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ENTEROHEPATIC CIRCULATION OF XENOBIOTICS AND
THE EFFECT OF BILE ACIDS AND DIET COMPOSITION
ON LIPID ABSORPTION IN
BILE DUCT CANNULATED CHICKENS

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David Leslie Pullen

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of the requirements for

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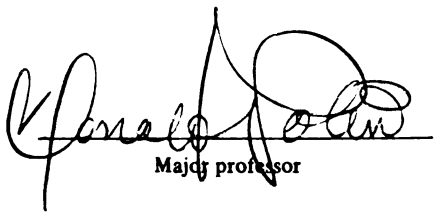

Major professor

Date February 14, 1983

ENTEROHEPATIC CIRCULATION OF XENOBIOTICS AND THE EFFECT OF BILE ACIDS AND DIET COMPOSITION ON LIPID ABSORPTION IN BILE DUCT CANNULATED CHICKENS

By

David Leslie Pullen

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ABSTRACT

ENTEROHEPATIC CIRCULATION OF XENOBIOTICS AND
THE EFFECT OF BILE ACIDS AND DIET COMPOSITION
ON LIPID ABSORPTION IN
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By

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Four experiments were conducted to study the effect of bile acids on lipid absorption and to determine percent pentachlorophenol (PCP), hexachlorobenzene (HCB) or polybrominated biphenyls (PBBs) excreted in the bile of broiler-type chickens, eight weeks of age with their bile ducts cannulated (BDC).

Birds were allowed free movement and were either sham-operated or had their cystic and hepatic ducts cannulated. Following PCP, HCB or PBBs dosage (30 mg/bird), bile was collected for 48 hours. HCB, PBBs and PCP recovery in bile was $< 1.0\%$, $< 1.0\%$ and $\sim 10\%$, respectively.

Sham operated birds absorbed 90-92% dietary fat and BDC birds 43 to 72%. Addition of 0.04, 0.08 or 0.16% cholic acid or 0.08% freeze-dried chicken bile did not improve lipid absorption significantly ($p > 0.05$). Neither practical nor purified diets, saturated or unsaturated fats significantly altered lipid absorption in BDC birds but percent digestibility was increased for purified diets ($p < 0.01$) compared to practical diets.

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TABLE OF CONTENTS

LIST OF TABLES

TABLE Page

LIST OF FIGURES

I. INTRODUCTION

Accidental contamination of animal feed with PBBs in 1973 resulted in the slaughter within Michigan of 29,800 dairy cattle, 1,470 sheep, 5,920 pigs and about 1.5 million chickens. In 1976, an explosion in a chemical factory in Italy sent clouds of noxious chemicals, among them dioxin, over the town of Séveso. In 1968 in Yusho, Japan, 1,200 people were struck by a disease which was eventually traced to PCB contamination of rice oil (Schneider 1979). Environmental contamination over the past 50 years has become an important social and political issue. Increased public awareness has spurred researchers in industry and universities to answer basic questions relating to the hazards and fate of these environmental contaminants.

Determining the metabolic fate of these compounds will someday enable victims of direct, indirect or accidental contamination to be treated and decontaminated. More research needs to be conducted to discover how these compounds are absorbed, distributed, metabolized and excreted within the physiological matrix.

This study will endeavor to provide insight on one aspect of the metabolic fate of three of these environmental contaminants, namely, pentachlorophenol (PCP), hexachlorobenzene (HCB), and polybrominated biphenyls (PBBs) by investigating the role of the enterohepatic circulation and biliary excretion

of these compounds in the chicken. The liver, acting as a type of physiological filter, plays an important role in the metabolism and excretion of many foreign substances and normal physiological metabolites. These are secreted along with the bile acids into the gallbladder or directly into the duodenum during the digestion process. Bile acids, manufactured in the liver, are known to play a very important role in the absorption of fats and other lipophilic compounds. Lipophilic compounds (i.e. those which tend to partition into fat) when introduced orally may be absorbed via the portal system along similar routes as the bile acids. This has indeed been shown to occur in other species but to what extent this occurs in the chicken will be considered in this study. Also the effect of bile acids on fat absorption will be investigated.

II. REVIEW OF LITERATURE

A. Bile Acid Metabolism

Bile is manufactured by the liver and is believed to play a very important role in the metabolism of fats. Biliary contents include breakdown products of hemoglobin (the so-called bile pigments), bile acids, mucoproteins, cholesterol, some free fatty acids, triglycerides and various lipophilic metabolites and toxins. Bile also plays a role in increasing the pH of the gizzard contents as they enter the duodenum. Studies by Lin et al. (1974) have shown the pH of chicken bile to be about 7.68. Farner (1942) showed a pH of about 5.88 in gallbladder bile of chickens. Avian bile also contains amylase which plays a role in the digestion of carbohydrates. Bile enters the duodenum of most poultry one of two ways. It may be released via the hepatic duct directly from the liver, or it may enter from the gallbladder via the cystic duct. Upon entering the distal end of the duodenum, its potent emulsifying properties play a major role in the digestive process. As digestion proceeds, the bile salts pass through the jejunum and upon reaching the ileum may be reabsorbed via the enterohepatic circulation to the liver where they are again recycled.

B. Synthesis

As stated previously, bile acids are synthesized in the liver and may be defined as either primary or secondary bile

acids. Primary bile acids are those that are formed from cholesterol in the liver while secondary bile acids are those formed from primary bile acids during the digestive process due to the action of intestinal microorganisms. The secondary bile acids may also be subjected to further change by the action of hepatic enzymes. The primary bile acids in the avian species include predominantly chenodeoxycholic acid and cholic acid with some allocholic acid found in carnivorous species. Deoxycholic acid, common in mammalian bile, has not been detected in the bile of chickens. Other primary bile acids such as α and β muricholic are present in the mouse and rat and hyocholic acid is present in the pig. Haslewood and Sjövall (1954) detected no glycine conjugates in the bile of eight species of birds and later Haslewood (1971) reported that the bile of germ free domestic fowl contains taurine conjugates of chenodeoxycholic (80%), cholic (17%), and allocholic acid (5%). Other bile acids reported in the bile of chickens include isolithocholic (Hosizima et al., 1930), tetrahydroxynorsterocholanic (Yamasaki, 1951), 3α hydroxy -7- oxocholanic (Wiggins, 1955a) and 3-oxochola-4-6-dienic (Wiggins, 1955b) acids.

The origin of bile acids from cholesterol was established in 1943 by Bloch et al. who examined the conversion of deuterium labeled cholesterol into cholic acid in the dog. Since that time, this has been shown to be true for many other species as well. Figure 1 shows in detail the changes that occur to the cholesterol molecule resulting in the formation of the various bile acids.

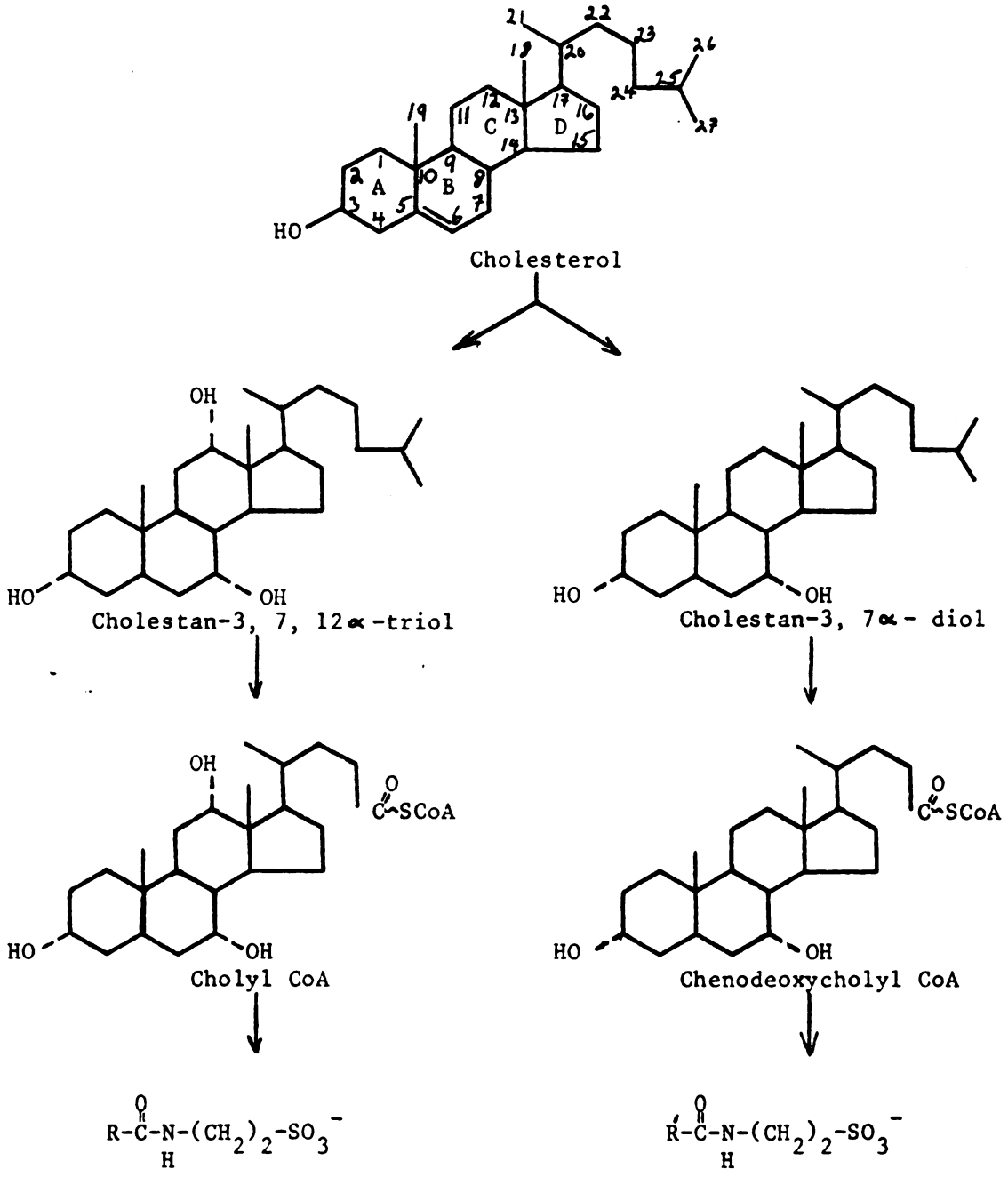


Fig. 1. Pathway for bile acid synthesis from cholesterol. (White et al. 1978)

As to the exact location, or hepatic precursor site, of bile acid production, Schwartz et al. (1977) showed that in man newly synthesized cholic and chenodeoxycholic acids, secreted by a bile fistula, arise from the same hepatic site. In the rat, however, chenodeoxycholic acid may be formed from a compartment of cholesterol different from that of cholic acid. Studies of this type have not yet been carried out in the chicken to determine the hepatic cholesterol precursor site.

According to Yeh and Leveille (1973) 64% of cholesterol synthesis takes place in the liver, 24% in the carcass and about 6% is in the intestine and skin. In the formation of bile acids the cholesterol in the liver is first converted to the cholestan-diol and-triol in the presence of a rate-limiting enzyme called cholesterol -7-α hydroxylase. This enzyme reduces the double bond in the B ring of cholesterol and adds either one or two hydroxyl groups. It also isomerizes the 3-β OH group to the α position. Next a thioester is formed between one of the terminal carbons and thio-CoA as indicated in the preceding figure (Figure 1). The terminal three carbons (25-27) come off as propionyl CoA and the thio-CoA is esterified to the C_{24}. This results in the formation of a dihydroxy thioester (cholyl CoA) and a trihydroxy thioester (chenodeoxycholyl CoA). Finally, before being released into the duodenum or gallbladder, they are conjugated. Almost all of the final product (i.e. bile acid) released is conjugated and in the chicken the only conjugates that have been identified as previously stated are taurine conjugates. Conjugation serves to prevent premature absorption

of the bile acids as they move down the digestive tract. When bile salts are deconjugated by abnormal bacterial growth, they yield free or unconjugated bile salts which are rapidly absorbed in the jejunum resulting in inefficient absorption of lipids (Dietschy et al. 1966). Conjugation lowers the ionization constant of unconjugated bile salts (pK_a about 6) to a pK_a 1.8-3.7 and they become less ionized at the luminal pH ranging between 5.7 - 6.4 in the chicken. According to Dietschy et al.(1966), ionized bile salts are absorbed in the jejunum and proximal ileum in rats by ionic diffusion at rates proportional to their intraluminal concentrations and activities. Deconjugation by bacteria under normal conditions is limited primarily to the colon and bile salts are usually excreted in the unconjugated form.

The secondary bile acids result from the action of intestinal microorganisms. These are no longer conjugated. Significant amounts of these secondary bile acids are also reabsorbed in the enterohepatic circulation. Lithocholate, a secondary bile acid has been shown to induce the ductular cell reactions (biliary-proliferation) in the chicken as well as other species (Hunt et al., 1963). Eyssen and De Somer (1963) showed lithocholate toxicity in chicks resulting in reduced feed intake (25-30%), decreased weight gain (50%), greatly enlarged livers and extensive bile duct cell proliferation.

C. Enterohepatic Circulation of Bile Salts

Absorption of bile acids appears to take place all along

the small intestine, preferentially in the distal ileum. Lindsay and March (1967) studied the rates of absorption of sodium glycocholate and taurocholate by different segments of the mesenteric small intestine. The absorptive capacity in general increased towards the distal end of the intestine and both glycocholate and taurocholate were readily absorbed. It should be noted that glycocholate is not a normal conjugate form in avian bile. Absorption of bile acids has been shown to be dependent on dietary nutrients as well. Fondacaro and Walcott (1981) showed that taurocholate absorption in the distal ileum is inhibited more by triglycerides than by protein (albumin) and carbohydrates (starch) both in vivo and in vitro. Corn oil significantly decreased uptake of taurocholate by the ileal villi. Garlich and Nesheim (1965) found that chicks fed raw soybean meal have increased excretion rates of labeled cholic acid compared to chicks fed diets containing heated soybean meal. Chicks fed raw soybean meal had significantly less bile in the gallbladder at necropsy. Lindsay et al. (1969) found that unsaturated dietary oils tend to increase and that saturated dietary oils decrease the levels of bile acids in the excreta of humans, rats and cockerels. Adult cockerels fed 15% coconut oil excreted lesser amounts of bile acids than did cockerels fed 15% of either corn oil or herring oil. These data indicate that diet plays a very important role in the absorption of lipophilic compounds via the enterohepatic circulation. Therefore, one must carefully consider the role of dietary constituents and their effect on the absorption of environmental contaminants from the intestine.

The enterohepatic circulation is very efficient in the absorption of bile acids thus the synthesis rate of new bile acids is relatively slow. Only under abnormal conditions such as dietary treatment of biliary sequestrants or in bile-fistulated birds would one expect rapid synthesis of new bile acids. Research by Clarkson et al. (1957) revealed that White Leghorn cockerels (14 weeks of age) secreted about 1 ml of bile per hour. Lin et al.(1974) reported a bile flow of about 1.2 ml/kg/1 hour or about 2 ml/hr. in White Leghorns age 10-18 weeks. These were the only studies to have been conducted on secretion rates of bile in the chicken.

The gallbladder functions as a storage and concentrating organ for hepatic bile. The gallbladder, a smooth muscle accessory organ, releases bile in response to neural and hormonal stimulation when food, especially fat, reaches the duodenum. Gallbladder bile, being more concentrated, contains less water and a greater concentration of bile acids than hepatic bile.

The primary biliary functions are emulsification of dietary lipids, alkalinization of chyme, digestion of dietary carbohydrates and the excretion of metabolites. The biliary function of primary importance to this review is related to the excretion of environmental toxins and their metabolites. A discussion of lipid absorption will permit a better understanding of the role of bile acids in relation to the absorption of dietary toxins which are of such vital concern today.

D. Lipid Absorption

Bile contains highly concentrated detergents which can quickly hydrolyze most dietary lipids. Their amphipathic structure makes them very potent emulsifiers, (i.e. contain both polar and nonpolar groups). Emulsification of fatty foods occurs in the distal portion of the duodenum where as previously stated, the hepatic and cystic duct enter. Triglycerides, small amounts of cholesterol and phosphoglycerides, are emulsified by the action of bile which makes them miscible with water forming lipid-bile salt micelles. These micelles allow for accelerated activity of pancreatic lipase on the fatty acids in the 1- and 3- position of the triglyceride projecting into the aqueous phase. A typical micelle is made of lecithin, cholesterol and conjugated bile salts. The formation of these micelles in addition to allowing action by water soluble enzymes provides a means for their presentation to the absorptive mucosa on the intestinal surface. Located along the intestinal mucosa within the folds of Kerkring are millions of needle-like projections called villi. Along the surfaces of these villi are even smaller projections called microvilli. Each intestinal epithelial cell contains approximately 1000 microvilli which serve to increase the surface area of the intestinal epithelial membrane by 15 to 25 fold (Scott et al. 1976), thus providing an absorptive surface capable of handling individual fatty acids, phosphoglycerides and other lipophilic substances. The initial phase of uptake is not energy dependent

as evident from studies showing uptake of fatty acids in the absence of oxygen, at low temperatures, in the presence of metabolic inhibitors and with boiled and dead tissues (Ockner, 1974). Thus, the passive rates of uptake depend on the concentration of free, unbound fatty acid external to the cell. It may be possible that there are specific receptor sites for fatty acids in the plasma membranes, but that has yet to be demonstrated. Recent investigation by Ockner (1972) points to a Fatty Acid Binding Protein (FABP) located within the intestinal mucosa. The existence of this protein was theorized as a means of explaining differences between saturated and unsaturated long chain fatty acids with regards to their intestinal absorption and esterification. Ockner (1974) isolated a soluble fatty acid binding protein (FABP) of molecular wt. 12,000 in tissues that utilize fatty acids including the intestinal mucosa, liver, myocardium, adipose and kidney of the rat. Katongle and March (1979) also demonstrated the existence of a FABP in the intestinal mucosa of the chicken. They also noted that FABP is present at the time of hatching and before any feed has been ingested and the relative concentration of FABP in different levels of the intestine vary depending upon the amount of fat in the diet. In a later paper, Katongle and March (1980) compared lipid absorption between various genetic sources and studied the significance of the concentration of FABP in relation to efficiency of fat utilization. New Hampshire chicks were able to utilize both tallow and corn oil more efficiently up to five weeks of age than broiler-type and White Leghorn chicks.

These breed differences were most evident at three weeks of age. After six weeks of age, there were no significant differences between breeds. In a second experiment, the concentrations of FABP in each of the breeds were studied. It was demonstrated that the level of FABP declined after hatching and increased at three weeks of age in all breeds. The FABP was lower at hatching and at one week of age in broiler-type chicks than in New Hampshire or White Leghorns. Tallow was absorbed less efficiently in broilers than in New Hampshire or White Leghorn breeds at two weeks of age. The intestine of broiler-type chicks at the time of hatching contained a FABP concentration of 18.8 mg/g and rose to 31.6 mg/g by five weeks of age. New Hampshire and White Leghorn chicks exhibited the highest concentration of FABP, 39.5 mg/g and 32.2 mg/g at hatching and never exceeded these amounts throughout the five weeks. Katongle concluded that perhaps FABP may be the limiting factor in birds not utilizing dietary fat efficiently in the presence of adequate amounts of bile.

Several studies have been conducted on the effect of supplemental bile acids on lipid absorption to see if efficiency can be improved in both young and mature chickens. It would appear that the ability of the young chick to absorb saturated fats is not fully developed until about four weeks of age, according to Duckworth et al. (1950). Fedde et al. (1960) obtained an increase in absorption of tallow from 47 to 69 percent in chicks when ox-bile at 0.5% was added to a diet containing 20% tallow. Gomez and Polin (1974) demonstrated a

slight improvement of absorption of tallow at high levels in purified diets fed to chicks. Gomez and Polin (1976) showed that apparent fat absorption in chicks four to seven days of age on an 8% tallow-based diet absorbed about 39.6% as compared to 51.2% after addition of chenodeoxycholic acid at 0.025%. Utilizing broiler chicks 14-19 days old and fed diets with 8% tallow, absorption of fats improved from 68.2% to 78.5% with 0.05% cholic acid added to the diet. Polin et al.(1980) also showed that lipase proved to be somewhat effective in improving lipid absorption in young chicks. Katongle and March (1980) found that broilers and White Leghorn chicks supplemented with .05% sodium taurocholate in tallow-based diets, showed a significant increase in absorption; whereas New Hampshire chicks did not. Serafin and Nesheim (1967) showed that endogenous bile acids are more readily excreted in young chicks. It may be that the lipase has an additive effect with these endogenous bile acids. Garrett and Young (1964) conducted a study on the absorption of fatty acids and triglycerides in bile-duct-cannulated chicks 8 to 10 weeks of age. Absorption of both triglycerides and their respective fatty acid mixtures were decreased to about the same degree. Apparently fatty acids such as oleic acid, which forms simple micelles, or less polar ones as palmitic acid, when solubilized in mixed micelles, show the greater decrease in absorption in the absence of bile. Thus micelle formation would appear to be essential for maximum absorption. Garrett and Young (1964) also showed that in the absence of bile 36-50% absorption of fatty acids occurred which

indicates that a lipid-bile salt-micelle is not the only prerequisite in the process of fatty acid absorption. Only limited information was available in the abstract (Garrett and Young, 1964) and nothing was said about the surgical procedure or the duration of the experiment.

Once the fatty acids have been transported to the intestinal membrane of mammals and chicks, the bile salts are released and returned to the lumen for use again in micelle formation. The fatty acids of less than 10 or 12 carbons along with the free glycerol are transported by the chicken's portal system to the liver. The long chain fatty acids and monoglycerides are re-esterified to triglycerides within the endoplasmic reticulum and along with cholesterol and a small amount of protein form chylomicrons covered by a phospholipid layer. In mammals these chylomicrons are transported via the lymph to the blood stream in contrast to the chicken where it has been shown by Bensadoun and Rothfeld (1972) that the chylomicrons are absorbed directly into the portal blood system and transported to the liver.

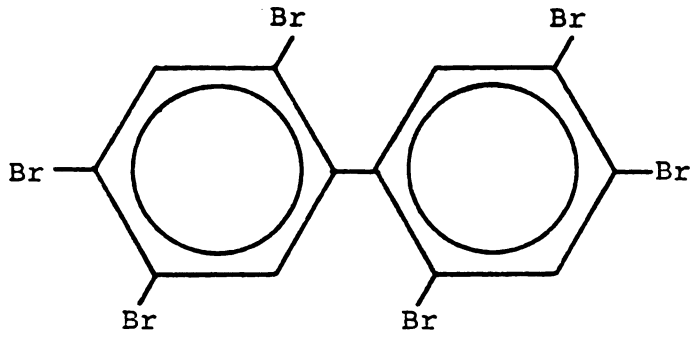
E. Environmental Contaminants

Absorption, distribution, metabolism and excretion of a particular toxin varies according to species, strain, age, sex, diet, health, chemical structure of the toxin and other factors, some perhaps yet undefined. This discussion concerns itself with the absorption and moreover the enterohepatic circulation of three very familiar environmental contaminants,

namely polybrominated biphenyls (PBBs), hexachlorobenzene (HCB) and pentachlorophenol (PCP).

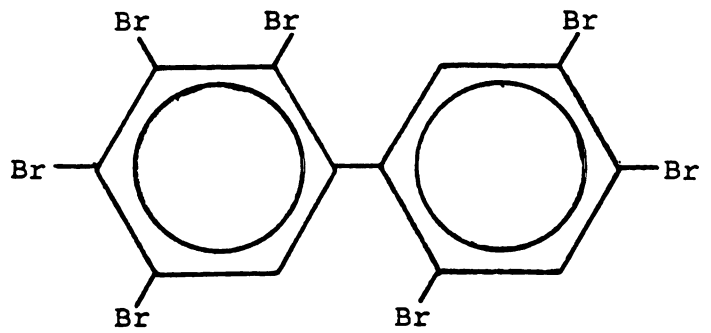
PBBs were manufactured for use as an industrial fire retardant. These PBBs were produced by Michigan Chemical Corporation, St. Louis, Michigan and sold as FireMaster(R) FF-1. The product, which looks much like a trace mineral additive (magnesium oxide) was accidentally shipped to a feed mill and used as magnesium oxide with the consequent contamination of the feed and much of Michigan's dairy, egg, and meat supplies. Ultimately, of course, it was ingested by a majority of Michigan's human population. The compound sold as FireMaster(R) FF-1 contains hexabromobiphenyl--62.8%, heptabromobiphenyl--13.8%, pentabromobiphenyl--10.6%, tetrabromobiphenyl--2.0%, other bromobiphenyls--11.4% (Kerst, 1974). PBBs are reported to have an acute oral LD_{50} in rats of 21.5 g/kg body wt. (Hill Top Research, 1970). In comparison to other environmental contaminants it would appear to be relatively nontoxic. However, as with all halogenated hydrocarbons, it accumulates in the adipose tissue and may be toxic at levels lower than that indicated by the LD_{50} for rats. According to Dent et al. (1976) PBBs are potent inducers of hepatic and kidney microsomal drug metabolizing enzymes. They cause a mixed type induction which mimics that caused by treatment with both phenobarbital and 3-methylcholanthrene. The two major components (see Figure 2 and 3) 2, 4, 5, 2', 4', 5' hexa (6BB-4), detected as peak 4 on a chromatogram, and 2, 3, 4, 5, 2', 4', 5' heptabromobiphenyl (7BB-8) detected as peak 8, have been shown by Moore et al. (1978) to be

strictly phenobarbital-type inducers.



mw 555.40

Fig. 2. Structure of 2, 4, 5, 2', 4', 5' hexabromobiphenyl.



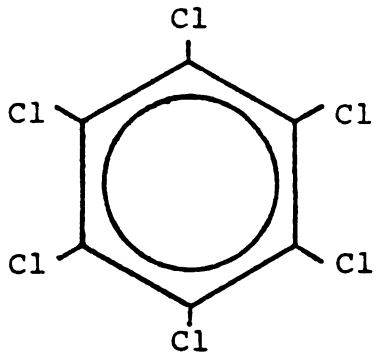
mw 634.30

Fig. 3. Structure of 2, 3, 4, 5, 2', 4', 5' heptabromobiphenyl.

Studies by Matthews et al.(1977) in rats have shown that the hexabromobiphenyl was readily absorbed from the intestine, initially distributed throughout the body and ultimately accumulated in adipose tissue. It was not appreciably metabolized and was excreted almost exclusively by the feces at a very slow rate. Approximately 90% of an oral dose was absorbed from the intestine. Biliary excretion was also studied

and 0.68 ± .19% of the total PBBs dose was excreted between 0 and 4 hours after iv administration. Rats cannulated 24 hours after an iv dose excreted 0.032 ± .004% of the total dose in bile in one hour. From this it is evident that biliary excretion is only a minor route of excretion, or that the elimination of the compound is extremely slow.

HCB was manufactured as a fungicide for use on seed grains such as wheat, barley, oats and rye. In 1959 HCB induced porphyria cutanea tarda (PCT) in some 5,000 people in Turkey. According to a review by Courtney (1979) and Schmid (1960), fungicide-treated wheat seeds were utilized for making bread because of a severe wheat shortage. As a result, the PCT was induced. HCB also occurs as an industrial waste product in the manufacture of perchlorethylene, chlorine, carbon tetrachloride and various pesticides. HCB bioaccumulates in both terrestrial and marine animals and is not easily metabolized.



hexachlorobenzene mw 284.80

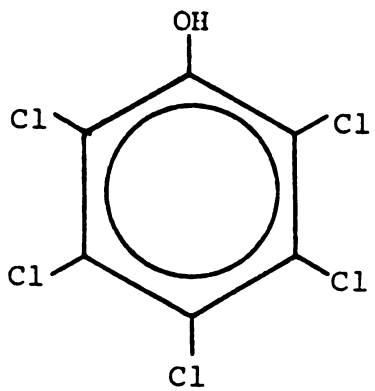
Fig. 4. Structure of HCB.

According to studies by Iatropoulos et al.(1975) HCB is absorbed more slowly than dichlorobiphenyl or dieldrin in rats. Portal venous transport of HCB to the liver is a minor route with the major portion being absorbed by the lymphatics

and deposited in adipose, by-passing systemic and excretory systems. Excretion of HCB in rats takes place primarily in the feces with a small percent being excreted in the urine. Ingerbrigtsen et al.(1981) found that less than four percent of the total dose was recovered in the bile within 48 hours in the rat. Chickens rapidly accumulate HCB in fat, liver, muscle and eggs in proportion to dietary intake of the compound (Courtney 1979). In laying hens, according to Hansen et al. (1978), at least 50% of the decline in HCB residues is due to elimination of the parent compound in egg yolk. Total excreta load was found to be only 3.8 to 5.2% of the amount excreted in the egg yolk at the end of the experiment. One cannot ascertain, however, how much of the HCB is being reabsorbed via the enterohepatic circulation in the chicken thus affecting the total amount excreted. Also it may be postulated that HCB would be absorbed into the bloodstream and flow directly to the liver in chickens in contrast to what occurs in mammals, in which absorption is via the lymphatic system and thence into general circulation.

PCP prepared by the chlorination of phenol in the presence of a catalyst, has been widely used as a fungicide, bactericide, herbicide, pre-harvest dessicant, molluscacide, insecticide and wood preservative. Herds of swine and dairy in Michigan were quarantined following identification of PCP in body fluids and tissues according to Thomas et al.(1977). In the production of PCP, a progressive increase in temperature maintains a fluid reaction mixture. This favors formation of various contaminants in PCP including chlorodibenzo-p-dioxins,

chlorinated phenoxy phenols (pre-dioxins) and chloro-dibenzofurans. These are among some of the most toxic chemicals known to man.



pentachlorophenol mw 266.35

Fig. 5. Structure of PCP

Stedman et al.(1980) showed a significant linear relationship between PCP accumulation in the tissues of birds 8 weeks old and the amount of PCP in the diet. Accumulation of PCP was greatest in the kidney followed by liver, heart, leg, breast, gizzard and fat. Mammals show similar results. Also fatty degeneration of the liver and proliferation of bile duct tissue occurred.

The chart below (Figure 6) from Stedman et al. (1980) suggests several hypotheses concerning the accumulation factor and half-life for several chlorinated hydrocarbons in the chicken. These molecules differ in structure at the six-position only and this may play an important role in the pharmacokinetics of these compounds.

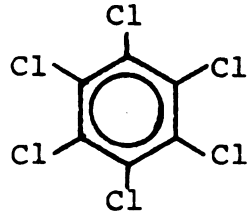
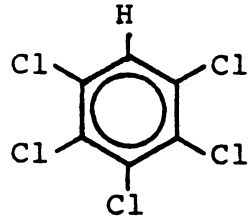
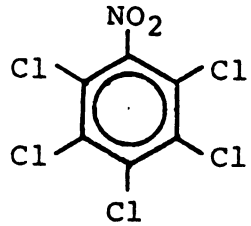
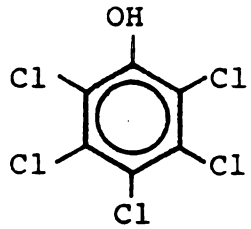
		Accumulation Factor	Half-Life	Reference
HCB		25 - 30	3.79 weeks	Reed (1976)
PCB		40.9	5.5 weeks	Dunn (1977)
PCNB		0.1	. . . .	Reed (1976)
PCP		.4214	1.78 weeks	Present study

Fig. 6. The accumulation factor and half life for several chlorinated aromatic hydrocarbons as compared with PCP. (Stedman et al. 1980).

Highly lipid soluble compounds may be reabsorbed from tubular urine while those with low lipid solubility or those that are highly ionized will be excreted (Baggot, 1977). As examples, HCB, pentachlorobenzene, and probably PBBs, which are highly lipid soluble, may be reabsorbed from tubular urine. Instead of being rapidly excreted, they may be deposited in lipid stores which are relatively inactive in physiological

processes of excretion or elimination. PCP which is ionized under normal physiological conditions would be more readily excreted in urine, though it can occur via bile as well.

F. Biliary Excretion of Foreign Compounds.

Observations of other compounds as conjugates seem to indicate that species, conjugation and molecular weight play a role in the biliary excretion of foreign compounds. According to Abu-El-Makaren et al.(1967), biliary excretion of compounds less than 300 m.w. (benzene derivatives) did not exceed 10% of the dose in the rat. Using ^{14}C benzene about 0.8% of the dose appeared in the bile. Metabolites of most of the compounds studied including some sixteen compounds of molecular weight less than 200 (benzene, toluene, aromatic acids, aromatic amines and phenols) appear in the bile as conjugates. Milburn et al.(1967) suggested that for appreciable biliary excretion in the rat a compound should have a polar anionic group and a molecular weight of about 350 or greater or be able to be metabolized to such a compound. The rate of metabolic change may also affect the amount of a metabolite in the bile. Milburn et al. (1967) also speculated that perhaps the normal transport mechanism is utilized in biliary excretion of endogenous compounds. Compounds such as glycocholic (m.w. 466 pK_A 4.54) and taurocholic acids (m.w. 516 pK_A 1.56) and bilirubin mono-and-di-glucuronides (m.w. 761 and 937, respectively, and pK_A 3-4), have a highly polar anionic group and a relatively high molecular weight. One would expect then that compounds similar to

these would, though exogenous in origin, be excreted via the bile. Sperber (1963) showed that renal tubules tend to secrete compounds within a range of 200-400 molecular weight; whereas compounds with 400 or greater molecular weight are more efficiently excreted in the bile.

Based on these results then one may expect that very little PBBs, HCB or PCP would be excreted by the bile in the chicken. This supposition is what is to be evaluated.

III. MATERIALS AND METHODS

A. Introduction

Experiment #1

This initial investigation was to develop a procedure for cannulating bile ducts and to permit bile collection in free-moving birds. In addition, the effect of supplemental cholic acid on fat absorption in broiler-type chickens with their bile ducts cannulated was evaluated.

Experiment #2

Based on the results of the first experiment, the effect of freeze-dried chicken bile was compared to cholic acid for their effect on fat absorption in broilers with cannulated bile ducts.

Experiment #3

Broilers were orally dosed with either (PCP)[1] (HCB)[2] or (PBBs)[3]. The bile was collected over 2 days as 2 periods: Period 1, (0-24 Hr); period 2, (24-48 hr).

[1]Monsanto Lot No. MB 538, Industry composite, obtained from Inorganic Research and Development, 800 N. Lindbergh Blvd., St. Louis, MO 63166.

[2]HO 2550, Industrial grade, PFALTZ and BAUER Inc., 375 Fairfield Avenue, Stanford, CN 06902.

[3]FireMaster(R) BP-6, Michigan Chemical Corporation.

Experiment # 4

Absorption of corn oil (unsaturated fat) and tallow (saturated fat) differ in chickens. This study was undertaken to evaluate the effect of practical-type or purified-type diets containing either corn oil or tallow on fat absorption in broilers with cannulated bile ducts.

B. General Procedure

All experiments were conducted in an environmental room at 23 $\pm$ 1°C. The lighting period for all the experiments was identical (14 hours light, 10 hours dark). Broiler-type males, eight weeks of age were housed individually in 20.3 x 40.6 cm wire cages for each experiment. Feed and water were provided ad libitum. All birds underwent surgery, some were sham operated and others had both the cystic and hepatic ducts cannulated with Clay Adams PE 90 (ID 0.86 mm 0D 1.27 mm) tubing.[4]

Pre-surgery

Feed intake was recorded daily. Feed was withdrawn from all birds at least 12 hours prior to surgery.

Surgery

The birds were anesthetized with approximately 1.5 ml pentabarbital solution per kg of body weight. The concentration was 25 mg pentabarbital per ml of saline. The birds were placed in a cradle ventral side up with the legs restrained and spread apart. A 2.5 cm incision was

[4]Fisher Scientific Company, 34401 Industrial Road, Livonia, MI. 48150

made in the right abdominal wall starting posterior to the juncture of the last rib and the sternal member of that rib and proceeding in a straight line to a point slightly dorsal to the caudal end of the sternum. The hepatic duct and cystic ducts were carefully exposed by blunt dissection with minimal bleeding and injury to the surrounding connective and pancreatic tissue (Figure 7). Though intrahepatic communication (anastamoses) between the bile duct system of both lobes has been demonstrated (Clarkson et al., 1957) both ducts were cannulated for total bile collection. The cannulae were exteriorized through the incision and sutured in a loop to the skin posterior to the incision. The cannulae were looped over the top of the leg and emptied into a 15 ml preformed collecting tube. The tubes, 16 x 125 mm culture tubes with screw caps, were heated with a propane torch just above midpoint and bent to an angle of about 115^{o}. The tubes were then taped securely to the right leg with orthaletic adhesive tape from Parke-Davis. Holes were drilled in the caps for the cannulae to pass through. The cannulae were taped on both sides of the cap in order to prevent leakage and to prevent the bird from pulling the cannulae out of the collecting tube. A protective device to prevent the chicken from pulling or twisting the cannulae consisted of specially fitted vests (see Figure 8 and Figure 9) which covered the incision site and most of the cannulae leading to the collecting tube. The vest allowed unrestricted movement within the cage and the birds could move about to reach food and water.

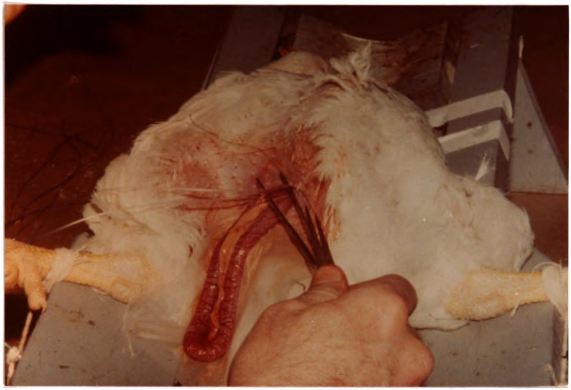


Fig. 7. Point of incision and cannulation of both the hepatic and cystic ducts.

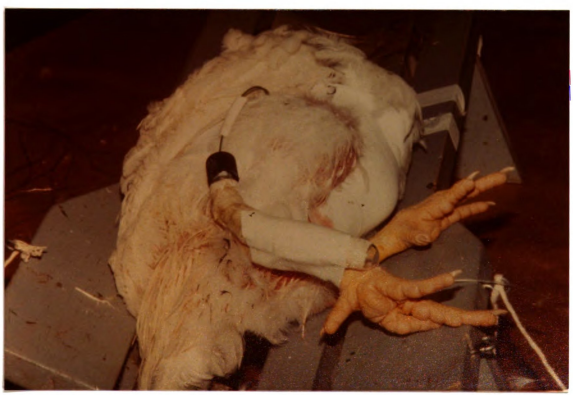


Fig. 8. Protective vest used to cover the incision and bile collection tube taped onto the right leg.

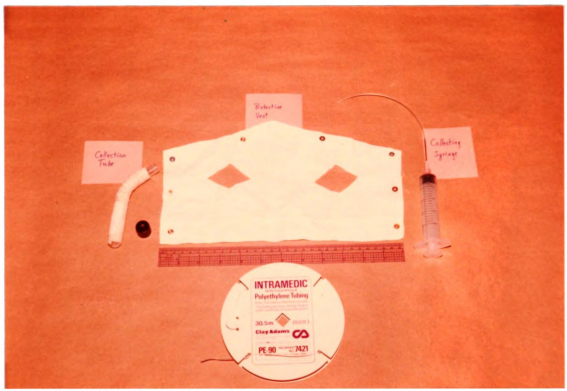


Fig. 9. Protective vest and collecting tube for chickens with their bile ducts cannulated.

Post Surgery

The birds were allowed at least 48 hours to recuperate after which the experiments were begun. Feed intake, general alertness and weight changes were monitored to evaluate recuperation following surgery. Feed intake and bile production were recorded immediately following surgery and throughout the experimental period. All birds were placed on the experimental diets at least 24 hours prior to the beginning of the experiment. The experimental period lasted three days, during which all excreta were collected for individual birds. The excreta were air-dried at room temperature and finely ground in a Wiley mill. The excreta were then analyzed for lipid content by hexane extraction (AOAC 1975) followed by 10% glacial acetic acid hydrolysis and re-extraction to determine % lipid saponified.

C. The Experiments

1. Experiment # 1

Twenty-eight broiler-type males, eight weeks of age were distributed among four dietary treatments of cholic acid. The control group consisted of sham operated birds (two per level of cholic acid). The treated group consisted of five birds with their bile ducts cannulated per level of cholic acid. A practical type (corn-soybean meal) diet (Table 1) supplemented with 0.0%, 0.04%, 0.08% and 0.16% cholic acid was given ad libitum to each of the four dietary

treatment groups. A second experiment was performed following the first 3-day experimental period in which all birds receiving dietary treatments of 0.0%, 0.04%, 0.08% were increased to 0.16% cholic acid and all birds on 0.16% cholic acid were changed to 0.0% cholic acid treatment. This experimental period lasted five days.

2. Experiment # 2

Eighteen broiler-type males eight weeks old were distributed among three dietary treatments and a control. The control group consisted of three intact non-operated birds and the treated group consisted of five birds with cannulated bile ducts per dietary treatment. A practical type (corn-soybean meal) diet (Table 1) was supplemented with either 0.0%, 0.08% cholic acid, or 0.08% freeze-dried chicken bile. The diet for the control birds contained no supplemental bile acids.

3. Experiment # 3

Twelve birds from experiment # 2 were selected and dosed with 80 mg of either PCP, HCB or PBBs. Bile was collected from 0-24 hr. and from 24-48 hr. in each of these birds. During this period, all birds received a corn soy diet (Table 1) with 0.08% supplemental bile acids. The bile was then analyzed to determine what percent of the xenobiotic had been excreted via the bile.

4. Experiment # 4

Twenty broiler-type males eight weeks old were

Table 1. Composition of diets in Experiment #1, #2 and #3.

Ingredients	g/kg
Corn, No. 2 yellow	502.1
Soybean meal, (48%)	310.0
Alfalfa leaf meal (17%)	50.0
Wheat bran	60.0
Corn oil, stable	40.0
DL-Methionine	0.9
Limestone	5.0
Dicalcium phosphate	22.0
Salt	3.0
Choline chloride, 50%	3.0
Vitamin mix[1]	3.0
Mineral mix[2]	0.5
Selenium mix[3]	0.5

[1]Supplied the following per kg of diet: Vitamin A, 11,000 I.U.; Vitamin D_3, 1,100 I.C.U.; Vitamin E, 11 I.U.; Vitamin K, 2.2 mg; Thiamin, 2.2 mg; Riboflavin, 4 mg; Panthothenic acid, 14.1 mg; Nicotinic acid, 31.5 mg; Pyridoxine, 4 mg; Biotin, 0.1 mg; Folic acid, 1.3 mg; Choline, 13.2 mg; Vitamin B_{12}, 0.01 mg; and Antioxidant (Santoquin), 12.5 mg.

[2]Supplied the following per kg of diet: Manganese, 60 mg; Zinc, 40 mg; Iron, 30 mg; Copper, 5 mg; Iodine, 0.5 mg.

[3]from Calcium Carbonate Company - supplied as 0.1 mg/kg of diet.

Table 2. Composition of diets 1 and 2 used in Experiment #4 (practical type diet).

Ingredients	Diet #1 g/kg	Diet #2 g/kg
Yellow corn, #2 dent	502.10	502.10
Soybean meal (48%)	310.00	310.00
Alfalfa leaf meal (17%)	50.00	50.00
Wheat bran	60.00	60.00
Tallow	40.00	0.00
Corn oil	0.00	40.00
Limestone	5.00	5.00
Dicalcium phosphate	22.00	22.00
Salt	3.00	3.00
DL-Methionine	0.90	0.90
Choline Chloride (50%)	3.00	3.00
Vitamin pre-mix[1]	3.00	3.00
Mineral pre-mix[2]	0.50	0.50
Selenium mix[3]	0.50	0.50

[1]Supplied the following per kg of diet: Vitamin A, 11,000 I.U.; Vitamin D_3, 1,100 I.C.U.; Vitamin E, 11, I.U.; Vitamin K, 2.2 mg; Thiamin, 2.2 mg; Riboflavin, 4 mg; Pantothenic acid, 14.1 mg; Nicotinic acid, 31.5 mg; Pyridoxine, 4 mg; Biotin, 0.1 mg; Folic acid, 1.3 mg; Choline, 13.2 mg; Vitamin B_{12}, 0.01 mg; and Antioxidant (Santoquin), 12.5 mg.

[2]Supplied the following per kg of diet: Manganese, 60 mg; Zinc, 40 mg; Iron, 30 mg; Copper, 5 mg; Iodine, 0.5 mg.

[3]from Calcium Carbonate Company - supplied as 0.1 mg/kg of diet.

Table 3. Composition of diets 3 and 4 used in Experiment #4 (purified-type diet).

Ingredients	Diet #3 g/kg	Diet #4 g/kg
Glucose monohydrate ("clintose")[1]	320.00	320.00
Corn starch	262.00	262.00
Cellulose	50.00	50.00
Tallow	75.00	0.00
Corn oil	0.00	75.00
Isolated soy protein (87%)	250.00	250.00
Methionine hydroxy analogue (93%)	2.40	2.40
Salt	5.00	5.00
Vitamin pre-mix[2]	5.00	5.00
Mineral pre-mix[3]	0.50	0.50
Limestone	10.00	10.00
Dicalcium phosphate	20.00	20.00
Selenium mix[4]	0.50	0.50

[1]Clinton Corn Processing Company, Clinton, Iowa.

[2]Supplied the following per kg of diet: Vitamin A, 11,000 I.U.; Vitamin D_3, 1,100 I.C.U.; Vitamin E, 11 I.U.; Vitamin K, 2.2 mg; Thiamin, 2.2 mg; Riboflavin, 4 mg; Pantothenic acid, 14.1 mg; Nicotinic acid, 31.5 mg; Pyridoxine, 4 mg; Biotin, 0.1 mg; Folic acid, 1.3 mg; Choline, 13.2 mg; Vitamin B_{12}, 0.01 mg; and Antioxidant (Santoquin), 12.5 mg.

[3]Supplied the following per kg of diet: Manganese, 60 mg; Zinc, 40 mg; Iron, 30 mg; Copper, 5 mg; Iodine, 0.5 mg.

[4]from Calcium Carbonate Company - supplied as 0.1 mg/kg of diet.

distributed five per treatment among four treatments. Treatment one consisted of a practical-type diet utilizing "fancy tallow" as the fat source. Treatment two consisted of a practical type diet utilizing corn oil as the fat source (Table 2). Treatment three was a purified type diet utilizing "fancy tallow" and treatment four substituted corn oil for tallow in a purified type diet (Table 3). All birds were cannulated but this time received no supplemental bile acids.

D. Analytical procedures.

All contaminated bile samples were collected and stored until extraction and analysis could be completed.

1). Analysis of HCB and PCP.

PCP and HCB extraction from bile (free determination)

a). Replicate 1 ml samples of the bile to be analyzed were placed in 16 x 125 mm culture tubes with teflon caps (Corning #982616X).

b). A 0.22 ml volume of 4N sulfuric acid was added to each set of replicate samples. The replicates were then vortexed together at a speed setting of one for five seconds. The next set of replicates in the text tube rack was treated in a similar manner and so on until all sets of replicates had been vortexed once. The speed setting of the vortex mixer was increased to five and the process was repeated.

A third vortexing was done at a speed setting of ten.

c). 3 ml of benzene was added to each tube using a 3 ml volumetric pipette. The samples were then placed on a Fischer Rotorack and allowed to extract for 15 minutes at 70 rpm after which the samples were allowed to stand 10 minutes at room temperature.

d). The samples were then centrifuged in a Sorvall (Glc-4) centrifuge for 20 minutes at 2000 rpm. Dilutions of 1:10 were made for PCP but it was not necessary for HCB.

e). Injections of the supernatant were then made into the gas chromatograph.

2). PCP extraction from bile (total determination).

a). Replicate 1 ml samples were placed in 16 x 125 mm culture tubes with teflon caps (Corning #982616X).

b). 0.1 ml of 18M H_2SO_4 was added to each tube and vortexed[5] at slow speed for five seconds.

c). All tubes were then placed in a water bath 85°C for 3 hours to hydrolyze and release any conjugated or protein-bound forms.

d). The tubes were then cooled to room temperature and 3 ml of benzene were added using a 3 ml volumetric pipette.

e). The tubes were then placed on a Fisher Rotorack

[5]Vortex - Genie, Model K-550G, Scientific Industries, Inc., Bohemia, New York.

and allowed to extract for 1 hour at 70 rpm and set at room temperature for an additional 8 hours.

f). The tubes were then centrifuged for 15 minutes at 2000 rpm after which the benzene layer was diluted 1:10 and then injected into the gas chromatograph.

The column conditions for PCP and HCB differed only in that the column temperature for HCB was 120°C and that for PCP was 160°C. All other parameters were similar. The column (6' x 2mm ID) was packed with 1% SP 1240DA on 100/120 Supelco. The injection temperature was 200°C and the electron capture detector was at 350°C. Nitrogen was used as the carrier gas with a flow rate of about 50 ml/min. The chart speed was set at 1.0 cm/min.

3). Analysis for PBBs (extraction from bile).

a). Shake container first to mix bile and then pipette one ml into 250 ml separator funnel.

b). Add 15 ml ethyl acetate/toluene (3:1) and shake 1 minute.

c). Drain bottom layer (bile) into small beaker.

d). Pour top layer (ethyl acetate/toluene) into round bottom flask through a small funnel containing sodium sulfate drying agent.

e). Pour bile back into separator funnel and repeat steps b-d. Do a total of three such extractions.

f). Concentrate ethyl acetate/toluene on roto-evaporator to about 5 ml and transfer to a 10 ml volumetric flask. Rinse 2x with 2 ml and bring volume to 10 ml.

Column Conditions:

The column (6' x 2 mm ID) was packed with 3% OV-1 gas chrom Q 100/120 mesh. Column temperature was 250°C. Injector temperature was 240°C and an electron capture detector was at 310°C. Nitrogen was used as the carrier gas with a flow rate of about 30 ml/min.

4). Validation of extraction procedures.

a). Recovery of known concentration of HCB.

i). Spiking experiments were carried out by extracting standards of HCB at 100 ppb, 250 ppb and 500 ppb in control bile. A recovery of 91.7% (± S.D.= 14.1) was obtained.

ii). Control bile was extracted and chromatographed and no HCB was detected.

b). Recovery of known concentration of PCP

i). Spiking experiments were carried out by extracting standards of PCP at 100 ppb, 250 ppb and 500 ppb in control bile. A recovery of 85% (± S.D. 9.0) was obtained.

ii). Control bile was extracted and chromatographed. Trace amounts (< 300 pcg/ml) were detectable.

c). Recovery of known concentration of PBBs.

i). Spiking experiments were carried out by extracting standards of PBBs at 10 ppm, 5 ppm, and 1 ppm in control bile. A recovery of 86.6% (± S.D. 8.4) was obtained.

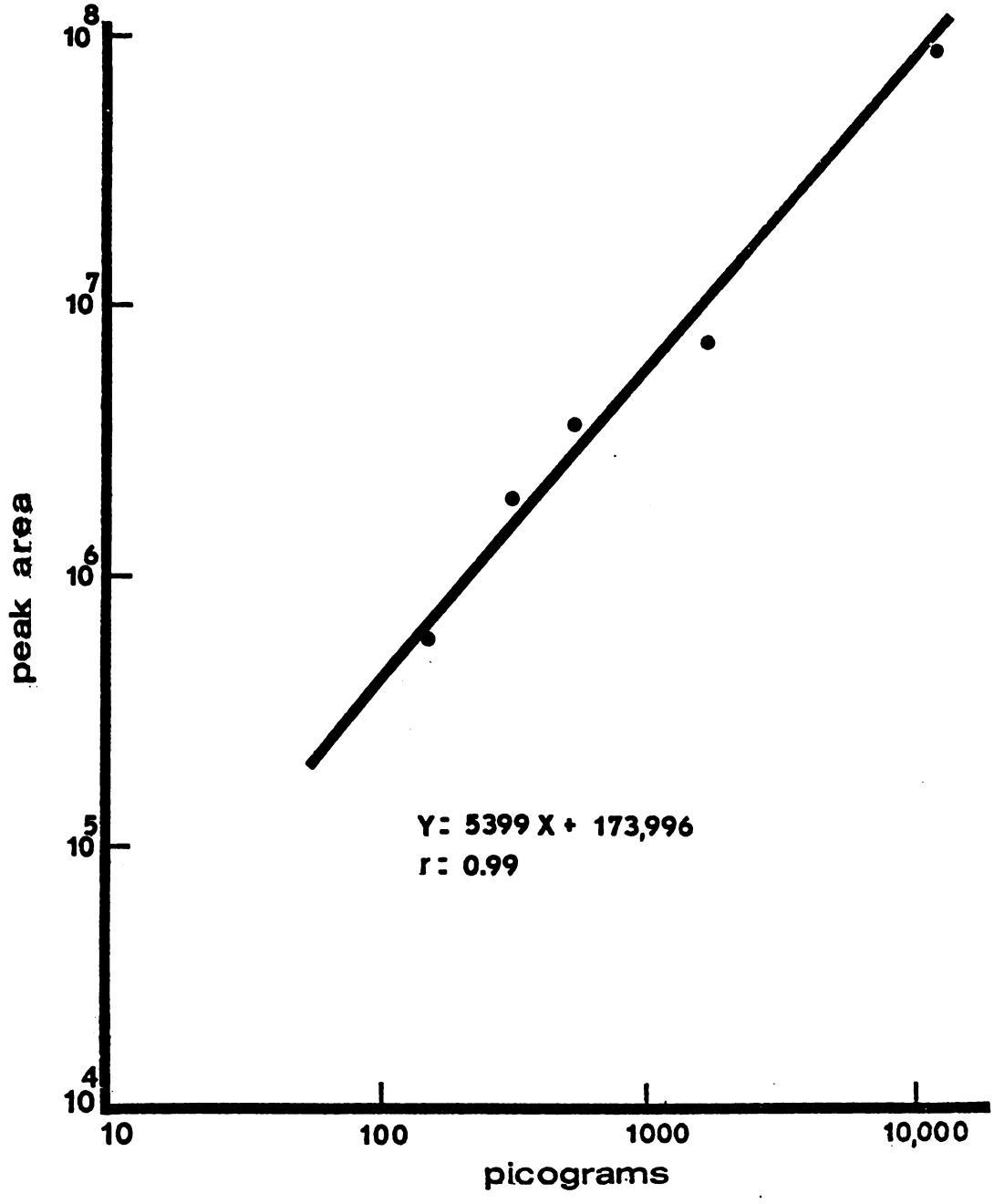


Fig. 10. Standard curve for PCP; (y = peak area), (X = amount in picograms)

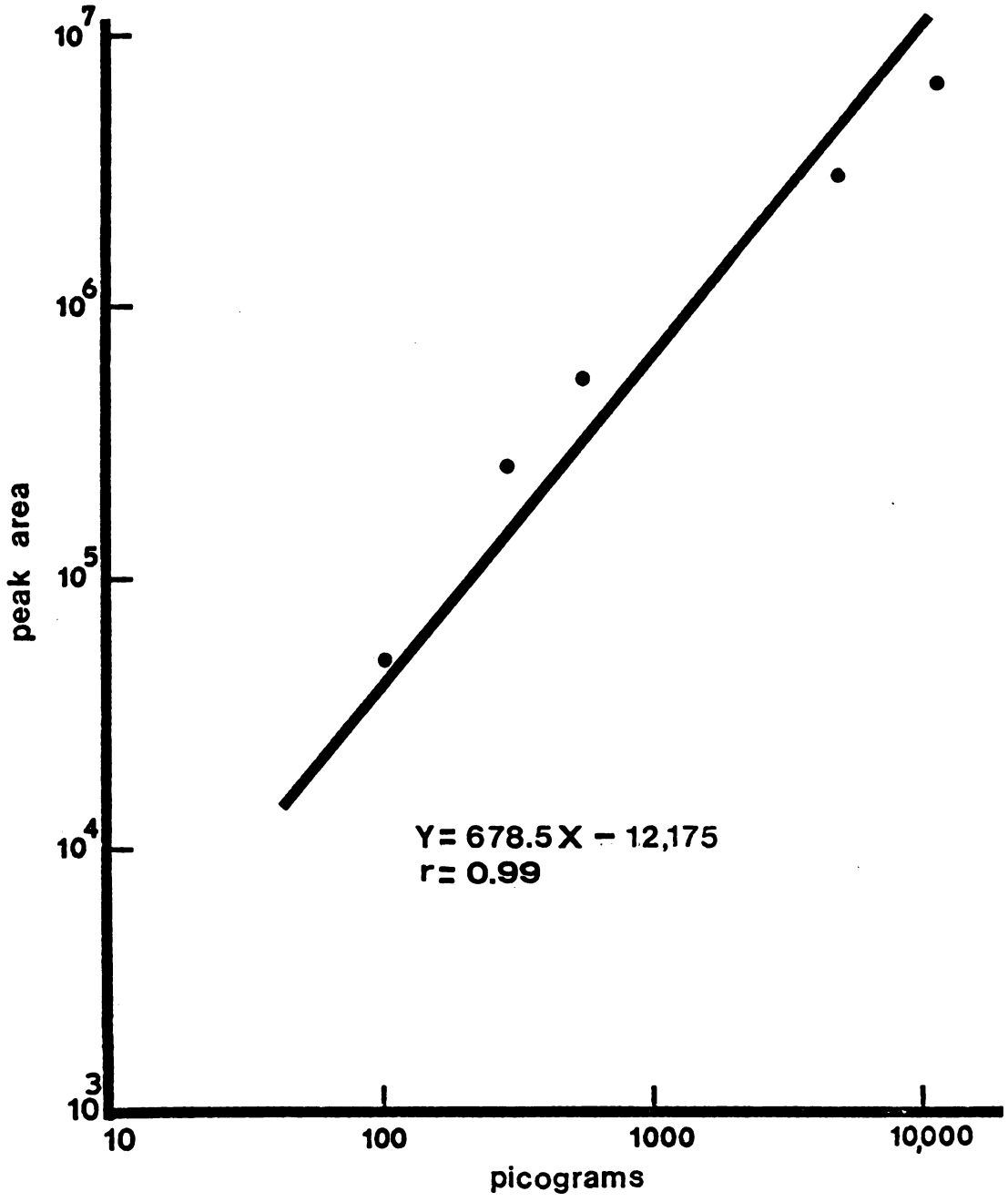


Fig. 11. Standard curve for HCB; (y = peak area), (X = amount in picograms)

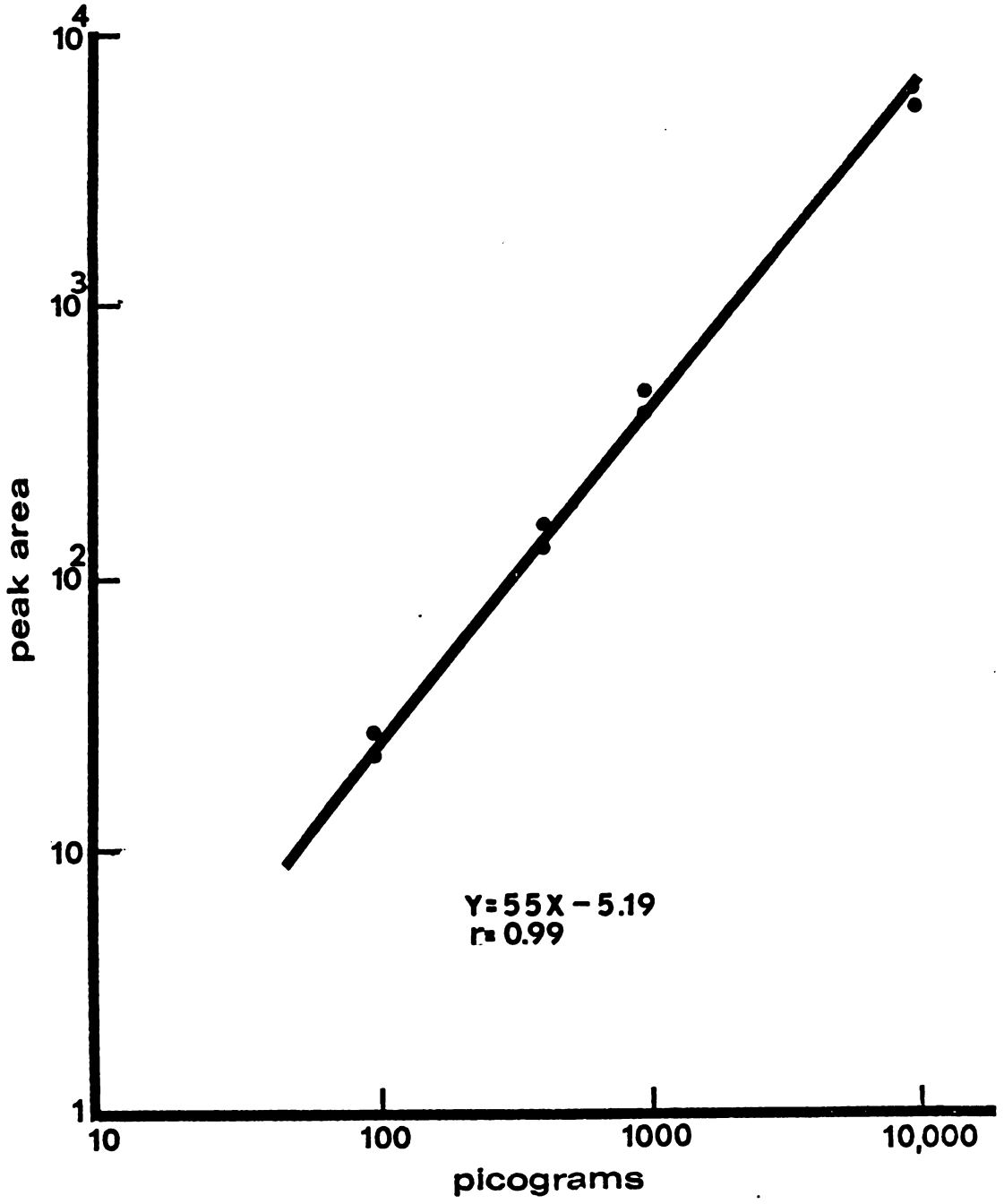


Fig. 12. Standard curve for PBBs; (y = peak area); (x = amount in picograms)

ii). Control bile was extracted and chromotographed and no PBBs were detected.

E. Statistical Analysis

All experimental data were analyzed statistically by the analysis of variance (Gill, 1978). Experiment # 1 was analyzed as a 2-way factorial; Experiment # 2 by Tukey's test for multiple comparisons of means; and Experiment # 4 by an orthogonal contrast. A level of $p \leq 0.05$ was considered as the level of significance.

IV. RESULTS

A. General Surgical Procedures

Due to the nature of the experiment and the surgical procedures involved, criteria were established in order to evaluate the performance of the birds following surgery. Feed intake, change in weight, general alertness, bile flow rate and color of the bile were all monitored in order to determine which birds would be used in the experiments. A daily feed intake of more than 50 grams, a bile flow rate of at least 10 ml per day and a positive weight gain over the experimental period of three days, were initially established to evaluate performance. General alertness and color of the bile were evaluated more subjectively.

Results in Table 4 indicate that % digestibility and % absorption were adversely affected in birds with cannulae. The sham operated birds did not appear to be affected by the stress of the operation. Several birds with cannulae were adversely affected perhaps because of the loss of the bile fluids or infection resulting from the cannulating operation itself. There was, however, a lot of variation between these cannulated birds indicating actual cannulation procedure needed to be improved. Several of the birds secreted blood through the cannulae, indicating that the cannulae had penetrated too far into the duct and perhaps even into the liver causing hemorrhage. Bile fluid changed color from the normally dark olive green, to various shades of yellow and brown

in several cannulated birds. This indicated a higher concentration of biliverdin and less bile acids per ml of fluid secreted. In some birds jaundice was induced perhaps as a result of obstruction of the bile ducts by the cannulae. These birds were observed to decrease their feed consumption and eventually they were removed from the experiment. Other problems included crimping or pecking of the cannulae but these could be repaired. Coating the cannulae with Anti-Pick lotion (Vineland Laboratories) reduced the frequency of these kinds of problems. Bile flow was sometimes erratic and there was a large variation in output between healthy birds and those that were slow to recover from the operation. By the end of the four experiments, the success rate for the operation with good recovery increased from about 50% (Experiment # 1) to 90% (Experiment # 4). The cloth vest served as an excellent protective device to prevent the bird from pulling the cannulae out and allowed for free movement within the cage. The birds were maintained as long as three weeks and although supplemental bile acids were added, they did not appear to compensate for loss in fluid bile as is apparent by the lipid absorption data in all trials. Both intact and sham operated birds were absorbing between 89-92% and the cannulated birds were absorbing anywhere from 43.4 - 72% with or without supplemental bile salts.

Necropsy revealed some distension of the gallbladder in birds that had cystic duct obstructions and the fluid in the bladder had become highly concentrated almost to the point of being a gel.

B. Experiment #1

The sham operated group on each of the four dietary treatments consumed almost two times the amount of feed as did those with cannulae in their bile ducts. The operation itself did not appear to stress the control group as they recovered rapidly and were consuming feed normally. The presence of cannulae, however, appeared to have an adverse effect on the health of about 30% of the birds. Not only was feed intake drastically reduced 50 to 60% in some cases, but % lipid absorption and digestibility were also decreased (Table 4). Factorial analysis, using the Feder-Zelen method for unbalanced data (Gill, 1978), indicated (Table 5) that feed intake, % digestibility and % lipid absorption were significantly ($p < 0.001$) different between the sham operated birds and the bile duct cannulated group. There were no significant differences between treatment levels of cholic acid or interaction between treatments and diets. It appeared that the level of supplemental cholic acid had no significant effect on feed intake, lipid absorption or digestibility. Bile flow ranged between 11 and 22.5 ml per bird per day and was highly correlated with the success of the operation and quick recovery of the birds. The second experiment (Table 6) indicated similar results to those in the first experiment. There were significant differences ($p < 0.001$) in feed intake, % lipid absorption and % digestibility between the sham operated birds and the cannulated birds (Table 7).

Table 4. The effect of bile duct cannulation and dietary supplementation of cholic acid on % absorption of lipids and digestibility in chickens: 1A.

	Sham operated					Bile duct cannulated					
		Diet[1]			Fat[1]			Diet[1]		Fat[1]		Bile
Treatment	n	Intake g/b/d	Digest. %	Diet %	Intake g/b/d	Absorp. %	n	Intake g/b/d	Digest. %	Intake g/b/d	Absorp. %	Collect. ml/b/d
None	2	153.60	67.00 (±0.22)[2]	9.6	14.80	90.00 (±0.50)	5	53.30	48.20 (±8.75)	6.20	43.40 (±7.06)	18.00
0.04% CA	2	141.30	65.70 (±1.12)	10.0	14.20	90.10 (±1.36)	6	66.90	58.00 (±1.28)	6.70	55.20 (±2.33)	15.90
0.08% CA	2	107.30	57.90 (±8.35)	10.3	11.00	88.90 (±1.29)	4	68.20	50.80 (±3.87)	6.40	57.70 (±5.10)	22.50
0.16% CA	2	160.10	63.60 (±0.71)	8.9	14.30	89.20 (±1.38)	5	114.70	58.60 (±0.94)	10.00	58.15 (±2.15)	20.50

[1] Dry wt. basis

[2] Mean (± S.E.)

Table 5. Analysis of variance of data on % lipid absorption and digestibility from Experiment #1A; Table 4.

Source of Variation	d.f.	Mean Square			f - ratio		
		feed intake	% lipid absorp.	% digest.	feed intake	% lipid absorp.	% digest.
Treatment (A)	1	1.75×10^8	57,235	9698	4054.68*	715.36*	72.81*
Diet (B)	3	28,866	90	74	0.67	1.13	0.55
(AB)	3	75,084	222	162	1.74	2.78	1.21
Error	20	43,160	80	133			

*$p < 0.001$

Table 6. The effect of bile duct cannulation and dietary supplementation of cholic acid on % absorption of lipids and digestibility in chickens: 1B.

	Sham operated						Bile duct cannulated					
		Diet[1]			Fat[1]			Diet[1]		Fat[1]		Bile
Treatment	n	Intake g/b/d	Digest. %	Diet %	Intake g/b/d	Absorp. %	n	Intake g/b/d	Digest. %	Intake g/b/d	Absorp. %	Collect. ml/b/d
None	2	134.0	64.20 (±3.86)[2]	9.6	12.91	89.90 (±2.16)	4	117.50	56.10 (±0.42)	11.32	49.40 (±4.82)	18.20
0.16 CA	6	136.0	64.40 (±0.69)	8.9	12.22	90.40 (±0.74)	4	78.30	54.80 (±3.61)	6.98	53.60 (±3.66)	19.60

[1] Dry st. basis

[2] Mean (± S.E.)

Table 7. Analysis of variance of data on % lipid absorption and digestibility from Experiment #1B; Table 6.

Source of Variation	d.f.	Mean Square			f - ratio		
		feed intake	% lipid absorp.	% digest.	feed intake	% lipid absorp.	% digest.
Treatment (A)	1	69,005,150	52,632	4242	261.64*	1339.6*	264.59*
Diet (B)	1	28,053	19	1	0.11	0.49	0.06
(AB)	1	76,484	25	4	0.29	0.64	0.28
Error	12	263,743	39	16			

*$p < 0.001$

C. Experiment # 2

As a result of Experiment # 1, a comparison was made between supplemental cholic acid and freeze-dried chicken bile acids to see if there were some hidden factors present in chicken bile which might improve lipid absorption over pure cholic acid. The birds were surgically prepared and their bile ducts cannulated. Lipid absorption ranged between 62-72% while digestibility, which appeared to improve over Experiment # 1, ranged between 60-62% (Table 8). The data were analyzed by Tukey's test (Gill, 1978) and there were no significant differences among the treatments. Bile flow ranged from 15 to 24 ml/b/d.

D. Experiment # 3

Twelve birds were selected to be given an 80 mg dose of either PBBs, HCB or PCP. As shown in Table 10 and 11, bile was collected for 2 periods from three birds on the PBBs dose, three birds on the HCB dose and four birds on the PCP dose. Each sample of bile was analyzed by gas chromatography and the results presented in Table 9 through 11. Total and free determination were carried out for PCP, while only total was carried out for HCB and PBBs. The results indicate that only small quantities of the xenobiotic are recovered via the enterohepatic circulation. As shown in Table 9, the chickens excreted in bile between 7.5 and 13.4% of the total dose of PCP in 0-24 hours (0.42 $\pm$ 0.077 mg/ml bile collected) and between 0.73 and 2.04% (0.05 $\pm$ 0.03 mg/ml bile collected) from

Table 8. The effects of dietary supplementation of freeze-dried chicken bile (FDCB) and cholic acid on lipid absorption and digestibility in bile duct cannulated chickens.

Treatment	n	Feed Intake[1] g/b/d	Fat Intake[1] g/b/d	Lipid Absorp. %	Digest. %	Bile flow ml/b/d
0.0% CA or FDCB	5	119.80	10.30	72.30 (±1.04)	60.10 (±2.50)	24.2
0.08% CA	5	73.60	6.80	61.70 (±6.31)	62.30 (±2.50)	18.2
0.08% FDCB	5	86.80	7.30	63.00 (±6.08)	61.30 (±2.91)	15.1

[1] Dry wt basis

[2] Mean (±S.E.)

Table 9. Recovery of pentachlorophenol from biliary fluid of chickens with their bile ducts cannulated.

Band No.	Period	ml bile collected	mg-recovered free[1]	mg-recovered conjugated	mg-recovered Total[2]	% of Dose free	% of Dose conjugated	% of Dose Total
14598	0-24	18.5	4.34	1.66	6.00	5.40	2.08	7.50
	24-48	15.0	0.56	0.02	0.58	0.70	0.03	0.73
No band	0-24	12.0	5.26	0.80	6.06	6.60	1.00	7.58
	24-48	17.0	1.39	0.25	1.64	1.73	0.31	2.04
14508	0-24	28.0	9.76	1.15	10.91	12.20	1.44	13.64
	24-48	25.0	0.61	0.14	0.75	0.76	0.18	0.94
14557	0-24	15.0	6.76	n.d.[3]	6.65	8.50	n.d.	8.31
	24-48	25.0	1.19	n.d.	0.96	1.50	n.d.	1.20
Mean	0-24	18.4 (±6.94)	6.53 (±2.37)	1.20 (±0.43)	7.41 (±2.36)	8.18 (±2.97)	1.50 (±0.54)	9.26 (±2.94)
	24-48	20.5 (±5.25)	0.94 (±0.42)	0.14 (±0.12)	0.98 (±0.47)	1.18 (±0.50)	0.18 (±0.15)	1.23 (±0.57)
Total		38.90 (±10.44)	7.46 (±2.30)	1.01 (±0.72)	8.38 (±2.24)	9.35 (±2.91)	1.26 (±0.90)	10.49 (±2.80)

[1] 85% recovery

[2] 86.5% recovery

[3] No difference detected between free and total (two different assays used for free and total).

Table 10. Recovery of HCB[1] from biliary fluid of chickens with their bile ducts cannulated.

Band No.	Period	ml bile collected	mg HCB in bile	% of dose
14563	0-24	9.0	0.100	0.125
	24-48	6.0	0.029	0.036
14505	0-24	17.0	0.447	0.560
	24-48	11.0	0.215	0.270
14509[2]	0-24	8.5	0.010	0.013
	24-48	0.0	n.d.	n.d.
14520	0-24	25.0	0.334	0.420
	24-48	26.0	0.232	0.29
Mean	0-24	14.9 (±7.8)	0.29 (±0.17)	0.37 (±0.22)
	24-48	14.3 (±10.4)	0.16 (±0.11)	0.20 (±0.14)
Total		25.6 (±18.75)	0.45 (±0.28)	0.57 (±0.36)

[1] 91.7% recovery

[2] no bile collected during second period, not included in average.

Table 11. Recovery of polybrominated[1] biphenyls (Peak #4) 2, 4, 5, 2', 4', 5' hexabromobiphenyl from biliary fluid of chickens with their bile ducts cannulated.

Band No.	Period	ml bile	mg PBBs in bile	% of dose
14577	0-24	23.0	0.029	0.035
	24-48	20.0	0.115	0.150
10928	0-24	9.0	0.047	0.577
	24-48	10.0	0.096	0.115
14535	0-24	23.5	0.179	0.230
	24-48	25.0	0.080	0.090
Mean	0-24	18.5 (±8.23)	0.09 (±0.08)	0.28 (±0.27)
	24-48	18.3 (±7.64)	0.10 (±0.02)	0.12 (±0.03)
Total		36.8 (±15.68)	0.18 (±0.06)	0.40 (±0.26)

[1] 86.6% recovery

24-48 hours. Also between 1 and 2.08% of the total dose was in the conjugated form during 0-24 hour period and 0.025-0.32 in the 24-48 hour period. Values for HCB (Table 10) ranged from 0.125-0.56% of total dose 0-24 hours and 0.036-0.29% between 24-48 hours. This corresponds to an average of 0.013 $\pm$ 0.01 mg/ml bile collected for all the birds in the 0-24 hour period and 0.011 $\pm$ 0.008 mg/ml bile collected for all the birds in the 24-48 hour period. As shown in Table 11, very little hexabromobiphenyl was excreted (0.03-0.20% of total dose) in 0-24 hours and (0.09-0.13% total) in 24-48 hours. Also more appeared to be excreted in the second period with two of the chickens, while the third excreted more in the first period. To what extent the impaired fat absorption mechanism affected these results cannot be determined. Supplemental cholic acid was added to each diet at 0.08%, but as indicated by experiments #1 and #2, fat absorption at this level of cholic acid does not compare to the normal physiological state of the intact chicken.

E. Experiment #4

Results in Table 12 indicate that lipid absorption ranged between 53-63% and digestibility ranged between 61-81%. An orthogonal contrast (Gill, 1978) revealed no significant differences in lipid absorption among treatments. The values for lipid absorption were not affected by either type of diet or type of fat added to the diet. However, there was a significant difference ($p < 0.01$) in the digestibility between

Table 12. Effect of type of diet (purified vs practical type) and dietary fat source (tallow vs corn oil) on lipid absorption and digestibility in chickens with their bile ducts cannulated.

Treatment	n	Feed Intake[1] g/b/d	Fat Intake[1] g/b/d	Lipid Absorp. %	Digest. %	Bile flow ml/b/d
Practical type diet + tallow	5	122.30	12.60	60.20 (±3.92)[2]	63.60 (±0.53)	24.40
Practical type diet + corn oil	5	89.90	8.10	63.40 (±3.57)	61.90 (±1.10)	20.40
Purified diet + tallow	5	77.00	6.00	53.50 (±4.57)	81.00 (±1.30)	16.10
Purified diet + corn oil	5	70.20	5.20	63.60 (±4.36)	78.00 (±1.04)	15.40

[1]Dry wt basis

[2]Mean (± S.E.)

Table 13. Analysis of variance - orthogonal contrast - on % lipid absorption and % digestibility of Experiment #4.

Contrasts	q_k	
	% lipid absorp.	% digest.
Practical type vs purified type	0.026	10.53*
Corn Oil vs tallow	0.108	0.21
Interaction	0.030	0.015

*$p < 0.01$

the practical type and the purified type diet (Table 13) with the purified diet being absorbed to a greater extent.

V. DISCUSSION

Influence of Cannulation and Surgery on Performance

Techniques for chronic collection of bile from restrained chickens have been reported by Paulson and Struble (1981). In these studies, which were published during the period of these experiments, Single Comb White Leghorn (SCWL) hens were surgically prepared either for total bile collection or intestinal perfusion of bile to replace that removed in order to insure normal physiological function. The birds were able to stand and move about (within the limits of the restraining harness) 1 or 2 hours after surgery. Within 24 hours after surgery, they were back to normal feed and water consumption levels. The rate of egg production after surgery (including the 1st and 2nd days after surgery) remained virtually unchanged for about 50% of the surgically modified birds. The rest of the birds eventually returned to normal production levels. No data were reported in that study on fat absorption in the surgically modified birds. The current study utilized a cloth vest in place of a restraining harness to prevent the birds from pulling out or twisting their cannulae. The birds were thus allowed more mobility and could turn around in the cage without problems. Some birds did, however, peck at the exposed areas of the cannulae from time to time. To remedy this, the exposed area of the cannulae were covered by tape and sometimes covered with an Anti-Pick fluid to discourage them from pecking at the cannulae. Also cannulae were repaired by sliding

larger diameter P.E. tubing over the areas that had been damaged. In Experiments # 2, # 3 and #4, the surgically modified birds absorbing between 60-70% appeared to be functioning normally, although feed consumption was reduced about 20%. The birds appeared healthy, alert and continued to gain weight over the period of the experiment. The duration of the longest experiment was about 2.5 weeks and the majority of the birds remained healthy. Those that became weak were sacrificed and necropsied revealing a distended gallbladder, enlarged liver, and other obvious signs of jaundice (color of sclera, etc). Infection had begun around the incision site in many cases and redness, edema and swelling were indications. Surgical technique could have been improved to help prevent infection by such changes as using 00 silk for securing the cannulae instead of cotton thread, more sterile procedures, using less suture at the incision site upon closure and dietary supplementation of antibiotics.

The initial experiment indicates that feed consumption was decreased drastically in the surgically modified birds (Experiment # 1). The previously mentioned study by Garrett and Young (1964) noted that birds lost weight during the experiment but that it did not appear to affect the results. Garrett showed that absorption of fatty acids was affected by the absence of bile in 8-10 week old chicks. Current theory on fat absorption would indicate that bile is needed for maximum absorption of fat, but as shown by this study and that of Garrett and others, that is not the only prerequisite for fat

absorption. This study showed that lipid absorption in cannulated birds ranged from between 43 to 72%, depending on the type of fat added to the diet and the type of diet consumed (practical type vs purified). Surgical skills improved over time and the health of the birds improved during the latter experiments as compared to the initial ones.

If bile is absent in the bird, then absorption of corn oil is not at its maximum, 89-90% as shown in Experiment #1 sham operated chickens. Thus any lipophilic compounds which are normally absorbed along with the lipids may not be absorbed as efficiently either. These may include fat soluble vitamins, cholesterol and even some environmental pollutants. This may have been a problem in Experiment #3, in which the chickens were dosed with PBBs, HCB and PCP. They may not have absorbed as much as a normal intact bird and thus the enterohepatic circulation rate of these compounds may not be representative of the intact bird. The only way to avoid this problem is to add enough bile acids to the diet in order to increase fat absorption to the normal level.

It is very difficult, if not impossible, to develop a representative model for the recovery of xenobiotics from the bile via the enterohepatic circulation. Ideally, the birds should be fed sufficient amounts of the xenobiotic to maximize uptake into the tissues. After the xenobiotic has been fed for a sufficient length of time, the birds should be cannulated and given clean feed. This would allow recovery of the xenobiotic as it is released from the tissues. In order to do

the surgery, the birds should be starved at least 12-24 hours prior to surgery but during this time they will begin to metabolize and excrete the xenobiotics. The procedure used in this study recovers any xenobiotic that was absorbed from the intestine, carried to the liver via the portal system, metabolized and excreted in the bile. The birds were eating clean feed (with supplemental bile acids to improve the absorption of the lipophilic compounds) and were not in a starvation state where they could be metabolizing the xenobiotic from their tissues. If there was any accumulation in the tissues, the xenobiotic may have remained in the tissues or else been in a steady state moving in and out of the tissues.

Effect of Supplemental Bile Acids on Intact and Cannulated Birds.

Garlich and Nesheim (1965), Edwards (1962), Fedde et al. (1960), and Gomez and Polin (1974 and 1976) showed that addition of bile acids to the diets of young chicks improved fat absorption. All of these studies were carried out with young chicks between day old and about 4 weeks. As reported previously, the ability of a young chick to absorb fat improves with age. According to Duckworth et al. (1950), the ability of the young chick to absorb fat is not fully developed until about 4 weeks of age. The exact mechanism that enables the young animal to improve the utilization of dietary fats with age is not clear. Jackson et al. (1971) and Smallwood et al. (1970, 1972) working with fetal dogs, attributed it to an enhanced ability in the synthesis rate of bile salts and a more efficient enterohepatic circulation of bile salts.

Experiments #1 and #2 in this study showed that addition of supplemental bile acids had no significant effect on lipid absorption. The sources used were cholic acid and freeze-dried chicken bile. Lipid absorption in the control group (no supplemental bile acids) was the same as that in the treated groups, so that the conclusion to be reached was that addition of bile acids at these experimental levels did not improve fat absorption to the level of the intact or sham operated chickens. An improvement in the ability to absorb fat with supplemental bile acids would be expected, based on the work with young chicks. This improvement would seem even more likely if the birds had their bile ducts cannulated. There appears to be something in the older birds which seems to indicate that bile becomes less important in the fat absorption mechanism. Fat absorption appeared to decrease about 30% in the birds with their bile ducts cannulated. They were still absorbing 70% of what the normal intact bird absorbs. There must still have been some micelle formation if the birds were absorbing this much. There may be other detergent like substances present in the duodenum and ileum which are not present at a younger age or there may be some other unidentified factor present which enables the older birds to absorb fats more efficiently. Studies by Webling and Holdsworth (1965) have shown that chicken bile contains about 112 mg bile acids per ml of bile. The flow rate of bile in chickens has been shown to be about 2 ml/hr which would mean the chickens were receiving about 220 mg/hr. If the diet is supplemented at 0.16%, the birds would consume

160 mg bile acids per 100 g diet (about 200 mg/day or 8.5 mg/hr.) It may be that higher levels of the bile acids would improve fat absorption. The highest level of cholic acid used in this study was 0.16% and this appeared to have no significant effect.

Influence of Type of Diet and Dietary Fat on Lipid Absorption.

The type of feedstuff, the type of diet (practical type vs purified) and the type of fat (unsaturated vs saturated) have been shown to affect absorption of lipids. Serafin and Nesheim (1967) found that young chicks fed raw soybeans nearly always had empty gallbladders, whereas those fed heated soybeans under identical conditions, had bile present. Further studies indicated that over a 6-day period, chicks 1-2 weeks old, fed unheated soybean meal, excreted 44% of a dose of ^{14}C labeled cholic acid as compared to 23% for chicks fed diets of heated soybean meal. They also found that chicks 42-48 days old fed diets containing either heated or unheated soybean meal appeared to have the same amount of bile acids in their bile acid pools when expressed on a mg/100 gm of body weight basis. Chicks receiving unheated soybean meal, however, excreted radioactive bile acids twice as rapidly as those receiving heated soybean meal. This is an indication that the chicks fed raw meal are probably synthesizing bile acids at a faster rate than control chicks, thereby providing enough bile in the lumen to permit normal fat absorption. Renner and Hill (1960) showed that lard was absorbed by young chicks 2 weeks to 8 weeks of age, at levels between 90-95% and that corn oil absorption

ranged from 94 to 98%. Tallow was shown to be absorbed poorly (70 to 82%) compared to corn oil and lard over the same age.

In this study, a practical type corn soy diet was compared to a purified diet to determine whether the type of diet would have any effect on lipid absorption in the cannulated birds, the thought being that there may be some natural detergent-like substances present in the practical type diets which would enhance lipid absorption in the cannulated birds. As indicated by the results, no significant difference was found between the purified type and practical type diets. There was, however, a significant difference in digestibility between the two diets. The purified diet was more efficiently digested. Tallow tended to be more poorly absorbed than corn oil, though there was no significant difference.

Enterohepatic Circulation of Xenobiotics

PCP, due to its less polar structure and more hydrophilic characteristics compared to HCB and PBBs, was found to be more readily excreted in the bile. Stedman et al. (1980) found the greatest accumulation of PCP in the kidney with lesser amounts in the liver and other tissues in the chicken. The lowest accumulation of PCP occurred in the fat. Also (Stedman et al., 1980) the half life of PCP was noted to be much shorter at 1.78 weeks than HCB and PBBs. The accumulation factor also is much lower. It would appear then that less PCP is accumulated in the tissues and that the clearance rate is more rapid for PCP than for HCB and PBBs. PCP, which is ionized under

normal physiological conditions, appears to be more readily excreted in the urine (Stedman et al., 1980). In this study, the chickens excreted in bile about 9.26% of the total dose of PCP in 0-24 hr. and about 1.23% between 24-48 hours.

HCB which has a much higher accumulation factor than PCP and a half life of 3.79 weeks (Stedman et al., 1980) was less readily excreted in the bile. Ingerbrigtsen et al. (1971) found that less than four percent of the total dose of HCB was recovered in the bile of rats within a 48 hour period. In this study, less than one percent of the total dose was recovered in the chicken bile. The highly lipophilic nature of HCB would tend to cause it to partition into adipose and be less readily metabolized and excreted.

PBBs, also due to their highly lipophilic nature, are accumulated into adipose (Polin and Ringer, 1978). Studies by Matthews et al. (1977) in rats have shown that the major component hexabromobiphenyl ultimately accumulated in the adipose tissue. It was also shown that hexabromobiphenyl was not appreciably metabolized and was excreted almost exclusively by the feces at a very slow rate. Biliary excretion was also studied and found to be 0.68 $\pm$ .19% of the total PBBs dose between 0 and 4 hours after iv administration. In this study less than one percent of the total dose of PBBs was excreted in the bile of chickens. Much of the PBBs may have accumulated into tissues.

It may be that the birds would be able to metabolize and excrete more of these compounds when feed is restricted. If that is the case, then more of the xenobiotic may be excreted

in the bile. The other consideration is that there may be some recirculation of the compounds back into the portal system after being excreted from the bile (enterohepatic circulation). Colestipol and cholestyramine have been shown to sequester bile acids to prevent their reabsorption by the enterohepatic circulation (Parkinson et al. 1970 ; Juul and VanderLinden, 1969). These same compounds could conceivably be used to sequester the xenobiotics to prevent their absorption or reabsorption and thence carry them out of the body via the excreta, ridding the body of xenobiotic.

VI. SUMMARY AND CONCLUSION

In this study, four experiments were conducted to: 1) evaluate the effect of bile acids on lipid absorption; and 2) quantitate the amount of PCP, HCB and PBBs absorbed via the portal circulation and excreted in the bile. In experiment #1, a technique was developed to cannulate and collect bile from 8 to 10 week old broilers. These birds were fitted with specially designed vests which allowed them to move about freely within their cages. The surgical procedures and bile collection technique were successful and the slight decrease in feed consumption did not appear to affect lipid absorption. The results obtained from Experiments #1 and # 2 revealed that the chickens were still able to absorb considerable amounts of fat even with their bile ducts cannulated. There appeared to be no improvement in fat absorption by dietary supplementation of cholic acid or freeze-dried chicken bile. Additional study needs to be done to see if higher levels of bile acids in the diet would improve fat absorption.

Twelve birds were dosed with 80 mg of a xenobiotic in Experiment #3 and their bile was collected and analyzed for the xenobiotic. Very little HCB or PBBs ($< 1\%$) were found to be excreted in the bile as compared to PCP (about 10%) over 48 hours. The PCP was excreted more rapidly from the body as compared to the more highly lipophilic compounds which were accumulated into the tissues.

The effect of type of diet (practical vs purified) and

dietary fat (tallow vs corn oil) on lipid absorption in birds with their bile ducts cannulated was evaluated in Experiment #4. The purified diet was found to be more digestible than the practical type diet but there was no difference in % lipid absorption. The type of dietary fat did not appear to have any significant effect on lipid absorption or digestibility.

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