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SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY ON PATHOLOGICAL EFFECTS OF PCBs (AROCLOR 1254) IN THE INTESTINAL MUCOSA OF CHICKENS

presented by

SHABAN RAHIMI

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in **Animal Science**

Major professor

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SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY ON PATHOLOGICAL EFFECTS OF PCBs (AROCLOR 1254) IN THE INTESTINAL MUCOSA OF CHICKENS VOLUME I

by

Shaban Rahimi

A DISSERTATION

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ABSTRACT

SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY ON PATHOLOGICAL EFFECTS OF PCBs (AROCLOR® 1254) IN THE INTESTINAL MUCOSA OF CHICKENS

by

Shaban Rahimi

The toxicopathological effects of Aroclor 1254 and the role of the intestine in its elimination were studied in immature egg-type chickens. A diet containing 10 ppm PCBs was fed for 14 days followed by 7 days of non-contaminated diet (days 0 to 7 of withdrawal). Approximately 4000 µg of PCBs were consumed by each chicken over the 14 days. The average body burden of PCBs in the chickens was 6.96 µg/g body weight at day 0 of withdrawal. Without any treatment, carcass PCB's declined by only 1.4% by day 5 of withdrawal. Treatments such as 5% mineral oil (MO) ad libitum, or 50% feed restriction (FR), or a combination of 50% feed restriction plus 10% MO, caused reductions of the PCBs by 7.5%, 24.5%, and 38.4%, respectively by 5 days of withdrawal. The intestine, particularly the ileum was the apparent site of increased transfer of PCBs from the blood into the luminal contents in MO treated birds, thus enhancing elimination.

Tissue samples of the jejunum and large intestine were taken for electron microscopic examination at days 0, 4, and 7 of withdrawal. In scanning electron microscopic (SEM) studies, blocks of epithelial cells were separated from the lamina propria of the jejunum. In addition, a loss of

morphology and degeneration of the villar structure of the large intestine were predominant lesions in PCB-treated chickens. In transmission electron microscopic (TEM) examinations, the lesions associated with PCB treatment were mild to moderate multifocal disruption and effacement of microvilli, dilation of endoplasmic reticulum, and vacuolization of the cristae mitochondriales leading to swelling and rupture of the mitochondria. Aroclor 1254 appears to be readily absorbed by the villal epithelial cells and toxic to brush border and energy producing systems involving mitochondria and endoplasmic reticulum. These adverse changes in the intestine are indicated to account for the possible "wasting" syndrome observed from higher PCB's concentration.

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[1] Aroclor® 1254 - Trade name for PCB containing 54% chlorine, Monsanto Chemical Company, St. Louis, Missouri.

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INTRODUCTION

Elimination of foreign compounds (xenobiotics) and their metabolites from the body is a key factor in the ultimate physiological effect or toxicity produced by these chemicals. The major routes for excretion of these substances are the renal, respiratory, and alimentary pathways. Of the three, the alimentary route has assumed greater importance. The presence of xenobiotics and their metabolic by-products within the gastrointestinal lumen arise from the following sources: (a) unabsorbed ingested compounds; (b) compounds previously absorbed by the gut or by other organs and secreted through the bile either intact, conjugated, or altered; (c) compounds that are actively or passively secreted in the saliva, in the gastric or intestinal mucosal secretory fluids, or in pancreatic secretions; (d) compounds that enter through gaseous exchange in the gut lumen; and (e) respiratory tract secretions that have been swallowed (Klaassen, 1986). Consequently, the character and quantity of those products that have actually been secreted into the gut lumen are influenced by many factors, including the quantity absorbed, the rate of biliary flow, and the level of hepatic activity in metabolizing the chemicals to an excretable form. Further, the physicochemical properties of the xenobiotics, their possible affinity for the tissues of the alimentary canal and its glands, and the metabolic functions of the respective tissues are factors contributing to excretion of the xenobiotic from the GI tract. To transfer a xenobiotic through the intestinal wall, the

lipophilic properties of the compound are very important. Active excretion requires specific metabolic processes in the mucosal, glandular, or ductal cells. There have been no studies that have firmly demonstrated facilitated diffusion as the mechanism by which any foreign compound is transferred out of or into the gastrointestinal tract (Levine and Pelikan, 1964).

Alimentary excretion may take place with an unaltered form of the agent or after metabolic biotransformation. The excreted compounds may also be reabsorbed in the aboral regions; this is especially the case when the compound has been excreted into the contents of the alimentary canal without biotransformation. Also, the metabolites may be taken up anew if microflora in the gastrointestinal tract hydrolyze conjugates of the excreted products.

Since this external route is the major pathway of excretion in some instances, it is important to study the alimentary excretion of ingested or parenterally absorbed compounds. Further, the toxicity or pharmacophysiological effects of the substance may be drastically altered by the metabolism of mucosal and/or hepatic systems. If the toxicity of the metabolite is enhanced, and if the agent is subsequently excreted through biliary secretion, severe toxicity may result after reabsorption.

This research emphasize the role of enhanced intestinal excretion as a means of detoxication to reduce the hazards

of environmental agents and the nutritional factors that influence its mode of action first in poultry and subsequently in other farm animals. The research also attempted to indicate which part of the intestine plays the greatest role in removal of the xenobiotics. In addition, the ultrastructural changes of the intestinal mucosa in the chicken due to Aroclor 1254 (PCBs) were studied.

LITERATURE REVIEW

I. INTESTINAL EXCRETION

The gastrointestinal tract may be divided into three major sections: the stomach, the small intestine, and the large intestine. Of these sections, the small intestine, with its unique morphological features of the mucosal surface, has the greatest available surface area because of existence of many folds and villi from which absorption can take place. Since there are no villi present in the mammalian stomach and large intestine, the available surface area in these two regions is smaller than that of the small intestine. The intestinal wall is also capable of secretion. The daily volume of intestinal secretions in humans is about 3 liters. The deep duodenal glands (Brunner's glands) produce an alkaline mucous secretion and in theory, acidic foreign compounds could be enriched in duodenal juice by passive transfer. The amount of fluid is, however, too small to have any significance. Active transport has not been studied, nor does it seem to be a very probable occurance. However, investigations have been made on the possible glandular metabolism of foreign compounds, but results have indicated that this tissue is practically devoid of drug-metabolizing enzyme activity (Toivonen and Hartiala, 1973).

A number of drugs are passively excreted from the blood through the wall and into the lumen of the gastrointestinal

tract. The steady-state concentration of the drugs in the lumen of the gastrointestinal tract and in the blood depend on the pH-gradient and the pK_a values of the drug. The intestinal blood barrier is essentially lipoid in nature and there is a correlation between the rate of transfer of a number of drugs and the lipid solubility of their nonionized form. Since the luminal contents are slightly acidic with respect to blood plasma, the concentration of, for example, aniline (weak base) is slightly higher in the gut lumen. Experimental results suggest that the mucosal surface has a pH of about 5.3, i.e. lower than the intestinal contents, and this has an effect on the partition, according to Hogben et al. (1958). The degree of ionization of an organic electrolite is an important factor in mucosal transfer of the compound through the intestinal wall (Hogben et al., 1958). The passage of salicylic acid and aniline through the wall of the small intestine was similar at different levels in spite of anatomical and physiological differences.

The diffusion of antipyrine into the gut is much more rapid than that of N-acetyl-4-aminoantipyrine. Both compounds can be used to measure body water and intraluminal water of the gastrointestinal tract of living cattle (Reid et al., 1957).

The fate of labeled diphenylhydantoin has been carefully studied (Noach et al., 1958). After intravenous injection, in high concentrations, it was found in liver, salivary glands,

and kidneys of rats. Free diphenylhydantoin appears rapidly in the gastrointestinal tract in concentrations higher than in urine. There is a considerable amount of free diphenylhydantoin in the gastrointestinal tract before any metabolites are found there. The free drug can also be found in the intestines of rats with ligated and cannulated bile ducts and in rats with ligated gastric cardia. This indicates that the drug can also pass through the gastrointestinal wall. The concentration of free diphenylhydantoin in bile is low. Noach and co-workers (1958) discussed the similarities of salivary glands and the exocrine pancreas, and suggested that pancreatic secretion might be responsible for the free gastrointestinal diphenylhydantoin. They also suggested that the drug might be secreted by the intestinal glands. The free diphenylhydantoin was absorbed from the gastrointestinal tract, since no free drug was excreted in the feces. Diphenylhydantoin was metabolized in the liver. Apparently the metabolites were secreted first into the gastrointestinal canal and were reabsorbed later, since in 48 h almost all of the radioactivity of the labeled compound was found in urine.

About 6% of parenterally administered, labeled digoxin enters the alimentary tract in dogs with a biliary fistula. Thus, some extrabiliary gastrointestinal excretion of the drug appears to take place (Harrison et al., 1966).

The Vermiform appendix of rabbits secretes electrolytes spontaneously at a relatively rapid rate, and studies by

Blackwood et al. (1973) suggest that secretion of some foreign compounds may take place actively. The antibiotic rifamycine B-diethylamine has been found in measurable amounts in the human appendix wall after intramuscular injections. Because the drug is excreted in bile, it is not known whether it is, in this case, transported to the appendix through the blood stream or through the intestinal contents (Acocella, 1967).

Foreign compounds can also pass through the colonic mucosa from blood to the lumen. In the steady state, the concentration of quinine is about seven times higher in the colonic lumen than in the plasma. Although the colonic perfusate has a pH of about 6.8, the effective pH in the mucosal surface must be lower to explain the difference between the expected concentration ratio (3.7) and that obtained (7.2). The pH in the colon is approximately 6.5, which is higher than the usual pH in the small intestine (pH, 5.3). Lipid solubility and the degree of ionization are the most important factors determining the rate of absorption of foreign compounds through the colonic wall, as in other parts of the gastrointestinal tract (Schanker, 1959).

There is some evidence to suggest that the quaternary ammonium compounds, benzomethamine and oxyphenonium, may be absorbed into the intestine by an active transport process and also may pass out of the intestine by some process other than passive diffusion (Levine, 1959; Levine et al., 1955; Levine and Clark, 1957).

FACTORS INFLUENCING INTESTINAL ABSORPTION AND EXCRETION

Transferring of the dissolved compounds through the intestinal wall depends on a wide variety of factors:

A. Interaction within the gastrointestinal lumen:

1) Excretion from respiratory tract to GI lumen. The respiratory tract excretes from the body gaseous waste products along with a significant amount of other compounds that are disposed of in the expired air. Furthermore, bronchi and the trachea are capable of secreting fluid. The mucociliary, bronchotracheal escalator takes the fluid and dust particles into the pharynx, whence they are swallowed or are expectorated in sputum. Thus, some of the foreign material that comes to the gastrointestinal tract enters the body primarly by way of inhaled air (Green, 1973).

2) Transfer of foreign compounds into gastric contents. Gastric juice is not usually considered a route of elimination of foreign compounds. However, a number of reports show that certain compounds are, in fact, excreted extensively into the gastric contents. The daily volume of gastric secretion is 2-3 liters in humans (Diem, 1970). The excreted compounds may, however, be reabsorbed in the gut after having passed the pylorus. A study by Short and his collaborators (1957) has shown that drugs cross the gastric epithelium in their nonionized form and that the ionized form penetrates very slowly, if at all. At the steady state, basic drugs like aniline, levorphan, aminopyrine, and quinine appeared in gastric juice in concentrations up to 40 times that of

plasma. In contrast, highly acidic drugs like salicylic acid, probenecid, and phenylbutazone appeared in gastric juice in very low concentrations compared to those of plasma. At the steady state, the concentrations of the nonionized drugs in plasma and gastric juice were equal.

The gastric secretion of antimicrobial agents may change the physiological conditions of the stomach. Sulfaethoxyphridazine, sulfadimidine, and sulfanilamide are excreted into the rumen of cows after intravenous administration. The drugs diminish microbial activity there and 2-3 days are needed to recovery (Egetoft and Rasmussen, 1963).

3) Pancreatic excretion. Scratcherd and Case (1973) have reviewed the mechanisms of secretion of electrolytes by the pancreas and Harper (1972) has reviewed the control of pancreatic secretion. A special characteristic of pancreatic juice, in addition to the presence of digestive enzymes, is its high bicarbonate concentration.

The amount of pancreatic secretion in humans is about 700 ml per day, so small amounts of foreign compounds can be excreted in this way into the small intestine. The excreted compounds may be reabsorbed later in the gut. Ampicillin has been shown to be secreted into the pancreatic juice, where the concentration is only about 30% of that in serum. Secretin administration does not affect its concentration in the pancreatic juice (Mandiola et al., 1972). Noach and

co-workers (1958) suggested that pancreatic secretion might be responsible for the appearance of free diphenylhydantoin in the intestine.

4) Biliary excretion. The molecular weight of the foreign compound affects its biliary excretion. Compounds with high molecular weight tend to be excreted in bile, and compounds with low molecular weight tend to be excreted in urine (Klaassen, 1986). The presence of a strongly polar group in a molecule appears to be a requirement for extensive biliary excretion (Smith, 1973). Since a small pH gradient exists between the plasma and bile, passively diffusing weak acids may have slightly higher concentrations in bile than in plasma, while weak bases have lower concentrations in bile than in plasma (Schanker, 1968). Despopoulos and Sonnenberg (1967) demonstrated that the compounds that were actively transported by the kidney were also highly concentrated in hepatic bile. Several compounds excreted in bile are reabsorbed from the gut.

5) Gut bacteria. Microorganisms, naturally present in the gastrointestinal tract, are more numerous in the aboral parts, especially in the large intestine, but there are no sterile regions in the gut. The microflora is composed of various species (Dubos et al., 1965; Gorbach et al., 1967). Gut bacteria can contribute to the metabolism of foreign compounds in several ways. Scheline (1968, 1973) reported that the hydrolysis of conjugates (especially that of glucuronides) by the gut microflora is of great practical

importance in the alimentary excretion of metabolites of foreign compounds. The intestinal microflora affect the morphology (Mickelsen, 1962) and the metabolic activity of the intestinal mucosa, including the metabolism of foreign compounds (Hietanen and Hanninen, 1971).

Intestinal microflora are responsible for the partial metabolism of insecticides in mammals. A number of intestinal bacteria are capable of the dehalogenation of DDT. An aromatic dechlorination does not, however, take place (Barker et al., 1965; Mendel and Walton, 1966; Stenersen, 1965; Wedemeyer, 1966).

6) Chelating agents. The excretion of foreign compounds via the gastrointestinal tract can be affected by formation of complexes. The rate of absorption of a drug complex is slower than the rate of absorption of a free drug (Reuning and Levy, 1969; Sugimoto, 1968). Strongly acidic compounds such as phenolsulfonphthalein (Levine and Pelikan, 1964) and heparinoid (Tidball and Lipman, 1962) have been found to be significantly better absorbed in the presence of EDTA than in its absence, but the calcium or magnesium salt of EDTA has not produced an increased absorption of heparinoid. The effect of EDTA in increasing absorption appears to be nonspecific and the degree or kind of ionization of the solute molecule does not determine whether its absorption can be increased by EDTA (Levine and Pelikan, 1964). EDTA may also

have an effect on the transfer of highly ionized compounds from the plasma to the intestinal lumen.

B. Mucosal Surface

Gastrointestinal mucous membranes are capable of detoxifying a number of foreign compounds (Hartiala, 1954; Shirai and Ohkubo, 1954; Wattenberg and Leong, 1962; Zini, 1952). This, in part, involves glucuronide synthesis in the gut, which is much more rapid in the proximal part (particularly the duodenum) than in the aboral part (Hanninen et al., 1968). Owing to the large mucosal surface area of the intestines, reabsorption of many compounds takes place at this site.

C. Blood Flow

After passing the intestinal epithelial cells, a substance is carried away by blood and lymph. Ther and Winne (1971) reported that the blood flow may influence the intestinal absorption in several ways. First, a "mechanical" or draining effect of the blood where a diminished blood flow causes an increase of the interstitial concentration and a decrease of the concentration gradient followed by a lowered absorption rate. This mechanical factor is important for substances passing the epithelium passively by diffusion. Second, a "biochemical" effect for substances passing the mucosal barrier by active mechanism. A sufficient oxygen supply is necessary to maintain this transport. If the blood flow falls below a critical limit, the active transport will stop. Third, "secondary morphological alterations" where a sufficiently

long period with decreased blood flow, for instance, 10 minutes ischemia or 20 minutes subnormal blood flow, will cause changes in the structure of the intestinal epithelium followed by a decrease of absorption.

D. Lymph Flow

The lymphatic system plays a minor role in absorption of the drugs from the intestinal lumen. Since villus capillary blood flow is about 500 times greater than lymph flow, the absorption of most drugs is via blood (Houston and Wood, 1980).

Efficient absorption of DDT through the gastrointestinal lymphatic system was reported by a number of investigators (Sieber and Farbo, 1974; Pocock and Vost, 1974). However, attempts to enhance the lymphatic absorption of lipophilic compounds by their incorporation into oil-in-water emulsions have met with little success (Noguchi et al., 1975, 1977a,b).

E. Electric-Potential Difference

The electric potential difference in the human small intestine is about 5 mv. This increases during glucose absorption and is reduced by low concentrations of sodium in the intestinal lumen. Potential differences of 40 to 60 mv were measured in the colon in various animals and man. The electric potential difference in the small intestine modifies the transfer of sodium, sugar, and amino acids (Geal and Summerskill, 1969).

F. Ionization

Hogben et al. (1958) reported the existence of an acid pH microclimate at the surface of the intestine, both in

the rat and humans. Excretion of acidic compounds is favored in the colon because of the higher pH as compared to that in the duodenum and jejunum (Robinson, 1976; Davenport, 1982). The results of experiments (Hogben et al., 1958) support the hypothesis that intestinal mucosa preferentially allows the absorption of the unionized form of a drug.

G. Lipid Solubility

The gastrointestinal epithelium has lipid-like barriers to the passage of many foreign organic compounds. Therefore, lipid and nonionized compounds pass through these lipophilic areas of the intestinal boundary rather than through small aqueous channels (Schanker and Lewis, 1961).

Hogben et al. (1958) reported that the lipid-soluble drugs are absorbed more readily than lipid-insoluble drugs. Intestinal excretion of highly lipid-soluble and nonmetabolized (or very slowly metabolized) compounds is more than renal excretion (due to extensive passive tubular reabsorption in the kidneys). Intestinal excretion of such compounds will increase with decreasing of plasma protein binding of them.

In summary, the lipophilic, nonpolar, nonionized, and small particles of xenobiotics have greater absorption from the intestinal mucosa than polar, ionized, and large molecules of xenobiotics (Klaassen, 1986).

H. Transfer System

Foreign compounds are absorbed by passive diffusion through the mucosa membrane. Also existing are active secretory processes, capable of transporting the drug from

the blood into the intestinal lumen against a concentration gradient (Houston and Wood, 1980).

I. Structure-Activity Consideration

There is a difference between the structure-activity relationship for intestinal excretion and biliary excretion. For biliary excretion to occur, physico-chemical characteristics of the compound such as weight, polarity, structural features, and biological factors such as species, sex, and genetics, as well as protein binding of the xenobiotic, renal excretion, and metabolism are considered (Smith, 1973). For intestinal absorption and excretion, lipid solubility may be a more important determinant. Therefore, in elimination of lipid soluble compounds, especially those with a long half-life, intestinal excretion should be considered as a significant route. Muhlebach and Bickel (1981) studied the pharmacokinetics of the unmetabolizable lipophilic compound 2,4,5,2',4',5'-hexachloro-biphenyl (6-CB) in rats. They found that about 75% of 6-CB given to the rat was irreversibly stored in adipose tissue. The urinary excretion (0.8% dose) was nearly complete after one week, but fecal elimination of 6-CB continued for 40 weeks and amounted to 16% of the dose.

The intestinal excretion of certain metabolites that are more lipid soluble than the parent compound would be quantitatively more significant than for the precursor. The intestinal excretion of DDT was increased by giving DDT-hydroxylated metabolite, DDOH, as the substrate to the

rat which resulted in DDT fatty acid conjugate formation (Leighty et al., 1980). Henderson et al. (1966) reported that the half-life of xenobiotics varied according to their structural configurations.

J. Intestinal Motility

Klaassen (1986) described that decreased intestinal motility tends to increase the overall rate of absorption and increased intestinal movement tends to decrease the overall rate.

K. Miscellaneous Factors

There are some other factors that affect the extent of intestinal excretion such as: (a) food, (b) dose of xenobiotic, (c) physicochemical properties of xenobiotics, (d) plasma level of xenobiotic, (e) gastrointestinal enzymes, (f) mucous, (g) quantity of feces, (h) pH of feces, and (i) solubility of xenobiotic in fecal water and its association with fecal solids. Changes in any other pathway of elimination will be reflected in the extent of intestinal excretion. For example, in the case of liver disease, in which biliary excretion is reduced, intestinal excretion of xenobiotics would be increased as an alternate excretory route.

TRANSPORT OF XENOBIOTICS WITH INTESTINAL EXCRETION

In humans, approximately 8.2 liters of fluids are secreted daily into the gastrointestinal tract. This includes 1.5 liters of saliva, 0.5 liters of bile, 2.5 liters of gastric juice, 0.7 liters of pancreatic juice, and 3.0 liters of

intestinal secretion (Boxenbam et al., 1979). Physical and chemical properties of meals, the external appearance of a meal (smell, taste), the nutrient composition of the meal, and the total volume of the meal are important to determine the magnitude and duration of gastrointestinal secretory response (Johnson et al., 1981). Many endogenous molecules and probably many foreign compounds are transported from plasma into the gastrointestinal lumen with the alimentary secretion. Some of these compounds may be reabsorbed from the GI tract but others are eliminated with the feces, for example, 25-30% of the urates are excreted daily via this route (Sorensen, 1960). The elimination of cholesterol with feces has been studied by Ferezou and his collaborators (1982). They have reported that the cholesterol secreted into the GI tract is the major source of the total fecal cholesterol excreted in humans. In this experiment they demonstrated that phytosterols (cytellin 9 g/day) enhanced the fecal excretion of plasma cholesterol while calcium (calcium gluconate, 3 g/day) lowered it.

The mechanisms of intestinal absorption have been reviewed carefully by Levine and Pelikan (1964). The role of the intestinal wall in the absorption of xenobiotics has been studied by Hayton (1980). In general, the mechanism of transferring xenobiotics from blood to the intestinal lumen is not well known. In a study in which the intestinal secretion was evaluated, Lauterbach (1975) demonstrated that most lipid soluble compounds transfer across the intestinal

wall by passive diffusion. On the other hand, Holland and Quay (1976) concluded that transfer of erythromycin from the blood to the intestine lumen was an active transport. Kolaassa and co-workers (1980) stated that the transfer of hypoxanthine and xanthine by the jejunum in guinea pigs is by active transport.

The location of xenobiotic transfer in the intestines also is not completely understood. In 1968, Berline and Hawkins found that the basal (or contralumenal) membrane of the small intestine is a major site of active secretion of the purines in hamsters. Welsh et al. (1982) reported that the crypts are the site of intestinal fluid and electrolyte secretion while the surface epithelium is the site of absorption in rabbits. The secretion of hydrophilic substances via the crypts and diffusion of lipophilic compounds across the intestinal epithelium have been confirmed by a number of studies, including the report of Parsons (1972) and Smyth (1972). DeBoer et al. (1982) described that the administration of certain drugs through the rectum is as effective as the oral route. The rectal dosage form seems to be a very critical factor in this matter. Aqueous or alcoholic solutions absorb rapidly, but suppositories absorb slowly.

To be transported from the serosa to the lumen of the intestines, a xenobiotic has to diffuse across the capillary endothelium; the longitudinal, circular, and submucosal muscles, and the mucosa (Bohlen, 1984). Consequently, a chemical may be metabolized in the mucosa before reaching

the intestinal lumen. Hartiala (1973) described the influence of various endogenous and exogenous compounds in the regulation of chemical metabolism in the gastrointestinal tract. In mammals, nonenzymatic decomposition, bacterial metabolism, and gastrointestinal epithelial metabolism of carbamates can occur simultaneously in the gastrointestinal tract (Pekas, 1980). The metabolism of several foreign compounds has been investigated in viable isolated liver and intestines of male Wistar albino rats (Shirkey et al., 1979). Glucuronic acid and sulphate conjugation were observed in both cell preparations in respect to phenol. Conjugation of 1-napthol was also observed in isolated intestinal cells. Acetanilide was the only metabolic product of aniline observed within the intestinal cells. The metabolism of hydralazine, isoniazid, p-aminosalicylic acid, and certain sulphonamides by the intestinal wall has been demonstrated (George, 1981). He found that the gastrointestinal mucosa represents an important site of Phase II drug metabolism in man. In some circumstances, the absorption of the drug from the GI tract may result in a high percentage of the drug being rendered inactive by metabolic transformation as it passes through the intestinal wall. In other circumstances, e.g. where the drug is given as a pro-drug, conversion to the active substance may occur during transit through the intestinal wall (Griffin, 1981).

The compound in the lumen of intestines may be reabsorbed, metabolized enzymatically or nonenzymatically by gut microflora, or become adsorbed to the fecal mass and

subsequently excreted (Boxenbaum et al., 1979). Several investigators demonstrated that gastrointestinal flora are capable of metabolizing foreign compounds via a variety of metabolic pathways resulting in the formation of more lipophilic derivatives. This process is influenced by the accessibility of agents to the microbial flora of the large intestine. Such lipophilic metabolites tend to be absorbed into the systemic circulation with possible pharmacological and toxicological effects.

EXAMPLES OF INTESTINAL EXCRETION OF SPECIFIC DRUGS AND XENOBIOTICS

The biliary and renal excretion are the main routes of elimination for many xenobiotics. However, for some compounds, secretion into the intestine is an important process (Rozman et al., 1982b; Polin et al., 1986; Klaassen, 1986).

Hatcher and Eggleston in 1919 discovered that digitoxin given parenterally to bileligated rats, was excreted in the feces. Another study demonstrated the presence of 10% of the parenteral dose of radioactive digitoxin in feces of dogs with bile fistulae (Israili and Dayton, 1984).

The secretion of drugs into the lumen of the gastrointestinal tract has its counterpart in ruminants, as described by Reid et al. (1957) while measuring extracellular volume with antipyrine.

Methyridine, an anthelmintic agent particularly effective against nematode infections of the gastrointestinal tract of mammals, is effective when given subcutaneously. In data

reported by Broome (1961), there was a close relationship between levels in blood and levels in the intestines after subcutaneous administration in sheep. It appears to be secreted along the entire length of the alimentary canal and especially high concentrations were found in the abomasum. Other injectable anthelmintics, such as disophenol and 2,6 diiodo-4-nitrophenol, were secreted in the intestines of fowl and dogs (Boisvenue, 1963; Wood et al., 1961).

Noach and VanRees (1964) ligated rat intestines at different sites and determined the intestinal excretion of phenytoin in jejunum, ileum, etc. 5 hours after i.v. injection of the compound. Another study by Henderson et al. (1980) reported that most of the i.p. injected dose of 4,4'-bistrifluoromethyl analogue of phenytoin to rats was excreted unchanged in feces during a period of 2 weeks and its renal excretion was less than 1% of the dose.

Many organochlorine compounds appear in the stools of animals after parenteral administration in quantities greater than can be accounted for by biliary excretion. Boylan and co-workers (1979) reported that an important route for the excretion of chlordecone into the intestinal lumen is from the intestinal wall rather than in bile. The intestinal excretion of chlordecone has been confirmed by a number of studies, including the report of Bungay et al. (1981) and Guzelian (1982a). The latter author stated that the gut is more important than the liver in transporting chlordecone

to the intestinal lumen. In 1976, Pittman et al. reported intestinal secretion of mirex (an organochlorine insecticide) in rhesus monkeys. The concentration of mirex in the large intestine was higher than that in the liver and the concentration of mirex in the bile was low, suggesting that relatively little excretion of mirex occurs by this route. Heath and Vandekar (1964) studied intestinal excretion of dieldrin by rats. They found a higher amount of dieldrin in feces than could be accounted for with biliary excretion. About 70% of the injected dose had been recovered from the feces. Therefore, fecal excretion seems to be an important route for the elimination of dieldrin. The rate of intestinal excretion of dieldrin differed between male and female rats. In males, intestinal mucosa contributed 10% or less of the total excreted, and about one third of the contribution in females. The intestinal excretion of dieldrin was confirmed by William and Smith (1965).

Experiments with bile-duct-cannulated rats have shown that most of the DDT metabolites found in the feces of rats were derived from biliary excretions (Blieberg and Larson, 1957; Datta, 1970; Hayes, 1965; Jensen, 1957; Judah, 1949). Leighty et al. (1980) reported that the alcohol metabolite of DDT conjugated with fatty acids was mainly secreted through the intestinal wall in rats.

Schanker (1959) studied the intestinal excretion of quinine in rats. He found that the level of quinine in the

colon was twice the plasma level. Mu et al. (1975) treated rats intravenously with mefloquine, a quinine-like antimalarial drug. Approximately 77% of the intravenous dose of the compound was excreted with feces. After bile diversion, about 14% of the dose was excreted in the feces, suggesting significant intestinal excretion for the drug. Intestinal excretion may be quite significant for an analogue of mefloquine, WR30,090, for which fecal excretion was also the major route of elimination in both rats and man.

Lee and co-workers (1956) reported that 37-43% of the intravenously injected erythromycin was discovered in the rat's intestinal tract. These authors found that in bile-cannulated rats, about 12% of the intravenously injected erythromycin was excreted in the feces.

The fate of emperonium in dogs has been studied by Sundwall et al. (1970). About 60% of the label was excreted in the feces during 7 days of observation. It was found that 27-66% was in the form of unchanged drug. In humans, 45% of an intramuscular dose of emepronium was found in the feces and 31% was excreted in the urine. This probably indicates extensive biliary excretion of the unaltered drug (Sundwall et al., 1973). The bile diversion experiments indicate that about 5-15% of an intravenous dose of emepronium in dogs is excreted through the gastrointestinal epithelium (Hallen et al., 1979).

The transepithelial permeation and the cellular uptake of n-methyl scopolamine (NMSCOP),n-methyl nicotinamide (NMN), and tetraethylammonium (TEA) were studied by Turnheim and Lauterbach (1977a) in the isolated jejunal mucosa of the guinea pig. The results indicated the existance of an active secretory system for monoquaternary ammonium compounds in the jejunal epithelium. Turnheim and co-workers (1977b) reported that NMSCOP is transferred across the jejunal epithelium by two transport mechanisms in series: one located in the basolateral and one in the luminal cell membrane.

Tissue distribution of radioactive cobalt has been studied in chickens (Lee and Wolterink, 1955). These researchers injected cobalt 60, as $^{60}CoSO_4$ intravenously and into the gizzard. They found that the radioactive material was able to traverse the intestinal wall in both directions and was highly concentrated in the intestinal tract, especially in the cecum. In similar study by Monroe et al. (1952), after oral or i.v. administration of radioactive cobalt, a significant amount of the compound was found within the mucosal wall of the small and large intestine and cecum of the chick. After i.v. administration to the chick, approximately 12% of the injected cobalt was found within the gut lumen during the first 24 hours, indicating significant gastrointestinal excretion (Monroe et al., 1952).

Supplementation of diet with choline chloride and inositol at the levels of 2.65 and 1.32 or 2.0 and 1.0 gm/kg of diet,

respectively, reduced carcass residue of DDT and metabolites in laying quail (Cregar and Kubena, 1971). They also indicated that same dietary treatment reduced body burdens of heptachlor in laying quails. Donaldson et al. (1971) presented data which suggested that starvation significantly decreased body burdens of DDT in chickens that previously were fed a diet containing 100 ppm DDT for 2 weeks.

Polin et al. (1986) reported that supplementation of the diet with mineral oil or colestipol, and feeding at 50% ad libitum were effective in reducing the level of hexachlorobenzene and pentachlorophenol in chickens.

METHODS FOR ALTERING CLEARANCE OF XENOBIOTICS FROM THE HOST

The intestinal excretion of foreign compounds has been studied by a number of investigators as a means of enhancing the elimination of persistent environmental agents and other undesirable compounds from the body. Several techniques have been used to hasten elimination of xenobiotics from the body.

A. Inhibition of Xenobiotic Adsorption From the GI Tract

The gastrointestinal tract is one of the most important routes in the absorption of drugs and xenobiotics. The absorption may take place along the entire GI lumen. Therefore, when appreciable amounts of potentially toxic compounds are ingested, their removal from the GI tract before absorption may reduce or prevent toxicity. Giving emetic agents or nonabsorbable lipids to the toxic-affected animal

could help the animal to remove the toxicant from the GI tract.

There are several determinants of the absorptive process, i.e. (1) the physical and chemical structure of the compound such as solubility in GI fluids, lipid solubility, and pKa, (2) the nonabsorptive physiological functions or state of the GI tract, that is, its motility and circulation, (3) dosage of xenobiotic and its concentration in the GI tract, (4) the structure of the absorbing surface (because of the very large surface area of the small intestine, most absorption occurs in this organ), (5) the presence of microorganisms in GI lumen [it has been calculated, for example, that bacteria comprise 65-70% of wet stool weight and that the fecal excretion of bacteria is 15 gram dry weight day^{-1} for those on a Western diet (Stephen and Gummings, 1980)], (6) the metabolic function of the absorbing cell, (7) nutritional requirements of the body, e.g. iron, calcium, etc., (8) miscellaneous factors such as the presence of other foreign compounds in the GI tract, [e.g. cadmium decreases the absorption of copper and zinc, calcium decreases the absorption of cadmium, ethylenediaminetetraacetic acid (EDTA) increases the solubility and absorption of metals, milk increases the absorption of lead, etc. (Klaassen, 1986; Levine, 1975)].

Specific antidotes are available for only a few of the numerous chemical products that have the potential to cause serious systemic toxicity. Since the first step in the

deposition of a toxic compound in animal tissue is its absorption from the intestine, one of the most important initial treatments is to try to minimize gastrointestinal absorption of the chemical (Klaassan, 1986). Oral administration of nonabsorbable lipids, or resins such as cholestyramine and colestipol, or activated charcoal either simultaneously or shortly after the ingestion of a foreign compound, was found useful in decreasing significantly gastrointestinal absorption of the chemical. These adsorbants or lipids trap and remove xenobiotics from the GI tract (more details under section V.B.4.). Some other procedures such as gastric lavage, gastrointestinal dilution of poisons, evaporated milk, and induced catharsis, which were used traditionally in the early management of poisoning to prevent the gastrointestinal absorption of chemical, will be discussed in this section.

B. Enhancement of Intestinal Excretion of Xenobiotics

Intestinal elimination of foreign compounds may be increased by one of the following procedures:

1. Increasing the blood flow to the intestines. The average splanchnic blood flow in humans is increased by about 10% after a high protein meal (Mclean et al., 1978). Increasing oxygen consumption due to increasing intestinal blood flow was reported by Gallavan et al. (1983). Also, blood circulation of the colon is enhanced (Kviety and Granger, 1982) after oral administration of drugs such as isoproterenol,

adenosine and glucagon. Vatner and co-workers (1970) have shown that the mesentric blood flow in dogs increased within 5 to 15 minutes of presentation of a high-protein meal, reached a maximum (from 115% to 300% of control) within 30 to 90 minutes of eating, and gradually returned to control values within 3 to 7 hours. Intestinal transportation was stimulated in cats by placing either a Tyrode solution (Solute-coupled volume absorption), Tyrode plus glucose, or Tyrode plus taurocholate into the ileal lumen. This was due predominately to an increased blood flow (Valleau et al., 1979). Sit and co-workers (1980) reported hyperemic reaction of intestinal circulation due to placement of glucose into the jejunal lumen. In 1979, Kvietys et al. demonstrated the influence of bile to increase the hyperemic effect of digested food in the jejunal lumen. They found that 33% bile altered the vascular effects of all nutrients tested, but 10% bile affected only free fatty acids.

2. Using agents which would displace a xenobiotic from its binding sites in tissues and/or plasma proteins.

3. Modification of the transport properties of the gastrointestinal mucosa. For example, lysolecithin increased the passive permeability of molecules through the walls of the ileum (Bolin et al., 1981).

4. Using nonabsorbable substances with a high affinity for the xenobiotic. Guzalian's (1982a) use of cholestyramine in patients with chlordecone accumulations showed for the

first time in humans that it was possible to enhance the rate of excretion of such toxic lipophiles (Cohn et al., 1978a). Studies with animals have suggested that other agents such as: (a) poorly absorbed oils, (b) activated charcoal, (c) specific antibodies (intact) or FAB fragments (antigen binding site of an antibody), and (d) miscellaneous factors such as fiber [a high-fiber diet lowers cholestrol levels in man (Vahouny et al., 1981)], enemas containing albumin, lipoproteins or other macromolecules might also favorably increase the rate of removal of persistent lipophiles from the body.

a) Nonabsorbable lipids. It is well known that nonabsorbable or poorly absorbed lipids are able to trap lipophilic substances in the GI tract and inhibit their absorption or reabsorption until they are finally excreted via feces. The use of nonabsorbable fats to decrease tissue stores of a toxic material is dependent on a delicate balance of hydrophobic and hydrophilic characteristics of the lipophile and its metabolites, as well as on the extent of metabolism. There are a number of reports of mineral oil absorption and resultant deposits of mineral oil in the liver and other tissue (Daniel et al., 1953; Ebert et al., 1966; Nochomovitz et al., 1975). Hepatic damage in association with deposits of mineral oil in man has been reported (Blewitt et al., 1977). Fat soluble vitamin status should also be considered in a long-term regimen of a nonabsorbable lipid.

Nonabsorbable or poorly absorbed lipids include mineral oil (paraffin oil), oil of the Limnanthes douglasii seed (Phillips et al., 1971), glyceryl triethers [triglyceride analogues with ether groups replacing esters (Mangold, 1979)], synthetic fat, sucrose polyester (SPE) (Jandacek et al., 1980). The type of dietary fat, consisting of hydrolyzable triglycerides, can also affect the extent of absorption of cholesterol and persumably other lipophiles (Sylven and Borgstron, 1969). Some components of normal diet such as fiber, gums, and pectin can influence intestinal absorption of lipophilic substances (Kritchevsky, 1977).

b) Activated charcoal. Activated charcoal is probably the most effective antidote with the potential to bind a broad spectrum of chemical compounds in the gastrointestinal tract and thus prevent their absorption. Activated charcoal is able to adsorb a wide variety of acidic, basic, and neutral substances, both water soluble and insoluble, mainly on its internal surface. In general, weak acids and bases are better adsorbed to charcoal in the nonionized form (Picchoni, 1970; Cooney, 1980).

The efficacy of activated charcoal as an adsorbant depends on its small particle size, large surface area, and low content of adsorbed substances, which are removed during the charcoal-activation process (Picchoni et al., 1974). Repeated small oral doses of activated charcoal enhanced the total body clearance of theophylline, and larger doses induced a

relatively small further increase (Park et al., 1983). Tsuchiya and Levy (1972) have shown by means of studies in humans that there is a difference in the adsorptive capacity of activated charcoal powder and activated tablets. They concluded that activated charcoal powder is more effective and is the preferred dosage form to use as an antidote. Formulating liquid preparations of activated charcoal may result in loss of its adsorptive capacity after storage in liquid form (Picchioni et al., 1974). Because of variations in the adsorptive capacity of charcoal for different chemicals, it is suggested to use the most effective charcoal product (Henschler, 1970). Picchioni et al. (1974) reported that the antidotal properties of charcoal against aspirin and chloroquine was improved by the combination of charcoal with $NaHCO_3$. Activated charcoal plus $NaHCO_3$ reduced peak salicylate levels in aspirin-treated animals by 51%; whereas the same amount of charcoal alone reduced it by only 16%.

In the case of acute poisoning, activated charcoal should be given orally as soon as possible, preferably within 30 minutes after the poison has been ingested, since a delay in the administration of the antidote tends to decrease its effectiveness (Picchioni, 1970). Chin et al. (1970) demonstrated that giving activated charcoal one minute after poisoning is markedly more effective in reducing the gastrointestinal absorption of drugs than the administration of charcoal 30 minutes after poisoning and, in some cases

of dog poisonings, administration of activated charcoal was ineffective in preventing death. Chin et al. (1973) concluded that the optimal activated charcoal-drug ratio for the treatment of pentobarbital, chloroquine and isoniazid is at least 8:1.

c) Cholestyramine and colestipol. Cholestyramine (CTR) and colestipol (CLP) are basic anion exchange agents, quite hydrophilic but insoluble in water, neither digested nor absorbed from the GI tract. They are cholesterol-lowering agents and commonly used to reduce serum cholesterol levels in humans. Both adsorb bile acids and inhibit their reabsorption in the GI tract to be eliminated via feces. Therefore, increasing fecal excretion of bile acids leads to increased hepatic oxidation of cholesterol to bile acids and decreases serum cholestrol (Israili and Dayton, 1984).

Cholestyramine and colestipol are frequently used in detoxication of specific xenobiotics in man and animals. The following are examples of CTR and CLP in preventing absorption of xenobiotics through the intestinal wall or enhancing elimination of them with feces. Caldwell et al. (1971) studied the influence of CTR on the enterohepatic circulation of digitoxin in rats, guinea pigs, and humans. They found that CTR binds to digitoxin and digoxin, then accelerates the metabolic disposition of those compounds. Bazzano (1972) reduced the plasma half-life of digitoxin from 9.3 days to an average of 2.75 days by using colestipol.

Goldfinger et al. (1970) described the situation that during CTR administration digoxin was poorly and erratically absorbed from the GI tract. CTR did not interfer in absorption or bioavailability of propranolol, an antianginal agent (Schwartz et al., 1982). Fecal elimination of chlordecone and its disappearance from the blood and other tissues increased in rats (Boylan et al., 1978) and humans (Cohn et al., 1978a) with the administration of CTR. Caldwell and Greenberger (1971) reported that CTR treatment afforded a significant degree of protection against lethal digitoxin intoxication in rats and guinea pigs. This was because digitoxin bound to CTR in the intestinal lumen and reduced its reabsorption, thereby increasing fecal elimination of the xenobiotics. Biliary and intestinal elimination of 2,4,5,2',4',5'-hexabromobiphenyl in rhesus monkeys was increased by the oral administration of mineral oil and cholestyramine (Rozman et al., 1982c). Elimination of digitoxin with CTR in man was studied by Cady et al. (1979), Carruthers and Dejoune (1980). The latter investigators reported that using a combination of spironolactone and CTR had additive effects and enhanced the clearance of digitoxin.

In speaking about the importance of bile excretion, it should be emphasized that the liver is in a very advantageous position for removing toxic materials from the blood after absorption by the gastrointestinal tract. Since the blood from the gastrointestinal tract passes through

the liver before it reaches the general systemic circulation, the liver can remove compounds from the blood and prevent their distribution to other parts of the body. In the liver, a foreign compound may be metabolized and reenter the bloodstream to be excreted by the kidney, or the metabolites may be excreted directly into the bile without returning to the blood. Once a compound is excreted into the bile and enters the intestine, it can be either reabsorbed or eliminated in the feces. So, biliary excretion is an important route in detoxification of xenobiotics. Polarity and molecular weight of the substance are major factors for biliary excretion.

Bile salts also play an important role as detergent into the intestinal lumen in formation of micelles and absorption of lipids from the intestinal wall. When bile is totally excluded form the intestinal tract, absorption of lipids and lipid soluble vitamins (A, D, E, and K) are markedly decreased.

d) Specific antibodies and FAB fragments.

Definition of the terms "antibody" and "FAB fragments":

Antibody: An immunoglobulin that will bind with an antigen or immunogen.

FAB fragment: An antibody is composed of two FAB fragments (antigen binding site) and one FC fragment (non-antigen binding site).

The results of studies of Butler et al. (1977), Lloyd and Smith (1978) demonstrate that FAB fragments are more rapidly distributed and eliminated than an intact antibody, resulting in a definite advantage to their potential use in advanced detoxification of specific xenobiotics.

Moolten et al. (1978) used a noncarcinogenic polycyclic aromatic hydrocarbon (PAH) analog to elicit nonspecific antibodies in guinea pigs against the enitre PAH class of compounds. Walker et al. (1972) reported that gastrointestinal mucosal antibodies in mice are capable of binding benzo[a]pyrene (BAP), and enhance the fecal excretion of orally administrated ^{3}H-BAP. These data showed that it was feasible to induce gastrointestinal mucosal antibodies capable of neutralizing an entire class of orally ingested carcinogens that are present in the environment at low concentrations. These data also point out the importance of using nonspecific antibodies for certain purposes. In another study with an environmental pollutant (Luster et al., 1978), passive immunization of mice with antisera against 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was shown to be ineffective in preventing or reversing the lethal effects of TCDD administration. The authors postulated that the inefficacy was the result of irreversible binding to the receptor.

In a study by Colburn and White (1979), mice were passively immunized with antisera against either 4-monochloroor 3,4,3',3'-tetrachlorobiphenyl following the oral absorption

and distribution of the respective chlorinated biphenyl. Immunization resulted in the redistribution of the chlorinated biphenyls into the vascular space with a concommitant reduction in tissue concentrations when compared to control animals. The results of this study indicated that the pharmacokinetics of chlorinated biphenyls can be altered by subsequent administration of antisera and that the tissue stores can be significantly reduced.

Antisera have been developed for other environmental contaminants including DDT, malathion, dieldrin, and aldrin (Haas and Guardia, 1968; Centeno et al., 1970; Langone and Vunakis, 1975), but they have not been used to reverse the toxic effects of these compounds. The use of antibodies and FAB fragments to reverse the untoward effects of environmental toxins should be an area of extensive study in the future because most of these compounds are not readily removed from the body by conventional methods, i.e. hemodialysis and hemoperfusion (Okinek et al., 1976).

Regarding the use of antibodies and FAB fragments to reverse toxic effects and to alter pharmacokinetics of a class of drugs, the most extensive and methodical research has been conducted with the digitalis glycosides (Colburn, 1980). A series of in vitro and in vivo experiments were preformed to determine the impact of antibodies and FAB fragments on quantifiable pharmacologic effects and the pharmacokinetics of digoxin, digitoxin, and ouabain. For

example, patients who did not respond to conventional therapy for a digoxin overdose were successfully treated with digoxin-specific FAB fragments to reverse the potentially lethal toxic effects of digoxin.

Using purified digoxin-specific FAB fragments appears to be a safe and effective means of reversing advanced, life-threating digitalis intoxication (Smith et al., 1976, 1982; Zucker et al., 1982; Harenberg et al., 1983).

The effects of fragments of high affinity specific antibodies were studied by Schmidt and Butler (1971a) in rabbits. All of the animals in these experiments that received specific FAB fragments survived; control animals died. The experiments revealed that FAB fragments of antibodies with high affinity for digitoxin were capable of rapid reversal of advanced, otherwise lethal, digitoxin toxicity, and were capable of reducing the plasma half-life while accelerating urinary excretion of digitoxin.

e) Miscellaneous agents. Dietary components that provide large surface areas tend to adsorb ions and decrease their absorption during intestinal transit. Fiber, clay, and tricalcium phosphate adsorb trace elements and tend to decrease their bioavailability. Besides the adsorption effect, these materials decrease intestinal passage time as well as impair access of the ions to mucosal receptors. The two major dietary factors that affect bioavailability are competitive antagonism among ions and the presence of chelators in foods. For

example, phytate, that is a natural chelating agent found in foods, forms insoluble complexes with many cations and could play a role to decrease toxic levels of metals which are excreted in the GI tract (O'Dell, 1983).

A high-fiber diet was shown to lower cholesterol levels in man (Vahouny et al., 1981). Data of this experiment indicate that low and high-viscosity grades of guar gum sequestered 15-23% of the monolein and 32-33% of the fatty acids, exhibiting a significant preference for linoleic acid in each case. Alfalfa fiber removed about 18% of the micellar monoglyceride and mixed fatty acids. Lignin, the hydrophobic component of dietary fibers, sequestered about 13% of the available lipids and displayed an apparent preference for oleic acid.

f) Methods of administration of nonabsorbable substances. To improve the efficiency of nonabsorbable substances (NAS) in removing the xenobiotics from the GI tract, administration of the nonabsorbable compounds directly at the place of the major excretory process is very important (Israeili and Dayton, 1984). For example, in clearance of lipophilic compounds from the GI tract, delivery of nonabsorbable lipids into the intestinal lumen is more effective than giving it orally. In cases of paracetamol overdose, introducing 50 gm of adsorbent, e.g. cholestyramine or activated charcoal, into the stomach (after it has been emptied by a wash out) is helpful (Dordoni et al., 1973). In another study of the

treatment of ulcerative colitis (Matts and Gaskell, 1961), administration of the solutions intrarectally was effective.

g) Potential risks associated with the use of nonabsorbable substances. Since most of the nonabsorbable substances are nonspecific materials, they might adsorb some essential nutrients or other endogenous compounds that are present in the GI tract. Prolonged unrestricted ingestion of mineral oil is dangerous and may cause structural and functional abnormalities which lead to severe malnutrition and death (Nochomovitz and Yus, 1975). These authors reported the deposition of mineral oil in the small intestine, abdominal lymph nodes, liver, spleen, and lungs of a patient who had ingested large amounts of liquid parafin over many years for laxative purpose. Hepatic damage in association with deposits of mineral oil is described by Blewitt et al. (1977).

Jackson et al. (1980) warned against overdose administrations of charcoal for the following reasons: (1) it may adsorb n-acetylcysteine, the "antidote", (2) it may cause oral and esophageal burns, and (3) neither methanol nor ethanol is significantly adsorbed by charcoal.

Use of the specific antibodies or FAB fragments has the advantage of the selective action of those materials on specific xenobiotics, removing them from the GI tract. They do not remove desirable substances and not cause side-effects as mentioned above.

C. Removal of Persistent Environmental Toxic Agents From Man and Animals

Most of the environmental toxic compounds that accumulate in human and animal tissues are lipophilic. Severe toxicity or even death due to exposure to the xenobiotics has been reported by many investigators. For example, chlordecone toxicity on the central nervous system, liver, and reproductive organs of humans and experimental animals were reported by Cohn et al. (1978b) and Guzelian (1982b). Toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin on cellular involution, division, and/or differentiation was described by Poland and Knutson (1982). The results of a study of a group of workers in a factory that manufactured the organochlorine pesticide, chlordecone, indicated that chlordecone produced adaptive changes in the liver that selectively affected the drug-metabolizing system (Guzelian et al., 1980). Hypothyroidism in workers who were exposed to polybrominated biphenyls (PBBs) was reported by Bahn et al. (1980). These authors described that PBBs are highly lipotropic and are not readily metabolized; therefore, they remain in adipose tissue for extended periods. Concern over the toxic effects of PBBs on humans arose when these compounds were accidentally fed to cattle in Michigan in 1973. Human exposure resulted due to ingestion of contaminated dairy food products. All of the exposed people showed symptoms and conditions related to toxicity from PBBs (Landrigan et al., 1979). In 1968, ingestion of rice oil contaminated with a commercial brand

of polychlorinated biphenyls (PCBs) produced 1057 patients with skin disease in Japan (Kuratsune et al., 1972).

The use of nonabsorbable substances (NAS) either alone or in combination with suitable procedures may be the treatment of choice in such intoxications. Fecal excretion of chlordecone was increased in humans (Cohn et al., 1978b) and rats (Boylan et al., 1978, 1979) by the administration of cholestyramine. Paraffin-treated rats excreted significantly more Kepone-^{14}C (Richter et al., 1979a) and 2,4,6,2',4'-pentachlorobi[^{14}C]phenyl (Richter et al., 1979b) in their feces compared to the control animals. Similar results were obtained in the treatment of hexachlorobenzene in rats with paraffin and/or squalane (Richter and Schafer, 1981). Elimination of hexachlorobenzene and pentachlorophenol from chickens was enhanced with the administration of mineral oil, colestipol, and/or restricted feeding (Polin et al., 1983, 1986). The results of other experiments (Polin and Leavitt, 1984; Polin et al., 1985) indicate that a combination of feed restriction and colestipol or mineral oil hastened withdrawal of polybrominated biphenyls (PBBs) and lowered body weight gain and carcass lipid in chickens. Rozman et al. (1982b) reported that hexadecane increased non-biliary, intestinal excretion of stored hexachlorobenzene in rats.

The use of active drug elimination procedures such as hemodialysis, hemoperfusion, hemodiafiltration, peritonal dialysis, and forced divresis may be either unsuitable or

ineffective in enhancing removal of xenobiotics (Guzelian, 1982b; Winchester, 1978). The results of studies on rats indicated that hemoperfusion is not an effective mode of therapy for parathion intoxication (Eigenberg et al., 1983). In overdoses of tricyclic antidepressants and other drugs with a large volume of distribution, the use of the hemoperfusion method is not helpful for elimination of the chemicals (Cohan et al., 1982). In other studies (Pond et al., 1979; Todd, 1984) data indicated that hemoperfusion is efficacious for drugs with low intrinsic clearance and a relatively small volume of distribution, such as phenobarbitone, theophylline, and tolbutamide. In contrast, hemoperfusion removes only a small fraction of drugs with high intrinsic clearance and a large volume of distribution, such as digoxin and amitriptyline. Holazo and Colburn (1982) compared contributions of hemodialysis, hemoperfusion, FAB fragment administration, and adsorption with cholestyramine to remove drugs in overdose situations and environmental exposure by using pharmacokinetic simulation technics and literature review. They described some advantages and disadvantages for those methods.

CONCLUSIONS

Excretion into the gastrointestinal tract by other than the biliary route had been considered to play a minor role in clearance of toxic compounds from the host. But recent studies by some investigators indicated that the intestinal

excretion of foreign compounds such as drugs and environmental contaminants is significant. The excretion into gastrointestinal lumen can take place by way of a direct passive transfer from the plasma through the mucous membrane, or by active transport system for certain compounds. In passive transfer, the mechanism is simple diffusion, and the lipophilic nature of the compound, molecular weight, pKa, and gradients of hydrogen ion concentration are important determinants in the excretion. Unchanged forms of the molecules pass the lipid-containing cell membranes rather easily. The gastrointestinal mucosa is also capable of biotransformation of foreign compounds prior to excretion into the lumen. The alimentary excretion of foreign compounds can be enhanced by a number of chemicals and it is possible for the foreign compounds excreted into the alimentary canal to be reabsorbed. The intestinal microflora have an important role in this process. The reabsorbed compounds are usually reexcreted into the bile and an enterohepatic circulation is established which may significantly prolong the half-life of the foreign compound in the body. Although the excretion of several xenobiotics with intestinal secretion has been confirmed by many scientists, still many aspects of intestinal excretion remain to be clarified; these include mucosal detoxification and microfloral activities. If excretion of xenobiotics by the intestinal route is to be made more efficient and reliable, then more investigations of an interdisciplinary nature are essential.

II. POLYCHLORINATED BIPHENYLS (PCBs)

Polychlorinated biphenyls are a class of toxic, highly stable industrial chemicals that were first synthetized more than 100 years ago (Peakall and Lineer, 1970). The commercial production of PCBs in the U.S. began in 1930 (Dustman et al., 1971). From 1930 through the 1970's, PCBs were a common component of manufacturing equipment, most extensively in electrical capacitors and transformers. Unfortunately their widespread use produced health hazards in humans and animals. Their residues were routinely detected in fish, wildlife, and human adipose tissue.

Studies of PCB toxicity in animals indicate that sensitivity to physiological intoxication by these compounds varies widely with species. Some animals are largely resistant to intoxication by PCBs, whereas some animals are somewhat sensitive. Human sensitivity to intoxication by PCBs appears to be related to the source of exposure. The results of several studies indicate that PCBs may be carcinogenic to laboratory animals.

The chemical structure of PCBs, sources, pharmacokinetics, and their toxic effects in various species will be reviewed in this section.

Chemical Composition

Polychlorinated biphenyls (PCBs) are commercial products which are prepared industrially by the chlorination of biphenyl and the commercial preparations are graded and marked according

to their chlorine content. The lower chlorinated materials are moderately viscous liquids, whereas the more highly chlorinated products are solids. The commercial PCBs have molecular weights from 188.7 to 398.5 (Zimmerman, 1982). Their boiling temperatures vary between 278 to 415°C. PCB materials are destroyed at a temperature of 800°C for 10 seconds or 1000°C for 3 seconds (Osweiler et al., 1985). There are a total of 210 isomers of PCB, but probably only 102 actually exist (Clesceri, 1984). Gas chromatography properties of the 209 PCB isomers were summarized by Safe and Mullin (1987).

Commercial PCBs have been produced in several countries and they are known by a variety of trade names such as Aroclor (USA), Phenochlor (France), Clophen (Federal Republic of Germany), Kanechlor (Japan), Fenchlor (Italy), and Sovol (USSR) (World Health Organization = WHO, 1976).

The properties of chemical stability, nonflammability, resistance to acids and alkalis, resistance to oxidation and degradation by other chemicals, low volatility under prolonged heating, low vapor pressures, low water solubility, and high dielectric constants make PCBs valuable and widely used industrial chemicals. They have been used as dielectric fluids in transformers and capacitors (i.e. fluorescent lighting ballasts, air conditioner motor capacitors, television capacitors, large power capacitors, and industrial furnace capacitors), plasticizers in paints, inks, printing, copying

paper, adhesives, sealants, plastics, and coatings, hydraulic systems, heat transformer systems, investment casting waxes, varnishes, gas turbines and vacuum pumps, marine antifouling paints, as "inert" ingredients for insecticides, microscope immersion oils, insulation, synthetic rubbers, polyvinyl chloride and acetate, celluloses, epoxy, fire retardant and flame proofing formulations, moulding processes, use in diesel and jet air craft fuels to improve flow characteristics and prevent water crystallization (Richardson and Waid, 1982).

Durfee (1976) reported that 95 percent of capacitors and 5 percent of transformers utilize PCBs. According to General Electric about 100 x 10^6 capacitors containing PCBs were made in the United States yearly. MacArthur and Nagy (1976) reported production of 5,000 transformers per year with an average of 5000 pounds PCBs per transformer. Life expectancy for capacitors is over 10 years for lighting applications and over 20 years in electric utility service. Life expectancy for transformers containing PCBs is over 30 years. There are two types of capacitors: small and big. The small kind used 9 to 13 million pounds of PCBs per year, and the big kind used 17 million pounds of PCBs per year.

Production

Polychlorinated biphenyls were under investigative development by Schmidt and Schultz as early as 1881 (Peakall and Lincer, 1970). They had gained wide use during the 1930's

in electrical transformers and condensers (Dustman et al., 1971). Pathological studies on laboratory animals were conducted (Bennett et al., 1938). In 1966, the Swedish scientist Sorben Jensen was studying the feathers of eagles that had been taken from museum collections. He identified commercial PCBs in feathers collected as early as 1944. In 1967 Widmark reported the presence of PCBs in pesticides. In 1968 Risebrough et al. discovered the effect of PCBs on the reproductive systems of wild birds. In the summer of 1968, in Yusho, Japan, PCBs from heat transfer fluid leaked into rice oil during the manufacturing process. Over 1,000 persons consumed the contaminated oil. Mild to severe symptoms of poisoning appeared (Rodericks et al., 1979). In 1971 thousands of chickens and eggs were destroyed in the U.S. because of PCB exposure to poultry feed. In 1969, the U.S. Food and Drug Administration (FDA) started routine sampling of foods for PCB contaminations. They isolated PCBs from milk, chicken, and poultry feed. Tolerance levels of PCBs in different animal products were established by the FDA as follows: 0.2 mg/kg in milk, 0.2 mg/kg in eggs, 5 mg/kg in fish, and 5 mg/kg in poultry. In 1979, a similar incident of Yusho disease occurred in Taiwan after consumption of PCB contaminated cooking oil (Chen et al., 1981).

About 99 percent of the PCBs used in the U.S. industry were produced by Monsanto Company. Annual PCB production of this company in 1970, was 85 million pounds. Approximately

70 percent of that were used in the production of capacitors and 30 percent in transformers. Imported PCBs (from Italy and France) were slightly over one percent of the U.S. usage, which were used in investment casting operations and semiclosed heat transformer applications.

Production of PCBs in the Monsanto Company was conducted in two steps: First, biphenyl (which was manufactured from pure benzene) was chlorinated with anhydrous chlorine in the presence of ferric chloride to produce crude PCBs. Then the crude PCBs were distilled to obtain the finished product. Almost one-half of the world PCBs were produced by Monsanto plants in the U.S. The company has reported domestic sales of 795 million pounds of PCBs for the years 1957 through 1974 (Durfee, 1976). Some other countries including Japan, West Germany, France, Italy, Spain, England, Czechoslovakia, and the Soviet Union were also involved in PCB production. Over 106 million pounds of PCBs were produced in the industrial countries of the world in 1971 (Karim, 1976).

Environment Contamination

Polychlorinated biphenyls are highly persistent environmental contaminants that bioaccumulate in food chains and produce direct, acute, and chronic effect on fish, birds, and mammals (Hutzinger et al., 1974). PCBs are transported throughout the ecosystem both aquatically and terrestrially. Industrial leakage, the flushing or international dumping of waste PCBs and destruction of materials containing PCBs

are known as primary sources of entrance of PCBs into the environment (Heath et al., 1972; Hesse, 1976; Wessel, 1976). PCB contamination of animal feed through heat exchange fluids, hydraulic fluid, plastic wrappers, paints, and sealants used on silo walls has been reported by many investigators. Richardson and Waid (1982) have explained the major routes of environmental entry of PCBs as follows:

- Vaporization, from open burning or incomplete incineration of solid wastes, and from paints, coatings, and plastics.
- Leaks and disposal of industrial fluids at sea or on land.
- Disposal in dumps and landfills, leaching, and volatilization into the environment from these dumps.
- Inclusion in pesticides.

Safe et al. (1987) reported that evaporation and exposition are the most important routes for the long range transport of polychlorinated hydrocarbons.

The presence of PCBs in air, water, and sediments of drainages in the United States and Puerto Rico has been reported by Kutz and Young (1976) and Dennis (1976). Kleinert (1976) reported that Great Lakes contain more than 100,000 pounds of PCBs solution (10 pptr) and probably much more than this amount in the sediments. The major discharge of PCBs from Wisconsin's drainage (about two pounds per day) to Lake Michigan was the wastewaters of pulp and paper mills

that recycle wastepaper. Nisbet and Sarofim (1972) estimated the presence of 84-100 tons of PCBs in water and sediments of Lake Michigan. They also mentioned that 4 to 5 x 10^3 tons/year of PCBs were discharged into fresh and constant waters in the peak year of PCB use. Shea (1973) estimated 353.8 million kg of polychlorinated biphenyls have entered the environment by various routes, but the majority of them has been directly dumped into aquatic environments by industries. Therefore, this is an important route in the PCB contamination of fish and other aquatic organisms. The total introduction of PCBs to the environment of North America from 1930 to 1970 has been estimated to be: atmosphere 30,000 tons; water (fresh and coastal) 60,000 tons; dumps and landfills 300,000 tons. World-wide environmental distribution of PCBs at that time was about 750,000 tons (Richardson and Waid, 1982).

PCBs have been rarely detected in agricultural soils, but they appear to be more prevalent in urban and industrialized areas. The most common encountered PCB was Arolcor 1254 (Carey and Gowen, 1976). The effect of PCBs on terrestrial plants is minimal. Even at high concentrations, no abnormalities or signs of chlorosis appear. However, the grazing of animals on these PCB-laden plants poses a serious threat to the environment (Clesceri, 1984).

PCBs have been isolated from fish, wildlife, domestic animals, rivers, lakes, oceans, and their sediments, aquatic

and marine flora, air, rain, food, feed, animals and human fats, milk, eggs, poultry, meat, and many other samples (Safe et al., 1987).

The public health significance of PCBs was brought to the front when over 1,000 Japanese were contaminated due to consumption of PCB-exposed rice oil. Clinical manifestation of the disease was called Yusho disease (Allen, 1975). Human exposure of the population to PCBs is limited to food, air, water, consumption of fresh-water fish, and to occupational exposure in industrial workers (Cordle et al., 1982).

Large amounts of PCBs still remain in the environment, in materials containing PCBs still in service (e.g. transformers), in landfills and dumps, in water and the bottom sediments of water bodies, in the soil and in the air. It is expected that land disposal of obsolete electrical equipment will constitute the primary source of PCB entry to the environment in the future (Gold, 1983).

Regulation

After the "Yusho" incident in Japan future productoin and import of polychlorinated biphenyls was prohibited in that country from January, 1972. Sweden banned the use of PCBs about the same time. When information became available regarding the health hazards of PCBs, use of these materials was discontinued in the U.S., except in closed electrical systems. In 1972, the sole American PCB producer voluntarily restricted the sale of PCBs to use only in closed electrical

systems. In February 1973, the Organization for Economic Cooperation and Development (OECD) announced their decision to prohibit the use of PCBs for industrial or commercial purposes except in certain closed systems (Train, 1976). The tolerance levels of PCBs in animal products including fish, eggs, dairy products, meat and poultry was regulated by FDA in 1973. Monsanto Company voluntarily stopped all of its PCB production in 1977. On July 2, 1979 production of PCBs was banned in the U.S. under the Toxic Substance Control Act. On August 25, 1982 a regulation was published by the Federal Register which prohibited the use of PCB transformers after October 1, 1985, because of their exposure risk to food or feed (Russo, 1983).

Although the production and sale of PCBs have been banned in the U.S., high concentrations of these compounds are still around in dump sites, fish, and wildlife. Also, workers who are responsible for the removal of PCB-containing equipment from the workplace may continue to be exposed to PCBs (Seegal, 1986). Hutzinger et al. (1985) reported that of the 1.4 billion pounds of polychlorinated biphenyls that have been produced in the U.S., it is estimated that 750 million pounds are still in use in some form, mostly in transformers and capacitors. Therefore, control of PCBs remaining in the environment should be considered to minimize the risk of contamination. The regulatory steps are of three types.

1. Reduce the flow of additional PCBs into the environment.

2. Reduce stocks of PCB in the environment.

3. Control Exposure of the population to PCBs.

Kopp (1975) described that high temperature incineration is the most effective way to completely destroy PCBs. The American National Standard Institute (ANSI) (1974) recommended the use of cement kilns of temperatures of 2200-2600°F for 16-18 seconds as being sufficient to reduce the PCBs.

PHARMACOKINETICS

Absorption

Tonabe (1981) reported that there is a relationship between molecular size of polychlorinated biphenyls and their rate of absorption by the host. As the number of chlorine atoms substituted in biphenyl molecule increased, the absorption rate decreased.

Metabolic studies in monkeys demonstrated that after the oral administration of PCBs to monkeys over 90% of the compound was absorbed by the gastrointestinal tract, and then distributed in high lipid content organs including the adipose tissue, skin, adrenal gland, ovaries, and the brain. The hydroxylated metabolites which formed in the liver were largely excreted through the biliary and in lesser amounts through the urinary tract. Lower chlorinated compounds were metabolized and eliminated faster than the higher chlorine metabolites. Approximately 75% of the biliary materials were reabsorbed by the gut and the non-absorbed material was recovered from the feces. Over 90% of the bile components were water soluble (Allen and Norback, 1976).

Results of an experiment concerning dermal absorption of PCBs in guinea pigs and rhesus monkeys showed that PCBs are rapidly and extensively absorbed through the skin, then distributed throughout the body and later slowly eliminated from the animals. Washing of the skin after contamination with PCBs could not remove all of the chemicals. An extensive washing immediately after dermal application of Aroclor 1242 only removed about 60% of the PCBs, the other 40% remained available for systemic absorption. A post 24-hour wash failed to remove any PCB (Wester et al., 1983).

Following oral administration of a PCB mixture of Aroclor 1254 to quail, all the components in the mixture were absorbed in equal proportions, but they were excreted at greatly different rates (Walker, 1976).

Distribution

There are several factors which affect PCB bioaccumulation. These include: concentration in the surrounding environment, duration of exposure, temperature, solubility of the pollutant, species, age, weight, feeding habits and lipid contents, trophic level variation and adsorption (Richardson and Waid, 1979; National Research Council, 1979).

Because of high lipid solubility and low water solubility polychlorinated biphenyls tend to accumulate and concentrate in the adipose tissue rather than being rapidly metabolized and excreted. The more highly chlorinated PCB homologs, which tend to be more chronically toxic are retained longer (Allen et al., 1974).

Hexachlorobiphenyl was found in the highest concentration of accumulation in human tissue after exposure to PCBs (Mathews and Anderson, 1976). Ringer et al. (1972) reported that PCBs seem to be more highly concentrated in the brain than in any other organs. Apparently the lipid soluble nature of PCBs causes it to concentrate in fat deposits and in tissue with a high lipid content. The high lipid content of the brain is the major reason for the very high PCB concentration found there. A significant level of PCBs has been found in the adipose tissues of humans as well. Price and Welch (1972), and Yobs (1972) estimated that levels of 1 ppm PCBs or more exist in 30 to 45% of the general U.S. population, In a few cases, levels as high as 200 to 600 ppm have been reported. In experimentally dosed birds, highest PCB levels were accumulated in adipose tissue, followed by kidney, liver, brain, muscle, and blood (Dahlgren et al., 1972: Lincer and Peakall, 1973). Experiments conducted by Richardson and Waid (1982) concluded that in mullets, most of the PCBs were retained in the head, bone, and gut of the fish with a large portion also being found in the liver. In dogfish, after intravascular injection of radioactivated PCB, approximately all of the dosed radioactivity was found in the liver until day 12 of the experiment. Small amounts were excreted in bile and trace amounts in urine (Bend et al., 1976). An accumulation of radioactive labeled PCBs in the bone marrow of monkeys and mice after intravenous injection was been reported by Beran et al. (1983).

Redistribution of PCBs from adipose tissue to the mammary glands during lactation in rats may be done by lower density lipoproteins (Vomachka and Vodicnick, 1984). In another experiment, Vomachka et al. (1984) reported that low density lipoprotein was a major transport vehicle for 2,3,5,2',4',5'-hexachlorobiphenyl in plasma of rats. Becker and Gamble (1982) have reported similar findings in the transport of PCBs to the cells by albumen and low density lipoproteins in humans.

Metabolism

Bailay and Bunyan (1972) reported that in pigeons and quails metabolism of polychlorinated biphenyls is dependent on the amount of chlorine in the molecule. They found that lower chlorinated isomers are metabolized faster than higher chlorinated compounds. They also reported half-life of PCBs in the fat of the quail and pigeon after cessation of dosing with Aroclor 1242 to be 50 days and with Aroclor 1254 to be 125 days. In chickens following the oral administration of 3,4,5,3',4',5'-HCB no metabolites were detected in the excreta. But in chickens which received 2,4,6,2',4',6'-HCB, the metabolites 2,4,6,2',3',4'-HCB and 2,4,6,2',3'-PCB were identified in feces (McKinney, 1976). Schnellmann et al. (1983) reported that 2,2',4,4',5,5'-hexachlorobiphenyl was not metabolized by the human liver, therefore it is the predominant PCB found in human adipose tissue.

Nebert and Felton (1976) reported that the regulatory and structural genes in mice may affect, directly or indirectly, the metabolism of xenobiotics. All of the highly chlorinated PCBs must be metabolized in order to be excreted through the biliary route (McKinney et al., 1985). The rate of metabolic attack on PCBs decreases with increasing chlorination (Berlin et al., 1975).

Matthews and Anderson (1976) injected polychlorinated biphenyls intravenously to rats. Fifteen minutes after injection approximately 90% of the given dose was removed from the blood and stored initially in the liver and muscles. Then it was redistributed and stored in adipose tissue and the skin. The results showed that the degree of redistribution and elimination of PCB compounds via bile and urine related to the degree and position of chlorine atoms in the biphenyl molecules. Only 10% of the excreted materials were as parent isomers, while about 90% were metabolites. Albro and Fishbein (1972) reported that less than 10% of the PCB fed to rats was recovered in feces. The rest of the PCB was absorbed and/or metabolized.

Excretion

The polarity of PCB metabolites is important in their excretion via bile or urine. These PCBs which are not metabolized are not readily excreted, and tend to concentrate in lipid-rich tissues such as adipose tissue (Matthews, 1983). In rats, bile has been recognized as a major route for the elimination of 1,4',5-trichlorobiphenyl (Bakke and Feil,

1983); 2,3,6,2',3',6'-HCB (Birnbaum, 1983); and 3,3',4,4'-tetrachlorobiphenyl (Clarke et al., 1983).

Sipes (1980) reported the elimination rate of 4,4'-dichlorobiphenyl in dose was 9 times higher than in monkeys. In an anesthetized dog, 33% of the dose was excreted into the bile in two hours. In the monkey the amount of excretion was 0.4%. In another study Sipes and his co-workers (1982a) demonstrated a clear difference in species in rate of metabolism of 2,3,6,2',3',6'-hexachlorobiphenyl and its subsequent excretion via the bile. Both dogs and monkeys eliminated the HCB as metabolites in the feces, but at different rates. The elimination constant for the dog was about three-fold greater than for monkeys. The dog excreted 26% of the dose into the bile in the first two hours, then 52% (feces 41%, urine 11%) during the first 24 hours, and 70% (feces 56%, urine 14%) in three days. The monkey excreted 2.4% of the dose in the first two hours, followed by 19% (nearly equal amounts in feces and urine) during the first 24 hours, and 61% (feces 46%, urine 15%) after 15 days. Sipes et al. (1982b) reported that dogs excreted 2,4,5,2',-4',5'-hexachlorobiphenyl (2,4,5-HCB) 10 times more rapidly than monkeys. Again, biliary excretion was the main route for removal of metabolites of 2,4,5-HCB from the bodies of dogs and monkeys. About 95% of the extracted materials in both animals was as metabolites. However, in the first 24 hours, monkeys excreted more than 50% of the compound as a parent dose.

Muhlebach and Bickel (1981) explained that 75% of the PCBs given to rats was stored in adipose tissue, and 18% was excreted mainly via feces. Urinary excretion was almost negligible. Abdel Hamid et al. (1981) found 3,4,3',4'-PCB excreted relatively fast in rats and monkeys. The major route of excretion was via the feces and mostly in the form of metabolites. The fecal excretion of 2,2',4,4',5,5'-hexachlorobiphenyl increased in rats after food restriction (25-50% diet) (Jondorf et al., 1983). In rats feeding 50% ad libitum, PCB residues declined to 38% and 7% of the dose in adipose tissue and skin, respectively, after 280 days. Fecal excretion was 43% and urinary excretion was 1.5% of the dose at this time (Wyss et al., 1986).

PCB may enter the gastrointestinal tract by back diffusion from the gut tissue into the lumenal contents. After PCB molecules are redistributed to other organs, such as adipose tissue, or metabolized and changed to hydrophilic material, then the concentration driving force into the gut contents is diminished and the mechanism of entry into the feces is reduced (Lutz and Dedrick, 1983). Yoshimura and Yamamoto (1975) found that unchanged 2,4,3',4'-tetrachlorobiphenyl was excreted through the intestine, when it was intravenously injected in rats with the bile duct ligated, while no metabolite of this isomer was excreted by this route. Kurachi and Mio (1981) suggested the use of active charcoal to accelerate the excretion of PCBs from contaminated animals.

McCormack et al. (1979) reported that the rate of PCB excretion via milk in lactating rats, was higher than PBB excretion, indicating that this route of excretion may be more important for PCBs. In mink, the elimination of PCB compounds through the milk has been reported by Aulerich et al. (1973) and Aulerich and Ringer (1977).

Transplacental passage of PCBs has been reported in humans and different species of animals including cattle, rats, mice, rabbits, and rhesus monkeys (Bleavins, 1980). In Japan after the "Yusho" incident, babies (occasionally still-born) born by PCB-poisoned mothers, showed abnormal characteristics. This indicates that PCB compounds are to pass through placenta and affect fetal life (Taki et al., 1969). Kuratsune (1976) reported symptoms of PCB poisoning in the neonates which received milk from PCB-contaminated mothers.

Kashimoto et al. (1981) reported that the major part of PCBs ingested by the "Yusho patients" were eliminated from the bodies, except the PCBs with longer retention time.

PCB TOXICITY IN ANIMALS

There is a considerable difference in the sensitivity of various species of animals to polychlorinated biphenyls. However, in most cases, young animals are more sensitive than adults, and females more sensitive than males. In general, chickens (and possibly other avian speices), guinea pigs and mink, are the most sensitive species of animals to intoxication with these classes of chemicals. In contrast

hamsters and amphibians appear to be fairly resistant to the toxic effects.

Commercial PCBs produce a wide variety of biological effects in animals. The most important of these include enzyme induction and inhibition, decreased reproductive efficiency changes in liver morphology, changes in plasma lipid concentrations, hepatic porphyria, decreased immunocompetence, dermatological effects, fetotoxic effects, tumor promotor, and carcinogenic effects (Neal, 1985; Zimmerman, 1982).

Hematological changes that occurred in all species of animals studied at high (lethal) exposure levels, appear to be directly related to lesions in the bone marrow (atrophy). In acute studies, thrombocytopenia, lymphopenia (Zinki et al., 1973), and anemia (McConnell, 1980) were reported in several species of laboratory animals.

PCB contamination of animals and humans is world-wide in distribution. The following are examples of species affected.

Humans

In response to PCB poisoning several adverse effects have been observed in humans. PCB exposure of industrial workers has been reported by Jones and Alden (1936), Schwartz (1936), and Meigs et al. (1954). Most human exposure to PCB in diet was in a PCB-contaminated fish, although there was some human exposure via other routes, such as milk, meat, and by inhalation from the air (Nisbet, 1976).

In 1968 in Yusho, Japan, over 1,000 people experienced PCB poisoning after consuming rice oil contaminated with Kanechlor 400 (Crow, 1970; Kuratsune et al., 1972). A few years later a similar accident occurred in Taiwan in which about 1,200 people were poisoned by consuming PCB contaminated rice oil (Chen et al., 1984).

The lowest total dose of Kanechlor producing an effect in the Japanese in the "Yusho incident" was 500 mg, and the average was 2000 mg (Highland, 1976). From three compounds, PCB, polychlorinated quaterphenyls (PCQs), and polychlorinated dibenzofurans (PCDFs) which were identified in the rice bran oil, that caused "Yusho" disease and in several tissues of Yusho patients, PCDFs were considered to be the main causative agents of pathological lesions (Nagayama et al., 1976, 1977; Kunita et al., 1984). The last authors explained that administration of a PCDF mixture consisting of PCDF compounds similar to those in the Yusho oil to cynomolgus monkeys produced similar dermal lesions in the monkeys, but administration of PCBs or PCQs alone failed to cause such lesions in the monkeys. Chen et al. (1984) reported that PCDF 2,3,4,7,8-pentachlorodibenzofuran was retained in the liver of a patient who was contaminated in the PCB incident in Taiwan. They explained that the PCDF compound may have had an important role in the etiology of the PCB poisoning in Taiwan. These authors also indicated that 3,4,3',4'-tetra-chlorobiphenyl (which is one of the most toxic PCBs) was

found in the toxic oil and one of the patient's blood and adipose tissue at an early stage of poisoning. Bickers et al. (1972) reported that toxicity of a PCB mixture positively related to the percentage of chlorine atoms present in that molecule.

Cattle

The presence of PCBs in milk constitutes an important health hazard because of the infancy of animals or humans consuming the contaminated milk, their strong lipophilic properties, the low elimination rate, and consequent strong tendency to accumulate in the body.

Platonow and Chen (1973) administered a single oral dose of Aroclor 1254 at 100 mg/kg of body weight to a Jersey cow. After 10 days, this cow was euthanized. During necropsy, a 2½-3 month-old normally developed male fetus was found. The researchers analysed the concentration of PCBs in various maternal and fetal tissues. They determined that concentrations of PCBs were higher in maternal than in the fetal tissues, except in the kidney where the opposite was noted. Brain PCB concentration of the cow was slightly higher than in the same organ of the fetus. However, the maternal liver and heart concentrations were three-fold higher than those in the fetus.

In 1969 in the U.S., PCBs were detected in milk of cows given feed treated with PCB mixed with herbicides. A year later in the same country, an occurrence of PCBs in milk

from cows given feed that had been stocked in silos coated with PCB-based products, was reported (O.E.C.D., 1973). In an accident in Kansas, steers were exposed to PCB compounds from a contaminated feedlot. Clinical symptoms such as anorexia, dyspnea, thirst, diarrhea, abdominal pain, and sternal recumbency were seen in the exposed animals (Robens and Anthony, 1980).

Sheep

Reduction of feed efficiency and weight gain in sheep after receiving low level PCB contaminated feed for a long time has been reported by Hansen et al. (1976b).

Pigs

Oral administration of polychlorinated biphenyls at 25 mg/kg body weight to the pigs caused inappetence, hemorrhagic diarrhea, retarded growth, incoordination, coma, and death (Miniats et al., 1978). Feeding PCB-contaminated diet to sows during nursing caused hypertrophy of the liver and erosion of the stomach. Slight atrophy of the spleen and thyroid gland were seen in pigs from treated sows (Hansen et al., 1975).

Dogs

A study by Sipes and Schnellmann (1987) reported that dogs eliminated PCBs more rapidly than other species, possess higher levels of a constitutive isozyme of cytochrome P450 with greater activity towards the ordinarily, slower metabolized 2,2',4,4',5,5'-hexachlorobiphenyl.

Following oral administration of PCB to dogs, reduction of body weight, increased liver weight, elevation of serum alkaline phosphatase, diffuse hyperplasia of the intestinal cells of the testes, and aspermatogenesis were observed (Wheeler, 1971).

Mink

The embryotoxic effects of polychlorinated biphenyls in mink have been reported (Platonow and Karstad, 1973; Aulerich and Ringer, 1977). No teratogenic effects of PCBs were reported in these studies.

Dietary dosages of one ppm Aroclor 1254 in mink reduced reproductive success. At 5 and 10 ppm no reproduction occurred. Ten ppm of PCB in the diet killed five of the six adults. Mixtures of PCBs at 30 ppm killed all of the adult mink (Aulerich et al., 1971). In another study, Ringer et al. (1981) found that PCB transfer via the milk to the newborn mink was greater than placental transfer. They also indicated that ferrets were less sensitive than mink to PCB mixtures.

Oral administration of Aroclor 1254 to mink caused gastrointestinal hemorrhage, hepatic fatty change, hepatic necrosis, cerebral edema, disseminated intravascular coagulation, and fibrosis of coronary arteries (Platonow and Karstad, 1973). Gillette et al. (1987) described severe necrosis of the epithelium of the small intestine with villous atrophy and fusion. Significant increases in smooth endoplasmic reticulum of hepatocytes, and symptoms such as

diarrhea, melena, and rough hair coat in mink which received PCB-treated diet.

Gastric ulceration in PCB-fed mink has been reported by Aulerich et al. (1973). Shull et al. (1982) indicated that Aroclor 1242 was a more potent inducer of liver microsomal mixed function oxidases than Aroclor 1016.

Rabbits

Vos and Notenboom-Ram (1972) administered Aroclor 1260 topically to rabbits upon a small shaved area of approximately 5 x 10 cm. After four weeks of Aroclor 1260 (20 applications of 120 mg), the rabbits showed chloracne clinical symptoms. Definitive hyperplasia and hyperkeratosis of the follicular epithelium of the skin were observed under a microscope. Liver weight was increased and histological degeneration of cell membranes, decrease of cytoplasmic glycogen, and damage of endoplasmic reticulum in hepatic cells were seen. In this experiment, in addition to the proliferative changes of the endoplasmic reticulum, degenerative changes of the hepatic cells was also reported.

Grant et al. (1971) explained placental transfer of PCBs in rabbits when administered orally during gestation. Abortions, maternal death, and stillbirths occurred in PCB-contaminated rabbits. Reduction of litter size in PCB-given pregnant rabbits was reported (Villeneuve et al., 1971). Necrosis of liver with destruction of the rough

endoplasmic reticulum in hepatic cells of PCB-received rabbits was seen (Koller and Zinki, 1973).

PCBs produce lymphoid atrophy and suppress humoral immune responses to several antigens in rabbits (Street and Sharma, 1975; Koller and Thigpen, 1973).

Guinea Pigs

McKinney et al. (1985) demonstrated that 2,4,5,2',4',5'-hexachlorobiphenyl is a relatively nontoxic compound, but 3,4,3',4'-tetrachlorobiphenyl is the most toxic PCB isomer in guinea pigs. Hedman et al. (1985) reported deterioration in the placental function by reducing its blood flow in guinea pigs dur to PCBs.

Rats

Following oral administration of Aroclor 1254 to Fischer rats, a decline of food intake, loss of body weight, atrophy of spleen and testes, enlargement of liver and kidney, and no weight change of lungs were reported by Carter and Mercer (1983). A significant increase in liver weight and a significant decrease in thymus weight in rats after receiving 3,3',4,4'-tetrachlorobiphenyl were observed (Clarke et al., 1984). Itokawa et al. (1976) explained enlargement of liver with dark-brown pigmentation in rats which were fed a PCB-mixed commercial diet.

Kimbrough (1974) reported ulceration of gastric and duodenal mucosa in rats after a single oral dose of 300 mg/kg PCBs. The incidences of stomach lesions which were

histologically identified as intestinal metaplasia characterized by architecture resembling that of crypts and goblet cells were seen in PCB-exposed rats (Morgan et al., 1981). Zimmerman (1982) reported that Aroclor 1260 and Kanechlor 500, 400, and 300 were carcinogenic in rats, producing tumors and hyperplastic nodules in liver. The neurological effect of PCBs in rats has been explained by Seegal et al. (1986).

Reproductive failure in female rats of Wistar strain following daily oral exposure to 30 mg/kg Aroclor 1254 for 1 month (Brezner et al., 1984), destruction of intestinal mucosa in infants, accumulation of blood in the digestive tract and amniotic fluid, a decrease of fetal size, and high late fetal death were seen in Sprague-Dawley rats after oral administration of 3,3',4,4'-tetrachlorobiphenyl at 1-10 mg/kg on days 6-18 of gestation (Waldell et al., 1982) or 5 mg/kg for 21 days (Clarke et al., 1984). When Gellert (1978) administered PCBs to female rats, he found there was an association between the estrogenic activity of the PCB and its effectiveness in altering neuroendocrine differentiation and induction of premature reproductive aging. Sager (1983) reported that seminal vesicle weights in PCB-exposed rats were significantly less than controls.

Alterations in liver fine structure in the rat following exposure to 3,4,3',4'-tetrachlorobiphenyl has been reported by Harris and Bradshaw (1984). They explained large increases

in the number and size of smooth endoplasmic membranes, extensive proliferation of smooth vesicles, and increase in rough endoplasmic membranes which were often distended and tended to encircle mitochondria or form large lamellar stacks of the cell periphery, and shrunken and condensed mitochondria with an atypical distribution of cristae in the liver of newborn pups. After oral administraion of PCBs to the rats, development of large membraneous concentric arrays, proliferation of SER, and an increase in lipid droplets within the cytoplasm of liver cells were detected (Allen and Abrahamson, 1973).

Induction of hepatic microsomal aryl hydrocarbon hydroxylase (AHH), and ethoxy resorufin o-deethylase (EROD) in immature male Wister rats after i.p. injection of 4-8 different doses of each compound 3,3',4,4',5-penta- and 3,3',4,4',5,5'-hexachlorobiphenyl have been reported by Leece et al. (1985). Kamohara et al. (1984) indicated that a decrease in glutathione peroxidase probably is the major reason for the increase of lipid oxidation in PCB-poisoned rats.

Serum levels of cholesterol, urinary ascorbic acid, norepinephrine, epinephrine, dopamine, and histamine were significantly increased in rats given PCBs (Nagaoka et al., 1986; Quazi et al., 1983). The binding of a 3,4,3',4'-tetrachlorobiphenyl to transthyretin (TTR) which interferes with the formation of the plasma protein complex carrying

both retinol and thyroxin reduces serum vitamin A transport in rats (Brouwer and Berg, 1986). In a study by Ebner et al. (1987), nitrogen deficiency due to increased excretion of urinary nitrogen in PCB-treated rats was reported.

Mouse

Aroclor 1254 and Kanechlor 500 are carcinogenic in mice. Both induce benign and malignant tumors in liver after oral administration of 300 mg/kg diet/11 months of Aroclor 1254 or 500 mg/kg/32 weeks of Kanechlor 300, 400, or 500 (Zimmerman, 1982). Atrophy of thymus in C57BL/6J and DBA/2J inbred mice after a single injection of 1500 μmol/kg 2,3,4,5,-tetrachloro- and 2,3,3',4,4',5-hexachlorobiphenyl has been reported by Robertson et al. (1984). Oral administration of 10 mg/kg polychlorinated biphenyls and tetrachlorodibenzo-p-dioxin in pregnant C57BL/6N mice caused ten-fold increase in cleft palates (Birnbaum et al., 1985).

Adult female mice after receiving PCB-contaminated milk (Clophen A 60, 20 mg/kg body weight), when mated, demonstrated a significant decrease in the frequency in implanted ova (Kihlstrom et al., 1973). This study shows the estrogenic effect of PCBs. In female mice, the estrous cycle length increased and the number of ova implanted following mating was reduced after receiving 0.025 mg Clophen A 60 (The European equivalent of Aroclor 1260) in 0.1 ml peanut oil/day (Orberg et al., 1972; Orberg and Kihlstrom, 1973). Orberg and Lundberg (1974) demonstrated the detrimental effect of Aroclor 1260 on secondary sex glands in male mice. Sanders and Kirkpatrick

(1975) reported a reduction of seminal vesicle weight and spermatogenesis in PCB-treated mice.

Certain isomers of PCB molecules cause immunosuppression in mice. This is dependent on activation of aromatic hydrocarbon (Ah) receptors by the PCB compounds. For example, isomers: 3,3',4,4'-TCB and 2,3,3',4,4',5-HCB, which are ligands for the Ah receptor and also induce hydrocarbon hydroxylase (AHH), produce immune toxic effects in B6 mice. While compounds 2,2',4,4'-TCB and 2,2',5,5'-TCB are not immunosuppressive.

Konat and Clausen (1973) reported that feeding 1500 ppm Aroclor 1254 to mice caused proliferation of liver endoplasmic reticulum, increased mixed function oxygenase, and enhanced liver microsomal cytochrome P-450 complex.

Avian

Some studies on the effects of PCBs in birds have appeared in the literature. In 1962, McCune and his co-workers explained symptoms similar to "chick edema" or "toxic fat" syndrome in chickens which were housed in wet epoxy-resin painted batteries. In 1970, 140,450 chickens were destroyed in New York State after showing high levels of PCB residues in their tissues (26.8 ppm on a fat basis). In 1971 in North Carolina, due to contamination of fish meal which was used for poultry feed, 123,000 pounds of egg products and 88,000 chickens were destroyed. Again in 1971 after detection of PCBs (20 ppm) in a turkey production company (Swift, USA)

approximately one million birds were kept from market (Wessel, 1976).

The toxicity of PCB materials increases in mallards, pheasants, bobwhite quail, and Japanese quail with the percentage increase of chlorine atoms, while an opposite effect is seen in domestic chickens. Based on the percentage of chlorination and duration of feeding, LC50 toxicities for birds appear to be between 100-400 ppm (Osweiler et al., 1985). The lack of effects of two ppm of dietary PCB on chicken reproduction was studied by a 39-week feeding test with Aroclor 1254 (Platonow and Reinhart, 1973). The scientists showed that 5 ppm Aroclor 1254 reduced egg production erratically over the 39-week period. With 50 ppm egg production fell sharply, hatchability dropped, and mortality occured. PCB residues in eggs was reduced after six weeks of clean food and hatchability returned to control values. Lillie et al. (1975) reported that the hatchability of eggs laid by White Leghorn pullets was decreased significantly after feeding 10 or 20 ppm Aroclor 1232, 1242, or 1248. In an experiment by Vos and Koeman (1970), they found that domestic chickens demonstrate an acute susceptibility to Aroclor intoxification. Liver enlargement occurs at dosages ranging from 100-10,000 ppm of Aroclor 1242, and mortality occurs at rates above 200 ppm. However, Aroclor 1258 causes mortality at dosages above 100 ppm and partial mortalities at dosages above 30 ppm when fed in a

diet for 4 to 5 weeks. One hundred percent mortality occurs in chickens fed Aroclor 1254 above 250 ppm. Hansen et al. (1976a) reported that PCBs (Aroclor 1242 and 1254) at 20 ppm did not affect growth rate or feed efficiency in broilers between one and nine weeks of age.

Lethal doses of polychlorinated biphenyls for birds are dependent on the species and age of the bird. The method of administration, the degree of chlorination, position of chlorine atoms, and the presence of impurities (Vos, 1972; Parkinson and Safe, 1987). The results of a study by Kimbrough (1974) indicated that the lower chlorinated biphenyls affect the reproductive system of birds more than the higher chlorinated biphenyls. In 1987, Hansen found that birds tend to be less resistant to higher chlorinated PCBs while rats and fish tend to be less resistant to lower chlorinated PCBs. Biocca and his co-workers (1976) studied the effect of different doses of 3,4,5,3',4',5'-hexachlorinated biphenyl (HCB), 2,3,4,2',3',4'-HCB, 2,4,5,2',4',5'-HCB, 2,3,6,2',3',6'-HCB, and 2,4,6,2',4',6'-HCB in cockerels and mice. They found that 3,4,5,3',4',5'-HCB was the most toxic isomer in both species. Moore et al. (1976) indicated the importance of tetrachlorodibenzo-dioxin in toxicity of 3,4,5,3',4',5'-HCB to chickens and mice. Brunstrom and Darnerud (1982) explained that the toxicity level of 3,3',4,4'-tetrachlorobiphenyl (3TCB) to chick embryos was 10,000 times higher than 2,2',4,5'-tetrachlorobiphenyl (2TCB).

PCBs stored in the adipose tissue of contaminated birds is readily mobilized during periods of stress that cause loss of body weight. Alternative starving and feeding of pheasants dosed with PCBs increased the toxicity of the PCBs (Dahlgren et al., 1972b). Birds that died from starvation had mobilized their fat reserves and had considerably higher PCBs in the brain and other tissues than did PCB-treated birds that were not starved.

Symptoms of the toxicity and pathological alterations in chickens due to polychlorinated biphenyls have been investigated by many scientists (McCune et al., 1962; Rehfeld et al., 1971; Harris et al., 1976; McKinney et al., 1976; Osweiler et al., 1985). Following are the important changes in chickens after exposure to PCB compounds: weight depression, gasping for breath, depression of secondary sexual characteristics, droppiness, ruffled feathers, defeathering, dermatitis, weakness, tremor, ataxia, generalized edema (accumulation of fluid around the heart, lungs, abdominal tract, and subcutaneous tissues including head and neck), internal hemorrhaging, increase in liver size as percentage of body weight, involution of thymus, atrophy of spleen, tubular changes of kidney, enteritis, cream-colored pancreas, enlarged adrenal gland, and increase of thyroid weight in the presence of low vitamin A diet (Spear and Moon, 1986). Firestone (1973) indicated that PCB-contaminated birds may show a terminal increase in body weight due to accumulation

of body fluids (subcutaneous edema, ascites, hydrothorax, and hydropericardium). Mechanism of action for generalized edema could be because of the increasing cell permeability with PCBs which affects the heart, resulting in primary hydropericardium, then pulmonary and systemic edema as a secondary change to decrease cardiac function (Flick et al., 1965; Osweiler et al., 1985).

McKinney et al. (1976) explained the microscopic changes in PCB-exposed chickens as: (1) marked depletion of lymphocytic cell types in the spleen and thymus; (2) congestion, mild necrosis and marked fatty infilteration in the liver, dilation of sinusoids containing blood; (3) congestion of mucosal surface of small and large intestine; (4) congestion and hemorrhage in the heart and thymus with infiltration of lymphocytes and heterophils on the pericardial surface; (5) marked degeneration, necrosis, fibrinoid and cystic changes, purulent exudation in the mucosal layer of the gizzard; (6) necrosis, cystic changes filled with mucus, and proliferation of reticuloendothelial cells in the lymphoid follicles of the bursa fabricius.

Hepatic histological changes and induction of mixed function oxidases by 3,4,5,3',4',5'-HCB were three to four times more severe than by 2,4,5,2',4',5'-HCB in chick embryos (Rifkind et al., 1983). In 1970, Vos and Koeman reported that oral administration of commercial PCB to chickens caused hepatic-type porphyria, which was characterized by excretion

and heptaic accumulation of uroporphyrin (Goldstein et al., 1974).

Blood changes such as depression in hemoglobin levels and packed cell volume in chickens fed PCB-contaminated diet has been reported by Rehfeld et al. (1972a). They indicated that as the amount of dietary PCBs was increased, the percentage of stored PCBs was elevated. Iturri et al. (1974) found significant reduction of heart rate in chickens due to Aroclor 1242 and Aroclor 1254 at $\geq$ 100 ppm. These authors mentioned a significant decline of hemoglobin concentration, HCT, and total erythrocyte in chickens exposed to Aroclor 1254 at $\geq$ 150 ppm. The same parameters were not affected by Aroclor 1221 or 1260 at any level used. Friend and Trainer (1970) reported that mallard ducklings that were fed PCB-mixed diet showed higher mortality after challanges with duck hepatitis virus than control birds. This is because of the immunosupression effect of PCB compounds.

PCBs can induce a variety of enzymes such as mixed function oxidases, demethylases, deethylases, enzymes involved in carcinogenesis and mutagenesis, metabolism of drugs, steroids, and other xenobiotics (Goldstein, 1980; Zimmerman, 1981; Poland and Knutson, 1982; Rifkind and Muschick, 1983). Mirando et al. (1987) reported that oral administration of PCB compounds to Japanese quail caused a significant increase in porphyrin content, significant increase of cytochrome P450, and delta-aminolevulinic acid synthetase (ALA-S) activity

in the small intestine and liver. A study by Goldstein et al. (1976) reported that 2,3,4,2',3',4'-HCB and 2,4,6,2',4',6'-HCB were the most active inducers of drug metabolism, 2,4,5,2',-4',5'-HCB was intermediate, and 2,3,6,2',3',6'-HCB was a poor inducer. Ehrich and Larsen (1983) reported that Aroclor 1254 was a more selective inducer of aniline hydroxylase activity in hens than in mice and rats.

Enzyme cytochrome P450 is the terminal oxidase of the microsomal electron transport pathway. Its mediated reactions are important for detoxification of xenobiotics, but it can also lead to activation or more toxic, mutagenic or teratogenic metabolites. Mercurio and Nemeth (1983) found cytochrome P450 and xytochrome B_5 to be present in five-day old chick embryo livers and to increase rapidly in concentration until day 11 of incubation. No difference has been found between the sexes concerning the activity of the hepatic cytochrome P450-mediated metabolism in chick embryos or newly hatched chicks (Drummond et al., 1972; Rifkind et al., 1979).

In birds, accumulation of a high dose of PCBs causes disruption of the normal breeding behavior and formation of thin-shelled eggs (Risebrough et al., 1968; Monsanto, 1970). Alteration of calcium metabolism in different avian species, resulting in thin egg shells, dead embryos, and subsequent reproductive failure have been reported by many investigators. The normal calciferol-mediated increase in intestinal calcium transport was significantly inhibited

by PCB pretreatment (Wong et al., 1974). PCBs interfere with two pathways that affect the shell thickness of birds' eggs: (1) they induce steroid hydroxylases and, therefore, lower the level of the steroid hormone estrogen, and decrease the calcium level in birds; (2) by interferring with the carbonic anhydrase system, the amount of calcium which flows in the blood stream to the oviduct decreases (Clesceri, 1984). Egg shell thinning in chickens (Nowicki and Norman, 1972), Japanese quail, and mallard ducks (Haegele and Tucker, 1974) due to PCBs, while no egg shell defects were detected in ring doves given Aroclor 1254 (Peakall, 1971). In chickens, dietary levels of as little as 10 ppm of Aroclor 1242 and 100 ppm of Aroclor 1254 caused thinning of egg shells, reduced egg production, and reduced hatchability (Keplinger et al., 1971). A report by Scott et al. (1975) indicated that PCB caused a decrease of egg production and decline of hatchability in chickens, but not in Japanese quail. Exposure of White Leghorn cockerels and pullets to PCBs enhanced the hepatic metabolism of several steroid sex-hormones (Nowicki and Norman, 1971). Platonow and Funnell (1971) reported that the size of combs, wattles, and testicles in male chicks that received 500 ppm PCB for 13 weeks, were smaller than controls. Aroclor 1254 produced a decline in sperm concentration but no change in semen volume in American Kestrels (Bird et al., 1983).

In the U.S., inadvertent leakage of PCBs into fish meal resulted in contamination of poultry feedstuffs consumed

by millions of chickens (Pichirallo, 1971). Hatchability of eggs from these chickens was noticeably reduced. The effects of PCBs on the reproductive system of chickens were investigated by Bush et al. (1974), Blazak and Marcum (1975), and Lillie et al. (1974).

The early maturation and inducibility of drug metabolism in chick embryos and the lack of material influence, make the chick embryo an important research tool for study of embryonic activition of xenobiotics. McLoughlin in 1963 demonstrated edema and beak deformation in chicken embryos after yolk sac-injection of 10 and 25 mg of Aroclor 1242 that caused 95 and 100 percent embryonic mortality, respectively. In a similar experiment by Brunstrom (1982) hatchability declined by 80 to 100% following the injection into eggs of 5 or 25 mg/kg Aroclor 1248.

Jensen (1966) identified PCBs in the feathers of eagles that had been taken from museum collections as early as 1944. Dekock and Randall (1984) reported PCB residues in eggs of coastal birds from the Eastern Cape, South Africa. In the U.S., PCBs were determined in the subcutaneous fat and breast muscle of fifty-five waterfowl collected in New York state during 1981 and 1982 (Kim et al., 1985). The mean PCB levels in fat were higher than in breast muscle on a wet weight basis.

Fish and Aquatic Organisms

Residues of PCBs in wildlife and fish samples in Holland, Germany, the Baltic Sea, the U.S.A., and many other countries in the world, have been reported (Koeman et al., 1969; Jensen et al., 1969; Nadeau, 1974). In the U.S.A., PCB contamination of sport fish and commercial fisheries in the Great Lakes (Hesse, 1976) and contamination of fish in the Hudson River of New York due to discharge of PCBs (approximately 30 pounds per day) from two General Electric plants (Nadeau and Davis, 1974) were reported. PCB residues in fish and aquatic invertebrates could be about 75,000 times of these compounds in water (Cordle et al., 1978). The major part of the human dietary intake of PCBs is from fish (Berglund, 1972; Hammond, 1972).

Polychlorinated biphenyls are toxic to freshwater fish and other aquatic organisms at very low levels and direct toxic effects are evident within a few hours, but much greater harm is caused by longer exposure at lower concentrations. Many aquatic invertebrates were affected at concentration ranges of 1 to 10 ppb PCB, including blue crabs, pink shrimp, and oysters (Duke et al., 1970). Hansen et al. (1971) found death in fish exposed to Aroclor 1254 at 5 µg/liter for 45 days. The results showed that the effect of PCBs was cumulative in fish and toxicity decreased with increasing

chlorination. Young fish appeared to be more sensitive to the PCB than their adults. A study shows that polychlorinated biphenyls such as Aroclor 1242 and 1254 are directly toxic to fish and aquatic invertebrates in the range of 0.1 to 10.0 µg/l (Nebeker, 1976).

Meyer et al. (1972) and Stalling and Mayer (1972) demonstrated effects of Aroclors 1242, 1248, and 1254 on aquatic organisms. They found that toxicology of these compounds increased at high temperature and at longer exposure periods but the toxicity decreased as the percentage of chlorine increased in amphipods against five PCB formulations, They also discovered that the most toxic PCB formulations appear to be those that contain four to five chlorine atoms. These predominated in Aroclor 1248, which was found to be the most toxic of all the Aroclors tested on fish.

Zimmerman (1982) explained that the toxicity of PCB compounds is dependent on the number of chlorine atoms located in positions 2 to 6 and/or 2' to 6'. Aroclor 1254 was found to be extremely toxic to immature pink shrimp of which more than 50% died during 15 days of continuous exposure to 0.94 mg/l (Nimmo et al., 1971). In another experiment, Duke et al. (1970) found that administration of Aroclor 1254 at 100 ppb for 48 hours was lethal for shrimp. Crustaceans and mollusks are extremely sensitive to PCBs.

Studies on plankton species in the marine environment have shown them to contain detectable levels of PCBs.

Zooplankton samples of the eastern continental shelf area were observed to contain PCB levels ranging between 0.07 and 3 ppm (Risebrough et al., 1972). Mosser et al. (1972) reported that Aroclor 1254 at 10-25 µg/liter inhibited growth of certain marine diatoms, but marine and freshwater algae showed more resistance. Toxicity of PCB compounds to a certain type of phytoplankton and diatom has been demonstrated by Fisher et al. (1972) and Keil et al. (1971). Wong and Kaiser (1975) reported that PCBs did not affect the growth of cultures of lake bacteria.

Pathological alterations were observed in the kidney, spleen, and liver of rainbow trout fed Aroclor 1254 at 10 or 100 mg/kg of diet for up to 330 days (Nestel and Budd, 1975). Ringer et al. (1972) reported that the administration of 10 µg/kg of Aroclor 1254 in coho salmon to mink caused 71% mortality in these animals.

The concentration of PCBs in fish tissues (whole fish calculated on a wet weight basis) should not exceed 0.1 micrograms per gram for the protection of fish-consuming birds and animals (Great Lake Water Quality Board, 1976).

Others

Lichtenstein et al. (1969) found that polychlorinated biphenyls were toxic to dipetran insects (flies), and toxicity increased as the percentage of chlorination decreased. In another expeirment by Rhee and Plapp (1973) in the house fly, the authors explained that Aroclor 1254 was a more

effective inducer of the microsomal enzyme aldrin epoxidase than Aroclors 1242 and 1221.

Treatment

PCB residues are gradually eliminated via by-products and waste materials from contaminated animals and humans. Low chlorinated PCBs are excreted much faster than highly chlorinated molecules. Urabe et al. (1979) reported that the skin symptoms of Yusho patients improved considerably and most of them, except those who were severely intoxicated, became symptom-free.

The treatment of PCB-poisoning in humans was dependent on the stage of poisoning and condition of the patient. If the PCBs were taken orally, emesis or lavage helps to remove the poisons from the digestive tract before further absorption. Then the use of sodium chloride cathertic or cholestyramine could be helpful. Cholestyramine combines with the bile salts (which are necessary for oil absorption) and inhibits PCB absorption. Giving barbiturates could stimulate hepatic enzyme systems and accelerate PCB hydroxylation. Most of the hydroxylated PCBs are excreted via bile into the intestine and eliminated by feces while part of them might be reabsorbed. Starvation could be helpful to remove the PCB residues from the fatty tissues if the patient is in sufficiently good condition to receive drastic therapy.

Experimental results (Rehfeld et al., 1972) showed that if growing chicks were accidently exposed to toxic levels

of PCBs for a short period of time, symptoms of toxicity of PCBs would be reversed or eliminated by feeding a non-contaminated diet.

In high doses of PCB contaminated birds, slaughtering and disposal of the animals are a more economical solution than maintaining the birds for long periods of time and feeding them a clean diet.

Polin et al. (1985) reported that a combination of feed restriction and colestipol were effective in reducing body burdens of PBBS in contaminated chickens. This method may be a useful technique for removing PCBs from contaminated birds.

SUMMARY

Polychlorinated biphenyls (PCBs) have been widely used in a variety of commercial products, including heat transfer agents, hydraulic fluids, plasticizers, adhesives, cutting oils, and inks. Because of their toxic effects in humans and animals, production and distribution of the chemicals have been banned in producer countries.

The material summarized here consists predominantly of more recently published information on toxic effects of PCBs in humans, monkeys, domestic mammals, avians, fish, and laboratory animals. There is some evidence that mink, chickens, and guinea pigs are more susceptible to the effects of PCBs than other animals. The effects observed include reproductive failure and death in mink, edema, embryotoxicity,

decrease of egg production in chickens, and congentivitis, skin pigmentation, and chloracne in primates.

There are some factors which affect determination of PCB function such as: type of PCB isomer, quantity of PCB used, species of the test animals, and duration of PCB exposure. In general, young animals may be more sensitive than adults and females and more sensitive than males.

STATEMENT OF THE PROBLEM

Chickens are considered to be one of the most sensitive animal species to polychlorinated biphenyls. They are, therefore, excellent models for describing the mechanisms of action of these substances and might point the way of possible harm to humans.

A reduction of body burdens of halogenated hydrocarbons in exposed animals due to feed restriction and dietary treatment with mineral oil were reported by many scientists, but the physiologic mechanisms of intestinal wall in excretion of the xenobiotics were not well understood.

Ulceration of the gastrointestinal mucosa and hemorrhagic enteritis in many species of animals after oral doses of PCBs have been reported by researchers. However, the effects of PCBs on the chicken's intestinal structure do not appear to have yet been investigated. It was thought that because of the pathotoxic effects of PCBs in various species of animals, they might alter the normal cellular structure of the intestinal wall after consuming the PCB contaminated diet.

MATERIALS AND METHODS

I. Experimental Methods

A. Husbandry

Two hundred and fifty seven White Leghorn Single Comb male chicks, 1 day of age, were obtained from a commercial hatchery and housed at Michigan State University Poultry Research and Teaching Center (PRTC). The chicks were individually wing banded, weighed, and raised in wire-floor, electrically-heated batteries using standard husbandry procedures. A starter-grower diet was fed for 21 days. Water and feed were provided ad libitum. At that time, they were weighed and sorted to obtain 168 chickens varying no more than approximately 5 grams in weight. They were divided into 2 groups of 48 chicks (controls) and 120 chicks (treatments). The chicks were transferred to growing batteries with wire floors and fed diets containing 0.2% chromic oxide (Cr_2O_3) and either 0 or 10 ppm Aroclor 1254 for 14 days. The controls and chickens fed PCBs were kept separate in two different houses to prevent any cross contamination. Diets and water were provided ad libitum.

B. Schedule

On the final day of treatment (day 0 of withdrawal), 16 controls and 40 chickens fed PCBs were randomly selected, weighed, and fasted overnight prior to transferring to the Department of Animal Science for necropsy or laboratory analysis.

When the chickens were brought to the Department of Animal Science (Anthony Hall - ATH), 8 controls and 20 chickens fed PCBs were randomly selected and sacrificed with excess CO_2 for whole-body analysis of PCB residues. Immediately after death, the carcasses were frozen and stored at -20°C.

The other 8 controls and 20 chickens fed PCBs were euthanized with an intravenous injection of sodium pentobarbital. The sodium pentobarbital reduced peristalic waves of the gastrointestinal tract thus preserving more of the contents in the intestinal segments. Necropsy of the euthanized chickens was performed immediately after the birds were dead according to the method employed by Schwartz and Bickford (1986). The loose skin between the medial surface of each thigh and abdomen was incised with a sharp knife. The legs were reflected laterally and the hip joints were disarticulated. The lateral skin and transverse skin across the middle of the abdomen were incised and reflected posteriorly, but the skin of the breast was reflected anteriorly. A longitudinal incision was made through the pectoral muscles on each side of the keel and over the costochondral junctions. The coracoid and clavicle bones were cut with heavy scissors. A transverse incision was made through the posterior part of the abdominal muscle on each side and the incision continued anteriorly through the costochondral junctions. The ventral abdominal wall and breast were removed as one piece.

After necropsy, the intestines were ligated at 5 different sites: the beginning and end of the duodenum, Meckel's diverticulum (attachment site of the yolk sac), the ileocecal junction, and the cloaca. The entire intestine was freed by cutting the mesenteric attachments and then removed after transecting the rectum. The duodenum was taken as the portion from the gizzard to the distal attachment of the pancreas; the jejunum from the duodenum to Meckel's diverticulum; the ileum from the diverticulum to the ileo-cecal-colic junction; the ceca and large intestine from the ileo-cecal-colic junction to the cloaca as described by Hurwitz et al. (1972). Each segment was cut separately and the contents were removed by manipulation and by gently washing with 0.9% saline solution through the lumen. The contents were collected into beakers, and immediately stored at -20°C.

For the electron microscopic study, the jejunum and large intestine of 4 controls and 6 PCB-chickens were quickly stretched out on a clean table and each one was entirely cut through the length with scissors. One-mm-long sections were taken from each of those segments. The tissue samples were immersed seperately in 2% glutaraldehyde solution fixative in small glass vials. All tissues for electron microscopy were placed in cold fixative within 3 minutes of the necropsy of the experimental animal (Hooper et al., 1979). After removing the entire intestines from the abdominal cavity, organs such as the liver, spleen, kidneys, heart, lungs,

etc. were examined for any gross pathological lesions. Finally, the carcasses of these chickens and the rest of their intestines were collected in disposable plastic bags, sealed in barrels, and disposed of in accordance with state and federal laws by Michigan State University's Laboratory Animal Care Service. The necropsy tools were rinsed with hexane to remove PCB residues. Droppings were collected and disposed of according to procedures formulated by MSU's Office of Radiation, Chemical, and Biological Safety.

The remaining chickens at the PRTC were divided and subjected to 4 different treatments from days 0 to 7 of withdrawal. One group each of controls and PCB-chickens were given clean diet ad libitum, while the others were fed diets containing 5% MO ad libitum or diets containing 10% MO at 50% ad libitum, or clean diet at 50% ad libitum. Half of the birds from each group were killed at day 4 of withdrawal and the remainder at day 7 of withdrawal. Their carcasses and intestines were handled in the same manner as described for those killed on day 0 of withdrawal.

The number of chickens euthanized, tissue samples, and intestinal contents taken at each period are presented in Table 1 (see Appendix A and B for detail).

C. Safety Procedures

Because of health hazards of polychlorinated biphenyls, the experimental room was isolated from other studies at the PRTC. Safety regulations were followed in every step,

Table 1. Experimental Schedule.

	Experimental days	No. birds	Treatments
	Pre-experimental		
Chickens 7/30-8/20/87	-21 to -1	168	None[1]
	Experimental		
Control chickens 8/21-9/3/87	0 to 14	48[2]	Non-contaminated diet
PCB chickens 8/21-9/3/87	0 to 14	120[3]	Aroclor 1254 contaminated diet
	Withdrawal period		
Control chickens 9/4-9/7/87	14 to 18[4]	32[2]	Non-contaminated diet
PCB chickens 9/4-9/7/87	14 to 18[4]	80[3]	Non-contaminated diet
Control chickens 9/8-9/10/87	18 to 21[5]	16[2]	Non-contaminated diet
PCB chickens 9/8-9/10/87	18 to 21[5]	40[3]	Non-contaminated diet

[1] Chickens received clean diet during this period.

[2] Sixteen control chickens were sacrificed on the final day of each specified time period, 8 of which were used for analysis of PCB residues in the whole body, 8 for intestinal content analysis of PCBs, and 4 of the latter 8 for electron microscopic examinations.

[3] Forty PCB chickens were sacrificed on the final day of each specified time period, 20 of which were used for analysis of PCB residues in the whole body, 20 for intestinal content analysis of PCBs, and 6 of the latter 20 for electron microscopic examinations.

[4] Days 0 to 4 of withdrawal.

[5] Days 5 to 7 of withdrawal.

all personnel who entered that room wore protective clothing, hair nets, masks, and plastic boots. Research equipment used in the room was rinsed with hexane to remove PCB residues. All wastes were collected and disposed of according to the state and federal safety laws by Michigan State University's Office of Radiation, Chemical, and Biological Safety.

II. Transmission Electron Microscopy (TEM)

Sections from the jejunum and large intestine were cut into 1-mm cubes and processed for standard TEM according to Hopper et al. (1979). After initial fixation of the samples in cold 5% buffered glutaraldehyde for 2 hours, samples were transferred from Anthony Hall (ATH) to the Center for Electron Optics (CEO) for further processing. The specimens were washed in two changes of 0.1 M phosphate buffer saline (PBS) and then immersed in 1% osmium tetroxide in buffer at 4°C overnight. Then the specimens were washed in a 0.1 M PBS for 10 minutes. The tissues were immersed in a graded series of cold ethanol:water mixture (25, 50, 75, and 100%) and stored refrigerated overnight in 100% ethanol. Transition to acetone was carried out by using ethanol:acetone solution in the ratio of 2:1 and then 1:2, fifteen minutes for each one. Samples were then rinsed two times in 100% acetone for 15-30 minutes each time.

Infiltration was done by using acetone:epoxy resin in a ratio of 3:1 and 1:1 for 3-4 hours for each solution (Hooper et al., 1979). Then samples were immersed in 100% epoxy

resin overnight. Specimens were transferred and embedded in fresh 100% resin in molds and dessicated for two hours. They were then polymerized at 65°C in an oven for 48 hours. Thin sections were cut with glass knives in an Sorval MT-2 ultramicrotome, mounted on grids, and stained with uranyl acetate and Reynolds' lead citrate. Grids were observed on a Philips 201 electron microscope and photographed on Kodak electron image film. Pictures were printed on Kodak polycontrast II rapid paper.

A total of 18 PCB-treated birds (6 birds in each on of days 0, 4, and 7 of withdrawal) and 12 control chickens (4 birds in each one of days 0, 4, and 7 of withdrawal) were used for transmission electron microscopic examinations. Two tissue samples of jejunum and large intestine were taken from each bird.

III. Scanning Electron Microscopy (SEM)

One 1-cm long section of jejunum and two 1-cm sections from the large intestine of 3 PCB-treated birds, and similar sections from 3 control birds were used for morphological studies of villi by scanning electron microscopy. The specimens were taken from those animals which were used for TEM examination.

The procedure was followed as outlined by Hooper et al. (1979). The desired intestinal tissue was removed, washed in cold saline to remove mucus, and placed in pools of cold 5% buffered glutaraldehyde for two hours. The samples then were brought to the Center for Electron Optics from Anthony Hall for further processing. They were rinsed in cold buffer (0.1 M sodium phosphate buffer pH 7.4) for 15 minutes and post-fixed in osmimum tetroxide for 3 hours. The tissues were then rinsed for 15 minutes in PBS, dehydrated in a graded ethanol series (25, 50, 75, 95, and 100%), critical point dried with CO_2, mounted on specimen stubs with conductive silver paint, and coated with gold in a "Film-VAC" coater for 3 minutes. The samples were observed on the JEOL JSM-35 CF SEM.

IV. Chemical Analysis

A. General Procedure (Batty et al., 1988)

A certain amount of tissue, intestinal content, excreta, or diet sample was weighed individually with a top-loading electronic analytical balance (Fisher Scientific Company,

FairLawn, NJ) into a 100 ml Erlenmeyer flask. The sample was homogenized in 25 ml toluene/ethylacetate (1:3) (Mallinckrodt, Inc., Paris, KY) per 1 gram of specimen in a Waring blender at low speed for 2 minutes. The fluid portion of the homogenized sample was decanted through a funnel containing Whatman #1 filter paper into a 250 ml flask under vacuum. The solid portion was added back into the blender and the process of blending and filtration was repeated two more times. Quantitative transfer of the solvents was followed by a rinse with the solvent. The combined filtrate was poured through a funnel containing glass wool and anhydrous granular Na_2SO_4 (Mallinckrodt, Inc., Paris, KY) and collected into a 250 ml flat bottom flask. The filter and funnel were rinsed with three 5 ml portions of the solvent. The total collection was evaporated on a Buchi vacuum rotorary evaporator to approximately 5 ml and transferred with rinsing into a 10 ml volumetric flask.

Each sample was loaded into an ABC Labs Gel Permeation chromatograph to isolate the PCB constitutents. Parameters for this elution were: column, 30 x 200 mm glass containing SX-3 Bio Beads, 200-400 mesh; flow, 5 ml ethyl acetate/toluene (3:1)/min; dump = 21; collect, 15 min; and wash, 5 min. The 100 ml collected were evaporated on a Buchi vacuum rotorry evaporator and transferred to a 10-ml volumetric flask for final analysis. The collection flask was rinsed several times, adding each portion to the volumetric flask. Finally the volume was brought to 10 ml.

Gas chromatographic (GC) analysis was done on a Varian 3700 instrument equipped with a 1.8 m x 2 mm ID glass column, and a ^{63}Ni electron capture detector. Column temperature was 270°C, injector temperature was 220°C, detector temperature was 270°C, and nitrogen carrier gas flow rate was 40 mg/min. GC data were output to a linear recorder with chart speed of 1.27 cm/min.

Quantitation was done by measuring the areas of the peaks from the Aroclor 1254 and converting the values to area/µl. The response line for each peak over a range of standards was graphed and calculated. The linearity of the response lines was determined and samples were injected at amounts to produce peak area/µl on the response lines. Concentration of PCBs was calculated from regression lines and corrected for percentage recovery of spiked samples.

B. Preparation and Storage of PCBs Standard Solution

Two ml of 598 ppm Aroclor 1254 were pipetted in a 100 ml volumetric flask and brought to full volume with toluene/ethyl acetate (3:1) to make a 12 ppm standard. Different concentrations of standard solutions were then prepared as follows:

1.2 ppm STD = 5 ml of 12 ppm STD brought up to 50 ml with 1:3 toluene/ethyl acetate.

0.84 ppm STD = 7 ml of 1.2 ppm STD brought up to 10 ml with 1:3 toluene/ethyl acetate.

0.48 ppm STD = 4 ml of 1.2 ppm STD brought up to 10 ml with 1:3 toluene/ethyl acetate.

0.24 ppm STD = 2 ml of 1.2 ppm STD brought up to 10 ml with 1:3 toluene/ethyl acetate.

Stock and standard solutions were stored in a refrigerator and brought to room temperature before use.

C. Preparation of Spike Samples

Spike samples were prepared by adding different concentrations of standard solutions (Aroclor 1254) to the control samples and then processed for GC analysis.

Procedure: In 2 grams of tissue:

1.2 ppm spiked sample = to get 1.2 ppm in 10 ml final solution, 1 ml of 12 ppm standard was added to the sample.

0.84 ppm spiked sample = to get .84 ppm in 10 ml final solution, 7 ml of 1.2 ppm standard was added to the sample.

0.48 ppm spiked sample = to get .48 ppm in 10 ml final solution, 4 ml of 1.2 ppm standard was added to the sample.

0.24 ppm spiked sample = to get .24 ppm in 10 ml final solution, 2 ml of 1.2 ppm standard was added to the sample.

D. Analysis of Whole-Body PCB Residues

Chickens chosen for whole body analysis were removed from the freezer, thawed at room temperature, and ground intact to hamburger consistency in a Hobart electrical grinder. Samples were put through the mixer 6 times to obtain a homogenous sample. Grab samples were removed and frozen in whirl pack bags at -20°C. These were later used for determination of carcass residues of PCBs (Batty et al., 1988).

GC analytical parameters for whole body samples were: (1) sample weight = 20 g; (2) injection time = 5 seconds; (3) attenuation = 16. Injector temperature, column temperature, detector temperature, and quantitation were as described in Part A, General Procedure for PCBs Analysis.

E. Analysis of PCB Residues in Intestinal Contents

The intestinal contents were removed from the freezer and freeze dried in a Virtis Freeze Dryer at a condenser temperature of -100°C and vacuum at 100 millitorr for 48 hours. They were kept again at -20°C until analyzed for PCB residues and CR_2O_3.

1. PCB Analysis

GC analytical parameters for intestinal components were: (1) sample weight = 2.0 g; (2) injection time = 5 seconds; (3) analysis time = 25 minutes; (4) attenuation = 16. Injector temperature, column temperature, and detector temperature were as cited in Part A, General Procedure for PCB Residues.

Quantitation was done according to Batty et al. (1988) which is described in Part A.

2. Chromic Oxide (Cr_2O_3) Analysis

The first method which was tried in this experiment to determine the CR_2O_3 concentrations in intestinal contents and excreta was the method of Czarnocki et al. (1960). A three gram sample was weighed on a top-loading electronic balance, wrapped in a filter paper, and then transferred to a 100-ml Kjeldahl flask. Ten ml of concentrated nitric acid (Mallinckrodt, Paris, KY) was added to the sample and allowed to stand overnight at room temperature. On the next day, the Kjeldahl flask was heated on a Labconco electrical heater to dryness. The Kjeldahl flask was connected to a vacuum system and all of the vapors were passed through a sodium bicarbonate solution (Fisher, FairLawn, NJ). After the sample was dried completly, the Kjeldahl flask was removed from the heater and cooled to room temperature. Fifteen ml of digestion mixture (see Appendix F) was added to the sample and the sample was heated again until complete digestion occured which was monitored by a color change from green to yellow or orange after 15 minutes. The heater was then turned off and the flask was cooled to room temperature. The digested sample was transferred to a 500 ml cylinder and brought to full volume with distilled water. After that it was transferred to a 500 ml flask and shaken well . Then the sample was allowed to stand overnight to precipitate

inorganic materials. Optical density of the sample was read on a Perkin-Elmer Spectrophotometer at 350 mμ. Double distilled water was used as a blank.

A standard curve was prepared at different quantities of Cr_2O_3 (Fisher Scientific Company, FairLawn, NJ) from 0.1, to 1.00 mg. A certain amount of Cr_2O_3 was weighed and wrapped in a filter paper, then digested with acid and diluted to 500 ml with distilled water as explained above. Optical densities were read at 350 mμ.

After running a few samples for Cr_2O_3 determination according to the procedure described above, it was realized that the amount of the intestinal contents was not enough for both Cr_2O_3 and PCB analysis. Therefore the continuation of this procedure for the rest of the samples was deemed impossible.

A second procedure, called "Inductive Coupled Plasma (ICP)" (Faires, 1982), was tried to determine Cr_2O_3 concentrations in the intestinal contents and excreta. In this method, the ICP excitation source is an electrodeless argon plasma operated at atmospheric pressure and sustained by inductive coupling to a radio frequency electromagnetic field. This method has many advantages including the use of a smaller amount of a specimen for specific analysis.

The problem with this method was non-digestibility of Cr_2O_3 in nitric acid and sulphuric acid. Therefore we could not prepare the sample to inject into the machine. An attempt for Cr_2O_3 analysis was unsuccessful.

The last choice was using the data on the percentage of Cr_2O_3 in different segments of the intestine that was presented by Bird (1968). In Bird's procedure, chromic oxide was fed as a marker to the chicken and the ratio of nitrogen to Cr_2O_3 was determined in feed, the crop, proventriculus, gizzard, and six sections of the small intestine (Table 2). These six sections consisted of the upper and lower halves of the duodenum, jejunum, and ileum. Data from that experiment indicated that the upper jejunum was the most active site of absorption of nitrogen from the intestine, the ileum had a minor role in nitrogen absorption, and the ratio of nitrogen to chromic oxide in the duodenum was 4 to 5 times that in the feed.

In the present study, to determine the ratio of PCBs to Cr_2O_3 in the duodenum and jejunum, the percentage of Aroclor 1254 in the contents of those segments was divided by the average percentage of Cr_2O_3 in those sections using data from Bird (1968). The percentage of Aroclor 1254 in the ileum and large intestine was separately divided by the average percentage of Cr_2O_3 in the ileum. The percentage of Cr_2O_3 in the ceca and large intestine was not given by Bird (1968).

E. Analysis of PCB Residues in Excreta

Excreta samples were collected for 14 days of PCB treatments and 7 days of withdrawal for each group of treatments. The samples were dried in a Thelco vacuum oven at 60°C for 24 hours. The dried excreta were homogenized

Table 2. Percent chromic oxide in the feed and portions of the gastrointestinal (GI) tract of 6-week-old chickens.[1]

Feed or intestinal segment	% Cr_2O_3	Ratio of Cr_2O_3 in the GI to Cr_2O_3 in feed	% Cr_2O_3
Feed	0.458	100	0.200
Crop	0.352	76.8	0.154
Proventriculus	0.192	41.9	0.084
Gizzard	0.189	41.3	0.083
Upper duodenum	0.230	50.2	0.100
Lower duodenum	0.282	61.6	0.123
Upper jejunum	0.700	152.8	0.306
Lower jejunum	1.437	313.7	0.627
Upper ileum	1.247	272.2	0.545
Lower ileum	0.811	177.0	0.354

[1] This table is adapted from F.H. Bird, 1968. Role of the avian small intestine in amino acid metabolism. Fed. Proc. 27(5): 1194-1198.

by a Waring blender at low speed for 1 minute and then kept in plastic bags at -20°C.

GC analytical parameters for excreta were: (1) sample weight = 10 g; (2) injection time - few seconds; (3) attenuation = 16; (4) analysis time = 25 minutes. Injector temperature, detector temperature, and column temperature were as cited in Part A. Quantitation was also done as explained in Part A.

F. Analysis of PCB Residues in Feed

Subsamples of diet were taken just after blending of experimental diets was completed. Samples were stored in plastic bags at -20°C until using them for PCB residue determination.

GC analytical parameters for feed were: (1) sample weight = 10 g; (2) injection time = few seconds; (3) attenuation = 16; (4) analysis time = 20 minutes. Injector temperature, detector temperature, and column temperature were as cited in Part A. Quantiation was also done as explained in Part A.

Raw data for PCB analysis in whole body, intestinal contents, and diet are presented in Table 4, Table 7, and Appendix E, respectively.

G. Pooling of Samples

As explained in Table 1, sixteen control chickens were sacrificed on each one of days 0, 4, and 7 of withdrawal (there were 4 different dietary treatments during withdrawal:

No PCB - no treatment; No PCB - 5% MO; No PCB - FR; No PCB - FR + 10% MO). Eight (2 from each treatment) of which were used for analysis of PCB residues in the whole body, 8 (2 from each treatment) for intestinal content analysis of PCBs, and 4 of the latter 8 for electron microscopic examinations.

Forty PCB chickens were also euthanized on each one of days 0, 4, and 7 of withdrawal; 20 (5 from each dietary treatment of: PCB - no treatment; PCB - 5% MO; PCB - FR; PCB - FR + 10% MO) of which were used for analysis of PCB residues in the whole body, 20 (5 from each dietary treatment) for intestinal content analysis of PCBs, and 6 of the latter 20 for electron microscopic studies.

Because of an inadequate amount of intestinal materials for chemical analysis, contents of duodenum and jejunum were pooled within treatments for days 0, 4, and 7 of withdrawal. Also intestinal contents for each segment on day 4 of withdrawal were pooled with the components of a similar segment of chickens on day 7 of withdrawal yielding a pool sample representative of day 5½. Statistical analysis was not performed because samples were pooled into one representing 20 birds.

Tissue samples for whole-body residues were also pooled for days 4 and 7 of withdrawal yielding a sample representing day 5½. Excreta for experimental days 0-4 of withdrawal and excreta for days 5-7 of withdrawal were pooled.

RESULTS

SCANNING ELECTRON MICROSCOPY

The intestinal tissues which were prepared thorough gentle washing steps, the villi of most specimens were remarkably free of debris. Sections not properly washed were covered with a layer of mucus and debris which in most cases made it difficult to view individual villi. Morphologic features of the villi varied slightly according to their location in the intestine. The noncontaminated villi of the jejunum from control chicks were generally apically squared off or finger-like (Figure 1), while the villi of the large intestine were wider, thicker, with a flattened tongue-shape (Figures 2 and 3). The villous surfaces were interrupted by numerous furrows and openings of goblet cells (Figures 2, 3, and 4). The brush border (fuzzy coat) was visible on the epithelial cells of the villi at higher magnification (Figure 5).

Dietary administration of 10 ppm Aroclor 1254 for 14 days to the chicken caused damage in both the jejunum and large intestinal regions. The epithelium of jejunal villi sloughed off exposing the laminae propria (Figure 6). In the large intestine, loss of morphology of villi and degrading of villar structure associated with debris were observed (Figures 7, 8, and 9).

FIGURE 1. Control chicken (no withdrawal treatment), age 35 days. Jejunum. There are finger-like villi with mucus (thick arrow) and opening of goblet cells (thin arrow). Glutaralydehyde and osmium tetroxide fixation. Gold coat. X520.

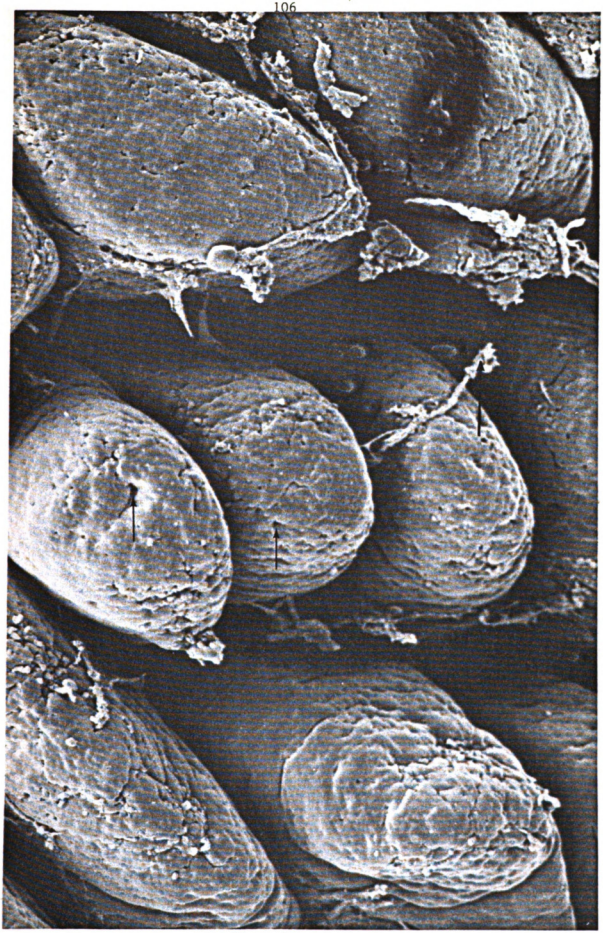

Fig. 1

FIGURE 2. Control chicken (no withdrawal treatment), age 35 days. Large intestine. There are tongue-shaped villi with prominent transverse furrows with mucus (arrow) at the tips of some villi. Glutaraldehyde and osmium tetroxide fixation. Cold coat. X516.

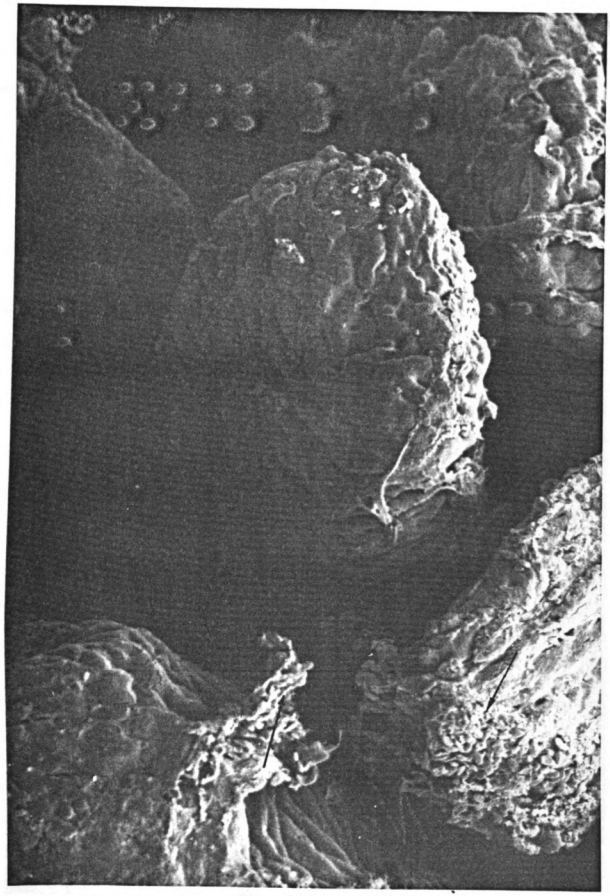

Fig. 2

FIGURE 3. Control chicken (no withdrawal treatment), age 35 days. Large intestine. Higher magnification from the same field as Figure 52. Showing a villous with opening of goblet cell (thin arrow) and mucus (thick arrow). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X1118.

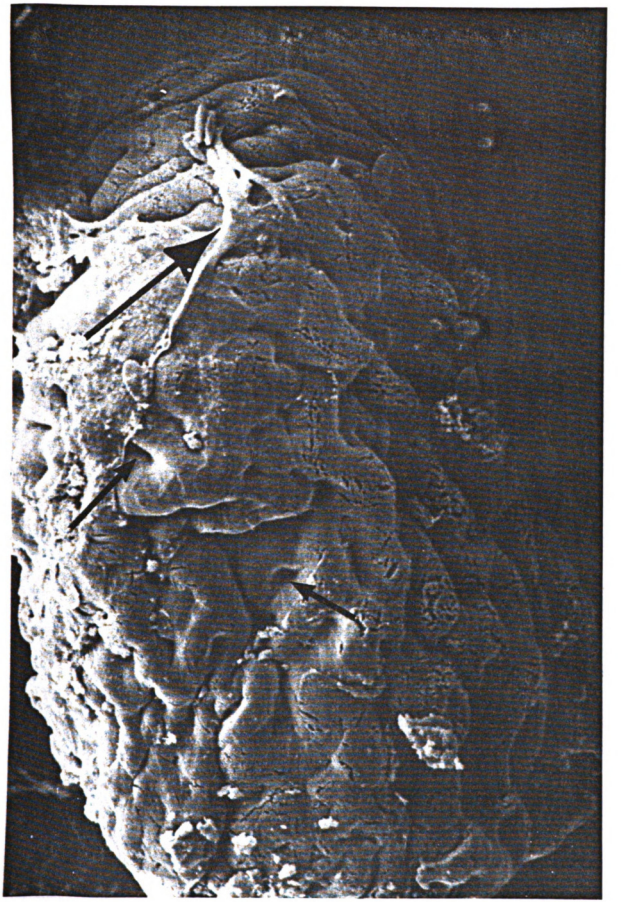

Fig. 3

FIGURE 4. Control chicken (no withdrawal treatment), age 35 days. Large intestine. Part of a villous with opening of goblet cells (arrow). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X4694.

Fig. 4

FIGURE 5. Control chicken (no withdrawal treatment), age 35 days. Large intestine. Tip of a villous showing brush border (fuzzy coat). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X8076.

Fig. 5

FIGURE 6. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Villi have denuded lateral and tips (arrow) with exposed lamina propria (LP). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X526.

Fig. 6

FIGURE 7. PCB-chicken (no withdrawal treatment), age 42 days. Large intestine. Note loss of morphology of villi and degraded villar structure associated with debries. Glutaraldehyde and osmium tetroxide fixation. Gold coat. X524.

Fig. 7

FIGURE 8. PCB-chicken (no withdrawal treatment), age 42 days. Large intestine. Higher magnification of a villous from Figure 57, showing the opening of goblet cells (thin arrow) and debris (thick arrow). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X1890.

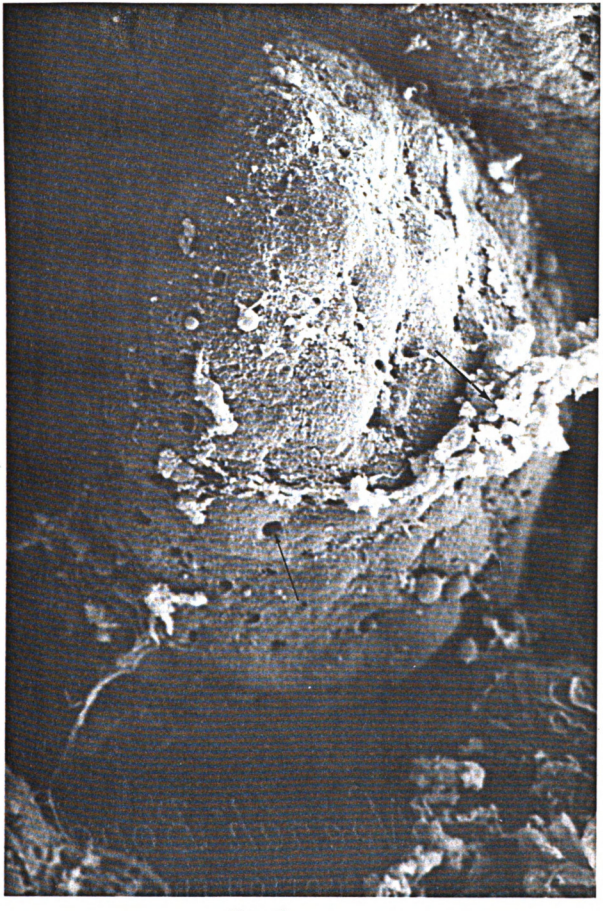

Fig. 8

FIGURE 9. PCB-chicken (no withdrawal treatment), age 42 days. Large intestine. Greater magnification from tip of villous. Note opening of goblet cells (thin arrow) and debris (thick arrow). Glutaraldehyde and osmium tetroxide fixation. Gold coat. X5214.

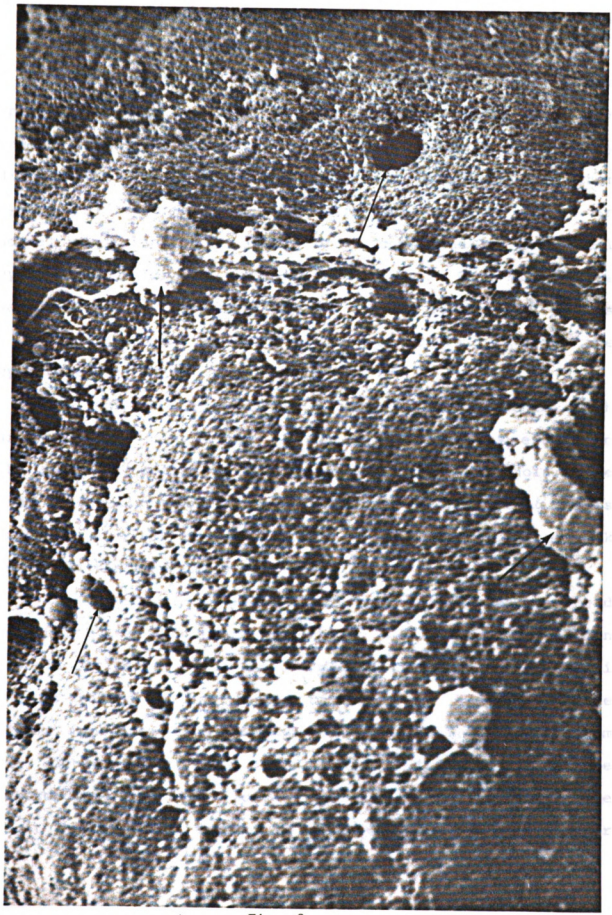

Fig. 9

TRANSMISSION ELECTRON MICROSCOPY

A. Control Chickens

Chickens not fed PCBs had the nuclei in the villous epithelial cells of the jejunum and the large intestine generally located centrally and the mitochondria were evenly distributed (Figure 10). The endoplasmic reticulum and other cytoplasmic organelles were present and appeared to be in normal condition. The brush border consisted of individual cylindrical microvilli (Figures 10 and 11). Each microvillus was seen as an extension of the apical plasmalemma and as a continuous membrane enveloping a structural complex, the cytoskeleton (Figure 10).

The core of the microvilli consists of filaments which are arranged in an axial fashion. The filaments extend to the apical cytoplasm of the enterocyte where they join those from neighboring microvilli in a network of interlacing fibers arranged parallel to the surface of the cell. This network is called the terminal web (Figure 10).

The lateral membranes of adjacent enterocytes are held together by various junctions (Figure 10). The tight junction (zonula occludens) is situated immediately below the microvilli and is formed by the fusion of the outer leaflets of the plasma membrane, thus sealing the intercellular spaces from the outer environment, i.e. the luminal space. The intermediate junction (zonula adherens) lies just below the tight junction and appears as an electron-dense small linear

FIGURE 10. Control chicken (withdrawal treatment of FR + 10% MO), age 35 days. Jejunum. Upper parts of two enterocytes (EN) facing to the lumen of the bowel, showing normal structure of the brush border and the cytoplasmic organells. Individual microvilli (MV) consist of a cylinder formed from the trilaminar membrane of the apical plasmalemma enclosing a structural complex, the cytoskeleton, terminating in a cytoplasmic condensation. The lateral or intercellular membranes (IM) and the three types of junctions seen as plaques of increased densities (T - tight junction; IJ - intermediate junction; TD - terminal desmosome). Extension of filaments of microvillous core into the cytoplasm below to form the network of fibrils known as the terminal wet (TW) are seen. The cristae mitochondrials (M) are of uniform width and are not vacuolated. Ribosomes are dispersed throughout the apical cytoplasm. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X50189.

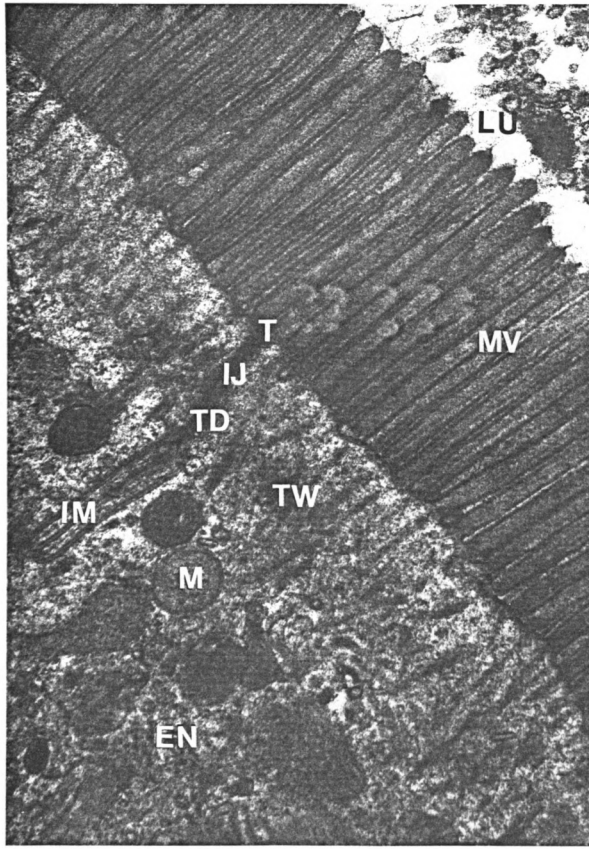

Fig. 10

FIGURE 11. Control chicken (no withdrawal treatment), age 35 days. Jejunum. Longitudinal section of several microvilli (MV) projecting from the apical portion of the enterocyte cytoplasm (EN) into the lumen (LU) of the bowel. Each microvilli is bound by unit membranes which are continuous with the surface membranes of the enterocyte (EN) and also with those of adjacent microvilli. Fillaments of glycocalyx (GC) are arising from the tips of the microvilli (MV). They formed the basal structure supporting the mucopolysaccharide layer that surrounds the outer surface of the microvilli. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X80000.

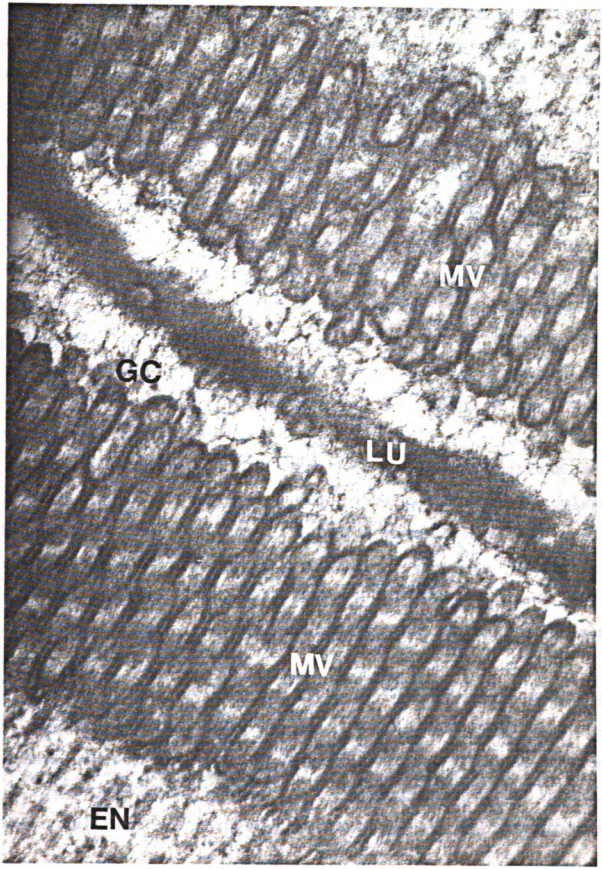

Fig. 11

area separated by a small gap and surrounded by fuzz extending into the adjacent cytoplasm. The third most prominent and numerous lateral cell junctions are the desmosomes (macula adherens). These are dense plaques composed of four membrane leaflets and a gap surrounded by dense cytoplasmic fuzz (Figure 10).

At high magnification of a longitudinal section of microvilli (Figure 11) one can note that microvilli are coated by glycocalyx. Glycocalyx, as well as many enzymes localized within the striated border, participate in the cell's absorptive processes (Benett, 1963). The fine filaments of microvilli also extend from the sides and tips of the free surface of their membrane into the lumen of the bowel to form part of the glycocalyx.

The mitochondria containing two layers of membrane and cristae are found as dense small bodies in the cytoplasm (Figures 10 and 12). The cristae mitochondriales of cells from normal chickens are conspicuous by their uniformity of width and lack of vacuolization (Figures 10 and 12). The ribosomes are present throughout the apical cytoplasm (Figure 10).

In the lamina propria (a histological term denoting the space occupied by connective tissue and its cellular content between the basal laminae of the villous epithelium and the muscularis mucosae) of a control chicken, a typical plasma cell with its special arrangement of the cytoplasmic

FIGURE 12. Control chicken (no withdrawal treatment), age 35 days. Jejunum. A plasma cell from lamina propria of the intestinal mucosa. The nucleus (N) is centrally located and the cytoplasmic cisternae (CY) occupy a large part of the cell and are arranged peripherally towards the cell membrane (CM). The cisternae are formed in parallel fashion to each other, are lined by rough endoplasmic reticulum = RER (arrow) and contain less dense finely granular material (the immunoglobulin). Two normal mitochondria (M) and few dense bodies (probably lysosomes) (L), plus some artifacts as (large black spots) (A) are seen in the cytoplasm. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X36600.

Fig. 12

cysternae and nucleus is present (Figures 12-14). Various cell types such as mast cell, fibrocyte, and few other cells of connective tissue plus fine strands of collagen are seen in the lamina propria of another control chicken (Figure 15).

The plasma cell has a centrally placed nucleus which shows well developed clumps of chromatin. Near the periphery of the cell is an abundance of cisternae lined with rough endoplasmic reticulum (RER). These cisternae are arranged in parallel fashion to each other and to the cell membrane (Figures 12-14). In these photomicrographs, few mitochondria are seen in intimate relation to the rough endoplasmic reticulum. The mitochondrial cristae are of uniform width and are not vacuolated. In Figures 12 and 14 are shown a few lysosomes (organelles that contain enzymes for intracellular digestion) just above the nucleus. They are less colored than the large black spots (artifacts). Also, a centriole lies just above the nucleus and below the lysosomes (Figures 12 and 13).

A mast cell with its distinguishing features of the cytoplasmic vacuoles, composed of smooth membrane, containing material which may be seen as granular, whorl-like or scroll-shaped is presented in Figures 15 and 16. The vacuoles are round bodies which take up most of the cytoplasm so that other organelles are sparsely represented.

FIGURE 13. Control chicken (no withdrawal treatment), age 35 days. Jejunum. Higher magnification of the plasma cell from the same bird's mucosa (Figure 12) showing the normal structure of the cytoplasmic organelles. The mitochondria (M) are not swollen, the cytoplasmic cisternae (CY) are parallel and surrounded by rough endoplasmic reticulum (RER) and the lysosomes (L) appear as dense bodies above the nucleus (N). The large black spots on the upper part of the photomicrograph are artifacts. A centriole (C) is seen just above the nucleus. Glutaraldehyde and osmium tetroxide fixation. Lead citrate uranyl acetate stain. X48750.

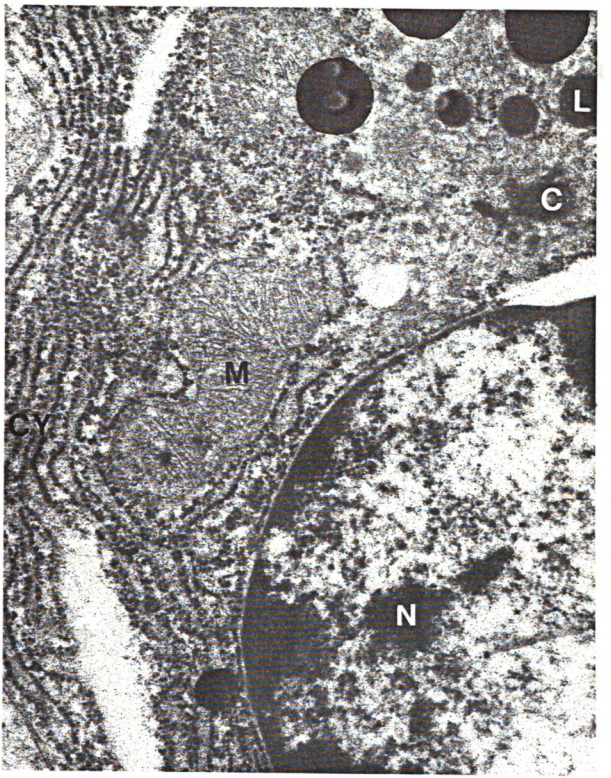

Fig. 13

FIGURE 14. Control chicken (no withdrawal treatment), age 35 days. Jejunum. A higher magnification of the same field as Figure 13. The photomicrograph shows nucleus (N), mitochrondria (M), and perinuclear areas of cytoplasm containing rough endoplasmic reticulum (RER). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X72857.

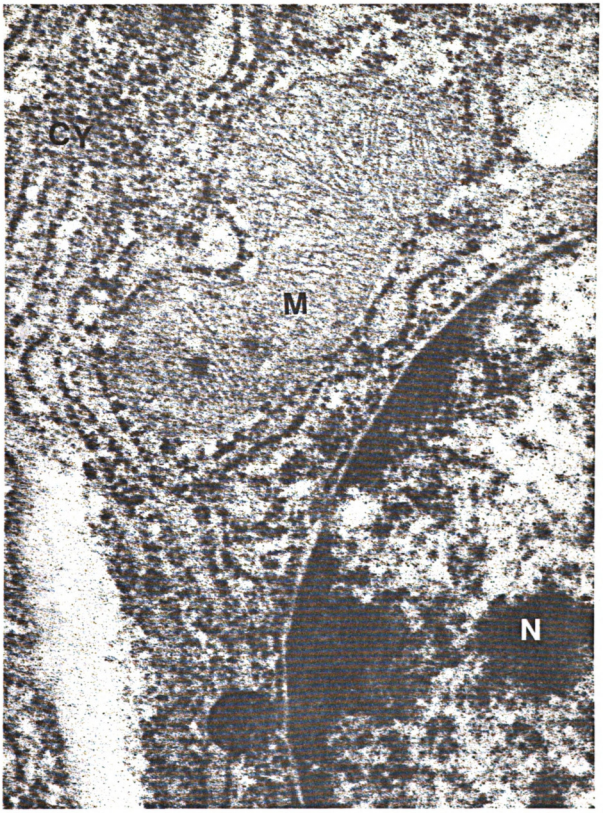

Fig. 14

The fibrocyte (forms both the fine connective tissue fibrils and the collagen fibers) has an elongated structure (Figure 15) and the cytoplasm may extend on either side of the nucleus to form long processes. The nucleus is elongated and sparse in chromatin material which is arranged close to the inner nuclear membrane (Figure 15). A few collagen fibers are seen in this picture.

FIGURE 15. Control chicken (no withdrawal treatment), age 39 days. Large intestine. Lamina propria of the intestine showing a typical mast cell with the large inclusion bodies (arrows) in the cytoplasm. A fibrocyte lies just left of the mast cell and has an elongated nucleus (N) with sparse chromatin close to the nuclear membrane. Several collagen fibers (CO) and cells of connective tissue are present in the lamina propria. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X26667.

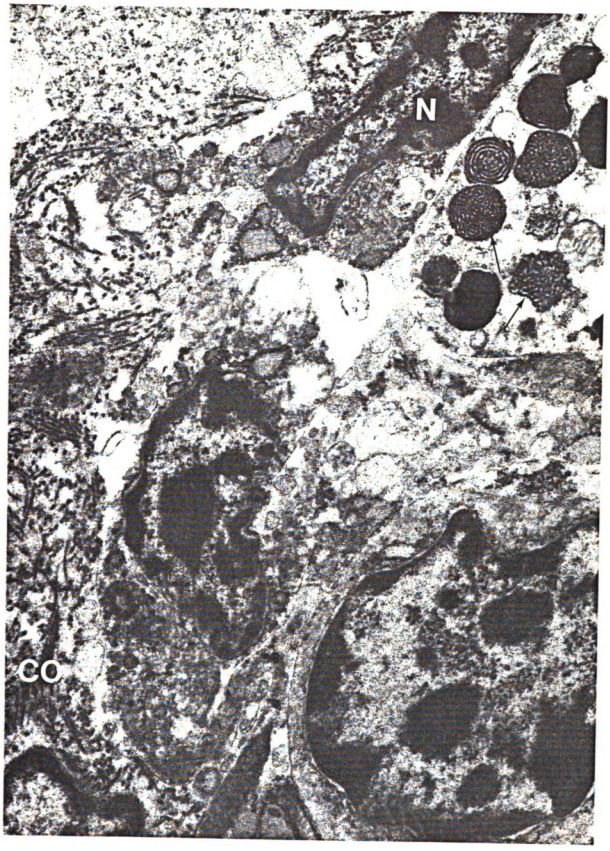

Fig. 15

FIGURE 16. Control chicken (withdrawal treatment of FR + 10% MO)., age 39 days. Large intestine. Higher magnification of the mast cell from the previous figure. The inclusion bodies vary in size and contain granular, whorl-like organelles (GR). Several of the vacuoles (VA) are clear of material while others are only half-filled, indicating that there is a constant discharge of their contents. Nucleus (N) and some cytoplasmic organelles such as rough endoplasmic reticulum (RER), are seen at the top of the photomicrograph. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X50909.

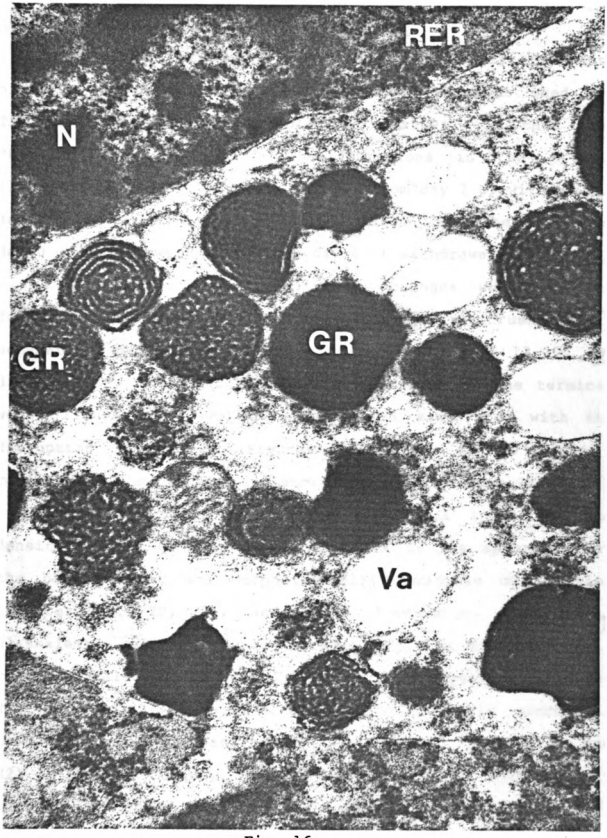

Fig. 16

B. PCB-Chickens

A total of 44% of the 18 (nine no withdrawal treatment, nine 10% MO + 50% FR) PCB-contaminated chickens used for TEM examination showed pathological changes. Of this 44%, 28% exhibited pathological manifestations in the jejunum (17% from day 0 of withdrawal and 11% from day 7 of withdrawal) and 16% showed the changes in the large intestine (11% from day 0 of withdrawal and 5% from day 4 of withdrawal).

Clear evidence of pathological changes was noted as follows: a variation in light and density of the brush border, separation and effacement of microvilli (Figures 17-20, 23, 35, and 38), irregularity and disintegration of the terminal webs (Figure 18), swollen mitochondria associated with the disruption of cristae (Figures 17-23, 25, and 27), and a remarkable dilation of endoplasmic reticulum (Figure 28).

There was variation of the microvilli in height and density and in grossly disrupted cells it was apparent that the brush border was morphologically incapable of function (Figures 18, 20, 23, and 26). Eventually, the apical plasmalemma was seen to be broken and the disrupted cell contents released to the lumen of the intestine (Figures 23, 26, and 27). A remarkable atrophy of the microvilli in a few individual entrocytes was observed (Figures 17 and 22). In some cases, the microvilli seen to be markedly shortened (Figures 19, 20, and 29), branched (Figure 30), and irregular (Figures 31 and 32), forms in which the

FIGURE 17. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Enterocytes are facing to the lumen of the bowel. Notice seperation, disintegration, and effacement of microvilli (arrow); destruction of terminal web (TW); and swelling of mitochondriales cristae (M). Various size of lipid-like droplets are seen within villus epithelial cells and lumen. Few goblet cells (GO) are opening to the lumen (LU). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7250.

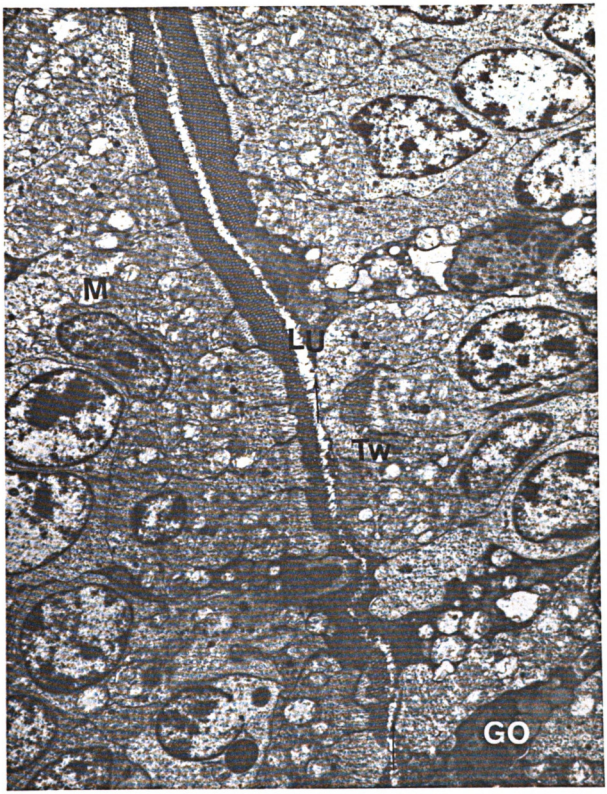

Fig. 17

FIGURE 18. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Longitudinal section of upper parts of few enterocytes (EN). The effacement and destruction of microvilli (arrow); loss of terminal web (TW); swelling of mitochondria (M); and disruption of the cristae mitochondriales are present. In goblet cells (GO), large numbers of secretory granules (GR) lined by smooth membrane and containing dark-colored granular material. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X15263.

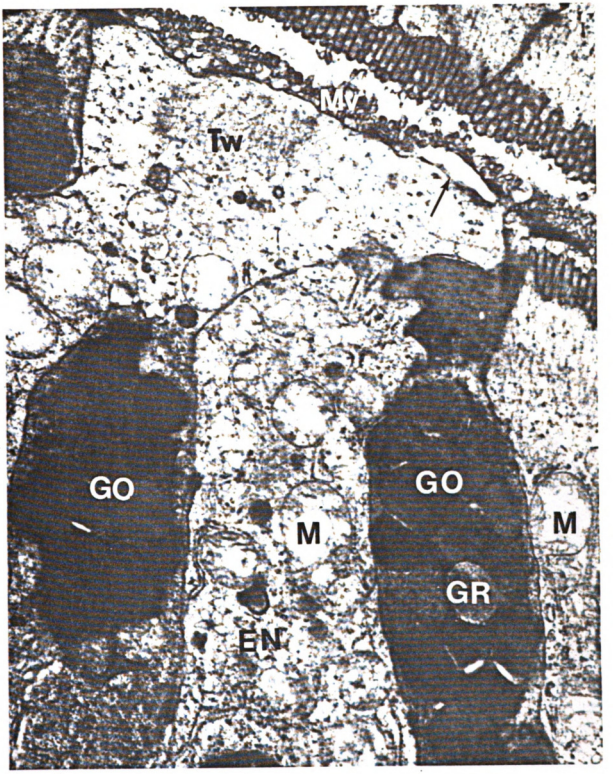

Fig. 18

FIGURE 19. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Upper half of few enterocytes, showing less organized cytoplasmic appearances with increase in vacuoles (VA) in entrocyte number 1 (EN1), and swelling of many mitochondria (M). Note the irregular, short and bulbous microvilli (MV). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X23571.

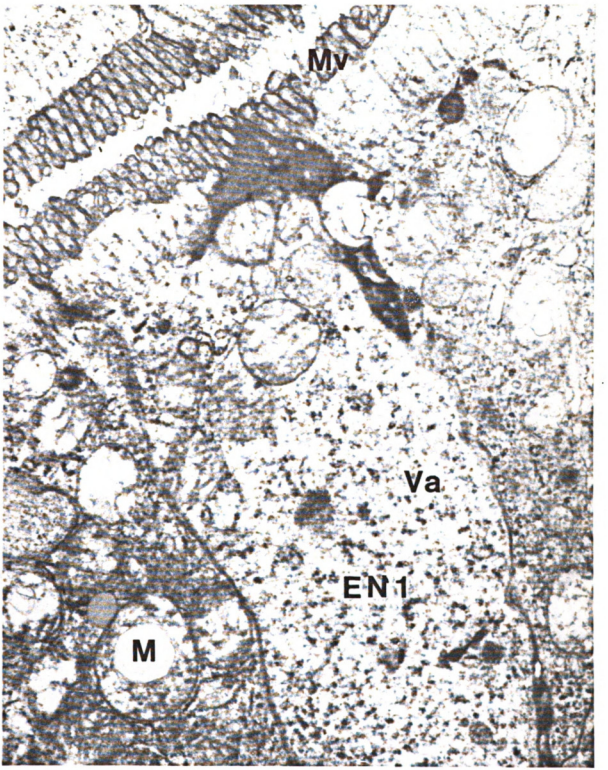

Fig. 19

FIGURE 20. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Longitudinal section of few enterocytes (EN). The microvilli (MV) are markedly atrophied, particularly in the upper portion. Several swollen mitochondria (M) are seen. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X23750.

Fig. 20

FIGUREZ 21. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Several enterocytes (EN) facing the lumen of the bowel (LU). The cytoplasmic changes are relatively minor except the mitochondria (M) which appeared swollen with disruption of their cristae. GO. Goblet cell. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7031.

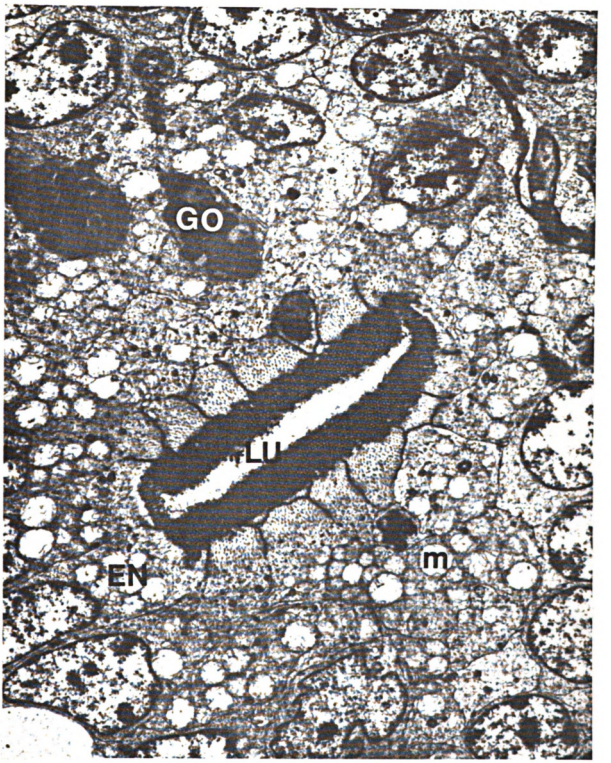

Fig. 21

FIGURE 22. PCB-chicken (no withdrawal treatment), age 35 days. Large intestine. Upper part of few entrocytes (EN) are facing from both right and left sides to the lumen (LU) of the bowel. Effacement and destruction of brush border from left enterocytes (arrow), swelling of the mitochondria (M), and disruption of the cristae (double arrow) are present. The enterocyte number 1 (EN1) shows lack of organization in its cytoplasm. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X25862.

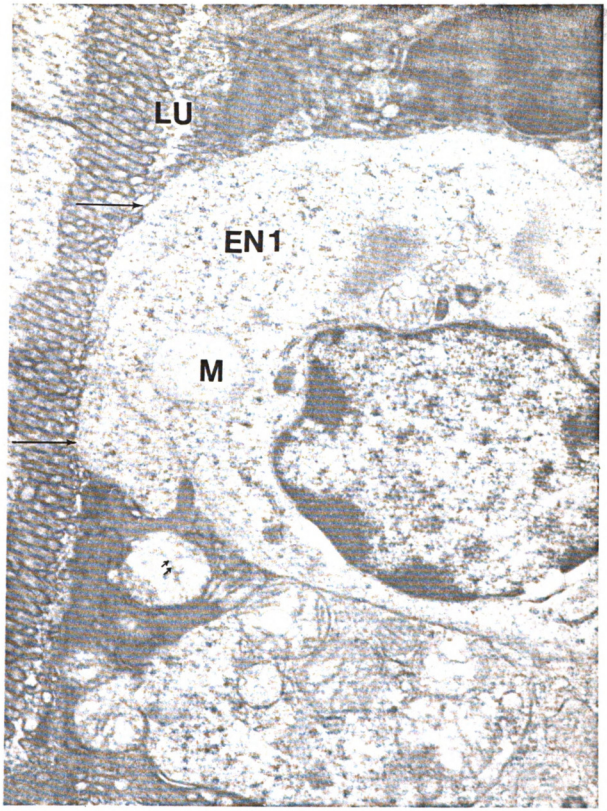

Fig. 22

FIGURE 23. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Notice degeneration of villus epithelial cell. Destruction and effacement of microvilli (MV), loss of cellular contents from cell into the lumen (arrow), and swollen mitochondria (M). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X9625.

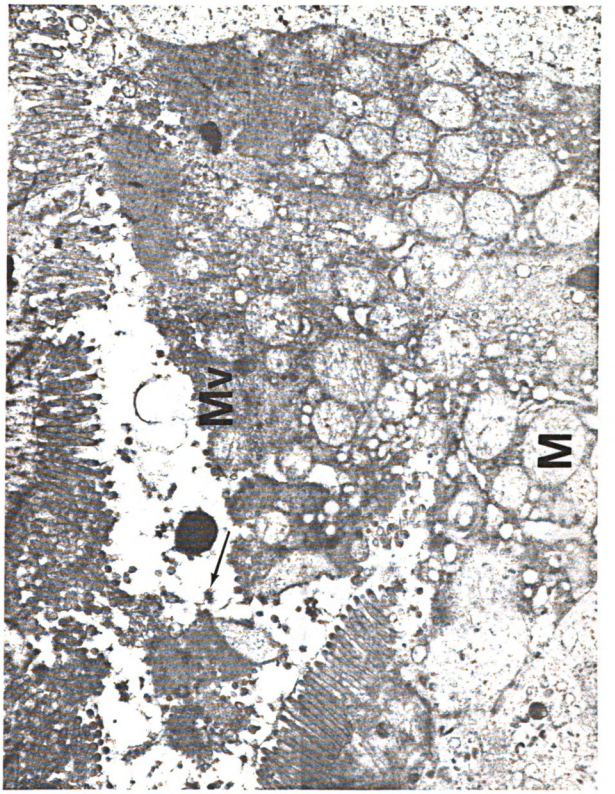

Fig. 23

FIGURE 24. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Part of an abnormal enterocyte from the same bird in the previous figure showing vacuolization (VA) of the cytoplasm and swelling of the mitochondria (M). The apical plasmalemma is seen to be broken and the disrupted cell contents released to the lumen of the intestine (arrow). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X25555.

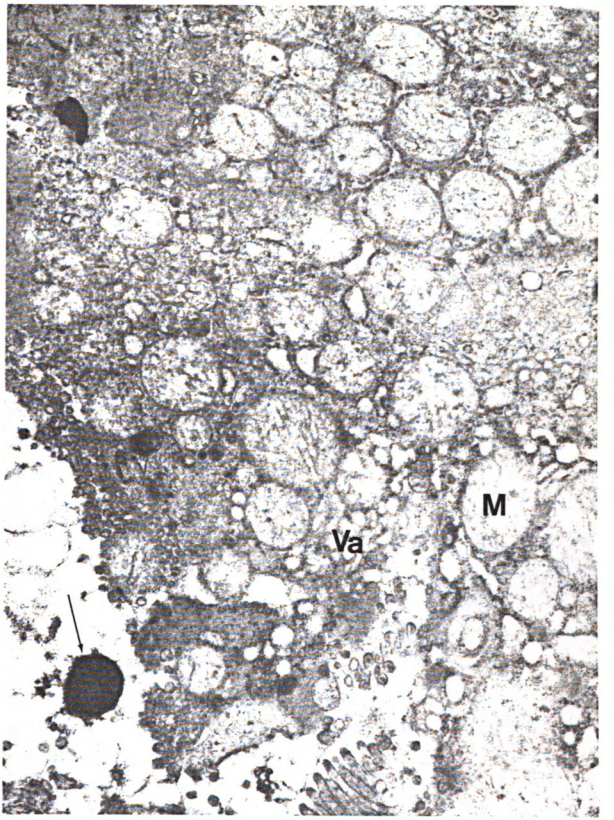

Fig. 24

FIGURE 25. PCB-chicken (withdrawal treatment FR + 10% MO), age 42 days. Jejunum. A higher magnification of few mitochondria (M), showing severe swelling and vacuolization of the mitochondriales cristae (arrow). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X71538.

Fig. 25

FIGURE 26. PCB-chicken (withdrawal tretment of FR + 10% MO), age 42 days. Jejunum. The microvilli (MV) on the right appear normal but those on the left are missing (arrow). The mitochondria (M) of the left entrocytes (EN) are slightly swollen. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X26000.

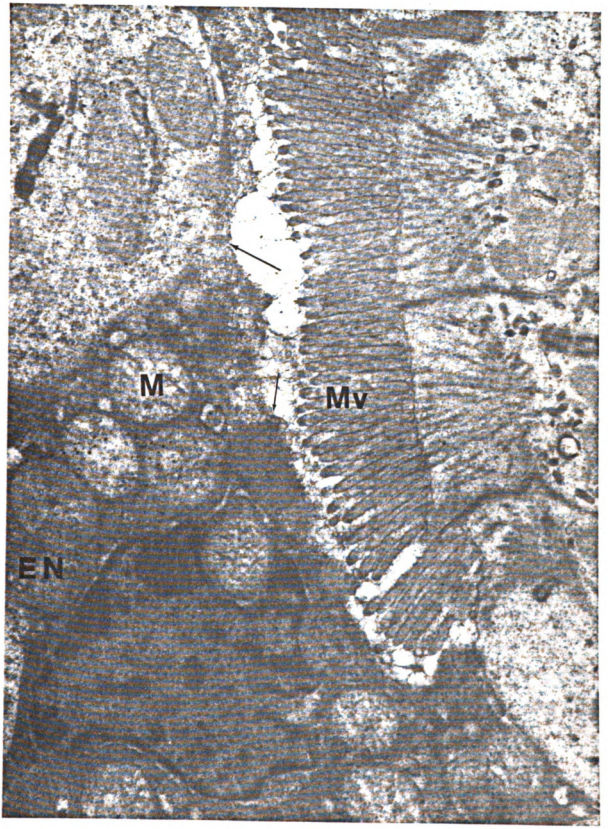

Fig. 26

FIGURE 27. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. This photomicrograph is showing rupture of the brush border and extrusion of the cell contents toward the lumen of the intestine (arrow). Swelling of the mitochondria and disruption of the cristae mitochondria (M) are seen. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X37000.

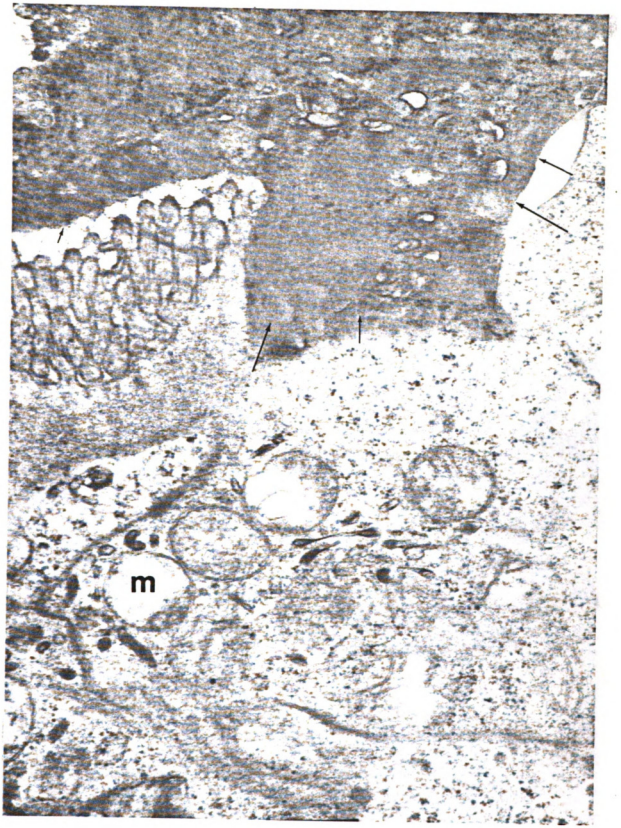

Fig. 27

FIGURE 28. PCB-chicken (no withdrawal treatment), age 39 days. Large intestine. Notice extensively expanded cisternae of rough endoplasmic reticulum (RER), and membrane bound cytoplasmic vacuole (VA). Eventually most of the cytoplasmic space is occupied by the vacuoles. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X15714.

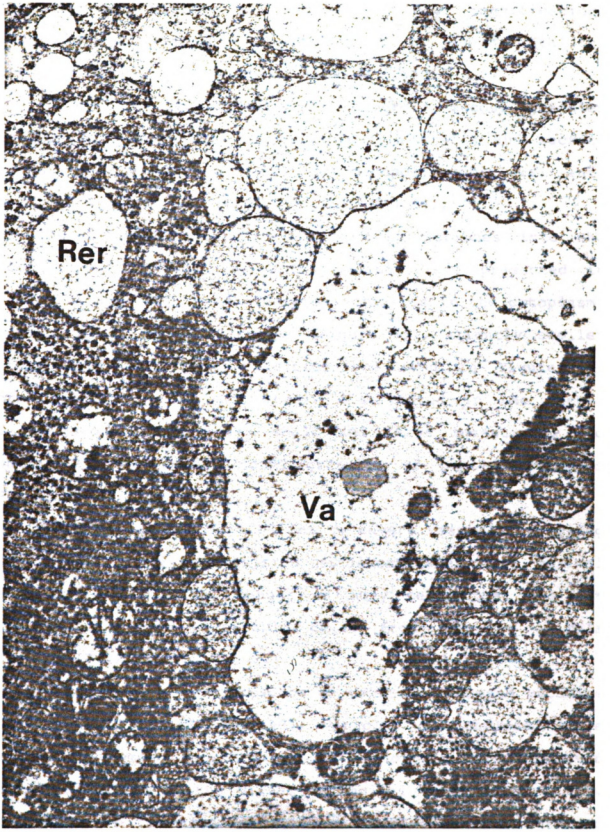

Fig. 28

glycocalyx was missing or reduced. At high magnification, a longitudinal section of microvilli is shown with the glycocalyx very much reduced or missing (Figure 33).

The goblet cells containing numerous mucous granules are placed between the villous epithelial cells and secrete mucous (the protective layer of mucopolysascharides) into the lumen (Figures 17, 18, 21, 31, 32, and 34).

The endocrine cells (APUD) secrete hormones into the blood stream through the basal laminae, are presented in Figures 35 and 36). Dense secretory granules in the cytoplasm, oval centrally situated nuclei (Figure 35), slightly swollen mitochondria and closed vacuoles are seen in Figure 36. Normally these cells are widely distributed in the mucosa of the stomach, pancreas, small and large intestines, and contain electron-dense, round granules of peptide hormones (Pearse, 1977).

There was an apparent slight to severe vascuolar swelling and disruption of the cristae mitochondriales, the maximal vascuolar degenerative changes of many cells that was not seen in otherwise comparable cells from PCB-free animals (Figures 17-22, 23-25, and 27).

In addition to the presence of membrane-enclosed vacuoles (Figures 37, 38, 39, and 40), vacuoles not enclosed by a membrane, termed "non-membranous vacuoles" were observed in the cytoplasm of the villal epithelial cells (Figure 41). In some cells the vacuoles were so large that they included

FIGURE 29. PCB-chicken (no withdrawal treatment), age 35 days. Large intestine. The microvilli (MV) are bulbous and irregular. The intracellular organelles show an increase in vacuoles (VA). Note the goblet cells (GO) with their secretory granules, and intercellular membranes (IM). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X11143.

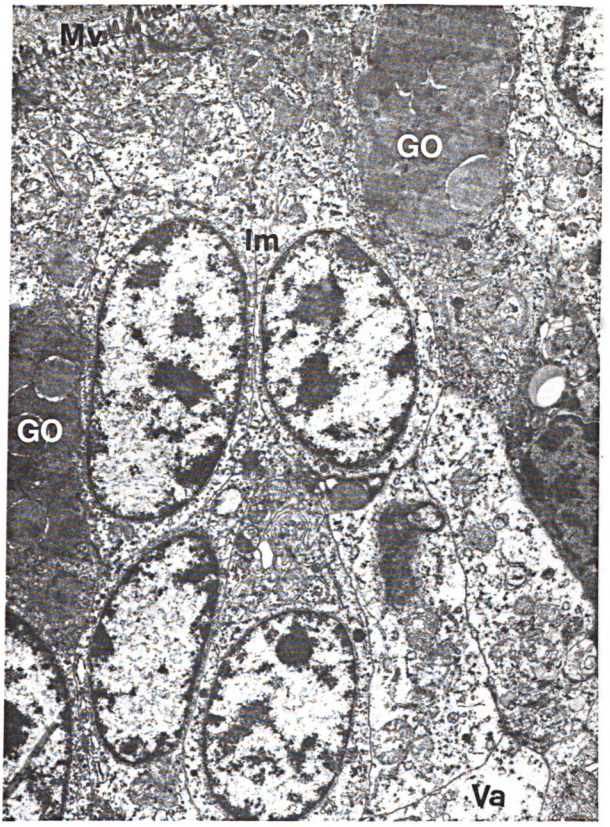

Fig. 29

FIGURE 30. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Few enterocytes (EN), of villi. The microvilli (MV) ar branched and irregular. The intracellular organells show numerous lysosomes (L) and normal looking mitochondria (M). A large vacuole (VA) is seen at the lower part of an entrocyte (EN). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X25652.

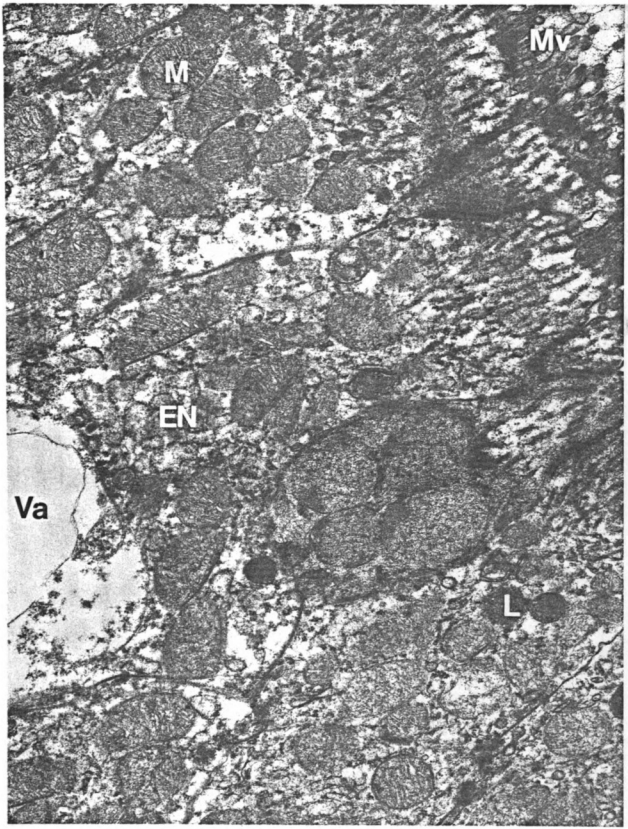

Fig. 30

FIGURE 31. PCB-chicken (withdrawal treatment of FR + 10% MO), age 39 days. Jejunum. Several enterocytes (EN) and goblet cells (GO) are facing to the lumen (LU) of the bowel. There is an apparent slight vacuolar swelling of the cristae mitochondriales (M). Microvilli (MV) are irregular. The basal membrane (BM) and basal laminae (BL) of the entrocytes plus connective tissue of laminae porpria (LP) are seen at the lower part of the photomicrograph. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X4933.

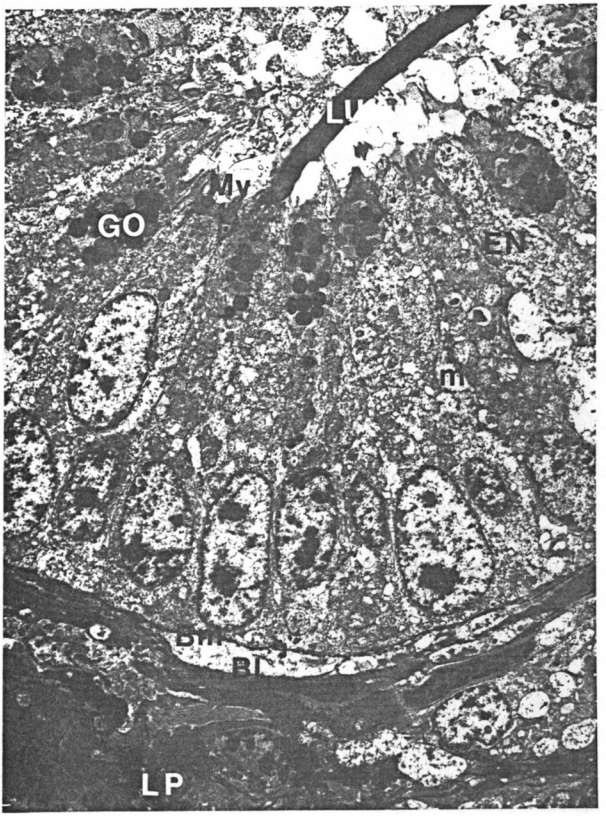

Fig. 31

FIGURE 32. PCB-chicken (withdrawal treatment of FR + 10% MO), age 39 days. Jejunum. Higher magnification of the same field as Figure 29. Note goblet cells (GO) and irregular microvilli (MV). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7500.

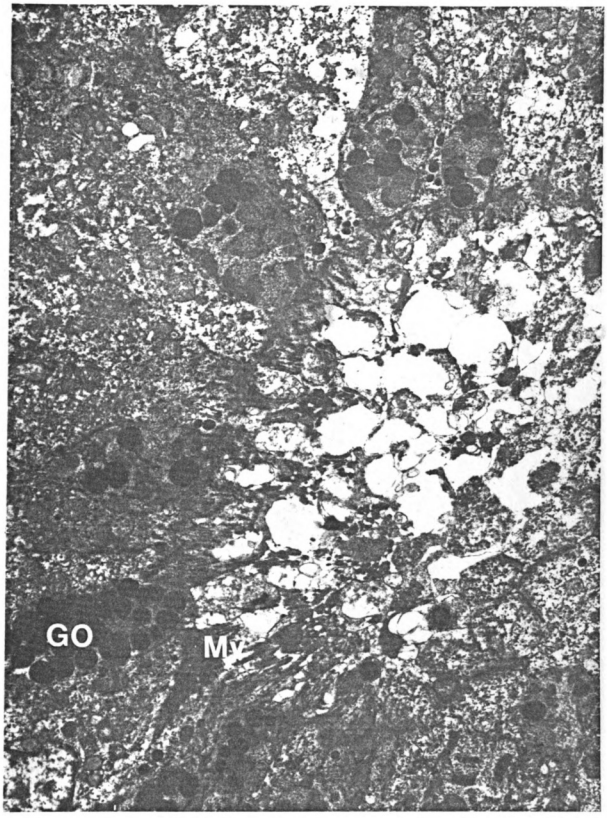

Fig. 32

FIGURE 33. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Longitudinal section of microvilli in higher magnification shows a marked reduction or absence of the glycocalyx (arrow). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X1010250.

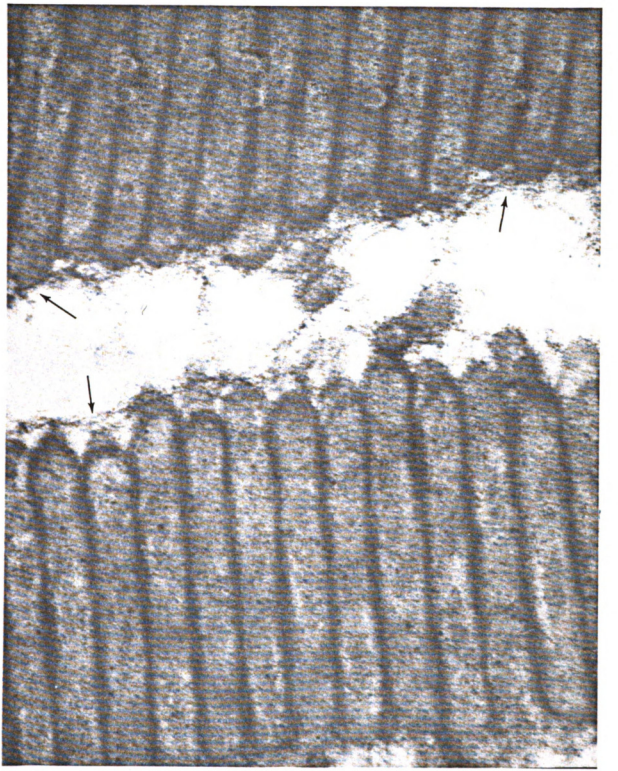

Fig. 33

FIGURE 34. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. Upper parts of few entrocytes (EN) facing to the lumen (LU) of the bowel. There is vacuolization of the cristae mitochondriales (M). IM. Intercelular membrane. GO. Goblet cell. Glutaraldehyde and osmium tetroxide fixation. Lead citrate uranyl acetate stain. X16333.

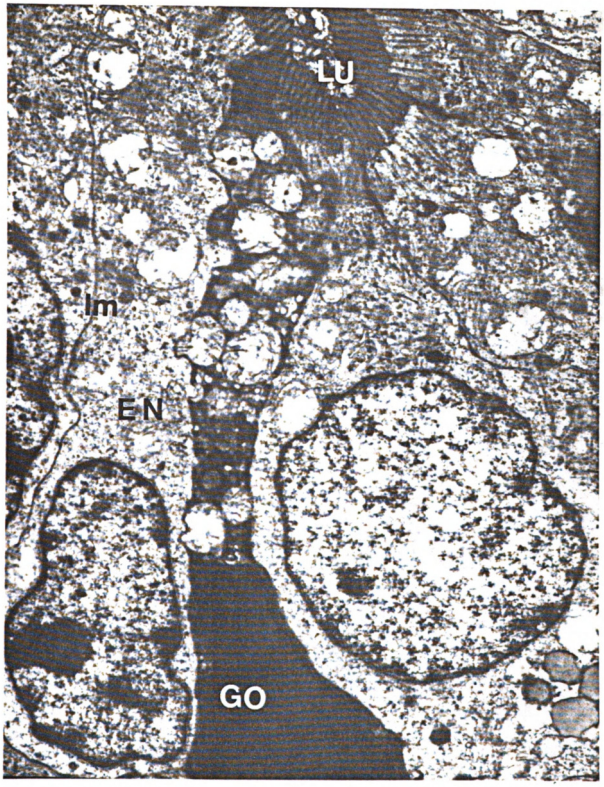

Fig. 34

FIGURE 35. PCB-chicken (no withdrawal treatment), age 35 days. Large intestine. An endocrine cell (E) with dense secretory granules (GR) in the cytoplasm and oval nucleus (N) situated above the granules in relation to the crypt lumen. The mitochondria (M) are swollen and broken and there is disruption and degeneration of the cristae mitochondriales. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X16970.

Fig. 35

FIGURE 36. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Lower portion of an endocrine cell (E) showing small cytoplasmic granules (GR), closed vacuoles (VA), and few slightly swollen mitochondria (M). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X49333.

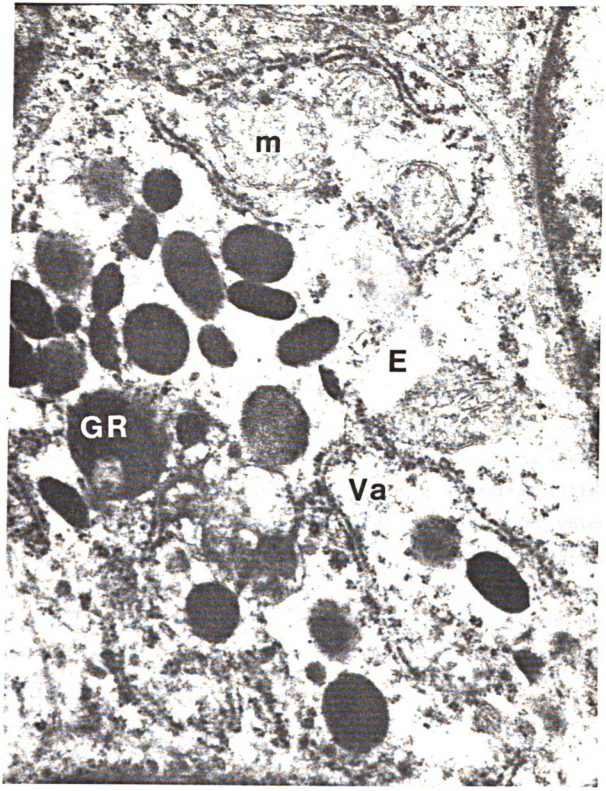

Fig. 36

almost the entire cytoplasmic space partly or completely enclosing membrane-lined vacuoles, and only traces of normal cytostructure remained (Figures 21, 28, 42, 43, and 41).

The lower half of several enterocytes and the lamino propria from the villi of a PCB-treated chicken is shown in Figure 44. Non-membranous vacuolization of an enterocyte, distribution of ribosomes and polyribosomes in entire cytoplasm of the entrocytes, and slight vacuolization of few mitochondriales cristae are seen (Figure 44). Basal membrane and basal laminae plus few fine collagen strands are present (Figure 44). A higher magnification of a fibrocyte shows vacuolization of the nucleus and few collagen fibres (Figure 45).

The plasma cells in PCB-chickens appeared moderately active. The most characteristic features of these cells were cisternal dilation, which varied from cell to cell. The cytoplasm often completely taken up with cisternae filled with a dense homogenous substance (the immunoglobulin) which indicates increased cell activity (Figures 46-48, 49-51, 52-56, 57-59, and 64). Under the electron microscope, the plasma cell was identified by its chromatin pattern of the nuclei or by the cisternal arrangement within the cytoplasm (Figures 46, 47, and 60).

FIGURE 37. PCB-chicken (no withdrawal treatment), age 39 days. Large intestine. Upper part of an entrocyte. The cytoplasmic organells show lack of organization with swelling of the mitochondria (M) and disruption of the cristae (arrows). There is increased vacuolization (VA) affecting both smooth endoplasmic reticulum and rough endoplasmic reticulum-lined vesicles and numerous free-lying ribosomes and polyribosomes (R). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X50000.

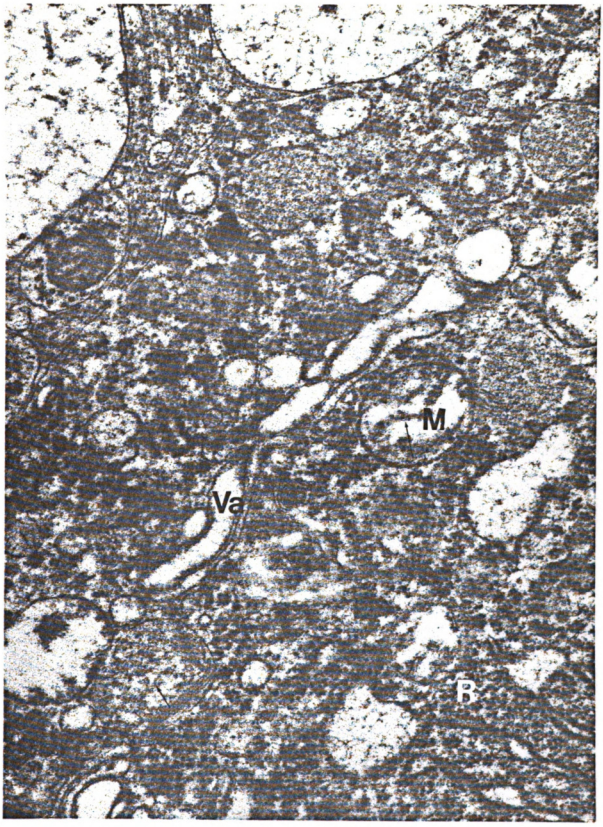

Fig. 37

FIGURE 38. PCB-chicken (no withdrawal treatment), age 39 days. Large intestine. Higher magnification from the same field as Figure 32. Numerous vacuolization (VA) is present in the cytoplasm. Marked internal vacuolization and disarray of the cristae of many of the mitochondria (M) are clearly apparent. Note numerous free-lying ribosomes and polyribosomes (R). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X76500.

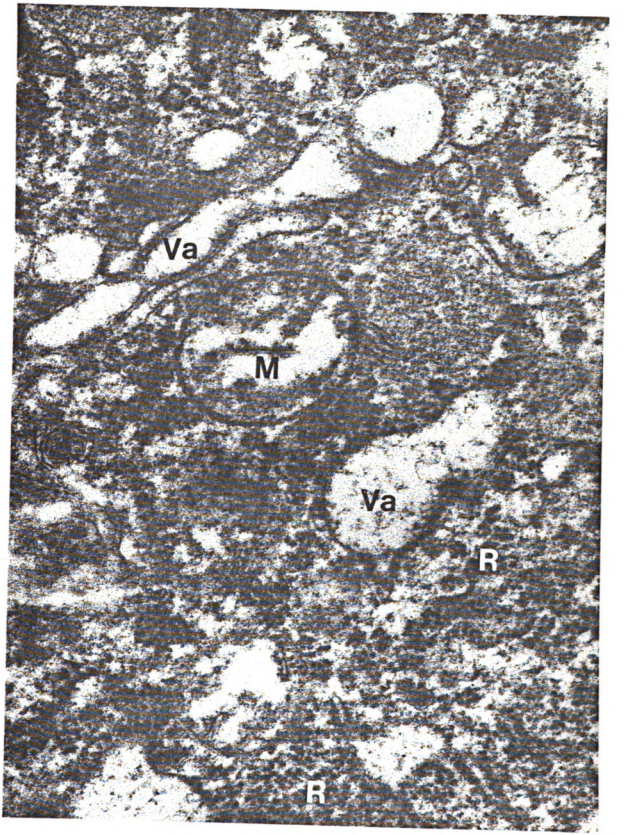

Fig. 38

FIGURE 39. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Villus epithelial cell. Notice numerous vacuolization (arrow) in the cytoplasm. M = mitochondria; L = lysosome; N = nucleus; IM = intercellular membrane. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X14000.

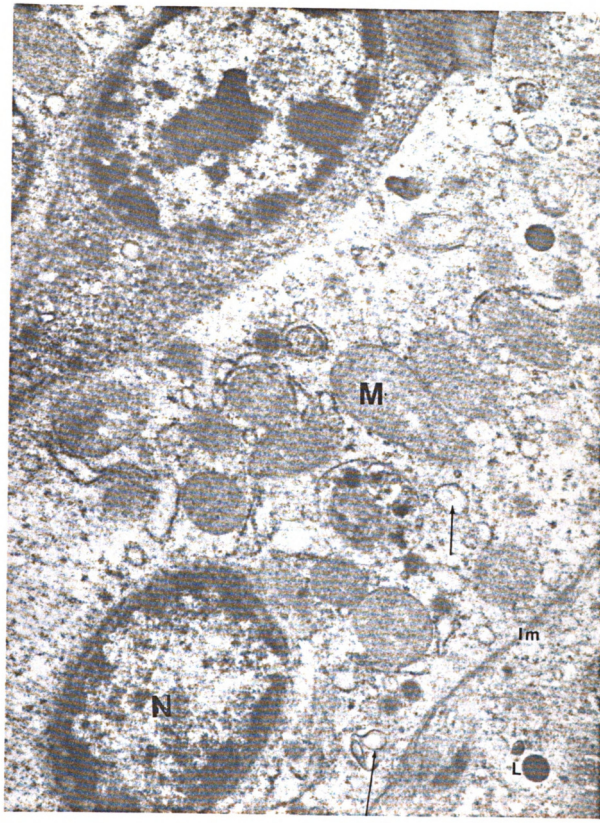

Fig. 39

FIGURE 40. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Several enterocytes (EN) and two goblet cells (GO) are facing toward the lumen of the bowel. Notice the cytoplasmic vacuolization (VA) of two enterocytes. There is an endocrine cell (E) at the upper left of the photomicrograph containing cytoplasmic granules. Some artifacts (A) as black dots are seen at the center and lower part of the picture. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7500.

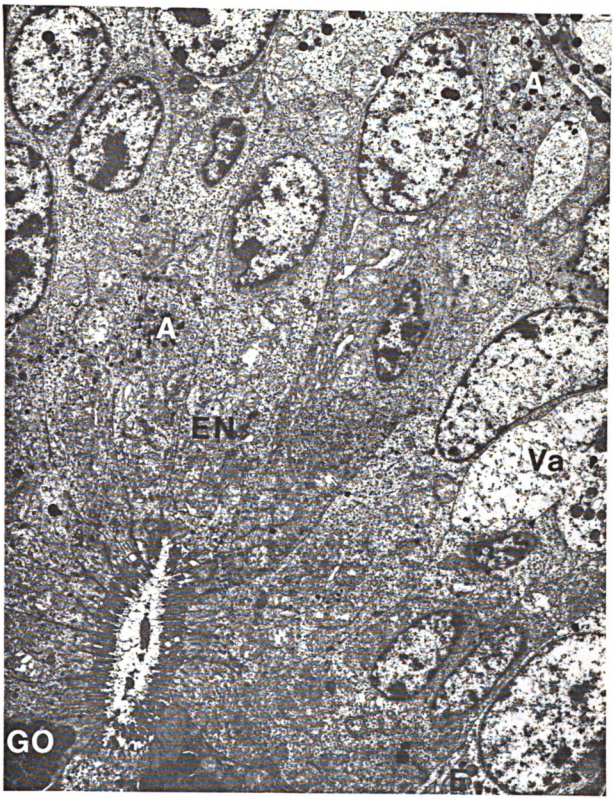

Fig. 40

FIGURE 41. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Nonmembraneous vacuolization (VA) of the entire cytoplasmic space includes cell debris, the nucleus, and the other organelles. M - mitochondria. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X23333.

Fig. 41

FIGURE 42. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Notice extensively expanded cisternae of rough endoplasmic reticulum (RER) in a villus epithelial cell. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X24667.

Fig. 42

FIGURE 43. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. The limiting membrane of the surface of the enterocytes (EN) appears heaped up (arrow). Notice degeneration of the villus epithelial cells and expanded cisternae of the endoplasmic reticulum (ER). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7421.

Fig. 43

FIGURE 44. PCB-chicken (withdrawal treatment of FR + 10% MO), age 39 days. Jejunum. The basal membrane (BM) and basal laminae (BL) of several entrocytes (EN). Fine strands of collagen fibres (arrow) are seen in lamina propria. Note the cytoplasmic vacuolization (VA) enterocytes. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X75000.

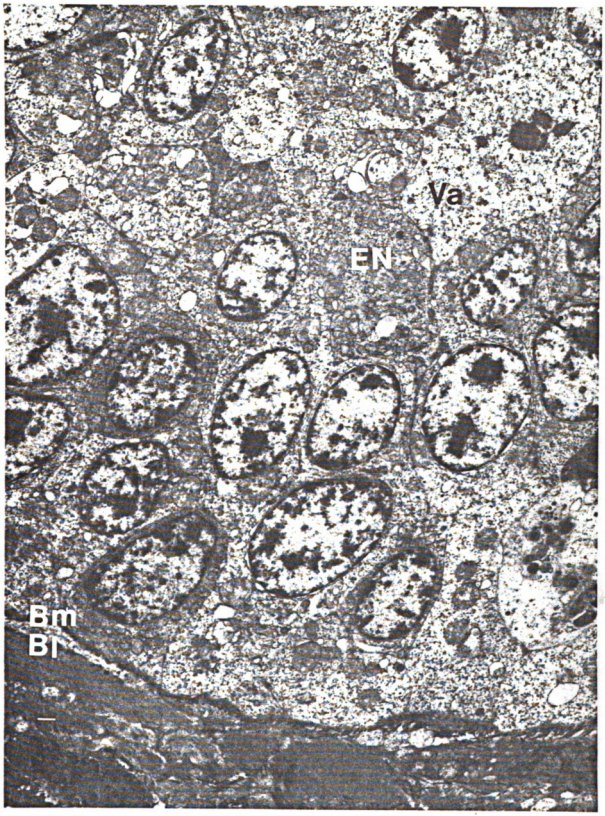

Fig. 44

FIGURE 45. PCB-chicken (no withdrawal treatment), age 35 days. Jejunum. The basal laminae (BL) of enterocytes (EN) appear as a wide layer composed of the fine fibrous tissues. Nucleus (N) of the fibrocyte shows vacuolization. The entrocyte also has membrane closed vacuoles (VA) in its cytoplasm. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X37826.

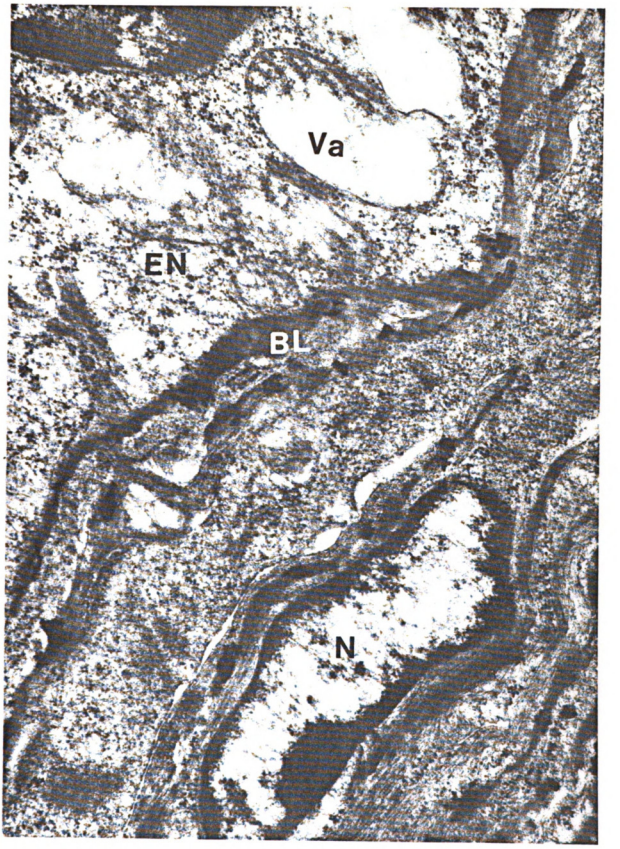

Fig. 45

FIGURE 46. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. A plasma cell from lamina propria. Nucleus (N) is centrally located, the cytoplasmic cisternae (CY) are slightly dilated, and fill most of the cytoplasmic spaces. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X27500.

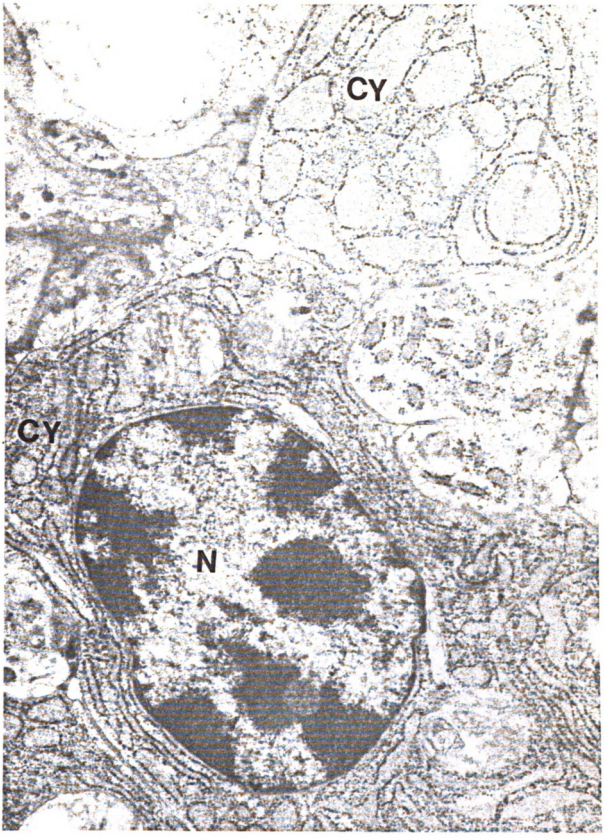

Fig. 46

FIGURE 47. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Higher magnification from same field as Figure 29. Note slightly dilated cytoplasmic cisternae (CY) which is surrounding the nucleus (N). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X52500.

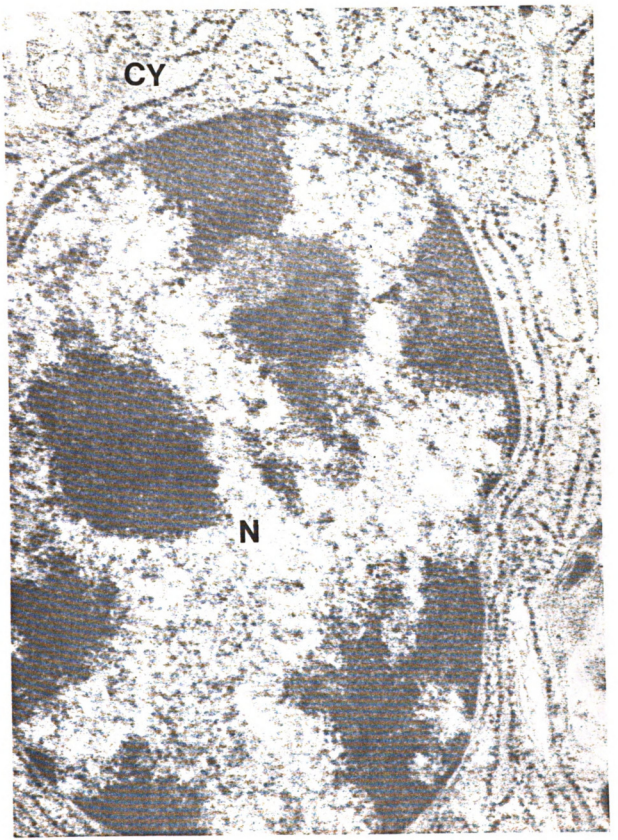

Fig. 47

FIGURE 48. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Part of the cytoplasm of a plasma cell showing dilated cisternae (CY) surrounded by rough endoplasmic reticulum (RER) and filled with fine granular material (arrow) which is more electron dense than the surrounding cytoplasm. Free ribosomes (R) can be observed. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X75000.

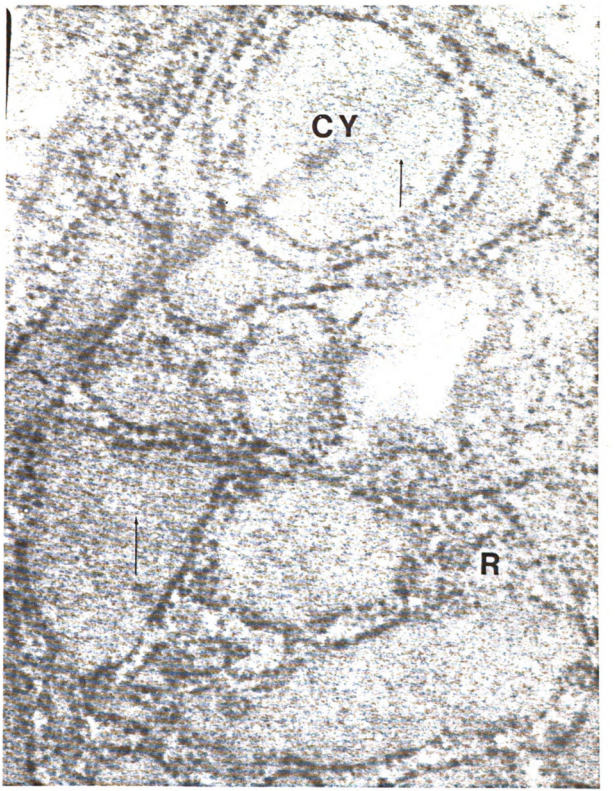

Fig. 48

FIGURE 49. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. The cytoplasm is composed of slightly dilated cisternae (CY) which are surrounded by the swollen mitochondria (M). There are numerous ribosomes (R) and few dense bodies, probably lysosomes (L). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X26667.

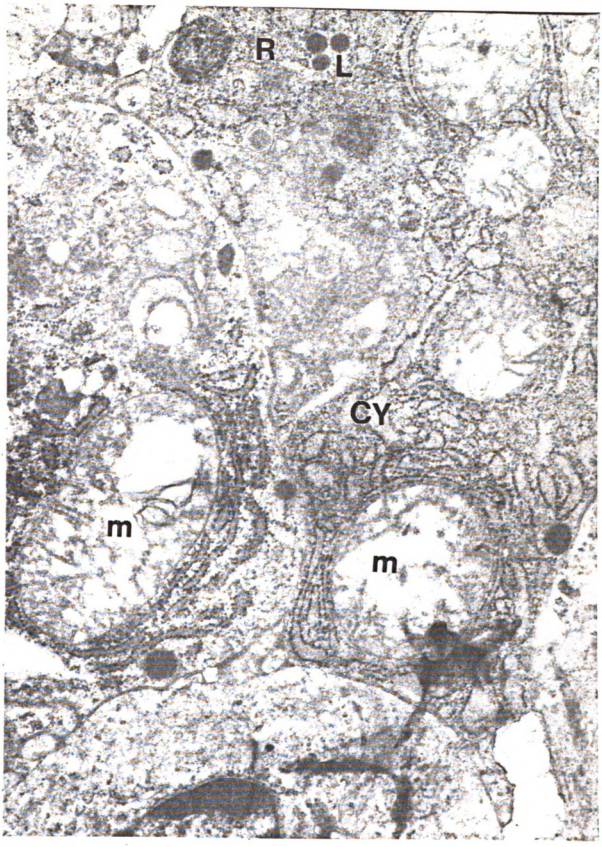

Fig. 49

FIGURE 50. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Part of the cytoplasm of the cell from the previous figure. Mitochondria (M) are swollen and cristae of mitochondria is disrupted. The cytoplasmic cisternae (CY) is slightly dilated and surrounded by RER. Notice free ribosomes (R) and lysosomes (L). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X50000.

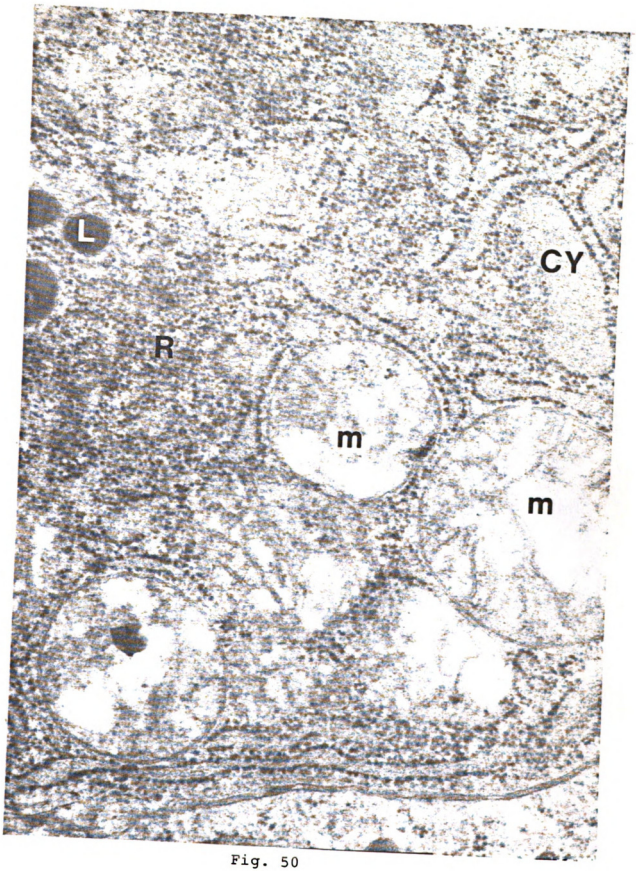

Fig. 50

FIGURE 51. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Higher magnification from the same field as Figure 34. The mitochondria (M) are severely damaged. Marked internal vacuolization (VA) and disarray of the cristae of the mitochondria is clearly apparent. Note ribosomes (R) and cytoplasmic cisternae (CY). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X49600.

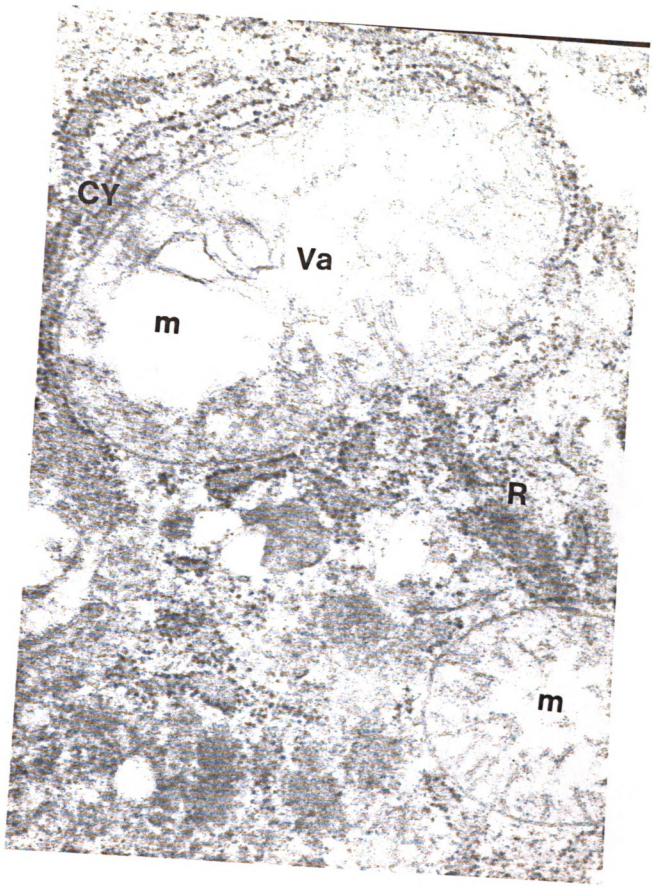

Fig. 51

FIGURE 52. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Part of the cytoplasm of a plasma cell showing the dilated cisternae (CY) containing fine granular material (arrow) surrounded by rough endoplasmic reticulum (RER). There is an artifact (a large black spot) at the center. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X35000.

Fig. 52

FIGURE 53. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Higher magnification from the same field as Figure 41 showing dilated cisternae (CY) surrounded by rough endoplasmic (RER) and filled with fine granular material (arrow) which is more electron dense than the surrounding cytoplasm. A - artifact (probably lead). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X40000.

Fig. 53

FIGURE 54. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Higher magnification of Figure 42. The cisternae (CY) are dilated and contain immunoglobulin. Note rough endoplasmic reticulum (RER) and free ribosomes (R). M - mitochondria. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X75000.

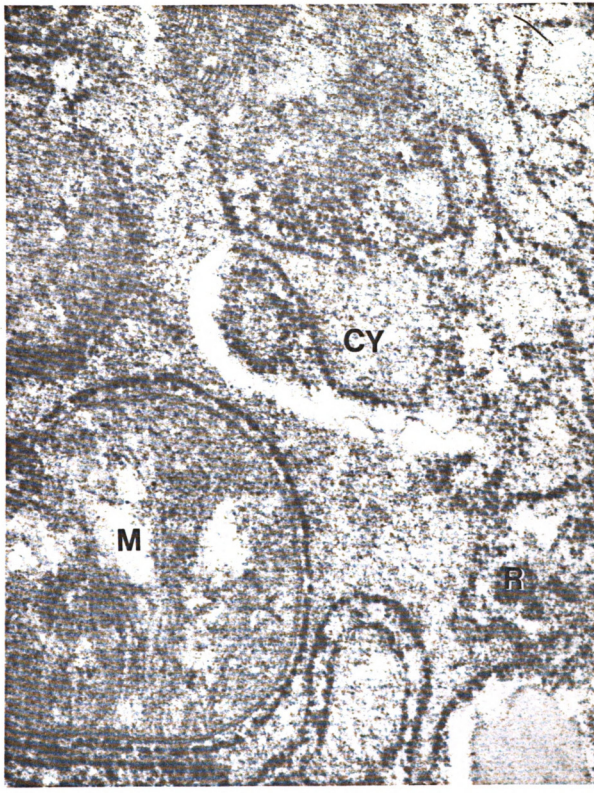

Fig. 54

FIGURE 55. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Part of the plasma cell from same bird as Figure 43. Showing a mitochondria (M) and slight dilation of the cytoplasmic cisternae (CY). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X109286.

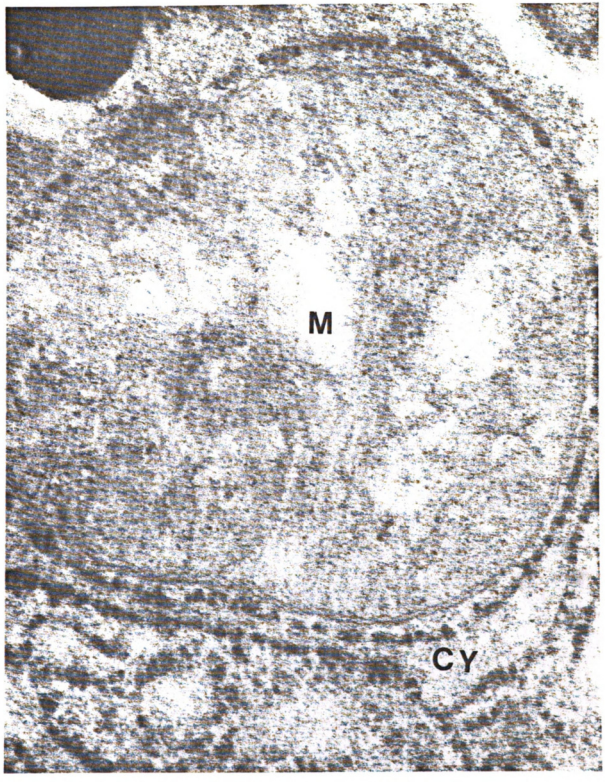

Fig. 55

FIGURE 56. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. Parts of two plasma cells showing the following features: two nuclei (N), dilated and irregularly arranged cytoplasmic cisternae (CY), and large vacuoles (VA). The mitochondria (M) are swollen. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X21250.

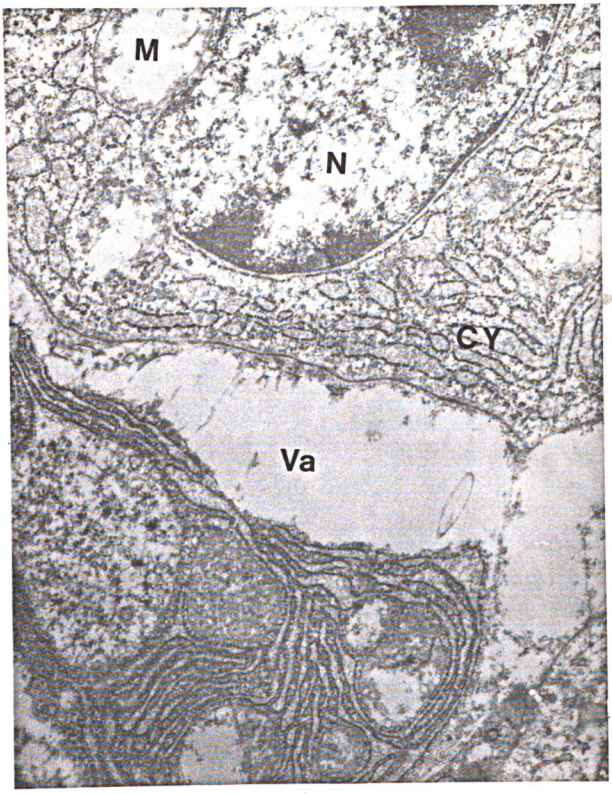

Fig. 56

FIGURE 57. PCB-chicken (withdrawal treastment of FR + 10% MO), age 42 days. Jejunum. Part of the cytoplasm of a plasma cell. The cytoplasm composed of dilated cisternae (CY) (containing immunoglobulin). The nucleus (N) of a neighboring cell is seen above and right. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X39000.

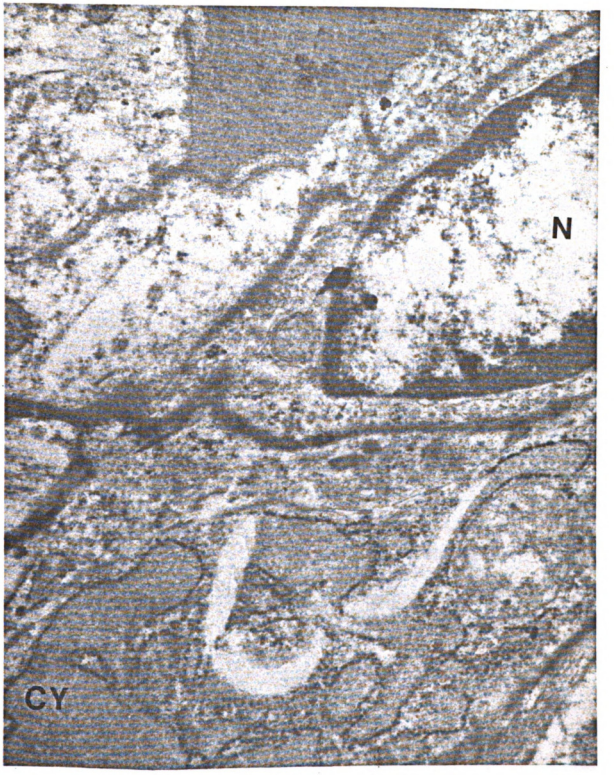

Fig. 57

FIGURE 58. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Part of the cytoplasm of a plasma cell. The cisternae (CY) is moderately dilated. Note the free ribosomes and polyribosomes (R). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X6000.

Fig. 58

FIGURE 59. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Jejunum. Higher magnification of plasma cell cisternae (CY) from Figure 48 is showing dilation of the cisternae. The dilated cisternae (CY) are surrounded by rough endoplasmic reticulum (RER). Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X112500.

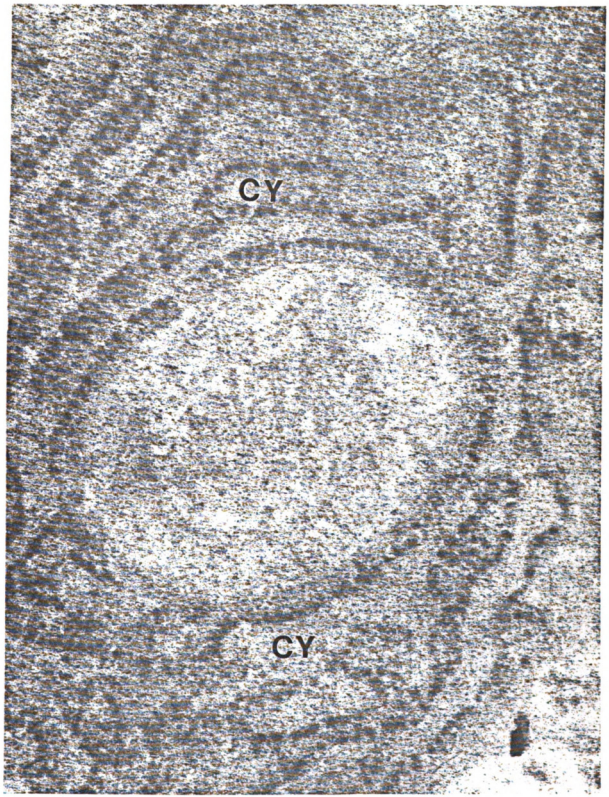

Fig. 59

FIGURE 60. PCB-chicken (no withdrawal treatment), age 42 days. Jejunum. A plasma cell containing a centrally placed nucleous (N) and slightly dilated cisternae (CY) which fill most of the cytoplasmic spaces. R - free ribosomes. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate strain. X4000.

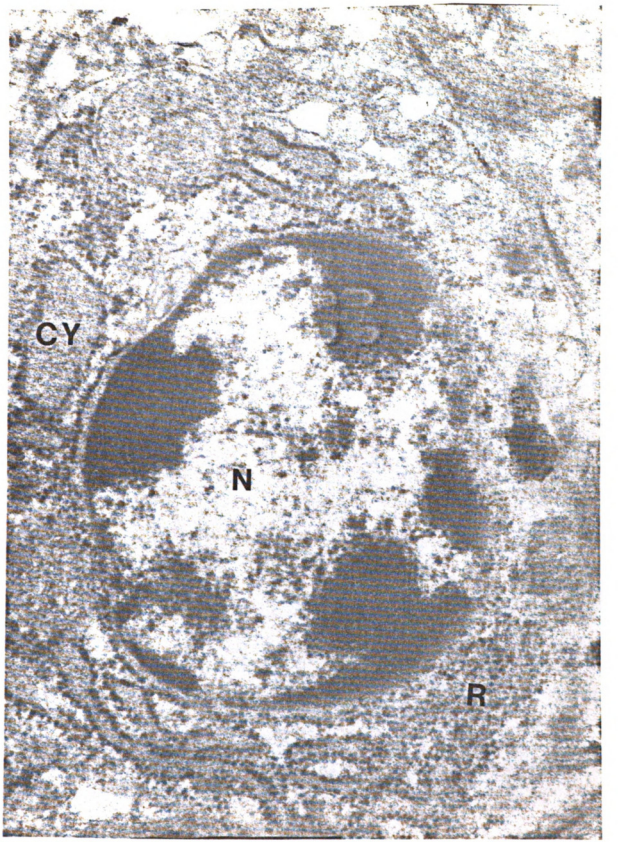

Fig. 60

FIGURE 61. PCB-chicken (withdrawal treatment of FR + 10% MO), age 35 days. Large intestine. Longitudinal section of an enterocyte (EN) and a goblet cell (GO). Notice that the goblet cell is well covered by microvilli (MV). During discharge of the granular material from the cell, the microvilli separate and the material in the form of mucous droplets escape into the lumen (LU). The cytoplasmic organelles within the enterocyte are not clearly visible and there is a reduction of the glycocalyx on brush border (arrow). N nucleus. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X23611.

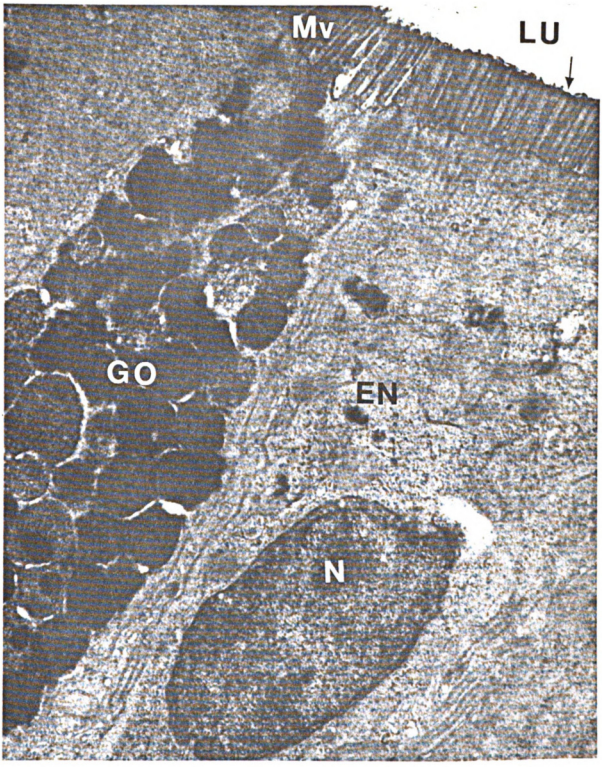

Fig. 61

FIGURE 62. PCB-chicken (no withdrawal treatment), age 35 days. Large intestine. Notice opening of many goblet cells (GO) to lumen (LU) of the bowel. E = endocrine cell; VA = vacuolization of the cytoplasm. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X7600.

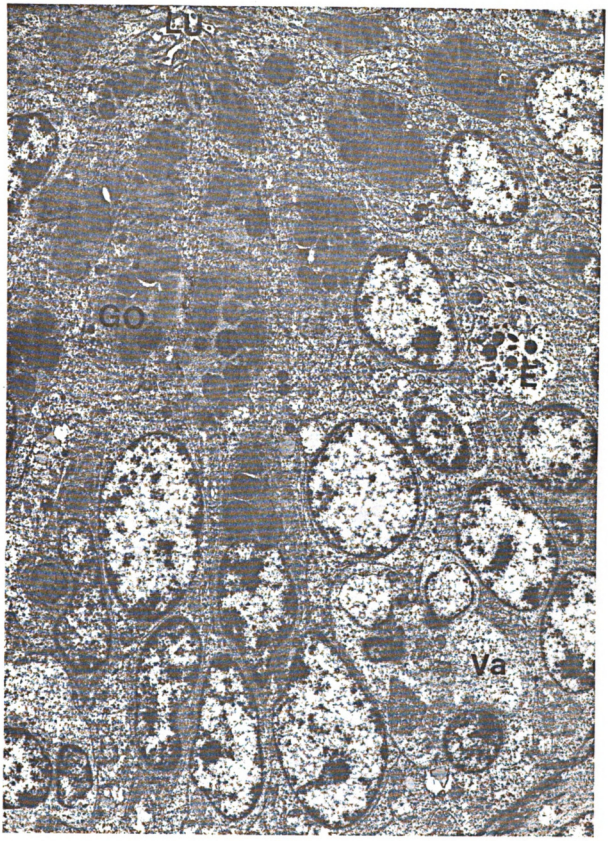

Fig. 62

FIGURE 63. PCB-chicken (withdrawal treatment of FR + 10% MO), age 35 days. Large intestine. Longitudinal section of two enterocytes (EN). Note the bulbous and shortened microvilli (MV). IM - intercellular membrane; N - nucleus; M mitochondria. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X25714.

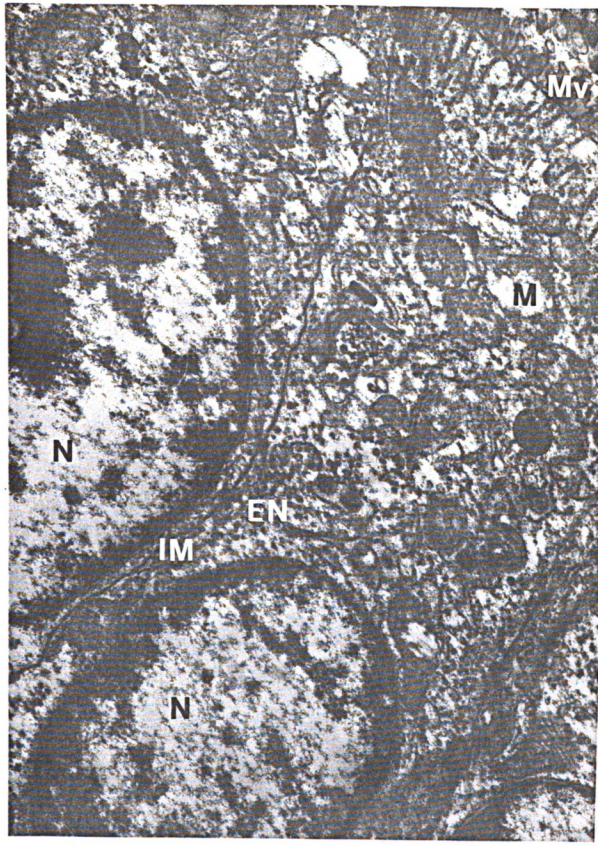

Fig. 63

FIGURE 64. PCB-chicken (withdrawal treatment of FR + 10% MO), age 42 days. Large intestine. Higher magnification of a cytoplasmic cisternae (CY). It is filled with fine granular material and surrounded by rough endoplasmic reticulum. R - ribosomes. Glutaraldehyde and osmium tetroxide fixation. Lead citrate, uranyl acetate stain. X110204.

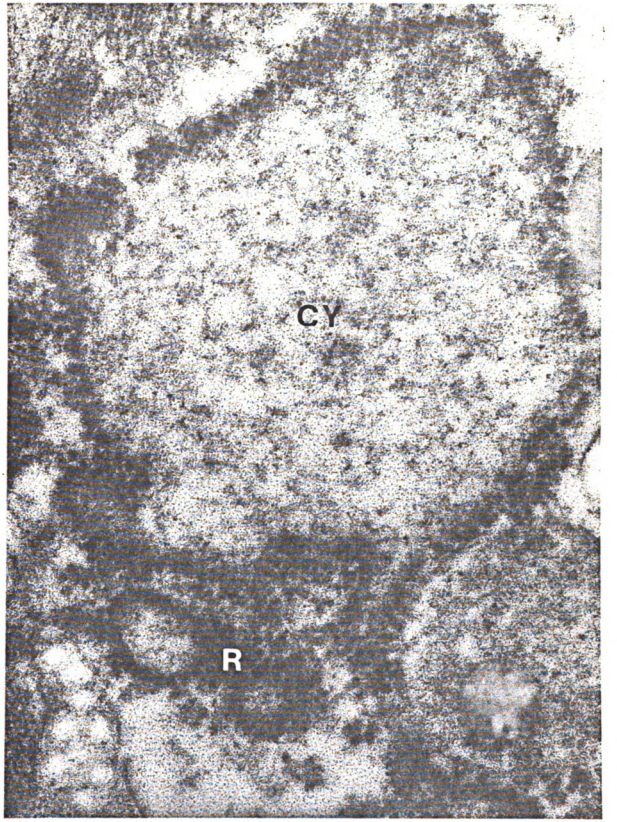

Fig. 64

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SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY ON PATHOLOGICAL EFFECTS OF PCBs (AROCLOR 1254) IN THE INTESTINAL MUCOSA OF CHICKENS VOLUME II

by

Shaban Rahimi

A DISSERTATION

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

DOCTOR OF PHILOSCOPHY

Department of Animal Science

1989

PCB ANALYSIS

In this experiment, the chickens fed 10 ppm Aroclor 1254 for 14 days had PCB residues in tissues equal to 6.96 µg/g at day 0 of withdrawal (Table 3). After 5 days of feeding clean diet ad libitum, the PCB residues averaged 6.23 µg/g, 10% lower than day 0. Restriction of feed to 50% of ad libitum for 5 days produced an 8% decline of body burdens in comparison to PCB-contaminated chickens fed clean diet ad libitum. Mineral oil at 5% in the diet reduced PCB concentrations to 5.33 µg/g, a 14% decline. However, the combination of 50% feed restriction and 10% mineral oil reduced PCBs by 23%.

The data in Table 3 indicate that, following PCB contamination, dietary administration of 5% mineral oil and 10% MO + 50% feed restriction increased the concentration of PCBs in excreta approximately 1.5 and 4 fold, respectively, as compared to chickens fed a clean diet for 5½ days. Values of PCBs in excreta were 3.3 µg/g for chickens not treated, while PCBs in excreta of chickens treated with 10% MO + 50% FR had excreta concentrations of 14.8 µg/g, a 4.4 fold increase (Table 3 and Figures 65 and 66).

The concentration of PCBs in various sections of the intestinal lumen at day 0 and day 5 of withdrawal are shown in Table 5. PCB concentrations increased from 3 to 4.5 fold in the intestinal contents in chickens restricted in feed and/or provided mineral oil (Table 5). There appeared to

Table 3. Effect of mineral oil and feed restriction on the body burdens of Aroclor 1254 and its concentration in dried excreta of chickens previously fed 10 ppm PCBs for 14 days.

Treatments	Day of withdrawal PCBs-µg/g whole-body		excreta		Day 5 of withdrawal PCBs - µg/g whole-body		excreta	
Ad libitum	(20)[1]	6.96	(120)	6.0	(10)	6.23	(20)	3.3
5% MO[2], ad lib[3]					(10)	5.33	(20)	4.4
Fed at 50% ad lib					(10)	5.73	(20)	7.5
10% MO, 50% ad lib					(10)	4.79	(20)	14.8

[1] No. of observation

[2] MO = mineral oil.

[3] Ad lib = ad libitum.

be a difference in the pattern of the PCB concentrations depending on whether MO or feed restriction was used. Mineral oil alone seemed to increase the concentration of PCBs in the intestinal contents. The effect of MO at 5% of the diet was somewhat less pronounced than at the 10% MO + 50% FR level in total amount of excreta elimination of PCBs (Fig. 67).

The ratio of PCBs to Cr_2O_3 in the intestinal contents of chickens given PCB-contaminated diet for 14 days was 1.4 times higher than those that received the clean diet ad libitum for 5 days after withdrawal (Table 6). On the other hand, the ratio of PCBs to Cr_2O_3 in the intestinal contents of chickens fed 50% ad libitum, 5% MO at 100% ad libitum, or 10% MO + 50% feed restriction was 2.3, 2.3, and 2.6 fold, respectively, higher in comparison to chickens that received clean diet ad libitum (Table 6).

The ileum played a major role in the excretion of PCBs in chickens fed 5% MO + ad libitum (Tables 5 and 7). The ratio of PCBs to Cr_2O_3 in that segment was 1.5 to 2 times that in the large intestine plus ceca, and duodenum plus jejunum, respectively (Table 6).

Statistical analysis of PCB residues (Aroclor 1254) in intestinal contents is presented in Table 8. There is a trend for a significant difference in effect of dietary treatments in amount of PCB secration from the intestinal wall. Also the difference among segments of intestine in rate of PCB secretion is not significant ($P > 0.05$).

Table 4. Raw data for predicted concentration of Aroclor 1254 in whole body of chickens fed 10 ppm Aroclor 1254 for 2 weeks.

Treatment	Day killed[1]	µg/g PCBs in sample	Chicken weight (gm) on kill day	µg PCBs in chicken	Percent PCBs removed
PCB - Ad libitum	0	6.96	(20)[2] 423	(20) 2944	
PCB - Ad libitum	5	6.23	(10) 466	(10) 2903	1.4
PCB - 5% MO[3]	5	5.33	(10) 511	(10) 2724	7.5
PCB - FR[4]	5	5.73	(10) 387	(10) 2217	24.5
PCB - FR + 10% MO	5	4.79	(10) 379	(10) 1815	38.4

[1] Represents day of withdrawal.
[2] No. of birds.
[3] MO = mineral oil.
[4] FR = feed restriction.

Table 5. Effect of mineral oil and feed restriction on the PCBs (Aroclor 1254) in intestinal contents (dried) of chickens previously administered 10 ppm PCBs for 14 days.

Treatments	Day 0 of withdrawal PCBs - µg/g intestinal contents			Day 5 of withdrawal PCBs - µg/g intestinal contents		
	Duodenum + jejunum	Ileum	Ceca + large intestine	Duodenum + jejunum	Ileum	Ceca + large intestine
Ad libitum	(20)[1] 4.7	(20) 3.7	(20) 1.4	(10) 2.9	(10) 2.2	(10) 2.1
5% MO[2], ad lib[3]				(10) 2.8	(10) 9.3	(10) 6.8
Fed at 50% ad lib				(10) 6.4	(10) 5.6	(10) 4.6
10% MO, 50% ad lib				(10) 6.4	(10) 7.7	(10) 5.4

[1] No. of birds pooled per sample.

[2] MO = mineral oil.

[3] Ad lib = ad libitum.

Table 6. Ratio of percent PCBs to percent chromic oxide in the intestinal contents (dried) at day 0 and day 5 of withdrawal from diets containing 10 ppm PCBs (Aroclor 1254).

Treatments	PCBs/ CR203 intestinal contents		
	Duodenum + jenunum	Ileum	Ceca + large intestine
	Day 0 of withdrawal		
Ad libitum	(20)[1] 0.00163	(20) 0.00082	(20) 0.00031
	Day 5 of withdrawal		
5% mineral oil, Ad libitum	(10) 0.00100	(10) 0.00049	(10) 0.00047
Fed at 50%, Ad libitum	(10) 0.00221	(10) 0.00125	(10) 0.00102
10% mineral oil, 50% Ad libitum	(10) 0.00221	(10) 0.00171	(10) 0.00120

[1] No. of birds pooled per sample.

Raw data for predicted concentration of Aroclor 1254 in whole body of PCB-chickens is shown in Table 4. As illustrated in this table, without any treatment carcass, PCB's declined only by 1.4% by day 5 of withdrawal. Treatments such as 5% MO + ad libitum, or 50% feed restriction or a combination of 50% feed restriction plus 10% MO, caused reduction of the PCBs by 7.5%, 24.5%, and 38.4%, respectively by 5 days of withdrawal.

Table 7 shows the raw data for predicted concentrations of Aroclor 1254 in intestinal contents of chickens. Ileum was the apparent site of increased transfer of PCBs from the blood into the luminal contents in MO treated birds.

Table 7. Raw data for predicted concentration of Aroclor 1254 in intestinal contents of chickens fed 10 ppm Aroclor 1254 for 2 weeks.

Treatments	Segments: Duodenum + jejunum	Ileum	Ceca + large intestine
	Day 0 of withdrawal		
PCB - Ad libitum (20)[1]			
µg/g PCBs in sample	4.7157	3.6730	1.3889
Intestinal content wt. (g)	2.71	0.93	2.29
µg PCBs in each segment	12.78	3.41	3.32
	Day 5 of withdrawal		
PCB - Ad libitum (10)[1]			
µg/g PCBs in sample	2.9443	2.2226	2.0779
Intestinal content wt. (g)	7.22	6.25	5.55
µg PCBs in each segment	21.26	13.89	11.53
PCB - 5% mineral oil, Ad libitum			
µg/g PCBs in sample	2.8438	9.3418	6.8290
Intestinal content wt. (g)	8.25	5.09	4.64
µg PCBs in each segment	23.46	47.55	31.69
PCB - 50% Ad libitum (10)[1]			
µg/g PCBs in sample	6.4496	5.6114	4.6151
Intestinal content wt. (g)	7.28	7.28	4.82
µg PCBs in each segment	46.95	40.85	22.24
PCB - 10% mineral oil, 50% Ad libitum			
µg/g PCBs in sample	6.4535	7.7404	5.4073
Intestinal content wt. (g)	7.62	7.30	7.20
µg PCBs in each segment	49.17	56.50	38.93

[1] No. of birds.

FEED CONSUMPTION AND BODY WEIGET

This study was not designed to determine the rate of feed consumption or effect of PCBs on body weight of chickens during 14 days of treatment. Therefore, statistical analysis of these were not performed (see Appendix A, B, and C for raw data). As shown in Figure 69, there is hardly any difference in body weight gain (gram/bird/day) of control and PCB-chickens during the 2nd week of PCB-treatment.

Variation of body weights during residue-withdrawal period is shown in Table 9 and Figure 68. The effect of different dietary treatments on body weights of both controls and PCB-chickens appears significant, while the difference between body weights at days 4 and 7 of withdrawal and the effect of PCBs on body weights are not significant (Table 10). During residue withdrawal, body weights of control chickens receiving clean diet ad libitum increased slightly faster than PCB-chickens on the same dietary treatment (Figure 70).

FIGURE 65. Concentration of PCB in whole body of chickens during withdrawal after being fed 10 ppm PCB for 14 days

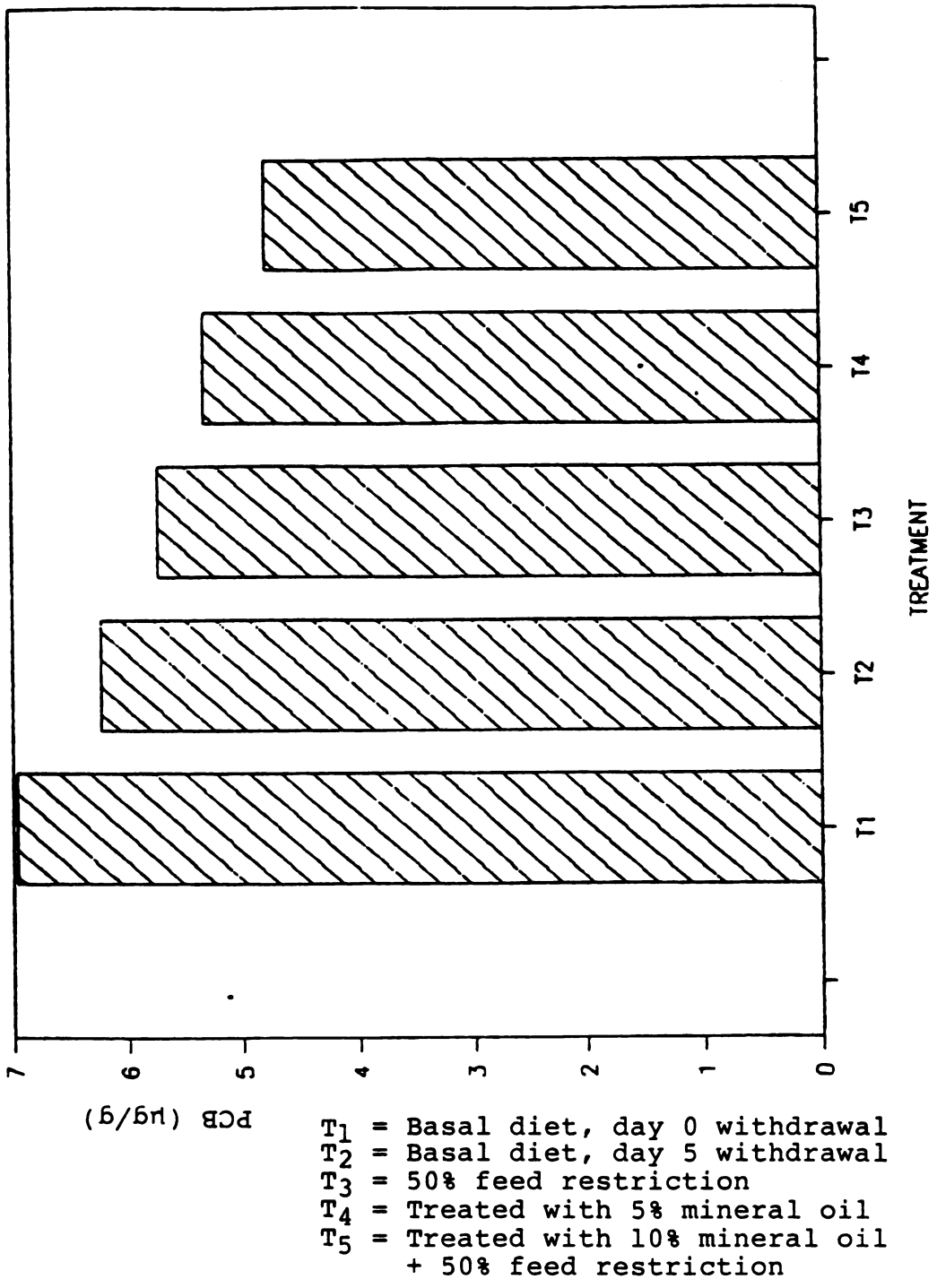


FIGURE 65. Concentration of PCB in whole body of chickens during withdrawal after being fed 10 ppm PCB for 14 days.

FIGURE 66. Concentration of PCBs in excreta of chickens during

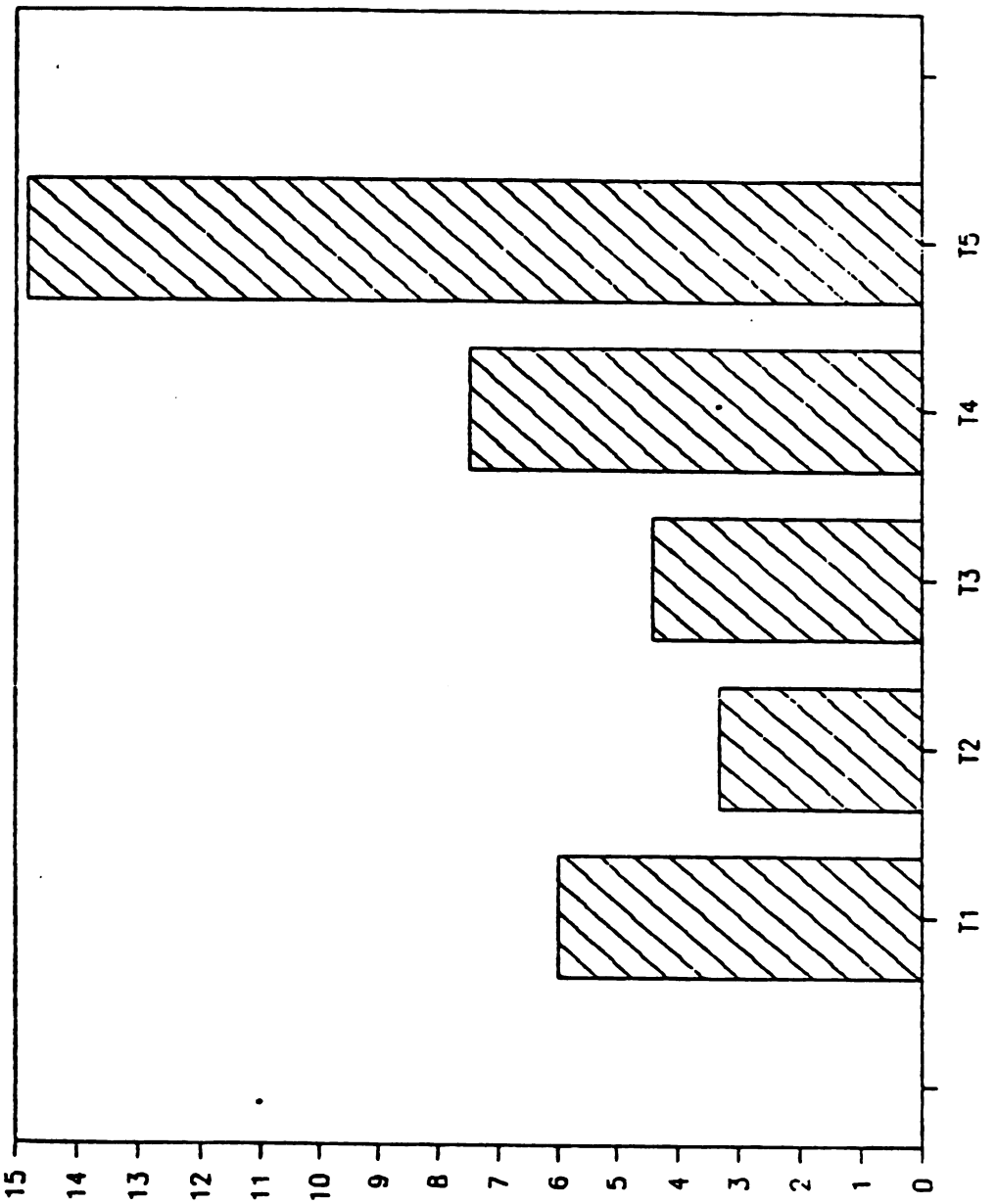


FIGURE 66. Concentration of PCBs in excreta of chickens during withdrawal after feeding 10 ppm PCBs for 14 days.

T_1 = Basal diet, day 0 withdrawal
T_2 = Basal diet, day 5 withdrawal
T_3 = 50% feed restriction
T_4 = Treated with 5% mineral oil
T_5 = Treated with 10% mineral oil + 50% feed restriction

FIGURE 67. Concentration of PCB in the intestinal content of chickens

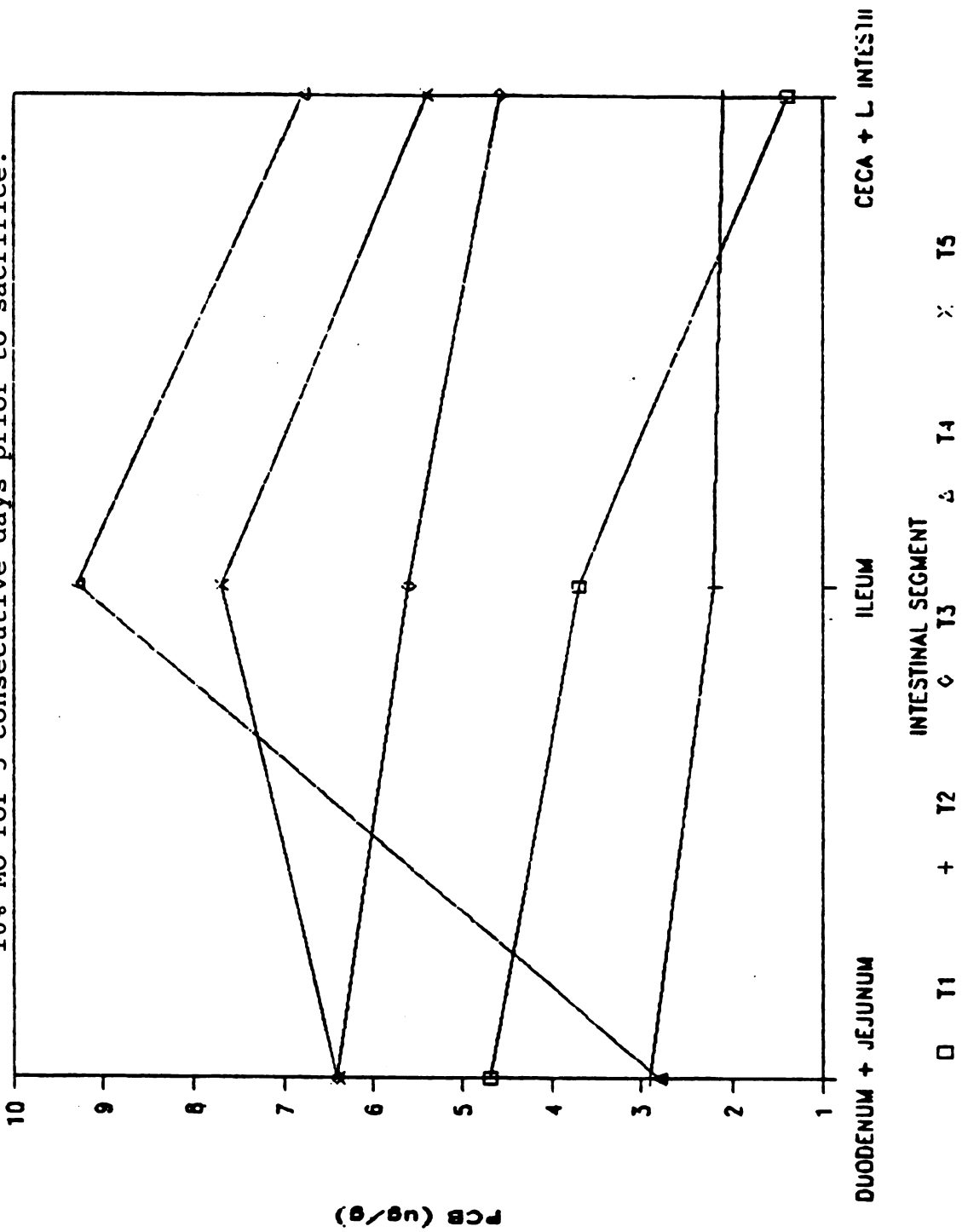
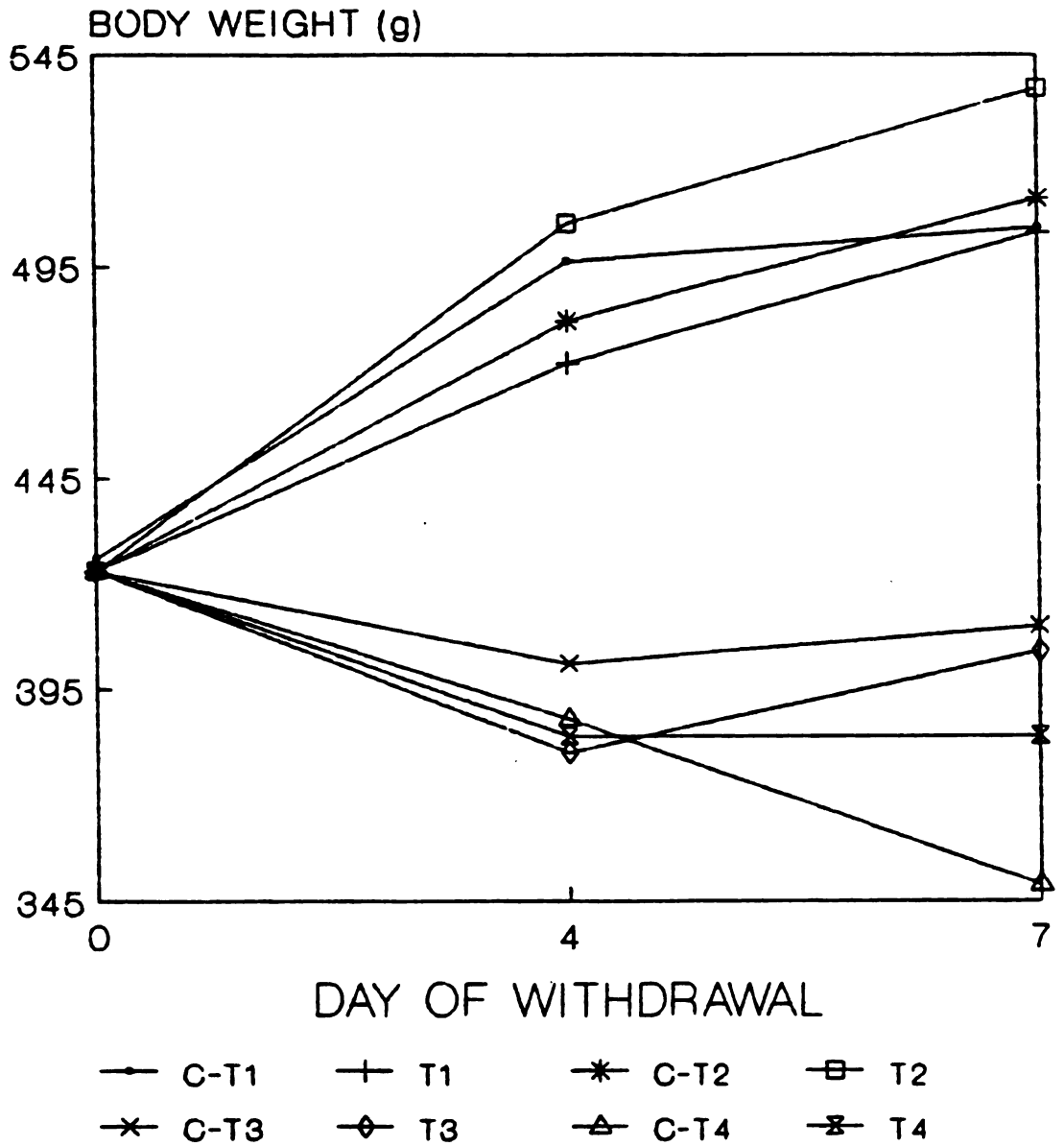


FIGURE 67. Concentration of PCB in the intestinal content of chickens dosed with 10 ppm PCB for 14 days prior to sacrifice. The diet of the treated animals was supplemented with 5% and 10% MO for 5 consecutive days prior to sacrifice.

T_1 = Basal diet, day 0 withdrawal
T_2 = Basal diet, day 5 withdrawal
T_3 = 50% feed restriction
T_4 = Treated with 5% mineral oil
T_5 = Treated with 10% mineral oil + 50% feed restriction
T_2-T_5 = Day 5 of withdrawal

FIGURE 68. Effect of mineral oil and feed restriction on the body weight of chickens previously receiving 0 or 10 ppm PCBs (Aroclor 1254) for 14 consecutive days.



T_1 = Clean diet, Ad libitum
T_2 = 5% mineral oil, Ad libitum
T_3 = Fed 50% Ad libitum
T_4 = 10% mineral oil, 50% Ad libitum
C = Control

FIGURE 69. Body weight gain (g/b/d) of chi

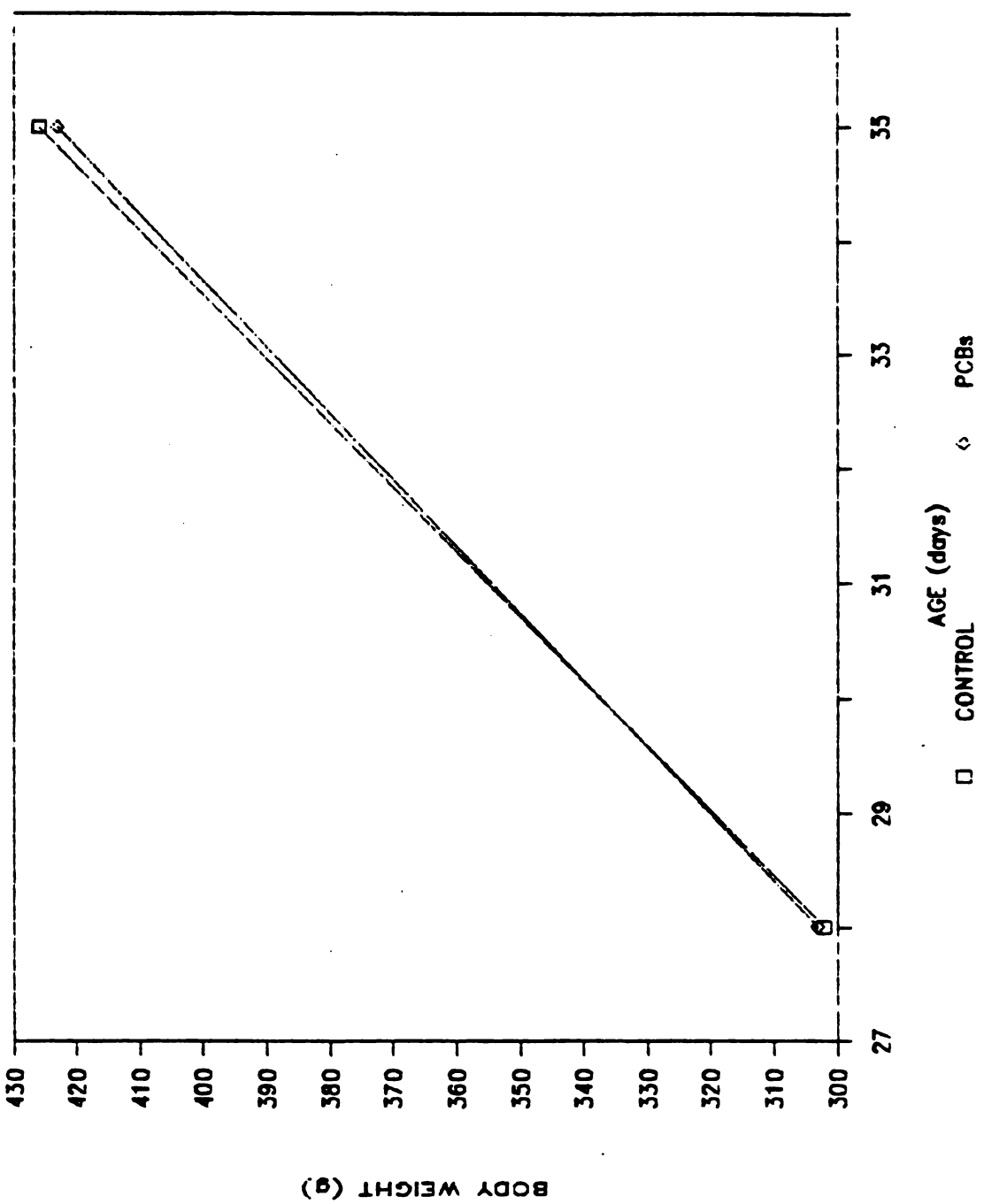


FIGURE 69. Body weight gain (g/b/d) of chickens during the 2nd week of PCBs treatment (days 28-35).

FIGURE 70. Variation of body weights in control chickens and PCB-chickens (previously fed 10 ppm Aroclor 1254 for 2 weeks) during residue withdrawal at the dietary treatment of

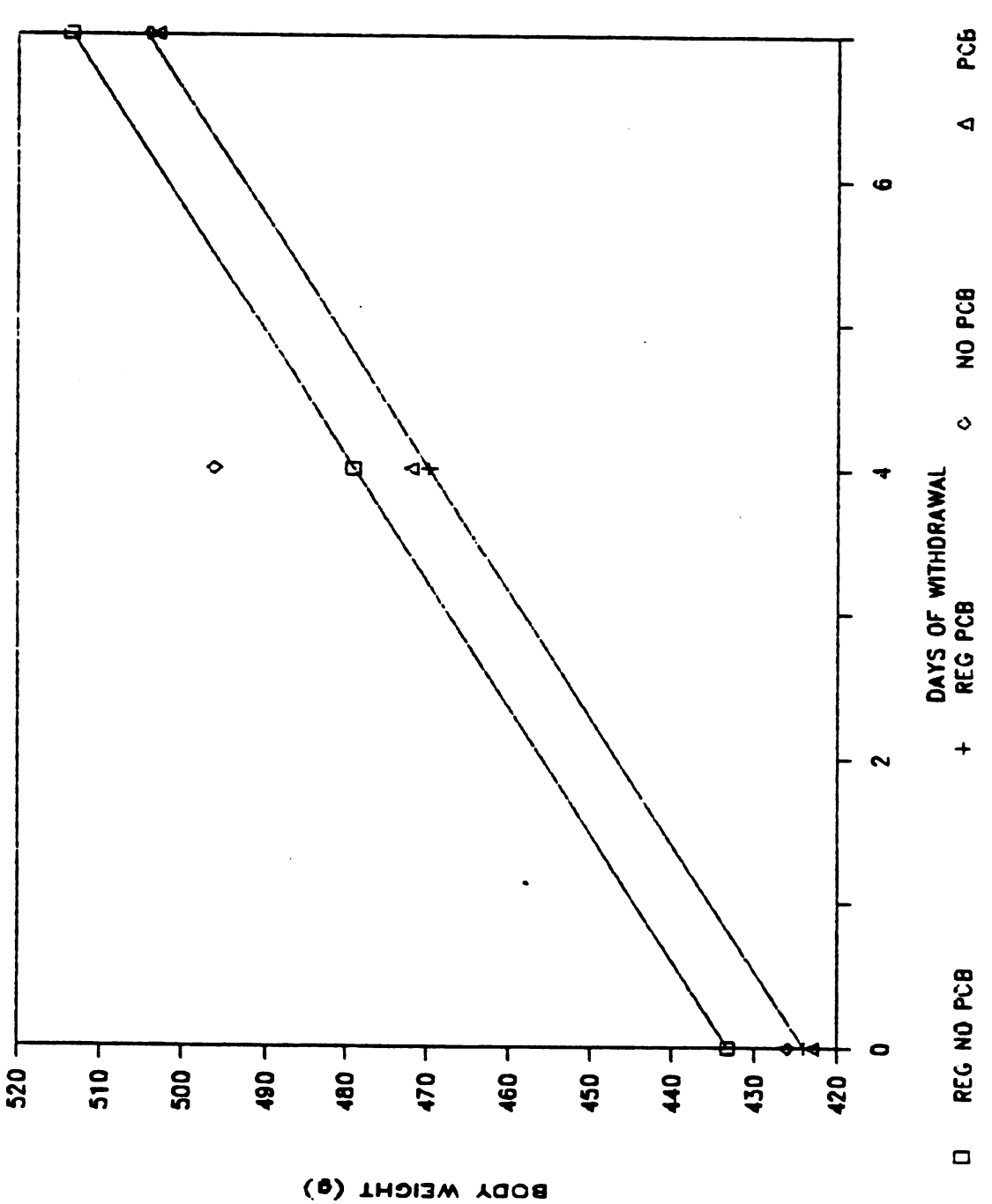


FIGURE 70. Variation of body weights in control chickens and PCB-chickens (previously fed 10 ppm Aroclor 1254 for 2 weeks) during residue withdrawal at the dietary treatment of clean feed Ad libitum.

Table 8. Statistical summary of data presented in Table 5.

Source of variation	Degrees of freedom (d.f.)	Sum square (SS)	Mean square (MS)	F ratio	Critical value
Segments	2	10.72	5.36	2.081	P = .07[1]
Treatments	3	32.55	10.85	4.22	---
Error	6	15.45	2.57	--	---

[1] Trend for significance.

Table 9. Effect of mineral oil and feed restriction on the body weight of chickens previously receiving 0 or 10 ppm PCBs (Aroclor 1254)

Table 9. Effect of mineral oil and feed restriction on the body weight of chickens previously receiving 0 or 10 ppm PCBs (Aroclor 1254) for 14 consecutive days.

Treatments	Day 0 of withdrawal body weight (g/bird) ± S.D.		Day 4 of withdrawal body weight (g/bird) ± S.D.		Day 7 of withdrawal body weight (g/bird) ± S.D.	
	No PCBs	10 ppm PCBs	No PCBs	10 ppm PCBs	No PCBs	10 ppm PCBs
Ad libitum	426 ± 37 (48)[1]	423 ± 42 (120)	496 ± 45 (8)	472 ± 26 (20)	504 ± 38 (4)	503 ± 26 (10)
5% MO[2], ad lib[3]			482 ± 22 (8)	505 ± 42 (20)	511 ± 17 (4)	537 ± 49 (10)
Fed at 50% ad lib			401 ± 75 (8)	380 ± 34 (20)	410 ± 34 (4)	404 ± 37 (10)
10% MO, 50% ad lib			388 ± 28 (8)	384 ± 38 (20)	349 ± 35 (4)	384 ± 45 (10)

[1] No. of birds.

[2] MO = mineral oil.

[3] Ad lib = ad libitum.

Table 10. Statistical analysis of body weights of the chickens during withdrawal period.

Source of variation	d.f.	SS	MS	F	F value	PR>F
Day (day 4 and day 7 of withdrawal)	1	3685.52	3685.52	3.00	0.0852	NS[1]
PCB (No PCB, with PCB)	1	546.44	546.44	0.44	0.5058	NS
TRT (dietary treatments during PCB withdrawal)	3	412244.43	137414.81	111.87	0.0001	S[2]
Day x PCB	1	2766.79	2766.79	2.25	0.1354	NS
Day x TRT	3	9083.60	3027.87	2.47	0.0644	NS
PCB x TRT	3	9144.7	3048.23	2.48	0.0631	NS
Error	155	190289.93	1228.32	--	---	

[1] NS = not significant.
[2] S = significant.

DISCUSSION

ELECTRON MICROSCOPY

Many studies in laboratory animals and accidental exposure of humans and other animals have shown that PCBs are toxic to a variety of biological targets (Neal, 1985; Zimmerman, 1982). Destruction of intestinal mucosa and accumulation of blood in the digestive tract of rats fed 3 ppm 3,3',4,4'-tetrachlorobiphenyl was reported (Wardell et al., 1982). Mink showed ulceration of gastric mucosa after receiving 10, 20, and 40 ppm of Aroclor 1242 in the diet (Bleavins et al., 1980). The pathological finding in the present study such as atrophy and destruction of microvilli swelling and vacuolization of mitochondria, destruction of cristae in mitochondria, and dilation of granular endoplasmic reticulum in villal epithelial cells of chickens which received 10 ppm Aroclor 1254 in diet for 2 weeks confirm the pathotoxicity of PCBs for chickens, also.

THE BRUSH BORDER

Maronpot and Whitehair (1967) reported that alteration of the brush border and a decrease of specific functional enzymes in intestinal epithelial cells cause significant effects on nutrient absorption. The importance of the brush border in digestion and absorption of intestinal contents has been described by Eichholz (1969). Crane (1969) stated that the brush border is a digestive-absorptive surface organelle less complex than mitochondria, but a structurally

integrated subcellular organelle, controlling and interacting with the internal environment of the villal epithelial cell as well as forming its luminal surface.

Enzymes catalyzing digestive and absorptive functions seem to be spatially arranged in the brush border so as to offer a kinetic advantage for absorption, not only of digested foodstuffs, but for sodium, and, possibly other ions by a mechanism of cooperative interaction between these and other absorbed molecules in the mobile carrier transport system (Crane, 1968, 1969).

In the present study, the microvilli within the brush border of the intestine in chickens fed PCBs were detected to undergo disintegration. Body weights of the chickens were measured weekly but other clinical effects were not determined. However, this information may be important with regard to chronic exposure to small amounts of PCBs and the possible malabsorption syndrome and chronic weight loss in humans and other species of animals (Wasserman et al., 1982; Carter and Mercer, 1983; Villeneuve et al., 1971; Spear and Moon, 1986). In addition, one could speculate that the decreased food intake observed in animals fed high concentrations of PCBs could arise from damage to the intestinal mucosa that, in turn, signals the neural center (hypothalamus) of the brain.

THE GLYCOCALYX

The glycocalyx is probably structurally layered with a surface component of intrinsic hydrolases closely applied to the trilaminar (Crane, 1969) and is required primarly for the terminal digestion of carbohydrates and proteins. Ugolev (1965) described this region of the plasma membrane as the most important site for terminal digestion. Adenosine triphosphatase and alkaline phosphatase has been shown to be localized in or near the brush border.

The function of the glycocalyx and its role in absorption, transport, electrical characteristics, immunological behavior, and the adhesive nature of the apical plasmalemma was reported by Ito (1969). This researcher found that in cat intestinal absorptive cells, alkaline phosphatase activity is localized in the deeper layer of the glycocalyx. He also reported that the glycocalyx is continuously synthesized by the villal epithelial cells and moved to the surface, a process in the cat that requires 30 to 60 minutes.

Stinson and Calhoun (1981) reported that the glycacalyx is present on the surface of all cells and it is particularly well developed on microvilli of epithelial cells. Therefore destruction of microvilli in chickens fed PCBs may cause major defects in absorption of the nutrients from intestinal lumen. In chronic dietary exposure to Aroclor 1254, decrease of body weight gain and egg production may be seen in exposed chickens due to malabsorption from intestinal wall.

THE TERMINAL WEB

There were no apparent changes observed in the terminal web of cells from chickens fed clean diet, but alteration of the terminal web was detected in cells of chickens fed Aroclor 1254 at 10 ppm.

ENDOPLASMIC RETICULUM

The endoplasmic reticulum (ER) has a number of metabolic activities which are characterized by the enzymes associated with its membrane component. The difference between the granular (site of protein synthesis for extracellular use) and agranular endoplasmic reticulum as seen by the electron microscopic is the presence of ribosomes in the limiting membranes of the former structure. Indeed, the two major types of endoplasmic reticulum are a continuous system of planar membranes and tubules. Their respective functions and enzyme content vary according to whether synthesis of protein "packaging" materials (in which ribosomes are required) and specialized transport enzymes and/or synthetase mechanisms, etc. are present (Staley et al., 1968).

Functionally, the smooth endoplasmic reticulum (SER) is the site of glycogen synthesis, lipid synthesis, and transport, biotransformation, and ion storage and transport. In cells lining the intestine, the resynthesis of fatty acids back into fats and their transport to the basal or lateral cellular borders occur within SER. The synthesis of steroid hormones and lipoproteins occurs within this organelle.

The free ribosomes and polyribosomes are responsible for the synthesis of proteins for intracellular use. But the proteins synthesized on the ribosomes of the rough endoplasmic reticulum are primarily for extracellular use (Alberts et al., 1983).

Dilation of the endoplasmic reticulum (ER) of the villal epithelial cells in PCB-contaminated chickens of this experiment could be because of alterations in ions and fluid transport in endoplasmic membrane that cause an accumulation of more fluid in the cisternae of ER (Rubin and Farber, 1988).

Prenatal exposure of virgin female Sprague-Dawley rats to 3,4,3',4'-tetrachlorobiphenyl at 3 mg/kg/day from day 6 through day 18 of gestation produced ultrastructural changes in the liver consisting of dilation of cisternae in the rough endoplasmic reticulum (RER), extensive proliferation of smooth endoplasmic reticulum (SER), and shrunken mitochondria with condensed matrix material (Harris and Bradshaw, 1984; Gillette et al., 1987a). Intraperitonal injection of 2,4,2',4'-tetrachlorobiphenyl at 50 mg/kg in 4 to 7 month-old pastel-raised female mink did not increase the relative volume of the SER in hepatocytes, but the same dose of 3,4,3',4'-congener in the animal caused severe necrosis of the intestinal mucosa (Gillette et al., 1987a). Dietary administration of Aroclor 1242 for five and one half months caused hyperplasia of the gastric mucosa and dilation of the RER in zymogenic cells of rhesus monkey's stomach (Bell, 1976). Hypertrophy of

endoplasmic reticulum, enlarged mitochondria, increase microbodies and lysosomes have been described in biopsy specimens of liver from humans who were accidentally contaminated during a 1968 PCB incident in Yusho, Japan (Hirayama, 1976; Kikuchi and Masuda, 1976). Ueda (1976) suggested that hypertropy of the SER may be attributable in part to an increased synthesis of membrane proteins by ribosomes attached to the RER. Rubin and Farber (1988) indicated that proliferation of SER is due to metabolic demand of detoxification by the mixed-function oxidase system residing there. The gastrointestinal hemorrhage following dietary application of Aroclor 1254 has been reported in mink by Platonow and Karstad (1973). However, the source of the enteric hemorrhage was not identified grossly and the histologic appearance of the intestine was not reported. Aulerich et al. (1985) demonstrated gastric ulcers in mink fed 0.1 or 0.5 ppm 3,4,5,3',4',5'-hexachlorobiphenyl. No histopathologic description was given in that report.

Aryl hydrocarbon (Ah) receptors of the host cells are important in rate of enzyme induction by the PCBs (Gillette et al., 1987b). Damage of the intestinal cells in mink (containing high levels of Ah receptors) has been reported by these researchers. One may expect such enzyme activity and damage of intestinal mucosa in the chickens treated with Aroclor 1254. This suggestion needs more study in the future.

MITOCHONDRIA

Mitochondria are major components of animal and plant cells that are generally cigar-shaped bodies of 0.5 by 3 μm. The major definable mitochondrial compartments are: (1) the outer membrane, (2) the intermembrane space, (3) the inner membrane, which lies between the intermembrane space, and (4) the matrix. The number of mitochondria varies with the size and energy requirements of the cell.

The mitochondria carry out most cellular oxidation and produce the bulk of the cell's ATP. The matrix space contains a large number of different enzymes.

The mitochondria are important cell organelles which are affected by many toxic materials. The swelling and disruption of mitochondria in the villal epithelial cells of chickens fed Aroclor 1254 in this study probably are caused by the dissipation of the energy-gradient and consequent impairment of the mitochondrial volume control.

INTESTINAL SECRETION

From the toxicological point of view, secretion through the small intestinal wall appears to be very important, because it may be common with that for the excretion of other compounds that are neutral, highly lipid-soluble, and metabolically stable, as PCBs. The route may also prove useful if it can be utilized clinically for promoting the excretion of PCBs from human tisuses.

Yoshimura and Yamamoto (1975) suggested that the gastrointestinal tract was an important site for the excretion

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of 2,4,3',4'tetrachlorobiphenyl in rats. Studies were made on the cumulative excretion rate of the unchanged compounds in different parts of the gastrointestinal tract of rats, with the bile duct ligated after intravenous injection. It was found that one hour after injection, unchanged compound began to appear in the content of the small intestine but not in that of other parts. During periods of 3 and 6 hours after injection, a comparable amount of the unchanged compound was excreted into the small intestine and trace amounts of 2,4,3',4'-tetrachlorobiphenyl were also detected in the contents of the cecum, colon, and rectum during these periods. These results indicated that 2,4,3',4'-tetrachlorobiphenyl was mainly excreted through the small intestine when bile was routed to the outside of rats. Thus the term applied for this phenomenon is "non-biliary" excretion.

Many other studies have shown that intestinal secretion has a major role in elimination of the xenobiotics from the exposed animals (Richter and Schafer, 1981; Rozman et al., 1983; Rozman and Rozman, 1983; Polin et al., 1985).

Mineral oil is liquid paraffin which has been widely used in human therapy (5 mg/kg) as a non-specific treatment for poisoning caused by lipophilic compounds (Forth et al., 1977; North et al., 1977). It consists of a large number of aliphatic hydrocarbons with chain lengths varying between C_{10} and C_{30}. Rozman et al. (1983) suggest that administration of large amounts of mineral oil during acute phases of

aliphatic hydrocarbon intoxications have therapeutic value, whereas small amounts are contradictive. However, during chronic toxicities, administration of small amounts of mineral oil represents a useful treatment, whereas large amounts of this oil are meaningless for this purpose. In the present study, administration of mineral oil at the level of 5% + 100% ad libitum, or 10% MO + 50% FR, which results in equivalent intake of MO, led to a correspondingly enhanced depletion of PCBs (Aroclor 1254) from the stored tissues of chickens. Elimination of PCBs was enhanced 1.5-4 fold in mineral oil-treated chickens. The data from this experiment supports the previous finding of Polin and Leavitt (1984), Polin et al. (1985), and Polin et al. (1986) in that a combination of feed restriction with mineral oil or colestipol has a greater effect in withdrawal of halogenated hydrocarbons from the exposed animals.

The exact mechanism by which aliphatic hydrocarbons enhance the fecal excretion of lipophilic compounds remains somewhat obscure. Richter et al. (1977) suggests that the effect of liquid paraffin may be due to a shift of the equilibrium of the lipophilic compounds from the gut wall to the lumen and/or decreased absorption. It seems logical that the presence of lipophilic material in the intestinal content lead to a shift of the PCB-equilibrium from the intestinal wall to the intestinal lumen.

In rats after a single oral administration of 2,4,3',4'-tetrachlorobiphenyl (TCB) at 25 mg/body weight, about 64% of the dose was excreted unchanged in feces, and 3.3% was excreted as metabolites during 14 days. Most of the elimination took place during the first day (Yoshimura and Yamamoto, 1974). To study the biotransformation of PCB-metabolites through a biliary system, 2,4,3',4'-TCB was administered to rats cannulated in the bile duct, and excretion of metabolites in bile was examined by gas chromatography (Yoshimura et al., 1974). The results indicated that about 0.4% of the dose was excreted in the bile as metabolites during 3 days after the administration. No unchanged compound was detected in the bile. In the case of mice administered 2,4,3',4'-TCB intraperitonally, approximately 4% of the dose (250 mg/kg) was excreted into the feces (both unchanged compound and its metabolites) during 7 days after the injection. In the present experiment, about 70% of Aroclor 1254 residues consumed were deposited into tissues. The remaining 30% was either absorbed and metabolized or not absorbed. However, since PCBs are fat soluble, it is likely that most of the residues consumed were absorbed in conjunction with fat.

The data from this experiment suggest that the intestinal wall of the chicken has an important role in secretion of Aroclor 1254 and combination treatment of mineral oil and feed restriction is more effective than the additive effect

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from either one alone. Because the bile may be a major route of excretion of Aroclor 1254 into the intestinal lumen, the concentration of PCBs should be greater in duodenum for all treated animals. However, the results indicate that application of MO to the chicken enhanced intestinal secretion of the xenobiotic in the ileum. In a similar study in the future, the contribution of bile to be excretion of PCBs into the intestinal lumen should be calculated from the concentration of PCBs in bile and the average bile flow in chickens.

It is appropriate to investigate in further whether PCB treatments in chickens impair the absorption of nutrients from the alimentary canal. Such action could explain the wasting of body weight or subsequent death of exposed animals to high doses of PCBs. A decrease in the intestine's ability to take up specific nutrients, as a result of the lesions caused by PCBs, may play a role in the debilitation and wasting that often follows exposure to PCBs. Extrapolating this concept further, one would include the wasting effect caused by other xenobiotics to be a result of intestinal lesions such as those revealed in this PCB study.

COMPARATIVE TOXICOLOGY

Many of the toxic symptoms caused by PCBs are similar to those observed for other toxic halogenated aromatics, including the polybrominated biphenyls (PBBs), polychlorinated dibenzofurans (PCDFs), chlorinated dibenzo-p-dioxins (CDD),

and chlorinated nephthalenes (CNs) (McConnell, 1985). The following are examples of toxicity of PCBs-related compounds in various species of animals.

Many studies indicate that PBBs are rapidly absorbed from the gastrointestinal tract of the exposed animals and then widely distributed throughout the tissues with major concentrations occuring in liver and adipose tissue. Excretion of PBBs is primarily via the bile into feces. Additional excretory routes for these chemicals are hair, skin oil, milk, and eggs (Damstra et al., 1982). The clinicopathologic syndrome from PBBs exposure is different in each species of animals. In monkeys, hyperplasia and ulceration of gastric mucosa were observed under light microscope following the feeding of 25 ppm PBBs in the diet (Allen et al., 1978). Dairy heifers fed 250 mg/day of fireMaster BP-6 for 202 days, showed no clinical evidence of hepatic, renal, or intestinal toxicity (Schanbacher et al., 1978). Dietary administration of 10 ppm 2,3',4,4',5,5'-hexabromobiphenyl (HBB) or 62 ppm 2,2',4,4',5,5'-HBB to the rat showed ultrastructural changes in hepatocytes consisting of vacuolization, increased smooth endoplasmic reticulum, swollen mitochondria, and disruption of mitochondrial cristae on transmission electron microscope (Dharam et al., 1982).

Moore et al. (1976) reported that 2,3,7,8-tetrachloro-dibenzo-p-furan (TCDF) is highly toxic at low doses to chicks. When one-day-old chicks were started on daily oral doses

of 5 µg/kg TCDF daily, most of them died within 11.5 days. Symptoms of toxicity consisted of weight loss, decreased food consumption, and general failure to thrive. At autopsy subcutaneous edema, ascites, hydropericardium, atrophy of thymus, congestion, and hemorrhage into the gastrointestinal tract was observed.

In chickens, ascite, hydrothorax, hydropericardium, infiltration of numerous fat droplets in the liver, and necrosis of the liver cells following oral administration of chlorinated dibenzo-p-dioxins (CDD) were observed under the light microscope (Norback and Allen, 1973). Enlarged hepatocytes with a proliferated smooth endoplasmic reticulum consisting of numerous vesicles and reorganization of the parallel cisternae of the rough endoplasmic reticulum to form large agranular concentric membrane arrays in rat liver following ingestion of CDD were demonstrated by using transmission electron microscopy (Norback and Allen, 1973). These researchers also reported gastric hyperplasia and ulceration (under light microscope), swelling of mitochondria with widely seperated cristae in the heart, hypertrophy of hepatocytes with numerous autophagosomes and fat droplets, an increase in the smooth endoplasmic reticulum with a decrease in the rough endoplasmic reticulum, and swollen mitochondria in liver cells (on transmission electrol microscope) in monkeys following ingestion of CDD. Colitis cystica profunda, characterized by the presence of non-neoplastic glands and

mucin-containing cysts in the submucosa of the large intestine was observed in rhesus monkeys (Macaca mulatta) by using light microscope after an oral dose of PCB (Scotti, 1975).

Mains and Kim (1980) reported that TCDD treatment in the rat stimulated aryl hydrocarbon hydroxylase (AHH) activity markedly in the second segment of intestine (5-10 cm from the pylorus) and the lowest activity was in the terminal ileum. Impairment of active intestinal absorption of glucose and leucine were observed in Sprague-Dawley CD strain rats, 2-3 weeks after oral treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin at the levels of 5 and 100 µg/kg body weight (Ball and Chhabra, 1981). Rats that received 5 µg/kg TCDD orally showed no significant different in weight gain or food consumption compared to their pair-fed controls or controls fed ad libitum over 3 weeks after dosing. At the higher dose, a severe reduction of food consumption was reported. These scientists concluded that malabsorption of specific nutrients may help occasion of body wasting seen in many animals after acute exposure to TCDD. In albino rats given an oral dose of 10.0 µg/kg body weight 2,3,7,8-TCDD for 26 days, necrosis and ulceration in the glandular part of the stomach, congestion, and hemorrhage in the intestine and increase number of goblet cells were observed under the light microscope. In other groups of the same species given a single oral dose of TCDD at 5.0 or 25.0 µg/kg body weight, the small intestine and mesenteric lymph nodes appeared slightly congested, and the intestinal contents were more mucinous than that of the control

rats (Gupta et al., 1973). These researchers also reported hemorrhages in the gastrointestinal tract of the guinea pig after it received weekly doses of 0.2, 0.04, or 0.008 µg TCDD/kg body weight.

Madge (1977) demonstrated effects of chlorodioxins on the function of the small intestine in mice. A single oral dose of 2,3,5-T (50 mg/kg body weight), TCDD (150 µg/kg), DCDD, and OCDD (2.0 mg/kg) were given to different groups of mice. DCDD, OCDD, and 2,4,5-T did not affect intestinal structure or its function. Light microscope examination of the specimens from TCDD-treated animals showed slight congestion of the superficial intestinal blood vessels, and in some cases superficial damage to the mucosal cells, infiltration of leucocytes in villi and swollen submucosa of intestine. Absorption from intesting of experimental animals apparently decreased following administration of dioxins. Absorption of D-galactose, L-arginine, and L-histidine were unaffected by the chemicals. However, damage to the intestinal glyocalyx or brush border was not studied.

The effect of phenoclor DP6 (hexachlorobiphenyl) on intestinal transport of hexoses in mice was studied by Madge (1976). He found that 7 days after a single oral dose of phenoclor DP6 at 1.0 g/kg body weight, absorption of D-glucose decreased significantly, and histology of the small intestine showed minimal changes under light microscopes.

Because of the similarity in the clinicopathologic syndrome associated with the PCBs, CDD, CDF, PBB, or CNs, the clinical diagnosis to differentiate between these classes of chemicals is almost impossible. Therefore, chemical analysis of tissue samples is required for a definitive etiological diagnosis. A careful post-mortum examination is also important to determine the severity of intoxication caused by a particular chemical.

The effect of PCB-related compounds on the histopathology, biochemistry, and physiology in different mammals and birds has been extensively studied. But the ultrastructural changes of intestinal mucosa due to PCB exposure do not appear to have yet been investigated and this was the main purpose of this work. The electron microscopic assessment of intestinal mucosal cells from PCB-exposed chickens in this experiment demonstrated degenerative changes in microvilli, mitochondria, and rough endoplasmic reticulum, with marked infiltration of plasma cells in lamina propria of the injured epithelium.

One hypothesis is that morphological alteration of the brush border and glycocalyx will adversely affect their function, so that the terminal digestive processes, particularly of proteins and sugars, essential for their absorption through the apical plasmalemma will be markedly reduced, or stopped. Additionally, however, because of the damage created in the mucusal lining, permeability of the

lining to foreign macromolecules might be increased. This is suggesting that the toxic impurity on the intestinal mucosa could be indirectly responsible for the development of malabsorption and malnutrition processes in PCB-contaminated animals.

The other hypothesis is that changes of the mitochondrial structure indicate alterations of the multienzyme systems of the mitochondrial membranes; and these facts would be indicative of an effect of the PCBs on cellular respiration and consequently on mitochondrial energy metabolism in intestinal absorptive cells, which would limit the availability of adenosine triphosphate (ATP) necessary for active absorption of nutrients. Destruction of mitochondrial matrix would have an adverse effect on the citric acid cycle, oxidative phosphorilation, energy production, fatty acid oxidation, and protein synthesis.

The changes of the endoplasmic reticulum would be probably accompanied by alterations of its function. It has been demonstrated that the smooth and rough endoplasmic reticulum interconnect to form a continuous system of cisterns and tubules spread throughout the cytoplasm of the intestinal absorptive cell, and that it is implicated in the synthetic activities of the cell (Trier, 1967): for example, the synthesis of enzymes found within the absorptive cell cytoplasm, and the synthesis of proteins and triglycerides. The plasma cell infiltration represents perhaps an auto-immune reaction to an epithelium altered by the action of PCBs.

In conclusion, the findings in the present experiment study are postulated to provide further evidence that the oral administration of PCB-related compounds exert a direct causative role in the pathogenesis of intestinal damage in animals. Furthermore, it can be speculated that similar morphologic changes in the intestinal epithelium could be responsible, at least in part, for the weight loss syndrome commonly found in chronic exposure to these chemicals. Obviously, important lesions to be investigated further are those of the basic energy-producing processes involving adenosine triphosphatase and its related organelles - the mitochondria, possibly the endoplasmic reticulum, and the brush border. The alterations of the glycocalyx are also obvious targets for intensive study and are likely to be important contributory factors in the pathotoxicity of PCB-related compounds.

APPENDICES

APPENDICES

APPENDIX A

Codes of control chickens used for electron microscopic examination and PCB (Aroclor 1254) analysis in whole body, intestinal contents, and excreta (E)[1].

Band #	Code #[2]	Body weight gram/bird[3] Day 0 of withdrawal	Day 4 of withdrawal	Day 7 of withdrawal	Residue analysis and electron microscopic (EM) examination
Dietary treatment (ad libitum)					
35501	1	483			WB[4],E
35581	2	410			WB,E
35717	3	392			IC[5],TEM[6] SEM[7],E
35728	4	412			IC,TEM,SEM,E
35733	5	381	469		WB,E
35734	6	420	516		WB,E
35736	7	436	523		IC,TEM,E
35746	8	450	572		IC,TEM,E
35747	9	412	517	546	WB,E
35732	10	400	488	524	WB,E
35755	11	360	450	480	IC,TEM,E
35756	12	355	432	465	IC,TEM,E
Sum body weights		4911	3967	2015	
No. of birds		12	8	4	
Mean		409.2	495.9	503.75	

[1] Excreta was collected in 3 periods: a) experimental days 0-13 from all 48 control birds; b) day 0 of withdrawal to day 4 of withdrawal from 8 birds/treatment; and c) day 5 of withdrawal to day 7 of withdrawal from 4 birds/treatment.

[2] These codes will be used to refer to chickens in appendices

[3] Birds (Code #1-4) were killed at day 0 of withdrawal; birds (Code #5-8) were killed at day 4 of withdrawal; birds (Code #9-12) were killed at day 7 of withdrawal.

[4] WB = whole body.

[5] IC = Intestinal contents used for PCB and Cr_2O_3 analysis.

[6] TEM = Transmission electron microscopy.

[7] Scanning eleceton microscopy.

(con't.)

Appendix A (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
5% mineral oil, (ad libitum)					
35636	13	403			WB,E
35653	14	436			WB,E
35660	15	455			IC,E
35706	16	500			IC,E
35716	17	442	523		WB,E
35731	18	375	450		WB,E
35732	19	403	475		IC,E
35744	20	395	480		IC,E
35749	21	398	480	512	WB,E
35750	22	384	463	488	WB,E
35760	23	415	500	528	IC,E
35761	24	404	489	515	IC,E
Sum body weights		5010	3860	2043	
No. of birds		12	8	4	
Mean		417.5	482.5	510.75	

[1] Birds (Code #13-16) were killed at day 0 of withdrawal.
[2] Birds (Code #17-20) were killed at day 4 of withdrawal.
[3] Birds (Code #21-24) were killed at day 7 of withdrawal.

(con't.)

Appendix A (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
Fed at 50%, (ad libitum)					
35634	25	390			WB,E
35649	26	433			WB,E
35675	27	360			IC,E
35702	28	440			IC,E
35703	29	466	413		WB,E
35718	30	457	413		WB,E
35721	31	423	370		IC,E
35722	32	430	380		IC,E
35723	33	460	422	428	WB,E
35730	34	430	387	386	WB,E
35739	35	510	448	449	IC,E
35742	36	423	379	376	IC,E
Sum body weights		5222	3212	1639	
No. of birds		12	8	4	
Mean		435.2	401.5	409.7	

[1] Birds (Code #25-28) were killed at day 0 of withdrawal.
[2] Birds (Code #29-32) were killed at day 4 of withdrawal.
[3] Birds (Code #33-36) were killed at day 7 of withdrawal.

(con't.)

Appendix A (con't).

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
10% mineral oil, 50%, (ad libitum)					
35573	37	480			WB,E
35591	38	433			WB,E
35638	39	474			IC,TEM,SEM,E
35646	40	399			IC,TEM,E
35688	41	420	382		WB,E
35693	42	424	372		WB,E
35701	43	472	410		IC,TEM,E
35727	44	490	435		IC,TEM,E
35740	45	424	374	316	WB,E
35743	46	421	377	350	WB,E
35745	47	407	347	334	IC,TEM,E
35754	48	461	407	398	IC,TEM,E
Sum body weights		5305	3104	1398	
No. of birds		12	8	4	
Mean		442.1	388	349.5	

[1] Birds (Code #37-40) were killed at day 0 of withdrawal.
[2] Birds (Code #41-44) were killed at day 4 of withdrawal.
[3] Birds (Code #45-48) were killed at day 7 of withdrawal.

APPENDIX B

Codes of PCB-chickens used for electron microscopic examination and PCB (Aroclor 1254) analysis in whole body, intestinal contents, and excreta (E)[1].

Band #	Code #[2]	Body weight gram/bird[3] Day 0 of withdrawal	Day 4 of withdrawal	Day 7 of withdrawal	Residue analysis and electron microscopic (EM) examination
Dietary treatment (ad libitum)					
35511	1	425			WB[4],E
35518	2	457			WB,E
35522	3	420			WB,E
35731	4	400			IC,TEM,SEM,E
35737	5	418			WB,E
35557	6	508			IC[5],TEM[6],E
35577	7	412			IC,TEM,SEM[7],E
35603	8	413			IC,TEM,E
35612	9	420			IC,E
35624	10	444			IC,E
35632	11	379	455		WB,E
35655	12	400	465		WB,E
35658	13	400	470		WB,E
35666	14	416	472		WB,E
35663	15	481	445		WB,E
35665	16	415	477		IC,TEM,E
35667	17	400	480		IC,TEM,E
35668	18	455	540		IC,TEM,E
35671	19	426	510		IC,E
35672	20	392	455		IC,E
35674	21	403	470	485	WB,E
35678	22	398	460	478	WB,E
35679	23	423	500	527	WB,E

(con't).

Appendix B (con't.)

Band #	Code #[2]	Body weight gram/bird[3] Day 0 of withdrawal	Day 4 of withdrawal	Day 7 of withdrawal	Residue analysis and electron microscopic (EM) examination
Dietary treatment (ad libitum)					
35681	24	398	460	490	WB,E
35695	25	411	480	512	WB,E
35697	26	414	465	494	IC,TEM,E
35698	27	424	490	514	IC,TEM,E
35699	28	451	520	547	IC,TEM,SEM,E
35709	29	374	440	463	IC
35726	30	413	490	524	IC
Sum body weights		12590	9544	5134	
No. of birds		30	20	10	
Mean		419.7	472.2	503.4	

[1] Excreta was collected in 3 periods: a) experimental days 0-13 from all 120 PCB-birds; b) day 0 of withdrawal to day 4 of withdrawal from 20 birds/treatment; and c) day 5 of withdrawal to day 7 of withdrawal from 10 birds/treatment.

[2] These codes will be used to refer to chickens in appendices

[3] Birds (Code #1-10) were killed at day 0 of withdrawal; birds (Code #11-20) were killed at day 5 of withdrawal; birds (Code #21-30) were killed at day 7 of withdrawal.

[4] WB = whole body.

[5] IC = Intestinal contents used for PCB and Cr_2O_3 analysis.

[6] TEM = Transmission electron microscopy.

[7] Scanning eleceton microscopy.

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
5% mineral oil, (ad libitum)					
35502	31	433			WB,E
35516	32	404			WB,E
35517	33	408			WB,E
35541	34	430			WB,E
35542	35	436			WB,E
35548	36	422			IC,E
35549	37	460			IC,E
35601	38	472			IC,E
35608	39	400			IC,E
35611	40	388			IC,E
35617	41	375	436		WB,E
35621	42	447	525		WB,E
35625	43	425	500		WB,E
35635	44	440	517		WB,E
35639	45	400	480		WB,E
35640	46	428	500		IC,E
35643	47	433	510		IC,E
35644	48	416	582		IC,E
35645	49	404	478		IC,E
35648	50	427	502		IC,E
35654	51	436	515	557	WB,E
35666	52	385	443	458	WB,E
35667	53	438	510	535	WB,E

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
5% mineral oil, (ad libitum)					
35685	54	497	582	624	WB,E
35686	55	391	470	496	WB,E
35691	56	462	534	563	IC,E
35692	57	450	518	560	IC,E
35694	58	454	532	569	IC,E
35700	59	431	510	534	IC,E
35704	60	383	453	475	IC,E
Sum body weights		12835	10097	5371	
No. of birds		30	20	10	
Mean		427.8	504.8	537.1	

[1] Birds (Code #31-40) were killed at day 0 of withdrawal.
[2] Birds (Code #41-50) were killed at day 4 of withdrawal.
[3] Birds (Code #51-60) were killed at day 7 of withdrawal.

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
Fed at 50%, (ad libitum)					
35504	61	447			WB,E
35507	62	423			WB,E
35521	63	436			WB,E
35524	64	451			WB,E
35525	65	463			WB,E
35530	66	441			IC,E
35551	67	407			IC,E
35563	68	420			IC,E
35567	69	473			IC,E
35572	70	450			IC,E
35607	71	360	338		WB,E
35613	72	399	374		WB,E
35616	73	420	390		WB,E
35618	74	410	373		WB,E
35620	75	406	369		WB,E
35622	76	494	372		IC,E
35626	77	354	349		IC,E
35629	78	425	420		IC,E
35631	79	380	359		IC,E
35633	80	383	328		IC,E
35642	81	450	433	459	WB,E
35647	82	407	373	379	WB,E
35650	83	504	465	465	WB,E

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
Fed at 50%, (ad libitum)					
35661	84	396	358	374	WB,E
35670	85	395	365	368	WB,E
35682	86	391	365	378	IC,E
35690	87	380	372	377	IC,E
35714	88	470	431	441	IC,E
35720	89	413	378	393	IC,E
35758	90	435	397	403	IC,E
Sum body weights		12683	7604	4039	
No. of birds		30	20	10	
Mean		422.8	380.4	403.9	

[1] Birds (Code #61-70) were killed at day 0 of withdrawal.
[2] Birds (Code #71-80) were killed at day 4 of withdrawal.
[3] Birds (Code #81-90) were killed at day 7 of withdrawal.

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
10% mineral oil, 50% (ad libitum)					
35512	91	445			WB,E
35515	92	488			WB,E
35527	93	416			WB,E
35533	94	450			WB,E
35543	95	457			WB,E
35544	96	447			IC,TEM,SEM,E
35571	97	410			IC,TEM,E
35583	98	410			IC,TEM,E
35602	99	408			IC,E
35605	100	420			IC,E
35606	101	395	360		WB,E
35610	102	407	371		WB,E
35614	103	350	330		WB,E
35619	104	420	397		WB,E
35526	105	482	448		WB,E
35628	106	399	378		IC,TEM,E
35637	107	368	343		IC,TEM,E
35684	108	430	393		IC,TEM,E
35651	109	430	390		IC,E
35659	110	420	405		IC,E
35664	111	376	345	328	WB,E
35676	112	400	362	357	WB,E
35683	113	407	370	358	WB,E

(con't.)

Appendix B (con't.)

Band #	Code #	Body weight gram/bird Day 0 of withdrawal[1]	Day 4 of withdrawal[2]	Day 7 of withdrawal[3]	Residue analysis and electron microscopic (EM) examination
10% mineral oil, 50% (ad libitum)					
35630	114	372	332	347	WB,E
35687	115	370	350	356	WB,E
35705	116	440	397	394	IC,TEM,E
35707	117	470	442	437	IC,TEM,E
35708	118	495	470	473	IC,TEM,E
35711	119	454	410	410	IC,E
35713	120	410	390	384	IC,E
Sum body weights		12647	7683	3844	
No. of birds		30	20	10	
Mean		421.5	384.1	384.4	

[1] Birds (Code #91-100) were killed at day 0 of withdrawal.
[2] Birds (Code #101-110) were killed at day 4 of withdrawal.
[3] Birds (Code #111-120) were killed at day 7 of withdrawal.

APPENDIX C

Raw data for feed consumption (gram/bird/day)[1]

	Experimental days	Treatment	Food consumption
Control chickens	0-13[2]	No contaminated diet	28.9
	14-20[3]	No contaminated diet	40.2
PCB-chickens	0-13[2]	10 ppm Aroclor 1254 contaminated diet	28.6
	14-20[3]	No contaminated diet	39.3

[1] Values represent feed consumption in chickens fed ad libitum. Feed restricted chickens fed at 50% ad libitum.

[2] Residue build-up period.

[3] Residue withdrawal period (day 0 to 7 of withdrawal).

APPENDIX D
STATISTICAL METHODOLOGY

A. Linear Model

$Y = B_o + B_1 X + E$

Y = Primary variable of interest

X = Fixed variable

B_1 = Slope (average change in Y per unit change in X)

B_2 Y-intercept = orgin (extrapolated value of Y when X is fixed at zero).

E = Random error of the relationship (failure to explain Y by the form $B_o + B_1 X$).

B. Estimation of Regression Parameters

$\hat{Y} = \hat{B}_o + \hat{B}_1 X$

$\hat{Y}$ = Predicted Y

$\hat{B}_o$ = Estimated $B_o = b_o$

$\hat{B}_1$ = Estimated $B_1 = b_1$

Predicted $X = \hat{X} = (Y-b_o)/b_1$

C. Partition of Variation

$SS_Y = \sum \sum \sum Y_{ikj} - (\sum \sum \sum Y_{ijk})^2/n \quad n=abr$

$SS_A = (\sum Y^2_{i..}/br) - (\sum \sum \sum Y_{ijk})^2/n$

$SS_B = (\sum Y^2_{.j.}/ar) - (\sum \sum \sum Y_{ijk})^2/n$

$SS_E = SSy_Y - SS_A - SS_B$

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[1] All formulas are taken from Volume 1 of the Design and Analysis of Experiments in the Animal and Medical Science (Gill, 1978).

Appendix D (con't.)

D. Hypothesis testing.

Source of variation	df	SS	MS	F ratio	Critical value
A	a-1	SS_A	MS_A	MS_A/MS_E	F V_A, V_E
B	b-1	SS_B	MS_B	MS_B/MS_E	F $, V_B, V_E$
Error	(a-1)(b-1)	SS_E	MS_E	---	---

APPENDIX E

Raw data for predicted concentration of Aroclor 1254 in diet.

µg/g PCBs in sample	Sample weight (gm)	µg PCBs in sample
11.00	10	110.00

APPENDIX F

Digestion mixture used for chromic oxide analysis in intestinal contents and excreta (Gzarnocki et al., 1960).

Reagents	Quantity
Sodium molybdate	20 gm
Concentrated sulphuric acid	300 ml
70% perchloric acid	400 ml
Distilled water	300 ml

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