerous short microvilli (arrows) extend from the apical plasma membrane. X 10,900
Fig 18
Figure 19. Cuboidal follicular cells with a reduced cytoplasmic area and few profiles of rough endoplasmic reticulum (E), a small Golgi apparatus (G), and round lysosomes (L). Apical vesicles (A) are absent and microvilli (arrows) are sparse. Rat administered 100 μg of thyroxine daily for 4 weeks. X 15,600
Figure 20. Scanning electron micrograph of flattened luminal surface of thyroid follicular cells in a rat administered 100 μg of thyroxine daily for 4 weeks. Microvilli are widely separated and short but of uniform length and width. X 5,000.
CHAPTER III

FINE STRUCTURAL LESIONS AND HORMONAL ALTERATIONS IN THYROID GLANDS OF PERINATAL RATS EXPOSED IN UTERO AND BY THE MILK TO POLYCHLORINATED BIPHENYLS

INTRODUCTION

The widespread contamination of the environment with polychlorinated biphenyls (PCB) has been well documented in several recent reports.¹⁻⁴ These compounds have gained use in industry as dielectric fluids in capacitors and transformers, hydraulic and heat transfer fluids, and plasticizers or solvents in adhesives and sealants because of their nonflammability and high dielectric constant. The escape of PCB into the environment through sewage outfalls and industrial disposal into waterways plus their long half-life have led to detectable levels in the adipose tissue of a large proportion of the human population.⁵ Polychlorinated biphenyl residues have been detected in rivers and oceans, and tissues of fish, wildlife, cattle and poultry.²,³,⁷ Escape of PCB into the air from plasticized materials, leakage of lubricants, hydraulic and heat transfer fluids, and leaching from waste dumps also may contribute significant amounts of PCB to the environment.

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The disease-producing capability of these compounds has been well documented in human beings, cattle, and poultry following accidental contamination of food stuffs.\textsuperscript{8-10} Intoxication with PCB results in the development of lesions in several organ systems. In the liver PCB is a potent inducer of microsomal enzymes, resulting in the proliferation of smooth endoplasmic reticulum in hepatocytes and may cause fatty degeneration and necrosis.\textsuperscript{11-13} Epidermal hyperplasia and hyperkeratosis, porphyria, and degeneration of lymphoid tissues and kidney have been reported following PCB intoxication.\textsuperscript{14-15}

Recent evidence indicates that PCB has an adverse effect on reproduction, growth, and development in several animal species\textsuperscript{16-18} and man.\textsuperscript{8} PCB administered in the feed before breeding and to pregnant mink or rats resulted in increased stillbirths, decreased litter sizes and birth weights, and decreased weight gain and survivability in weanling animals.\textsuperscript{19-23} The feeding of PCB to sows throughout gestation and nursing significantly reduced the numbers of live pigs farrowed and increased the number of mummified fetuses.\textsuperscript{17} Infants born to mothers who had ingested PCB in rice oil had decreased birth weights and growth rates over a 3-year period.\textsuperscript{24} Reduced weight gain and feed efficiency also have been reported in weanling sheep and pigs fed concentrations of PCB as low as 20 ppm.\textsuperscript{16} PCB has been reported to be transferred by the placenta to the fetus\textsuperscript{25,26} and residues have been found in mother's milk.\textsuperscript{24,27}

Decreased reproductive performance and an interference in growth and development have been reported in man and animals made hypothyroid by agenesis or removal of the fetal thyroid gland, ablation of the fetal or neonatal
thyroid with $^{131}$ or by interference in the synthesis of thyroid hormone by certain drugs. Previous studies from our laboratory provided evidence that PCB intoxication in adult rats produces fine structural lesions in thyroid follicular cells suggesting an interference in lysosome-colloid droplet interaction necessary for the secretion of thyroid hormones thereby contributing to the significant lowering of serum thyroxine. In addition PCB enhances the peripheral metabolism and excretion of thyroxine-glucuronide in the bile. These findings suggest that some of the disturbances in reproduction, growth, and development reported in animals and man intoxicated with PCB may be related to alterations in the structure and function of the thyroid gland in the dam, fetus or neonate. Therefore, the objectives of this investigation were: (1) to investigate the fine structural alterations produced by the administration of PCB on thyroid glands of rats exposed in utero and by the milk during early postnatal life, and (2) to correlate ultrastructural lesions in the thyroid with changes in circulating levels of thyroxine and triiodothyronine in order to determine the effects of PCB on thyroid structure and function in utero and during the perinatal period.

**MATERIALS AND METHODS**

Forty five, 12-week-old, female, Osborne-Mendel rats were divided into three groups of 15 animals. The female rats were bred and the initiation of pregnancy was determined by the presence of sperm in the vagina. Upon confirmation of mating, female rats were fed rations containing either 0, 50 or 500 ppm PCB. The rations were prepared by mixing PCB (Aroclor 1254) into
powdered Purina rat chow using corn oil as a vehicle. Control rats received a similar diet with 3% corn oil but without PCB.

Thyroid glands were collected from the fetuses or pups from 3 dams in each exposure group at the 18th day after conception, at parturition and at 7, 14 and 21 days of age. Serum was collected at parturition and at 7, 14, and 21 days of age and pooled from half of each litter of rat pups. Fetuses and neonatal rats were perfused with 1% glutaraldehyde through the left ventricle of the heart. Rat pups from the older age groups were perfused through the abdominal aorta. Thyroid glands were collected after perfusion and minced into 1 mm$^3$ blocks in cold 3% glutaraldehyde. Tissue was collected and processed for histopathologic evaluation and sections were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) or both.

Thyroid for electron microscopic evaluation was fixed in ice-cold 3% glutaraldehyde (E.M. Grade, Electron Microscopy Sciences, Box 251, Fort Washington, PA., 19034) in 0.1 M sodium cacodylate buffer for 2 hours, washed twice in 0.1 M cacodylate buffer, and post-fixed in 1.33% osmium tetroxide in s-collidine buffer at pH 7.4 for 1 hour. Six tissue blocks from each rat (3 left lobe, 3 right lobe) were dehydrated through ascending concentrations of ethyl alcohol, transferred to propylene oxide, and embedded in Epon 812 (Shell Oil Co., New York, N.Y.). One micron thick-sections were cut from each block and stained with toluidine blue for light microscopic evaluation and selection of the most appropriate area of the block for sectioning. Thin sections were cut at 600 to 800 Å on a Reichert Om-U2 ultramicrotome and mounted on 300 mesh copper grids. The sections were stained with uranyl acetate and lead citrate, and examined with a Philips 200 or 300 microscope.
Serum thyroxine and triiodothyronine were determined by radioimmunoassay (V.K. GanJam, Unpublished data).

**RESULTS**

**Body Weight and Litter Size**

Litter size was significantly decreased (7.7 pups/litter) in rats fed 500 ppm PCB compared to control rats (8.9 pups/litter) (Table 1). Body weights were reduced significantly in rats exposed to 50 and 500 ppm PCB in utero and by the milk at 21 days of age but not at parturition or at 7 and 14 days of age. (Table 1).

**Electron Microscopy**

**Thyroid Glands of 18 day Rat Fetuses**

**Control Rats.** The thyroid glands were composed of solid nests or cords of cells arranged to form follicles. These primitive follicles were formed either by the coalescence of two or more intracellular microfollicles or between several adjacent thyroid epithelial cells. Both intracellular and extracellular follicles contained a finely granular, electron-dense material similar to colloid. The lumens of both types of follicles were lined by numerous microvilli of uniform length and width (Figure 1). The cytoplasm contained abundant free ribosomes and the rough endoplasmic reticulum was present as individual profiles with narrow or occasionally dilated cisternae. The Golgi apparatus was small and associated with a few vesicles. Mitochondria were prominent and distributed throughout the cytoplasm. Apical vesicles were absent in the zone just below
the microvilli in most follicular cells. Colloid droplets were not present in the cytoplasm and there was only an occasional small lysosome. The nucleus was large with a peripheral condensation of chromatin. Cells were observed frequently in various states of mitosis. The nests and cords of cells were limited by a thin basement membrane.

**Low (50 ppm) and High (500 ppm) Dose Polychlorinated Biphenyls.** The thyroid glands of 18 day old fetuses from dams exposed to 50 or 500 ppm PCB since conception were similar and will be described together. Thyroids consisted of solid nests of cells, cords forming primitive follicles, and immature follicles. Primitive follicles were similar to those in control fetuses and were formed either by the coalescence of intracellular microfollicles or extracellularly between two or more cells. Small developing follicles were lined by several immature thyroid epithelial cells. Follicles in all stages of development contained a finely granular, electron-dense material similar to colloid.

Follicular cells in fetuses exposed to PCB contained abundant free ribosomes and the rough endoplasmic reticulum was increased compared to controls and appeared as large dilated profiles or lamellar arrays with narrow cisternae. Numerous ribosomes were attached to membranes of the endoplasmic reticulum. Mitochondria were large, numerous, and many were swollen with disrupted cristae (Figure 2). The Golgi apparatus was small as in controls and consisted of flat sacs with few associated vesicles. Apical vesicles, colloid droplets, and lysosomes were absent in cells forming solid nests or primitive follicles. A few apical vesicles and occasional lysosomes but not
colloid droplets were present in cells lining immature follicles. The nucleus was often irregular in shape with a condensation of chromatin near the nuclear membrane. Mitotic figures were common.

**Thyroid Glands of Neonatal Rats at Parturition**

**Control Rats.** Follicles were more mature in rat pups at parturition and only occasional solid nests of cells were present in the thyroids. Most of the follicular cells contained intracellular microfollicles or were lining immature or mature follicles containing colloid (Figure 3). Numerous long microvilli of uniform length and width were present on the luminal surface of follicular cells. The cytoplasm contained an increase in rough endoplasmic reticulum compared to 18 day rat fetuses that appeared as individual profiles with narrow tubular or infrequent dilated cisternae. The Golgi apparatus was more prominent and consisted of pairs of smooth membranes and associated vesicles. A narrow zone of apical vesicles was present in the area immediately beneath the microvilli. Occasional small lysosomes but no colloid droplets were present in the cytoplasm. Mitochondria with transverse cristae were distributed throughout the cytoplasm.

**Low (50 ppm) and High (500 ppm) Dose Polychlorinated Biphenyls.** Follicular cells either had microfollicles in the cytoplasm or lined developing follicles that contained colloid in both the low and high dose group. However, mature follicles predominated in neonatal rats at parturition whose dams were exposed to 500 ppm PCB (Figure 4). This was in contrast to control rats where immature follicles and solid nests of cells were still numerous. Long microvilli
of uniform length and width extended into the colloid. The rough endoplasmic reticulum was abundant and was present as lamellar arrays often with dilated cisternae. Mitochondria were swollen and vacuolated with disruption of cristae (Figure 4). The Golgi apparatus was small and consisted of a few pairs of smooth membranes associated with infrequent vesicles. A narrow layer of apical vesicles were present in the zone immediately beneath microvilli and were most numerous in cells lining mature follicles. Occasional small lysosomes were observed in both groups. No colloid droplets were observed in follicular cells.

Neonatal Rats - 7 Days of Age

Control rats. Mature follicles and follicles forming by the coalescence of microfollicles were lined by cuboidal cells (Figure 5). The cytoplasm of follicular cells contained abundant free ribosomes and individual profiles of rough endoplasmic reticulum with narrow tubular cisternae. The Golgi apparatus was more prominent than in rat pups at parturition and composed of numerous pairs of smooth membranes and associated vesicles. A narrow layer of apical vesicles was present in the zone beneath microvilli, especially in cells lining mature follicles. Microvilli were numerous and uniform in length and width. Occasional lysosomes were present but colloid droplets were not observed. Mitochondria were large and distributed throughout the cytoplasm. The nucleus occupied much of the cell area and had peripherally condensed chromatin.

Low (50 ppm) and High (500 ppm) Dose Polychlorinated Biphenyls. Thyroid glands of 7-day-old rats exposed both in utero and by the milk of dams ingesting
either 50 or 500 ppm PCB in the feed were composed of mature follicles lined by cuboidal follicular cells. The follicular cells were larger than in controls and contained increased rough endoplasmic reticulum that appeared as extensive lamellar arrays with with narrow or dilated cisternae (Figure 6). The Golgi apparatus was small in most follicular cells and consisted of a few pairs of smooth membranes with associated vesicles. A narrow layer of apical vesicles was present beneath microvilli. Occasional vacuolated mitochondria were present in follicular cells. Lysosomes were infrequent and colloid droplets were not present in follicular cells. Microvilli were numerous and of uniform length and width.

**Neonatal Rats - 14 Days of Age**

**Controls.** Cuboidal follicular cells lined the well developed follicles in thyroids of 14 day-old rat pups. Individual profiles and lamellar arrays of rough endoplasmic reticulum were present and the Golgi apparatus was more extensive than in younger rats. A narrow layer of apical vesicles were present immediately beneath the microvilli. Large mitochondria were distributed throughout the cytoplasm. Lysosomes were round, more numerous, and larger than in 7 day-old rats. No colloid droplets were observed in follicular cells.

**Low (50 ppm) and High (500 ppm) Dose Polychlorinated Biphenyls.** Tall cuboidal to columnar epithelial cells lined thyroid follicles of 14 day-old rats exposed to PCB in utero and by the milk. The cytoplasm contained extensive lamellar arrays of rough endoplasmic reticulum. The cisternae were narrow or moderately dilated by a finely granular material. The Golgi apparatus was
small in the hypertrophied follicular cells, associated with few small granules, and apical vesicles were reduced compared to controls. Vacuolated mitochondria with disrupted cristae were present in follicular cells (Figure 7). Microvilli were numerous and uniform in length and width. Lysosomes were present as in controls but there were no colloid droplets in follicular cells of neonatal rats in the low dose group. There were increased colloid droplets of variable size in follicular cells of rats in the high dose group compared with control rats (Figure 7). They were present in the apical part of the cell, bounded by a single membrane, and contained a finely granular electron-dense material. The colloid droplets were more electron-lucent than the colloid in the follicular lumen. Lysosomes were increased compared with controls, but there was little evidence of colloid droplet-lysosome interaction.

Neonatal Rats - 21 Days of Age

Controls. Thyroid glands of 21 day-old rats were composed of mature follicules lined by cuboidal follicular cells. The cytoplasm contained individual profiles and lamellar arrays of rough endoplasmic reticulum with narrow cisternae. Free ribosomes were present but were less numerous than observed in younger age groups. The Golgi apparatus consisted of flattened sacs with associated vesicles and granules. Mitochondria were large and distributed throughout the cytoplasm. Apical vesicles were present as a narrow zone beneath microvilli. Microvilli projecting into the follicular lumen were of uniform length and width. Rare colloid droplets were observed in follicular cells. Lysosomes were round and present in approximately similar numbers as the 14 day old rats.
**Low (50 ppm) and High (500 ppm) Dose Polychlorinated Biphenyls.**

Follicular cells in thyroids of 21 day-old rats exposed in utero and by the milk to 50 or 500 ppm PCB were more columnar than in controls. The increased cytoplasmic area contained extensive lamellar arrays of rough endoplasmic reticulum with narrow or dilated cisternae which occupied much of the cytoplasmic area in rats exposed to 500 ppm (Figure 8). There were numerous large colloid droplets of similar electron density as luminal colloid within follicular cells. Myelin figures and other membranous debris were present in some colloid droplets. Lysosomes were present as in controls and occasionally were heterogeneous in density but there was little evidence of interaction with colloid droplets. Mitochondria were vacuolated with disruption of cristae. The Golgi apparatus was small and consisted of a few membranous sacs and associated vesicles. Apical vesicles were present as a narrow zone beneath microvilli. Microvilli were of uniform length and width in the low dose group. In rats exposed to 500 ppm, microvilli were shorter than normal with occasional club-shaped or branching forms.

**Radioimmunoassay of Serum Thyroxine and Triiodothyronine**

The serum thyroxine and triiodothyronine concentrations of control and rats exposed to PCB were determined by radioimmunoassay and similar age groups were compared statistically using the Student t test. Thyroxine levels were significantly reduced (P<0.001) at birth and at 7, 14 and 21 days of age in rats exposed in utero and by the milk to 50 and 500 ppm PCB (Figure 9). There was no significant difference in serum thyroxine levels between different age
groups of rats exposed to either 50 or 500 ppm PCB. Triiodothyronine levels were reduced significantly (P<0.01) in neonatal rats at birth and at 7 and 14 days of age following exposure to 50 and 500 ppm PCB (Figure 10). There were no significant differences in triiodothyronine levels between 21 day old control and experimental rats exposed to 50 or 500 ppm PCB. The lowering of serum triiodothyronine in experimental rats was not significantly different between the 2 dose levels of PCB.

DISCUSSION

Exposure of fetal and neonatal rats to PCB in utero and by the milk resulted in dose-dependent and age-related ultrastructural changes in thyroid follicular cells and significant reductions in serum thyroxine and triiodothyronine. The electron microscopic alterations attributed to the direct effects of PCB were characterized by vacuolation of mitochondria with disruption of cristae, alterations in microvilli, and an interference in colloid droplet-lysosome interaction. Similar alterations have been reported in thyroid follicular cells of adult Osborne-Mendel and Holtzman rats fed PCB for 4 weeks.\textsuperscript{31,36} In addition to these changes in common with perinatal rats, follicular cells of adult rats had striking accumulations of irregular lysosomes and colloid droplets also with little evidence of interaction between these organelles that is necessary for the secretion of thyroid hormones.

Colloid droplets did accumulate in thyroid follicular cells of 14- and 21-day-old rat pups but there was no change in the ultrastructure of lysosomes. The accumulation of colloid droplets in these age groups of rat pups was
interpreted to be the result of increased stimulation of follicular cells by thyrotropin in response to the reduced levels of thyroid hormones. This stimulation of follicular cells and accumulation of colloid droplets in neonatal rats corresponded to the time of hypothalamic-pituitary maturation in the normal rat. At this age the hypothalamus and pituitary are capable of responding to decreased circulating thyroid hormone levels by increased synthesis of thyrotropin-releasing hormone and thyrotropin. In contrast, follicular cells in adult rats were capable of responding to stimulation by thyrotropin after exposure to PCB as indicated by an increased uptake of $^{131}$I by the thyroid gland. The increased development and dilatation of rough endoplasmic reticulum in perinatal rats exposed to PCB also was probably related to a sustained thyrotropin stimulation in response to the lowered circulating levels of thyroid hormones.

The differences in thyroid lesions between the various age groups of perinatal and adult rats may be due to the sensitivity of follicular cells or to the inability of young rats to metabolize PCB as occurs in adults. PCB is metabolized 3 weeks of age, Perinatal rats are deficient in these and other drug-metabolizing enzymes at birth and appreciable levels are not observed until 2 to 3 weeks of age; however, exposure to PCB in utero and by the milk may have resulted in the induction of these enzymes at an earlier age. Certain metabolites of PCB which are present in adult rats and responsible for the abnormal accumulation of lysosomes in follicular cells may not be present in the perinatal rat.

Ultrastructural changes in thyroid follicular cells similar to those in the 14- and 21-day-old neonatal rats have been observed in homozygous Gunn rat
fed PCB for 4 weeks. The homozygous Gunn rat being deficient in the hepatic microsomal enzyme UDP-glucuronyltransferase, may be unable to convert PCB to other potentially more toxic metabolites.

Perinatal rats exposed in utero and by the milk to PCB had a highly significant reduction in serum thyroxine. The lowering was not dose-dependent with the levels of 50 and 500 ppm PCB. The reduction in thyroid hormones appeared to be primarily the result of a direct effect of PCB on the secretion of thyroid hormones in 14 and 21 day old rats. The induction of hepatic microsomal thyroxine UDP-glucuronyltransferase by PCB at an early age may contribute to the lowering of thyroid hormone levels particularly in the 18 day fetuses and rat pups at parturition and 7 days of age. However, other investigations using DDT (another potent hepatic microsomal enzyme inducer) failed to demonstrate an increased enzyme activity in rat pups exposed in utero or by the milk until 4 days of age.

**SUMMARY**

Polychlorinated biphenyls (PCB) produced ultrastructural lesions in thyroid follicular cells and a reduction in serum levels of thyroid hormones in neonatal (0, 7, 14, and 21 days of age) Osborne-Mendel rats exposed to 50 or 500 ppm PCB in utero and by the milk. Litter size was decreased significantly in rats fed 500 ppm PCB. Body weights at 21 days of age were reduced in rats exposed to 50 and 500 ppm PCB. The ultrastructural lesions in follicular cells were dose- and age-dependent but were less extensive than in adult rats of the same strain. At all ages the lesions in thyroid follicular cells were characterized by
increased development of rough endoplasmic reticulum and vacuolization of mitochondria. There was an increase of colloid droplets and lysosomes in the older age groups (14 and 21 days) but little evidence for colloid droplet-lysosome interaction necessary for the secretion of thyroid hormones. Shortening of microvilli with the formation of club-shaped or branching forms were observed only in 21-day-old rat pups. These ultrastructural alterations in follicular cells exposed to PCB were associated with a significant reduction in serum thyroxine in the rats at birth and at 7, 14, and 21 days of age. Serum triiodothyronine was reduced significantly in 7- and 14-day-old rat pups. The ultrastructural alterations in follicular cells appeared to contribute to the significant lowering of serum thyroid hormone levels in 14 and 21 day old rats exposed to PCB. These findings suggest that alterations in thyroid structure and function may be important in the pathogenesis of certain metabolic disorders associated with PCB intoxication.
Figure 1. Fetal rat thyroid (18 days gestation) illustrating epithelial cells arranged around a developing follicle (F). The follicular cells contain many free ribosomes (R), individual profiles of rough endoplasmic reticulum (E), Golgi apparatus (G), and mitochondria (M). Microvilli (arrow) projecting into the lumen of the microfollicle between follicular cells are short but uniform in shape. (X 13,200)
Figure 2. Thyroid epithelial cells of a fetal rat (18 days gestation) from a
dam fed a diet containing 50 ppm PCB since conception. An
intracellular microfollicle (F) has prominent microvilli (arrow)
extending into the lumen. The rough endoplasmic reticulum (E) is
increased compared to control rats and cisternae are dilated by a
finely granular material. Mitochondria (M) are swollen and cristae
disrupted. There are numerous free ribosomes (R) and a small
Golgi apparatus (G). (X 12,000)
Figure 3. Immature thyroid follicle from a control rat at birth lined by cuboidal follicular cells. There are numerous free ribosomes and profiles of rough endoplasmic reticulum (E) with narrow cisternae. Mitochondria (M) are distributed throughout the cytoplasm. Numerous microvilli (arrows) extend into the follicular colloid (FC). (X 9,200)
Figure 4. Developing thyroid follicle in a rat at birth whose dam ingested a diet containing 500 ppm PCB since conception. The increased rough endoplasmic reticulum (E) has dilated cisternae and mitochondria (M) are swollen with disrupted cristae. The Golgi apparatus (G) is small and a few apical vesicles are present beneath the microvilli (arrow). (X 9,000)
Figure 5. Thyroid follicle from a control rat 7 days of age lined by cuboidal cells containing individual profiles of rough endoplasmic reticulum (E) with narrow cisternae, scattered mitochondria (M), and numerous apical vesicles (A) beneath the microvilli. An occasional lysosome is present. Microvilli (arrows) are long and uniform in length and width. (X 16,600)
Figure 6. Thyroid follicular cells containing greatly increased rough endoplasmic reticulum (E) with tubular cisternae in a 7 day rat from a dam fed a diet with 500 ppm PCB. The Golgi apparatus (G) is small, apical vesicles (A) are reduced, and an occasional lysosome (L) is present. Microvilli (arrows) extend into the luminal colloid. (X 13,200)
Figure 7. Columnar follicular cells lining thyroid follicle of a 14-day-old rat pup whose dam was fed a diet containing 500 ppm PCB. Numerous lamellar arrays of rough endoplasmic reticulum (E) fill the cytoplasm. Mitochondria (M) are swollen and have disrupted cristae. The Golgi apparatus (G) is prominent but there is a reduction in apical vesicles (A). Microvilli are long and uniform in shape. (X 13,200)
Figure 8. Thyroid follicular cell with numerous lamellar arrays of rough endoplasmic reticulum (E) and colloid droplets (C) from a 21-day-old rat pup whose dam was fed 500 ppm PCB since conception. The Golgi apparatus (G) is small, apical vesicles (A) are reduced, and an occasional lysosome (L) is present. Microvilli are short but numerous. There are occasional club-shaped microvilli (arrow). (X 16,700)
Figure 9. Serum thyroxine (mean ± standard error) in neonatal rat pups exposed in utero and by the milk to PCB. The dams were fed diets with 0 (control), 50 or 500 ppm PCB since conception. There was a significant reduction in serum thyroxine in rats of all age groups exposed to PCB.
SERUM THYROXINE (μg/dl)

Fig 9
Figure 10. Serum Triiodothyronine (mean ± standard error) in neonatal rats exposed in utero and by the milk to PCB. There was a significant reduction in triiodothyronine levels in 7- and 14-day-old rat pups.
SERUM TRIIODOTHYRONINE (ng/dl)

Fig 10
Table 1 - Body Weight and Litter Size of Rat Pups Exposed In Utero and by the Milk to 50 and 500 ppm PCB.

<table>
<thead>
<tr>
<th>Group</th>
<th>Litter Size</th>
<th>Parturition</th>
<th>7 Days</th>
<th>14 days</th>
<th>21 days</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>8.9 ± .2 (12)</td>
<td>5.4 ± .1 (29)</td>
<td>9.6 ± .2 (27)</td>
<td>20.2 ± .2 (24)</td>
<td>33.5 ± .4 (28)</td>
</tr>
<tr>
<td>50 ppm PCB</td>
<td>8.3 ± .2 (12)</td>
<td>5.3 ± .4 (25)</td>
<td>9.5 ± .4 (26)</td>
<td>20.3 ± .2 (24)</td>
<td>31.3 ± .4* (25)</td>
</tr>
<tr>
<td>500 ppm PCB</td>
<td>7.7 ± .1* (11)</td>
<td>5.3 ± .2 (23)</td>
<td>9.5 ± .9 (16)</td>
<td>19.8 ± .2 (23)</td>
<td>29.0 ± .7* (23)</td>
</tr>
</tbody>
</table>

( ) = Number of litters or number of rat pups (for body weights).
* p = < .001
CHAPTER IV

BILIARY EXCRETION OF $^{125}$I-THYROXINE AND FINE STRUCTURAL ALTERATIONS IN THE THYROID GLANDS OF GUNN RATS FED POLYCHLORINATED BIPHENYLS (PCB)

INTRODUCTION

Polychlorinated biphenyls (PCB) are commonly used industrial compounds which have escaped into the environment and caused widespread contamination.\(^1\)-\(^4\) The disease-producing capability of these compounds has been well documented and includes alterations in reproduction, growth and development.\(^5\)-\(^10\) Some of these alterations may be related to the recent evidence that exposure to PCB causes a significant reduction of serum levels of thyroxine and triiodothyronine.\(^11\)-\(^15\) The lowering of thyroid hormone levels appears to be due to alterations in thyroid structure and thyroxine metabolism. In rats administered PCB there is an enlargement of the thyroid gland and thyroid follicular cells accumulate large numbers of colloid droplets and large abnormally shaped lysosomes, suggesting there is an interference in lysosome-
colloid droplet interaction. Ultrastructurally, colloid droplets do not appear to fuse with lysosomes, thus interfering with the release of thyroid hormones from the thyroglobulin molecule by acid hydrolases.

In addition to alterations in follicular cell structure, PCB is a potent inducer of hepatic microsomal enzymes. Exposure to PCB results in the induction of thyroxine UDP-glucuronyltransferase and increased conjugation and excretion of thyroxine-glucuronide in the bile.

The significance of PCB-induced lesions in thyroid follicular cells and their effects on the secretion of thyroid hormones are not known since the induction of thyroxine UDP-glucuronyltransferase and increased excretion of thyroxine-glucuronide may contribute to the lowering of serum thyroxine levels. In order to assess directly the effect of PCB on the thyroid gland the homozygous Gunn rat (which is deficient in UDP-glucuronyltransferase needed for the conjugation of thyroxine) and the heterozygous Gunn rat (which has this hepatic microsomal enzyme but in less than normal levels) were used as an animal model. The objectives of this investigation were: 1) to evaluate the ultrastructural changes in thyroid follicular cells in heterozygous and homozygous of rats fed 0 and 500 ppm PCB, 2) to compare these structural alterations with serum levels of thyroxine and triiodothyronine, and 3) to determine the biliary excretion rates of exogenous I-thyroxine in homozygous and heterozygous Gunn rats.
MATERIALS AND METHODS

Male 300-400 gm. heterozygous and homozygous Gunn rats were divided into two groups. One group of homozygous (6 rats) and one group of heterozygous (6 rats) Gunn rats were fed powdered Purina Rat Chow mixed with Aroclor 1254 (PCB) at a concentration of 500 parts per million (ppm) for 42 days. The PCB was mixed into the pulverized feed using corn oil as a vehicle. The other two groups (6 rats each) served as controls and were fed a similar diet for 42 days with 3% corn oil but without PCB. At the end of the experiment 1.0 ml of blood was drawn from the retroorbital sinus from each rat and serum collected for assay of thyroid hormones before the injection of $^{125}$I-thyroxine.

The biliary clearance rates of $^{125}$I-thyroxine ($^{125}$I-$T_4$) were determined by the method of Bastomsky. Each rat was injected intravenously with 20 $\mu$Ci $^{125}$I-$T_4$ in 0.2 ml 0.9 NaCl 18 hours before biliary cannulation. The rats were anesthetized with pentobarbital (60 mg/kg intraperitoneally supplemented by additional 40 mg/kg doses subcutaneously as needed to maintain surgical anesthesia). A celiotomy was performed and the common bile duct was cannulated with the shaft of a 25-gauge hypodermic needle affixed to a 14 cm length of polyethylene tubing with an internal diameter of 0.5 mm. Bile was collected into plastic counting tubes for 80 minutes divided into 20 minute periods. At the midpoint of each period, 0.25 ml blood was withdrawn from the jugular vein and added to tubes containing 10 $\mu$l of a heparin solution. The plasma was collected and the radioactivity of 50 $\mu$l aliquots of plasma and 100 $\mu$l aliquots of bile was measured a liquid scintillation spectrometer, (Packard
Tri-Carb #3375). The ratio of radioactivity was calculated in equal volumes (0.1 nl) of bile and plasma. The volume of bile obtained in each period was calculated from the radioactivity in the remainder of the sample. Clearance in mL./hr./kg. was calculated from formula: B/P X bile volume (ml.) X 3 X body weight (kg.).

After the collection of bile, rats from both groups were exsanguinated and perfused with 1% buffered glutaraldehyde. One thyroid lobe was collected for electron microscopy and minced into 1 mm³ pieces in cold 3% buffered glutaraldehyde. The opposite thyroid lobe was collected for histopathologic evaluation and stained with either hematoxylin and eosin, periodic acid-Schiff or both.

Thyroids for electron microscopic evaluation were fixed in ice cold 3% glutaraldehyde (E.M. Grade, Electron Microscopy Sciences, Box 251, Fort Washington, Pa. 19034) in 0.1 M sodium cacodylate buffer for 2 hours, washed twice in 0.1 M cacodylate buffer, and post-fixed in 1.33% osmium tetroxide in s-collidine buffer at pH 7.4 for 1 hour. Six tissue blocks for transmission electron microscopy from each rat were dehydrated through ascending concentrations of ethyl alcohol, transferred to propylene oxide, and embedded in Epon 812 (Shell Oil Co., New York, N.Y.). One micron, thick-sections were cut from each block and stained with toluidine blue for light microscopic evaluation and selection of the most appropriate area of the block for sectioning. Thin sections for electron microscopic evaluation were cut at 600 to 800 Å on a Reichert Om-U2 ultramicrotome and mounted on 300 mesh copper grids. The sections from each rat were stained with uranyl acetate and lead citrate, and examined with a Philips 200 or 300 electron microscope.
Serum thyroxine and triiodothyronine were determined for each rat at the termination of the experiment. Serum collected was frozen (-20°C) until completion of the experiment when all samples were assayed at the same time. Serum thyroxine and triiodothyronine were determined by radioimmunoassay, (V.K. GanJam, Unpublished data).

RESULTS

Light Microscopy

Homozygous and heterozygous Gunn rats - 0 ppm PCB. The thyroid glands of both genotypes were composed of both large and small follicles lined by a single layer of cuboidal to low columnar follicular cells (Fig. 1). The cytoplasm was weakly PAS-positive and homogenous. The nucleus was situated between the central and basal parts of the cell. The colloid was strongly PAS-positive and homogenous. Thyroid C- cells were present in small clumps of three to four cells between follicles or as individual cells within follicular walls. Interfollicular capillaries were separated from follicles by a thin PAS-positive basement membrane.

Homozygous and heterozygous Gunn rats - 500 ppm PCB. The thyroid glands in both genotypes were similar histologically and will be described together. They were composed of follicles lined by low to tall columnar follicular cells. The follicular lumen was reduced compared to controls due to the hypertrophy of follicular cells. The cytoplasm of follicular cells was more strongly PAS-positive and granular than in rats receiving no PCB, and contained
basally located nuclei (Fig. 2). The colloid did not react as strongly with PAS as in rats receiving 0 ppm PCB, was less uniformly homogeneous, and contained numerous vacuoles immediately adjacent to the apical surface of follicular cells.

**Electron Microscopy**

**Homozygous and heterozygous Gunn rats - 0 ppm PCB.** Follicular cells in both genotypes were cuboidal to low columnar and situated on a thin basement membrane (Fig. 3 and 4). The cytoplasm contained both lamellar arrays and individual profiles of rough endoplasmic reticulum with a finely granular electron-dense material. The Golgi apparatus was located in a perinuclear position, was moderate in size, and consisted of smooth membranes associated with small electron-dense granules. Mitochondria with transverse cristae were dispersed throughout the cytoplasm of follicular cells. The nucleus was basally to centrally positioned and had peripherally clumped chromatin. A narrow layer of electron-dense apical vesicles was present immediately beneath the microvilli along the luminal surface of follicular cells. Numerous finger-like microvilli of uniform length and width extended into the luminal colloid. A few round electron-dense lysosomal bodies and an occasional membrane-bound colloid droplet were present in the cytoplasm, particularly in the apical portion of follicular cells (Fig. 4). Many interfollicular capillaries lined by endothelial cells with fenestrae were present between thyroid follicles.

**Heterozygous Gunn rat - 500 ppm PCB.** Follicular cells of heterozygous rats ingesting 500 ppm PCB were more columnar than in control rats. There
was extensive dilatation of the rough endoplasmic reticulum (Fig. 5). The cisternae were irregularly enlarged and contained a finely granular electron-dense material but ribosomes remained attached to the membranes. Only an occasional round colloid droplet was present in the cytoplasm between the apical surface and nucleus. Round, uniformly electron-dense lysosomes were present in similar number as heterozygous Gunn rats receiving no PCB. There was little evidence of colloid droplet-lysosome interaction necessary for the secretion of thyroid hormones. The Golgi apparatus was of a similar size as in follicular cells of rats receiving no PCB. Only occasional mitochondria were vacuolated with disruption of cristae. The nucleus was basally located and had peripherally clumped chromatin. Apical vesicles immediately beneath the microvilli were variable being increased in some follicular cells but present in normal or reduced number in other cells. Microvilli were similar to those in rats which did not ingest PCB. They were numerous and present as uniform finger-like projections extending into the luminal colloid.

**Homozygous Gunn rat - 500 ppm PCB.** Tall columnar follicular cells lined thyroid follicles of homozygous Gunn rats fed 500 ppm PCB (Fig. 6 and 7). Large colloid droplets were markedly increased in number compared to heterozygous Gunn rats, round or irregularly shaped, and filled much of the apical cytoplasm of follicular cells (Fig. 7). Lysosomal bodies also were present in follicular cells but were less numerous than in heterozygous rats (Fig. 8). There was little evidence of colloid droplet-lysosome interaction necessary for the secretion of thyroid hormones. The cytoplasm contained rough endoplasmic reticulum that was present as dilated profiles or lamellar arrays with narrow
Many mitochondria were vacuolated with extensive disruption of cristae and often were displaced peripherally in follicular cells by the striking accumulation of colloid droplets (Fig. 6). The nucleus was basally located in follicular cells. Electron-dense apical vesicles were present in variable numbers in the narrow zone beneath microvilli. The Golgi apparatus was diminished in size and apical vesicles were greatly reduced in number in follicular cells filled with colloid droplets. Microvilli were shortened in follicular cells distended with colloid droplets compared to rats receiving no PCB.

**Serum Thyroxine and Triiodothyronine.** The serum thyroxine (T₄) and triiodothyronine (T₃) concentrations were determined by radioimmunoassay and were compared statistically using the Student-t test (Table 1). Thyroxine levels were significantly (p<0.001) reduced in both heterozygous (0.50±0.02 μg/dl) and homozygous (0.60±0.02 μg/dl) Gunn rats fed 500 ppm PCB daily for 6 weeks (Table 1). There was no significant difference in the reduction in T₄ in heterozygous compared to homozygous Gunn rats fed 500 ppm PCB. Triiodothyronine levels were significantly (p<0.001) reduced in heterozygous (55.90±1.48 ng/dl) Gunn rats fed 500 ppm but there was no significant change in homozygous Gunn rats fed the same diet.

**Clearance of ¹²⁵I-Thyroxine.** The bile:plasma (B:P) was increased more than five-fold to 7.82±0.34 in heterozygous Gunn rats ingesting 500 ppm PCB for 6 weeks (Fig. 9). Bile flow was unaltered by PCB. The biliary clearance of plasma ¹²⁵I, reflecting the elevated B:P, also was significantly (p<0.001)
increased more than 5-fold from $3.70 \pm 0.30$ to $19.24 \pm 1.63$ ml./hr./kg (Fig. 10). The B:P in homozygous Gunn rats ingesting no PCB ($0.89 \pm 0.05$) was about one-half that of heterozygous Gunn rats administered 0 ppm PCB (Fig. 9). Bile flow was similar to that of heterozygous rats; therefore, biliary clearance of $^{125}\text{I}$ was markedly less ($2.20 \pm 0.17$ ml./hr./kg.) in homozygous Gunn rats (Fig. 10). PCB significantly raised the B:P two-fold to $2.30 \pm 0.19$ ml./hr./kg. in homozygous Gunn rats and increased the biliary clearance of plasma $^{125}\text{I}$ to $5.29 \pm 0.52$ ml./hr./kg.

**DISCUSSION**

The chronic ingestion of PCB by the homozygous Gunn rat, which is deficient in thyroxine UDP-glucuronyltransferase, resulted in a significant decrease in serum levels of thyroxine. This reduction in serum thyroxine was interpreted to be due to an interference in the secretion of thyroid hormones from the gland since there was only a slight increase in the biliary excretion of $^{125}\text{I}$-thyroxine after chronic PCB exposure. Thyroid follicular cells in homozygous Gunn rats had striking accumulations of colloid droplets with little evidence of interaction with lysosomes, vacuolization of mitochondria, and atrophy of the Golgi apparatus, and a reduction in the number of apical vesicles. The biliary excretion of $^{125}\text{I}-\text{T}_4$ was elevated only to levels observed in the heterozygous Gunn rat. The small increase in $^{125}\text{I}-\text{T}_4$ clearance may have contributed to the reduction in serum thyroxine but did not appear to be a major factor since the bile to plasma ratio was not significantly different from heterozygous Gunn rats receiving no PCB. Bile flow was unchanged and, therefore, should not have contributed to the decrease in the serum thyroxine.
Serum thyroxine also was significantly reduced in the heterozygous Gunn rat by the chronic administration of PCB. The heterozygous Gunn rat has a capability of glucuronide formation in vivo and in vitro that is intermediate between that observed in normal and homozygous littermates.\textsuperscript{21,22} The biliary clearance of $^{125}$I-T$_4$ was in close agreement with the values reported by Bastomsky\textsuperscript{20} in the normal male Sprague-Dawley rat. The chronic ingestion of PCB produced more than a five-fold increase in biliary $^{125}$I-T$_4$ clearance in heterozygous Gunn rats. This increase was similar to the degree of induction of UDP glucuronyltransferase by PCB in the normal rat.\textsuperscript{12} The decrease in serum T$_4$ in this group was approximately the same as in homozygous Gunn rats. These findings suggest that in the absence of alterations in the synthesis and secretion of thyroxine, the production of thyroid hormones should keep up with the increased rate of excretion stimulated by PCB. The ultrastructural lesions in thyroid follicular cells of heterozygous Gunn rats were different from those in the homozygous rats. The extensive dilatation of rough endoplasmic reticulum probably reflected a long-term stimulation of follicular cells by an increased thyrotropin secretion in response to the lowered circulating levels of thyroid hormones. Colloid droplets were infrequent but there were numerous lysosomes in heterozygous Gunn rats.

The variation in ultrastructural lesions observed in thyroid follicular cells between normal (Osborne-Mendel and Holtzman rats)\textsuperscript{11,23} and heterozygous or homozygous Gunn rats may be the result of differences in the ability to metabolize PCB. Considerable evidence suggests that phenolic and biphenolic compounds such as PCB are detoxified and excreted by hydroxylation and
conjugation with glucuronic acid.\textsuperscript{24,25} Homozygous Gunn rats with deficient UDP-glucuronyltransferase and a reduced ability to conjugate PCB or products from the detoxification are exposed to different metabolic forms of PCB than those present in the heterozygous Gunn rat or normal rat.

\textbf{SUMMARY}

This investigation was designed to assess the direct effects of PCB on thyroid structure and function in the homozygous Gunn rat (which is deficient in thyroxine UDP-glucuronyltransferase) compared with the heterozygous Gunn rat which has this hepatic microsomal enzyme. The feeding of 500 ppm PCB for 6 weeks produced ultrastructural alterations in thyroid glands and a significant reduction in serum thyroxine in both heterozygous and homozygous Gunn rats, and a decrease in serum triiodothyronine in heterozygous Gunn rats. In homozygous Gunn rats there was hypertrophy of follicular cells containing vacuolated mitochondria, abnormal accumulations of colloid droplets with little evidence of interaction with lysosomes, shortening of microvilli, and lamellar arrays of rough endoplasmic reticulum. The ultrastructural changes in heterozygous Gunn rats included hypertrophy of follicular cells with extensive dilatation of rough endoplasmic reticulum, only occasional colloid droplets, and limited lysosome-colloid droplet interaction. There was a 5-fold increase in the bile to plasma (B:P) ratio and biliary clearance of $^{125}\text{I}$-thyroxine in heterozygous Gunn rats but only a 2-fold increase of these parameters in homozygous Gunn rats. In spite of the difference in B:P and biliary clearance of $^{125}\text{I}$-thyroxine the reduction in serum thyroid hormone levels was similar
between heterozygous and homozygous Gunn rats. The lowering of thyroxine levels in the heterozygous Gunn rat appeared to be a combined effect of increased biliary excretion and a direct effect on thyroid follicular cells. The ultrastructural alterations in follicular cells, suggesting an interference in secretion of thyroid hormones, appeared to be primarily responsible for the reduction in serum levels of thyroxine and triiodothyronine following ingestion of PCB in homozygous Gunn rats.
Table 1. Thyroxine ($T_4$) and Triiodothyronine ($T_3$) Levels in Gunn Rats Exposed to PCB

<table>
<thead>
<tr>
<th>Group</th>
<th>$T_4$ (µg/dl)</th>
<th>$T_3$ (ng/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous - Control</td>
<td>2.39 ± 0.22</td>
<td>68.51 ± 2.77</td>
</tr>
<tr>
<td>Heterozygous - 500 ppm PCB</td>
<td>0.50 ± 0.02*</td>
<td>55.90 ± 1.48*</td>
</tr>
<tr>
<td>Homozygous - Control</td>
<td>2.02 ± 0.18</td>
<td>56.72 ± 3.31</td>
</tr>
<tr>
<td>Homozygous - 500 ppm PCB</td>
<td>0.60 ± 0.02*</td>
<td>56.08 ± 3.25 NS</td>
</tr>
</tbody>
</table>

$N = 6$ Mean ± standard error of mean

* $P<0.001$, NS = Not Significant
Figure 1. Thyroid follicles lined by tall cuboidal to low columnar epithelium in a heterozygous Gunn rat receiving 0 ppm PCB. The cytoplasm of follicular cells stained weakly PAS-positive and there were few vacuoles in the colloid near the apical surface of follicular cells. Periodic acid-Schiff; X 315.
Figure 2. Irregularly shaped thyroid follicles lined by columnar epithelial cells from a homozygous Gunn rat fed 500 ppm PCB for 6 weeks. The cytoplasm of follicular cells was strongly PAS-positive and granular, and the colloid had many vacuoles (arrows). Periodic acid-Schiff; X 315.
Figure 3. Thyroid follicular cell in a heterozygous Gunn rat fed 0 ppm PCB for 6 weeks with lamellar arrays of rough endoplasmic reticulum (E), a Golgi apparatus (G) with associated granules, and scattered lysosomes (L) and mitochondria (M). Numerous long microvilli (arrows) extend into the follicular lumen. X 13,800.
Figure 4. Thyroid follicular cells from a homozygous Gunn rat fed 0 ppm PCB for 6 weeks. The cytoplasm contained scattered profiles of rough endoplasmic reticulum (E), a Golgi apparatus (G) with associated granules, mitochondria (M), and scattered round lysosomes. Microvilli (arrows) of uniform length and width extend as finger-like projections into the colloid. X 10,600.
Figure 5. Extensive dilatation of rough endoplasmic reticulum (E) in follicular cells of a heterozygous Gunn rat fed 500 ppm PCB for 6 weeks. A small Golgi apparatus (G) with associated granules and scattered mitochondria (M) are present in the cytoplasm. Microvilli extend as uniform finger-like projections from the apical surface of follicular cells (arrows) into the luminal colloid. Apical vesicles are reduced immediately beneath microvilli. X 12,300.
Figure 6. Hypertrophied follicular cell in thyroid gland from homozygous Gunn rat fed 500 ppm PCB for 6 weeks. The cytoplasm contains numerous colloid droplets (C), a small Golgi apparatus (G), vacuolated mitochondria, and extensive profiles of rough endoplasmic reticulum (E). Microvilli were short and blunt compared to controls. X 14,500.
Figure 7. Hypertrophied thyroid follicular cells in a homozygous Gunn rat fed 500 ppm PCB for 6 weeks. The apical cytoplasm is filled with many large, irregular colloid droplets (C). Mitochondria (M) are vacuolated and there are reduced numbers of apical vesicles (A). Microvilli are short and blunt. X 14,000.
Figure 8. Numerous, irregular, membrane-bound colloid droplets (C) in the cytoplasm of a thyroid follicular cell from a homozygous Gunn rat fed 500 ppm PCB for 6 weeks. Lysosomes (L) contained granular or membranous debris (arrows) and mitochondria (M) were vacuolated with disrupted cristae. X 23,600.
Figure 9. Bile:plasma $^{125}$I-thyroxine in heterozygous and homozygous Gunn rats fed 0 or 500 ppm PCB. Rats were injected with $^{125}$I-T$_4$ 18 hours previously.
Fig 9

Heterozygous Gunn Rat (500ppm PCB)

Homozygous Gunn Rat

Heterozygous Gunn Rat

Homozygous Gunn Rat
Figure 10. Biliary clearance of $^{125}$I-thyroxine in heterozygous and homozygous Gunn rats fed 0 or 500 ppm PCB. Rats were injected with $^{125}$I-thyroxine 18 hours previously.
Heterozygous Gunn Rat (500ppm PCB).

Fig 10
Chapter I


Chapter II


Chapter III


23. Keplinger ML: Toxicological studies with polychlorinated biphenyls. PCB Conference, Quail Roost Conference Center, Rougemont, NC. Dec. 1971


Chapter IV


