MATURATION OF HEPATIC EXCRETORY FUNCTION: INFLUENCE OF CARBON TETRACHLORIDE AND POLYBROMINATED BIPHENYLS

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This is to certify that the

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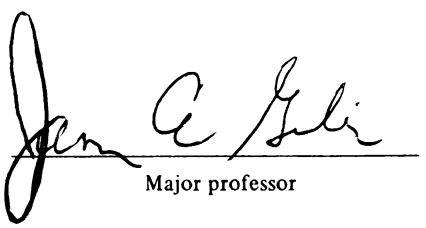

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ABSTRACT

Maturation of Hepatic Excretory Function: Influence of Carbon Tetrachloride and Polybrominated Biphenyls

by

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The biliary tract is a major route for elimination of xenobiotics from the body and the ability of the newborn to excrete chemicals into bile is low with respect to adult excretory capacity. The purpose of this investigation was to characterize the maturation of hepatic excretory function in rats and to determine the effect of carbon tetrachloride and polybrominated biphenyls on liver excretory function in developing rats.

Maturation of the hepatic excretory system was determined in developing rats by measuring the plasma disappearance and hepatic and intestinal (biliary) appearance of intravenously administered ouabain. Cumulative (40 minute) intestinal ouabain content was lower in 15 day old rats than in 21, 25, 35, and 45 day old animals and reached adult levels when rats were 35 days old. Decreased ouabain excretion in young rats resulted in retention of ouabain in plasma when compared to plasma ouabain concentrations in older animals.

Biliary excretion of sulfobromophthalein (BSP) is dependent on hepatic uptake from plasma and intrahepatic conjugation to glutathione (GSH). Enzymatic conjugation of BSP to glutathione *in vitro* was low

in young rats, however, elimination of BSP and conjugated BSP (BSP-GSH) from plasma was more rapid in adults than in 15 day old rats. Impaired elimination of ouabain and BSP from plasma of rat neonates correlated to low initial concentration of these drugs in liver. The inability of young rats to accumulate BSP and ouabain in liver may be the most important determinant for functional insufficiency.

Studies were undertaken to determine whether the rate-limiting step in the excretion of drugs by liver is age dependent. Bile duct ligation and bile salt infusion, treatments that primarily depress and enhance (respectively) excretion of BSP from liver into bile, markedly altered the disappearance of BSP from plasma of adult rats but did not appreciably affect BSP disappearance from blood of 15 day old rats. The effect of bile duct ligation on ouabain transport in 15 day old rats was also not as dramatic as the effect produced in adult rats. Hepatic uptake is rapid in adult rats and overall excretion is limited by a slower rate of transport from liver into bile. The lower rate of uptake in 15 day old rats may limit overall transport function in these animals.

Carbon tetrachloride (CCl_4) depressed hepatic excretory function in adult and developing rats and plasma ouabain concentration was significantly higher and biliary (intestinal) ouabain content was significantly lower in treated animals when compared to controls. Hepatic ouabain was significantly lower than controls in CCl_4 treated young rats (decreased uptake into liver), but significantly higher than controls in treated older rats (decreased excretion from liver into bile). CCl_4 may disrupt all mechanisms in the drug elimination

process. Since the rate-limiting step in drug transport changes with age, CCl_4 may disrupt transport function in accordance with existing age differences.

Exposure of developing rats to polybrominated biphenyls (PBBs) did not produce overt toxicity when compared to controls over a 49 day postnatal period. However, prenatal and postnatal dietary exposure to PBBs resulted in elevated liver weight. In 15 day old rats, increased liver weight following PBBs correlated to enhanced ouabain excretion into bile. Liver weight was also elevated in 21, 35, and 49 day old rats treated with PBBs but this effect was not associated with stimulation of ouabain transport in these animals. The mechanism for stimulation of ouabain transport following PBBs in 15 day old rats was increased hepatic uptake of ouabain. The selective stimulation in only young rats may be attributed to the relative importance of uptake for overall transport in 15 day old rats.

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TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF FIGURES

LIST OF FIGURES (continued)

LIST OF TABLES

LIST OF TABLES (continued)

INTRODUCTION

Objectives

Functional immaturity of the liver is exemplified by the inability of newborn animals to effectively excrete xenobiotics into bile (Klaassen, 1972). The mechanisms responsible for the maturation of hepatic excretory function are not precisely known, however, determination of the development of the excretory system may be complicated by the multifaceted nature of the drug elimination process. Transport of drugs from blood into bile requires a number of steps including specific uptake into the hepatic parenchyma, intrahepatic storage and metabolism, and finally secretion into bile. Thus, the deficiency in the young, for drug elimination may be due to a combination of any or all of these factors (Klaassen, 1975).

The low capacity for chemical excretion in newborns has toxicological consequences, for when compared to adults, young animals may be particularly susceptible to chemical-induced toxicity, including lethality (Klaassen, 1972,1973a). An equally important area of concern is the influence that foreign agents may have on the liver as an excretory organ. In adults, hepatic excretory function is well known to be disrupted following exposure to a variety of xenobiotics, but the influence of such substances on liver excretory function during development has not been extensively studied.

Since chemical excretion by the liver is a multifaceted process, the influence of chemical agents on overall drug transport may be a reflection of specific and distinct dysfunction at any transport step. In adults, the rate-limiting step in drug elimination into bile is hepatic secretion. Disruptions in hepatic function, therefore, are most evident when the hepatic secretory mechanisms are altered. Although drug transport into bile is low in the neonate when compared to the adult, the relative importance of the various transport steps has not been elucidated.

Thus, the objectives of this investigation were two-fold, both of which relate to hepatic excretory function and dysfunction during development. The first objective was to characterize the development of hepatic excretory function in rats and determine the relative importance of the various transport steps as a function of age. The second objective of this research focused on chemical-induced alterations in function. In particular, the objective was to determine the effects of carbon tetrachloride and polybrominated biphenyls on hepatic excretory function in both developing and adult rats.

Liver Development and Maturation of Hepatic Enzyme Systems

During embryonic development, the liver arises as an outgrowth of the endodermal wall of the primitive gut. The original outgrowth gives rise to a hollow hepatic diverticulum which soon differentiates into two parts: an anterior portion which proliferates to become the large glandular mass of the liver and its bile ducts (hepatic bile ducts); and a posterior part which gives rise to the gall bladder and cystic duct. The stalk connecting these two parts to the gastrointestinal tract becomes the common bile duct (Weichert, 1970).

In most mammalian species, the fetal liver, once organogenesis is complete, contains two major cellular components; hematopoietic tissue and hepatocytes (Jacquot et al., 1973). In the very young rat fetus, the hematopoietic component is by far the major one and hepatocytes are dispersed in it. The normal ontogenic pattern is a shift in the relative preponderance of these two tissue types. Thus, at birth, hepatocytes form a well organized parenchyma including dispersed erythropoietic cells.

In order to maintain homeostasis and permit normal growth, marked variations in many metabolic functions occur in the liver of mammals during development. These changes are particularly dramatic at birth, when the abrupt passage from intrauterine to extrauterine environment occurs, and during the weaning period, when great variations in nutrition takes place (Serini and Principi, 1971). Greengard (1974) noted that, with few exceptions, the many enzymes that have been studied in rat liver conform to one of four developmental patterns. The enzymes of one group (termed cluster I) appear to be mandatory for growth and are present in all fetal tissues and decrease in amount toward the end of gestation. More specific to hepatic functions are enzymes associated with the other three clusters. A late fetal cluster (II) emerges between the 17th and 21st day of gestation and includes the enzymes for the urea cycle and glycogen synthesis. The next cluster (III), which is brought forth during the early postnatal period, involves enzymes needed for gluconeogenesis and xenobiotic detoxication. Finally, the enzymes (cluster IV) involved in fatty acid synthesis and in the subtle regulation of amino acid and glucose levels in the blood emerge in the third postnatal week or late suckling period (Greengard, 1971,1974).

One aspect of fetal and neonatal development which has been studied in great detail is the enzyme system which acts to metabolize chemicals. The metabolism of drugs and other foreign compounds (xenobiotics) occurs to a considerable extent in the smooth endoplasmic reticulum (microsomes) of liver. Metabolism of drugs by this microsomal system results in more polar and water soluble products and often leads to reduction of pharmacological activity and more rapid drug excretion (Mandel, 1971).

Newborn human infants appear to be immature in their ability to metabolize drugs (Done, 1964). This immaturity has been best documented clinically in the case of the "grey baby syndrome" in premature and newborn infants following treatment with chloramphenicol (Burns et al., 1959; Lambden et al., 1960; Weiss et al., 1960). The antibiotic is extensively metabolized in adult liver and undergoes nitroreduction (Fouts and Brodie, 1957) and glucuronidation (Glazko et al., 1950) catalyzed by hepatic microsomal enzymes. However, during the neonatal period, the activities of enzymes catalyzing these reactions are low with respect to adult levels.

Low activity in the fetus and newborn for hepatic drug metabolism is now well documented in laboratory animal studies (Fouts and Adamson, 1959; Jondorf et al., 1959; Jacquot et al., 1973; Telegy, 1973; MacLeod et al., 1972) as well as in studies with the human fetus and newborn infant (Rane et al., 1973). Increases in the activitiy of drug metabolizing enzymes to adult levels occurs, for the most part, during the postnatal and suckling period.

Low activities of enzymes in the fetus and newborn may be partially related to the presence of hematopoietic cells in the developing liver (Henderson, 1971). Thus, increases in enzymatic activity would, to some degree, reflect the emergence of the hepatocyte population. Henderson (1971) found that 20% of all liver cells on the third postnatal day were still hematopoietic in rat liver and that the developing blood cells did not disappear from liver until the third postnatal week. However, Greengard et al. (1972) determined that the mean volume of individual hepatic parenchymal cells undergoes a three-fold rise during late fetal life, declines slightly in the early neonatal period, and doubles between the 12th and 28th postnatal days. From these data, it was calculated that only increases in enzyme concentration of less than two-fold would be attributed to the enrichment of parenchymal tissue at the expense of hematopoietic cells (Greengard, 1972). Thus, MacLeod et al. (1972) maintained that the magnitude of change in liver cell type after birth would be insufficient to contribute significantly to the pattern of microsomal enzyme development. Consistent with this hypothesis is the observation that smooth endoplasmic reticulum, the intracellular site of drug metabolism, is less prevalant in fetal and newborn hepatocytes when compared to adult liver cells (Fouts, 1962; Peters et al., 1963; Palmer et al., 1966; Koenzig et al., 1976). Thus, increases in enzymatic activity reflect maturation of the liver cell.

Hepatic Excretory Function During Development

Immaturity of the hepatic excretory system in human neonates is exemplified by the condition of newborn jaundice. Jaundice appears in

about 50% of newborn infants and can be very pronounced (Lathe, 1974). Newborn jaundice is characterized by high plasma concentration of unconjugated bilirubin, a metabolic product of the nonprotein component of hemoglobin (heme). If bilirubin in blood of the newborn is displaced from plasma proteins to which it is bound, or if plasma bilirubin concentration is exceedingly high, bilirubin will enter the central nervous system and produce a type of brain damage called kernicterus (Lathe et al., 1958).

The high incidence of newborn jaundice attracted the attention of physicians at the turn of the century and systematic studies were undertaken to identify the etiology of this condition. From these studies, it was generally held that the precipitating factor of newborn jaundice was increased hemolysis at birth. This was suggested by two observations: 1) the high hematocrit at birth usually falls dramatically shortly after birth, and 2) by the occurence of extreme jaundice in neonates with known hemolytic disease (Lathe, 1974). In 1947, however, Weech reviewed the subject and could detect no direct relationship between the amount of hemoglobin destroyed and the intensity of hyperbilirubinemia. As an alternative, Weech (1947) suggested that, in general, maturity of the neonate determined the extent of hyperbilirubinemia. This contention was supported when jaundice was found to be most extreme in premature infants (Hsia et al., 1953) and that the severity of jaundice in these infants was inversely related to the length of gestation and birth weight (Billings, et al., 1954). Since the major route for bilirubin detoxication and excretion is the liver and biliary tract, attention was focused on the inability of the liver, in the young, to excrete circulating levels of bilirubin (Zuelzer and Brough, 1969).

While most laboratory animals do not develop neonatal jaundice, many have shown impaired ability to excrete an exogenous load of bilirubin when compared to the adult (Schenker et al., 1964; Catz and Yaffe, 1968; Gartner and Arias, 1969; Klaassen, 1976). Moreover, hepatic excretion of xenobiotics that are primarily excreted into bile in the adult, are excreted into bile to a lesser extent in the newborn rat and guinea pig (Klaassen, 1972,1973b; Hwang and Dixon, 1973). In newborn rats, decreased biliary excretion leads to accumulation of certain drugs in plasma and results in high sensitivity, in the young, to drug-induced lethality (Klaassen, 1973a).

Excretion of bilirubin and other endogenous and exogenous compounds from blood into bile requires a number of transport steps: compounds are taken up from plasma into the hepatic parenchyma, metabolized within the liver, and finally secreted from liver into bile. Since the ability of the liver to metabolize drugs is depressed in the neonate, the excretion of agents that require intrahepatic metabolism prior to biliary excretion may be limited, in the young, by the degree to which the compound is metabolized.

In mammalian species, bilirubin is conjugated to glucuronic acid in hepatic microsomes prior to excretion into bile. The reaction is catalyzed by the enzyme UDP-glucuronyl transferase. In vitro, the ability of microsomal fractions of liver to conjugate bilirubin to glucuronic acid (UDP-glucuronyl transferase activity) is low in the newborn human (Lathe, 1974), monkey (Gartner and Lane, 1972), guinea pig (Gartner and Arias, 1969), and rat (Bakken, 1969; Catz and Yaffe, 1968; Vaisman et al., 1976) when compared to conjugation capacity in adults. Thus, low

hepatic metabolism may be an important mechanism for accumulation of bilirubin in plasma in the young.

Depressed rate of drug metabolism may also be a factor in retention of sulfobromophthalein (BSP) in plasma of the newborn. BSP is the prototype used for studying biliary excretion of organic acids. Prior to biliary excretion BSP is enzymatically conjugated in the liver to glutathione (Combes and Stakelum, 1960). A number of studies have shown that BSP disappears more slowly from plasma of newborn infants (Mollison and Cutarsh, 1949; Yudkin et al., 1949), guinea pigs (Goldstein et al., 1965; Whelan et al., 1970a) and rats (Klaassen, 1973b) than in their respective adults. Inasmuch as the capacity for BSP conjugation to glutathione is lower in the newborn than in the adult (Combes and Stakelum, 1962; Krasner and Yaffe, 1968; Whelan et al., 1970a) the slower rate of BSP elimination from plasma may be a reflection of the deficiency for BSP metabolism.

Other processes involved in hepatic excretion of drugs are also considered to be immature in newborn animals. Hwang and Dixon (1973) examined hepatic excretion of indocyanine green (ICG) in developing and adult guinea pigs and observed the ability of the newborn to clear ICG from plasma into bile was less than in the adults. Since ICG is not metabolized in the passage from blood to bile, this age difference was considered to represent a deficiency in the newborn in the absolute maximum capacity for biliary excretion. Similarly, hepatic excretion of ouabain, ICG, and conjugated BSP (BSP-GSH) is lower in newborn rats than in adults (Klaassen, 1972,1973b). These drugs do not require metabolism prior to biliary excretion and thus the depressed rate

of biliary excretion cannot be attributed to a deficiency in conjugating capacity.

The newborn may also be immature in the ability to take up drugs into liver from plasma. The initial rate of elimination of BSP from plasma, which primarily represents hepatic uptake capacity, is rapid in the adult and in older children ($t_{\frac{1}{2}}$ = 5.5 min) but is significantly slower ($t_{\frac{1}{2}}$ = 9.6 min) in human newborns (Wichmen et al., 1968). Cumulative hepatic uptake of bilirubin by liver was reduced during the neonatal period in guinea pigs and achieved adult capacity at approximately 15 days of age (Gartner and Arias, 1969). Hwang (1975) determined uptake capacity in vitro by measuring accumulation of ICG into liver slices. Liver preparations obtained from newborn guinea pigs could not effectively accumulate ICG, however, accumulation of ICG was high in slices obtained from adult liver (Hwang, 1975). In rats, the low rate of clearance of BSP and ouabain in newborns was related to a lesser ability relative to adults in the uptake of these compounds into the liver cell (Klaassen, 1972,1973b). Although the concentration of ouabain in liver of an adult reaches 50 times that of plasma, liver of the newborn rat cannot concentrate ouabain at all (Klaassen, 1972). The ability of the liver to extract ouabain from plasma in rats develops concurrently with the decrease in ouabain toxicity that occurs with development (Klaassen, 1972).

Little is known about the cellular mechanisms responsible for hepatic uptake of drugs. However, it has been suggested that a cytosolic protein, ligandin (Litwack et al., 1971; Levi et al., 1969a,b) may play an important role in hepatic uptake of organic anions (such as bilirubin, BSP, and ICG). Hepatic content of ligandin is low in early

life and increases with age in rats (Klaassen, 1975), guinea pigs (Levi et al., 1969a), and monkeys (Levi et al., 1970). In rats, the increase in hepatic concentration of ligandin appears to correspond with the age related increase in the ability to accumulate organic anions into the liver (Klaassen, 1975). The relative deficiency of ligandin has been suggested to be important in the etiology of unconjugated hyperbilirubinemia in human infants (Levi et al., 1970).

Whether the transport system that carries drugs from the hepatocyte into bile is also immature in young animals is difficult to determine, since its function may be limited by the concentration of drugs available to it. In guinea pigs, however, the maximum biliary transport capacity (Tm) of ICG in newborns is one-third that in the adult (Hwang and Dixon, 1973) even though bile flow rate in the young is the same as the adult value when calculated on a body weight basis (Hwang and Dixon, 1973).

The ability of newborn rats to excrete drugs from liver into bile cannot be directly elucidated since it is difficult to obtain bile samples from the small animals. However, DeWolf-Peeters et al. (1972) have described the morphological development of the biliary tract in rats as an indirect means to estimate function. Using electron microscopic and histochemical techniques, these authors examined the differentiation of the bile canaliculus, the primary site of both bile formation and drug excretion into bile. From 16 until 19 days of fetal age, the canaliculus is forming and is defined by an intracellular invagination of two adjunct cell membranes into one of the two neighboring hepatocytes. At birth, the canaliculus has a distinct lumen, however, the structure is irregular in form. The lumen of the canaliculus widens during the first 3 postnatal days but contains few microvilli. Following 3 days after birth, the lumen becomes progressively smaller and

gradually fills with microvilli leading, at 10 days after birth, to a normal adult canalicular structure (DeWolf-Peeters et al., 1972). These authors suggested that underdeveloped canalicular morphology may be an important factor in different conditions of human neonatal jaundice. However, in the rat, 10 days after birth, overall hepatic excretory function remains depressed (Klaassen, 1972). Therefore, if the adult-like canalicular structure reflects secretory function of the liver of the 10 day old rat, the maturation of hepatic excretory function following postnatal day 10 must be due to the development of uptake and/or conjugation capacity.

Since uptake, metabolism, and biliary excretion of drugs may all be depressed in the newborn relative to the adult, it is difficult to ascertain which deficiency in the newborn is most important in the immaturity of overall transport function. The solution to this problem may also be dependent on the animal species and the transported compound. Depressed hepatic transport of both BSP and conjugated BSP (BSP-GSH) is apparent in rat neonates relative to adults (Klaassen, 1973b). Newborn guinea pigs, however, are deficient only in the transport of free BSP and are adult-like in their ability to excrete BSP-GSH (Whelan et al., 1970a). Therefore, even though uptake capacity for bilirubin and ICG is low in newborn guinea pigs (Gartner and Arias, 1969; Hwang, 1975), depressed BSP uptake may not be as important for functional insufficiency as the inability of the newborn to conjugate BSP. In the newborn rat, however, hepatic uptake may represent the primary deficient step.

Hepatic Excretion of Xenobiotics

The liver occupies an anatomical site that facilitates the elimination of drugs and other environmental chemicals. Blood is directly received from the intestinal tract via the portal vein. Thus, orally administered compounds must first pass through the liver before reaching the systemic circulation.

Perhaps the first to point out a possible role of bile in the elimination of foreign compounds was M.J.B. Orfila who, in his work Traite de toxicologie generale (1813-1815), pointed to the fact that many metallic poisons are taken up by the liver and are either retained there or are excreted into the bile. Subsequently, Claude Bernard found that copper sulfate, potassium iodide, and turpentine spirits, when injected into the blood, rapidly pass into the bile (Smith, 1973). The role of bile, however, as a route for chemical elimination has not, until relatively recently, received much attention. In the past 25 years it has become increasingly apparent that the biliary tract is a major route for excretion of numerous drugs and other foreign compounds.

The extent of biliary excretion of a compound is influenced by two physico-chemical factors: 1) molecular weight, and 2) polarity (Smith, 1973). Evidence in support of the view that molecular weight has an important bearing on biliary excretion comes from two sources. First, from a consideration of the molecular weights of both endogenous and exogenous compounds which are excreted into bile, and secondly, from systematic studies on the relationship between molecular weight and the extent of biliary excretion of various groups of chemicals. Brauer (1959) suggested that substances which are highly concentrated in bile are usually organic carboxylic acids with molecular weights of 300 or greater. Sperber (1963) made a similar generalization stating that "the

majority of the compounds efficiently secreted by the renal tubules have a relatively low molecular weight (200-400); whereas, the substances excreted into the bile usually have larger molecules (molecular weight above 400)". Based on correlations between molecular weight and the extent of biliary excretion, a threshold molecular weight was determined for organic anion excretion. Compounds with molecular weights below this limit were only minimally (less than 10% of dose) excreted into bile. The molecular weight threshold varies with species and ranges from about 325 in rats to approximately 500 in man (Millburn et al., 1967; Millburn, 1970, 1976; Hirom et al., 1972).

The presence of a strongly polar group on a molecule also appears to be a requirement for extensive biliary excretion to occur. Smith (1973) has suggested that the presence of a potentially ionizable moiety, such as a carboxylic acid or quaternary ammonium group, augments biliary excretion. Such groups allow a molecule to exist at physiological pH as water-soluble anions or cations. Occasionally, as in the case of the cardiac glycosides which may be highly excreted in bile, there is no charged anionic or cationic center, but this may be compensated for by the presence of one or more water-soluble sugar residues in the molecule (Smith, 1973).

A number of compounds are eliminated from plasma and excreted into bile in an unchanged form. These include ouabain (Cox and Wright, 1959), indocyanine green (Wheeler et al., 1958) and some azo dyes (Ryans and Wright, 1961). However, many drugs that are excreted into bile are eliminated in the form of metabolites. With respect to biliary excretion, the most important metabolic reactions involve conjugation to certain endogenous substrates. In the liver there have been identified

eight conjugation mechanisms which utilize glucuronic acid, sulphate, glycine, glutamine, glutathione, methyl, acetyl, or thio groups as conjugating substrates. The most common of these conjugation reactions involve glucuronic acid and glutathione. In essence, conjugation to these substrates may augment biliary excretion for two reasons: 1) they introduce a polar center into the molecule, and 2) they increase the molecular weight of the compound (Smith, 1973).

Based on selective competition studies, it has been suggested that the liver has at least three transport systems for the excretion of organic compounds into the bile (Kupferberg and Schanker, 1968). These include an organic anion transporting system, for compounds such as indocyanine green, bilirubin, sulfobromophthalein and glucuronide conjugated compounds; an organic cation system, for which procaine amide ethobromide has become the prototype; and a third transport system for neutral compounds including cardiac glycosides, such as ouabain. An additional anionic transporting system may exist for bile acids (Alpert et al., 1969; Paumgartner and Reichen, 1975,1976).

In 1909 Abel and Rowntree published an important paper showing that a number of phthalein dyes undergo extensive biliary excretion. This observation was of considerable significance since it laid the basis for the development of diagnostic agents for the hepatobiliary system. Graham et al. (1925) conceived the idea of using the substance tetraiodophenolphthalein, which is extensively excreted in bile and opaque to x-rays, for the x-ray visualization of the gall bladder. Equally significant, however, was the introduction by Rosenthal and White (1925) of sulfobromophthalein (BSP) for a simple test of liver excretory function. As used clinically, the hepatic transport of BSP is evaluated by

determination of the rate of disappearance of the dye from plasma. Retention of BSP in plasma has been a good indication of various forms of adult and newborn hepatic disease (Leevy et al., 1963). In the time since the introduction of BSP for clinical evaluation of liver function, parallel studies have been undertaken to determine mechanisms of hepatic disposition and biliary excretion of BSP and similar prototype compounds.

The movement of drugs from blood to bile may be described in terms of four steps: 1) hepatic uptake, 2) intrahepatic storage, 3) conjugation, and 4) biliary excretion. The uptake of drugs into liver represents the first transport step for biliary excretion. Krebs and Brauer (1949) demonstrated by means of autoradiography that BSP uptake appeared to be a function of hepatic parenchymal cells and not Kupffer cells. This observation has been verified experimentally with cell preparations in which isolated parenchymal but not Kupffer cells accumulated BSP from an incubation medium (Stege et al., 1975).

Although little is known about the exact mechanisms by which drugs are accumulated into hepatic parenchymal cells, the process is very rapid. Compounds that are excreted into the bile show a marked tendency to initially accumulate in liver. Within 5 minutes after an intravenous injection of bilirubin in the rat, over 50% of the injected dose was in the liver (Brown et al., 1964). For this reason it has been assumed that the initial rate of elimination of drugs from plasma represents hepatic uptake (Paumgartner et al., 1970; Scharschmidt et al., 1975). With this assumption, Paumgartner and co-workers (1970,1976) and others (Scharschmidt et al., 1975) have characterized hepatic uptake of organic anions by single injection multiple dose techniques. From the plasma half-time of the initial exponential line a rate constant, K

(functional clearance), was determined such that $K = .693/t_{\frac{1}{2}}$. By examining the removal rate (V; V=K times D; where D = the injected dose) as a function of dose (D) the investigators could express uptake in terms of Michaelis-Menten constants (K_m and V_{max}) which demonstrates saturability. Moreover, hepatic uptake of bilirubin, indocyanine green, and sulfobromophthalein showed selective mutually competitive inhibition, suggesting these anions are accumulated into liver by the same mechanism (Scharschmidt et al., 1975). These data are therefore compatible with the existence of a carrier mediated transport process for uptake of organic anions. Uptake of bilirubin was not altered in the presence of the bile acid taurocholate (Paumgartner and Reichen, 1976), and it was therefore suggested that bile acids are accumulated in liver by a separate mechanism.

Scharschmidt et al. (1975) discussed the limitations of the single injection multiple dose technique and cautioned that several variables may complicate interpretation of values for K_m and V_{max}. Particularly, the rate of hepatic blood flow may be rate-limiting with low doses of the drugs and thus K_m values (but not V_{max}) would vary in accordance with hepatic perfusion. It is noteworthy that reduction in hepatic blood flow during hypothermia resulted in diminished uptake of BSP in the anesthetized dog (Brokaw and Penrod, 1949). In addition, enhanced hepatic blood flow following treatment with phenobarbital has been interpreted by some (Neis et al., 1976; Branch et al., 1974) to be a major mechanism for enhanced uptake of compounds following phenobarbital pretreatment. Thus, under non-saturating conditions (below V_{max}), hepatic uptake may be dependent on the intrinsic capacity of the liver

to remove compounds from blood, and also on the load presented to the liver (plasma flow times plasma concentration; Keiding, 1976).

The hepatic slice technique has also been utilized to characterize the uptake of compounds into hepatic parenchyma. Using this method, Kupferberg and Schanker (1968) concluded that the glycoside, ouabain, is taken up by the liver by an active process and that uptake is independent of the process which transports organic anions and cations. These investigators noted that accumulation of ouabain into rat liver slices was saturable and that the extent of ouabain accumulation into slices was depressed under nitrogen atmosphere or in media containing metabolic inhibitors or other cardiac glycosides. Ouabain accumulation into slices was not inhibited by p-acetylaminohippuric acid (anion) or the cation procaine amide ethobromide (Kupferberg and Schanker, 1968). However, inhibition of ouabain uptake was observed following addition of several naturally occurring and synthetic steroids and it was suggested that the cyclopentanophenanthrene steroid nucleus of these compounds may be important in transport specificity (Kupferberg, 1969). In similar experiments, Hwang and Schanker (1973) observed saturable uptake of the cation n-acetyl procaine amide ethobromide into rat liver slices which could be inhibited with a series of metabolic inhibitors or by other cations. However, accumulation of BSP into rat liver slices could not be depressed by metabolic inhibitors (Brauer and Pessotti, 1949). Thus, in contrast to transport of organic cations and cardiac glycosides, organic anions may not accumulate into the liver by an active transport system.

An alternative mechanism for uptake of anions was suggested by Arias and co-workers (Levi et al., 1969b). The authors described an

important role of the cytoplasmic anion binding protein, ligandin, for hepatic uptake of anions. The purported mechanism was that ligandin influences net uptake of organic anions into liver specifically by binding to, and thus regulating anion efflux from the cell into plasma (Arias et al., 1976). Thus, ligandin was proposed to act as intracellular receptor for free ions which had crossed the sinusoidal membrane. Several lines of evidence support the contention that ligandin may be important for hepatic uptake of organic anions. In vitro competition for binding to ligandin among many organic anions correlates with in vivo competition for hepatic uptake (Levi et al., 1969b). In newborn guinea pig, rat, monkey, and man and in teleosts and elasmobranchs, absence of ligandin in liver supernatant correlates well with impaired hepatic uptake of BSP and bilirubin (Arias, 1970; Levi et al., 1970; Levine et al., 1971). Stimulation of hepatic uptake of anions following administration of phenobarbital is associated with increased hepatic content of ligandin (Reyes et al., 1971).

A close relationship between ligandin and glutathione (GSH)-S-transferase activity in liver cytosol has been proposed on the basis of finding an identical elution volume in gel filtration for both GSH-S-transferase activity and BSP binding (Kaplowitz et al., 1973). The GSH-S-transferases are a major group of soluble liver proteins and six transferases have been separated from rat liver and are designated E, D, C, B, A and AA in order of their elution from carboxymethyl cellulose columns (Habig et al., 1974,1976). The GSH-S-transferases are known to have specific enzymatic functions in catalyzing glutathione conjugation with certain xenobiotics including BSP (Habig et al., 1974; Kaplowitz et al., 1975; Smith et al., 1977). GSH-S-transferase B has been shown to

be immunologically identical to ligandin (Habig et al., 1974). Thus, the GSH-S-transferase system may have a dual purpose for hepatic drug excretion; by both mediating organic anion uptake and providing a catalytic site for glutathione conjugation (Kaplowtiz et al., 1975).

The importance of transferase-mediated glutathione conjugation for excretion of drugs into bile has been studied almost exclusively for hepatic elimination of BSP. Glutathione conjugation to BSP was first suggested in 1959 when Combes reported the existence of metabolites of BSP in rat bile. Before this time it was generally believed that biliary excretion of BSP depended only upon hepatic uptake into liver cells and transport from liver into bile. In rat bile, about 70-85 percent of BSP appears in conjugated form (Combes, 1959). By comparing the biliary excretion rate of BSP and conjugated BSP (BSP-GSH), it has become apparent that BSP-GSH is excreted from liver into bile more rapidly than is BSP (Whelan et al., 1970b). This observation has been subjected to a number of interpretations as to the relative importance of intrahepatic conjugation for BSP excretion. Whelan et al. (1970b) have suggested that the conjugation of BSP facilitates dye transport into bile and moreover, in vivo may be the rate-limiting step in overall transport of injected free BSP. Klaassen and Plaa (1967), however, compared the maximal biliary excretion rate (T_m) for BSP in rat, rabbit and dog, and determined that for each of these species, the theoretical in vitro conjugation capacity greatly exceeded the observed in vivo excretory rate. Thus, biliary excretion was suggested to be rate-limiting (Klaassen and Plaa, 1967). By plotting hepatic concentration of BSP and BSP-GSH against drug excretion rate, Varga et al. (1974) determined that BSP-GSH has a 10-13 fold greater affinity for the

biliary transport system than does BSP. These studies suggest that conjugation of BSP to glutathione, although perhaps not rate-limiting, converts BSP to a compound that may be more readily excreted into bile. Although it is not clear whether conjugation is rate-limiting in BSP elimination into bile, the rate of hepatic uptake of BSP-GSH is slower than that for unconjugated BSP (Krebs, 1959; Melter et al., 1959; Whelan et al., 1970a,b). Since BSP-GSH is excreted into bile more rapidly than is unconjugated BSP, it is likely that hepatic uptake is not rate limiting in overall excretion.

The time lag between hepatic uptake and biliary excretion of BSP suggested that the dye was stored within the hepatocyte prior to excretion (Wirts and Cantarow, 1942). Following the intravenous administration of BSP to dogs it was shown by Wirts and Cantarow (1942) that the output of BSP in bile continued for over three hours after its virtual disappearance from plasma, indicating that BSP is first rapidly taken up by the liver and then gradually excreted into bile. Wheeler et al. (1960) quantified this phenomenon as the difference between the amount of BSP removed from plasma and the amount appearing in the bile and was termed "the relative storage capacity". The existence of hepatic storage of BSP may be most easily interpreted as a manifestation of the difference between hepatic uptake and biliary excretion of the dye. Thus, rate of uptake of BSP from plasma may be very rapid and storage of the dye within the liver would reflect a slower rate of biliary excretion.

The final step for drug elimination into bile is transport from the liver cell into the bile canaliculus. It is generally assumed that

substances excreted into bile are actively transported (Schanker, 1968). The evidence for this is indirect and not entirely rigorous. The principle findings suggestive of active secretion are concentration, saturation, and competition. Drugs may be concentrated into bile several hundred, and as much as one thousand, fold above plama levels (Brauer, 1959). Saturation of biliary excretory capacity has been shown for several compounds (Schanker and Solomon, 1963; Wheeler, 1969). The term used to designate the maximum velocity of biliary secretion is the transport maximum (T_m). Finally, specific competitive inhibitors may depress biliary excretion (Wheeler *et al.*, 1960; Schanker and Solomon, 1963; Wheeler, 1969).

Since hepatic uptake mechanisms are also concentrative, saturable, and specific, it may be suggested that carrier mediated drug transport from liver into bile is a manifestation of carrier mediated drug accumulation into liver. However, concentration of drugs in bile may exceed concentration in liver (Kupferberg and Schanker, 1968; Wheeler, 1969; Russell and Klaassen, 1972). In addition, when maximal uptake velocities are compared to the steady state excretory transport maximum (T_m), uptake capacity (V_{max}) exceeds excretory T_m by a factor as high as 60-fold (Goresky, 1964; Paumgartner, 1974; Paumgartner, 1975; Scharschmidt *et al.*, 1975; Paumgartner and Reichen, 1976). Thus, the excretory mechanism may be saturated before saturation of hepatic uptake which suggests carrier mediated uptake and secretion. These data may also demonstrate that uptake is not rate-limiting in overall drug transport from blood into bile.

The Importance of Bile Flow in Hepatic Drug Excretion

The formation of primary bile occurs at bile canaliculi, minute (1 μm in diameter) channels located between 2, or sometimes 3 hepatocytes. The canaliculi are closed at one end and are connected at the other end to bile ductules which in turn are connected to bile ducts (Popper and Schaffner, 1957; Steiner and Corruthers, 1961). Sperber (1959) suggested that secretion of bile acids into biliary canaliculi provides an osmotic driving force for water and electrolytes and thereby initiates bile flow. This view is supported by the fact that the choleretic potency of bile acids is roughly proportional to their osmotic activity, and that other osmotically active compounds demonstrate a choleretic effect (Preisig et al., 1962). There is increasing evidence, however, that excretion of bile acids may not be the only factor responsible for the output of canalicular bile. In the isolated perfused rat liver, bile flow persists when bile acid excretion is minimal or absent (Boyer, 1971; Boyer and Klatskin, 1970). After interruption of the enterohepatic circulation and depletion of the bile acid pool in the rat, bile flow decreased less than did bile acid excretion (Klaassen, 1971a). In studies of the correlation between bile flow and bile acid excretion, a positive intercept appears when bile acid excretion is extrapolated to zero (Erlinger et al., 1970; Boyer and Klatskin, 1970). Thus, a bile acid independent fraction of canalicular bile flow was postulated.

A number of studies have suggested that active sodium transport, possibly mediated by a canalicular membrane Na-K-ATPase, into bile may be the mechanism for the bile-acid independent fraction of canalicular bile flow (Erlinger et al., 1970; Boyer and Klatskin, 1970; Boyer,

1971; Boyer et al., 1976; Layden and Boyer, 1976). The evidence in favor of this hypothesis is, for the most part, indirect, since primary bile (canalicular) cannot, at present, be collected. Nonetheless, Boyer and co-workers (1970,1976), demonstrated a positive correlation between the activity of plasma membrane Na-K-ATPase and canalicular bile acid independent bile flow. Included in these investigations was demonstration of bile flow inhibition in the isolated perfused liver preparation following ouabain induced inhibition of Na-K-ATPase (Boyer, 1971; Boyer et al., 1976). However, low concentrations of ouabain may increase bile flow in the isolated perfused liver preparation (Graf et al., 1973; Graf and Peterlik, 1976). Graf et al. (1973) have suggested that the Na-K-ATPase-dependent mechanism regulating bile flow might be located in the sinusoidal side of the liver cell. Bile flow, thus, would be regulated by the effects of the sodium pump on intracellular Na.

Bile salt dependent and independent components of canalicular bile flow are not necessarily mutually exclusive. For example, bile salts and certain nonionic detergents activate Na-K-ATPase in vitro (Emmelot et al., 1966). It has been suggested that bile acids secreted across the canalicular membrane could cooperatively stimulate the secretion of Na^+ by a Na-K-ATPase pump (Plaa and Priestly, 1977).

Following formation of "primary" bile at the canaliculus, secretory and reabsorptive mechanisms may result in modifications of bile during passage through the biliary tract. The choleresis produced by secretin is independent of bile acid secretion (Preisig et al., 1962) and of total canalicular bile formation (Wheeler et al., 1968).

Secretin-induced choleresis involves the net addition to bile of a solution rich in bicarbonate and chloride, and the site for this inorganic ion secretion appears to be the lower part of the biliary tree (Wheeler and Mancusi-Ungaro, 1966).

Early studies of O'Maille et al. (1966) and of Ritt and Combes (1967) demonstrated that BSP transport maximum (T_m) could be enhanced significantly in dogs by infusion of sodium taurocholate and dehydrocholate. The effect of these bile salts (acids) on BSP excretion was associated with the increase in bile flow that results from bile acid infusion. Thus, the increase in BSP excretion was attributed to the increase in canalicular bile flow, which, by diluting BSP in canalicular bile, permitted the excretion of additional dye without exceeding a putative concentration maximum. Increased biliary excretion of pentobarbital metabolites (Knodell and Hollowing, 1976), digitoxin (Greenberger and Thomas, 1973), propylthiouracil (Papapetrores et al., 1972), and cholecystography contrast agents (Dunn and Beck, 1972) has also been observed following infusion of bile acids.

These effects are similar to the correlation between bile flow and BSP elimination that results from treatment with microsomal enzyme stimulators. Microsomal enzyme stimulators such as phenobarbital, pregnenolone-16α-carbonitrile and spironolactone increase bile flow and drug excretion into bile (Klaassen, 1974a; Zsigmond and Solymoss, 1972); whereas other microsomal enzyme stimulators, such as 3-methylcholanthrene and 3,4-benzypyrene, which do not increase bile flow also do not enhance BSP excretion (Klaassen, 1970).

The increase in bile flow following phenobarbital was not due to an increase in bile salt excretion and thus was attributed to enhanced

formation of the bile acid-independent fraction of canalicular bile production (Berthelot et al., 1970; Paumgartner et al., 1971). Since enhanced drug excretion associated with bile salt infusion would increase bile flow by increasing the bile salt-dependent fraction of canalicular bile, it appears that bile flow per se might be a determining factor in biliary excretion of drugs. In support of this contention is the relationship between body temperature, bile flow, and drug excretion. During anesthesia induced alteration of rectal body temperature, a linear relationship was found to exist between body temperature and the rate of excretion of BSP and bilirubin (Roberts et al., 1967). The maximum biliary excretion rate (T_m) of bilirubin and BSP was 1.5-2.0 times greater in rats whose body temperatures were 39°C compared to rats with a 31°C body temperature. Inasmuch as bile flow rates diminished with decreasing body temperature, hypothermia-induced alteration in drug transport was considered to be secondary to altered bile flow (Roberts et al., 1967). Therefore, these results would be consistent with the hypothesis that bile flow per se regulates biliary excretory capacity.

This hypothesis was questioned recently when canalicular bile flow, increased acutely with drugs such as theophylline, 4-methylumbelliferone and β-(2,4-dimethoxy-5-cyclohexylbenzoyl)propionic acid (SC-2644), was not associated with enhanced biliary excretion of BSP in the dog (Erlinger and Dumont, 1973; Barnhart et al., 1973; Forker and Gibson, 1973; Gibson and Forker, 1974; Barnhart and Combes, 1974). It has been suggested that bile flux proper (i.e., water flow) might not be the determining factor in excretion of compounds across the

canalicular membrane. As an alternative, the suggestion was made that bile acids exert specific, as yet undefined, effects on the BSP excretory mechanism, and that the increase in BSP excretion during bile acid infusion is not the result of the increase in canalicular bile production (Forker and Gibson, 1973; Gibson and Forker, 1974). However, this mechanism would not explain the effects of phenobarbital on BSP excretion since phenobarbital does not enhance bile-salt dependent bile flow (Klaassen, 1971b). The relationship between hepatic drug excretion and bile flow may be more complicated than was previously considered.

The importance of bile flow in the elimination of compounds from blood is most easily recognized with bile duct ligation. Acute extrahepatic bile duct ligation is a commonly employed procedure used to ascertain the relative importance of the biliary route for the elimination of xenobiotics from the body. Moreover, bile duct ligation resulted in enhanced susceptibility to drug induced lethality for particular compounds that are normally preferentially excreted by the liver (Gibson and Becker, 1967; Klaassen, 1973a). Studies undertaken to exclusively quantify drug-induced or age related differences in the uptake of drugs into liver employ the bile duct ligation procedure in order to minimize biliary excretion (Whelan et al., 1970a; Reyes et al., 1971). Thus, it has been assumed that bile duct ligation would specifically diminish bile flow and drug transport from liver into bile. However, bile duct ligation has recently been demonstrated to influence hepatic uptake of drugs in rats (Yam et al., 1977). Extrahepatic cholestasis produced by acute bile duct ligation decreased net hepatic uptake of BSP, BSP-GSH, phenol-3,6-dibromophthalein, and

ouabain (Yam et al., 1977). The effect of bile duct ligation on hepatic uptake of BSP was apparent when determined as early as 2 hours following bile duct ligation. Alteration of uptake capacity following bile duct ligation was returned to normal function after recannulation of the bile duct and subsequent release of bile, suggesting the effect was readily reversible. These results suggest that the mechanism for impaired uptake was not liver damage but rather competition between endogenous bile constituents and transported test drug (Yam et al., 1977).

Examples of Agents that Alter Hepatic Excretory Function

Microsomal Enzyme Stimulators

The liver can respond to increases in functional demand by changes in size, involving cellular hypertrophy and hyperplasia, and by quantitative and qualitative changes in cell organelles (Feinman et al., 1972). Perhaps the type of response that has received the most attention recently is stimulation of the activity of xenobiotic metabolizing enzymes following exposure to many drugs and environmental chemicals (Conney, 1967; Conney et al., 1967; Atio, 1973; Parke, 1975). The stimulation produced by these chemicals varies in accordance with the particular stimulating agent. Gillette et al. (1972) suggested that stimulators of microsomal drug metabolism be classified according to their effect on various components of the system. Phenobarbital and 3-methylcholanthrene represent two distinct types of inducing agent and these compounds have been utilized as prototypes for characterizing alterations in microsomal drug metabolism. Phenobarbital-like stimulating agents increase cytochrome P450, NADPH-cytochrome c-reductase concentration, and a wide range of microsomal

enzymes; whereas 3-methylcholanthrene-like agents increase cytochrome P_1450 and a more specific group of enzymes but not the reductase.

Since the excretion of many compounds into bile is dependent upon intrahepatic metabolism, exposure to hepatic enzyme stimulators often results in enhanced drug excretion into bile. The increase in drug elimination may therefore be a function of the enhanced rate of metabolism. However, many hepatic microsomal enzyme stimulators increase biliary excretion of compounds that do not require biotransformation prior to hepatic excretion. Thus, induction of hepatic excretory capacity may not be merely a reflection of increased biotransformation (Klaassen, 1970).

Phenobarbital accelerates the elimination of a variety of compounds from plasma into bile (Klaassen, 1970). Enhanced biliary excretion is not dependent on increased biotransformation as it occurs with indocyanine green, phenol-3,6-dibromphthalein disulfonate, amaranth and ouabain, compounds which are not metabolized before biliary excretion (Klaassen, 1970).

One of the mechanisms by which phenobarbital may enhance excretory function is to stimulate the uptake step for the transfer of compounds from plasma into liver. Acceleration of uptake of BSP from plasma into hepatic storage has been demonstrated following phenobarbital in the rat (Reyes et al., 1971), mouse (Fujimoto et al., 1965), and man (Capron et al., 1975). The reports that an increase in hepatic content of the organic binding protein, ligandin, also occurred following phenobarbital treatment suggested that increased ligandin content in liver might be the mechanism for stimulation of hepatic uptake of BSP (Reyes et al., 1971). In support of this contention was the observation

that microsomal enzyme stimulators, 3-methylcholanthrene and 3,4-benzpyrene, increase both ligandin content and BSP uptake in rats (Reyes et al., 1971). Klaassen (1975), however, suggested that increases in ligandin content might not be the only mechanism for stimulation of hepatic uptake. Increased hepatic uptake of the neutral compound, ouabain, was observed in young rats following treatment with phenobarbital, spironolactone, and pregnenolone-16α-carbonitrile, however, ligandin does not bind to ouabain and thus stimulation of ouabain uptake could not be attributed to increased ligandin (Klaassen, 1975). Moreover, no direct relationship existed between the ability of these inducers to increase ligandin content and BSP excretion even though BSP avidly binds to ligandin (Klaassen, 1975). Phenobarbital increases liver blood flow in the rat (Ohnhaus et al., 1971; Ohnhaus and Locher, 1975; Neis et al., 1976) and rhesus monkey (Branch et al., 1976) and it has been suggested that the increase in blood flow alone may contribute significantly to stimulation of uptake capacity (Branch et al., 1974). The increase in liver blood flow may be attributed to the large increase in liver mass produced by treatment with phenobarbital (Branch et al., 1974; Neis et. al., 1976). Therefore, the mechanism(s) for the stimulation of uptake following many microsomal enzyme stimulators is not known but may be attributed in part to changes in hepatic blood flow or, for some compounds, increases in ligandin content (Klaassen, 1975).

Another mechanism by which microsomal enzyme stimulators may enhance hepatic drug transport is to increase the rate of canalicular bile flow. Phenobarbital, when administered for 3-15 days, increases bile flow in the rat (Roberts and Plaa, 1967; Klaassen and Plaa, 1968a;

Hart et al., 1969) and rhesus monkey (Redinger and Small, 1973). Since treatment with phenobarbital does not result in increased bile salt excretion (Berthelot et al., 1970; Klaassen, 1971b; Paumgartner et al., 1971), the increase in canalicular bile flow may be attributed to stimulation of the canalicular bile salt-independent fraction of bile flow. Several lines of evidence suggest that the effect of microsomal enzyme stimulators on bile flow is not directly related to their influence on the hepatic drug metabolizing system. Among several compounds that stimulate hepatic mixed function oxidase activity and increase liver mass, only phenobarbital significantly increased bile flow (Klaassen, 1969). In the hamster, phenobarbital increased liver weight, hepatic cytochrome P450 content, and produced a proliferation of the smooth endoplasmic reticulum, but did not alter bile flow (Capron et al., 1974). In the rat, pentobarbital increased bile flow but did not increase liver weight or cytochrome P450 (Capron, 1974).

Although no relationship exists between microsomal enzyme stimulation and bile flow, a direct correlation can be made between the ability of enzyme inducers to increase bile flow and enhance drug elimination into bile (Klaassen, 1970,1975; Zsigmond and Solymoss, 1972). Treatment with 3-methylcholanthrene and 3,4-benzpyrene does not result in increased bile flow or enhanced excretion of BSP into bile (Klaassen, 1969,1970). However, phenobarbital, spironolactone, and pregnenolone-16α-carbonitrile increase bile flow and BSP excretion, and, moreover, a positive correlation exists between the ability of these agents to increase drug excretion and bile flow (Zsigmond and Solymoss, 1972; Klaassen, 1969,1970,1974a). Pregnenolone-16α-carbonitrile was most effective in increasing both bile flow and

excretion of ouabain and BSP into bile (Zsigmond and Solymoss, 1972; Klaassen, 1974a).

It is noteworthy that some compounds, such as 3-methylcholanthrene and 3,4-benzpyrene, enhance hepatic uptake of BSP but do not increase bile flow (Klaassen, 1969; Reyes et al., 1971). Therefore, phenobarbital may enhance all components of the hepatic excretory system (hepatic uptake, metabolism, and biliary excretion), but the transport steps may be mutually exclusive and selective stimulation is possible. However, hepatic uptake is not rate-limiting in biliary excretion of drugs and thus selective stimulation in uptake capacity may not be important for enhanced overall excretion following hepatic stimulation. Thus, even though 3-methylcholanthrene and 3,4-benzpyrene enhance hepatic uptake of BSP (Reyes et al., 1971), overall hepatic excretion remains unchanged (Klaassen, 1970).

Carbon Tetrachloride

Carbon tetrachloride (CCl_4) is an agent widely used to produce experimental liver damage in laboratory animals (Recknagel, 1967). The hepatic damage produced by this compound is not specific and all elements of the hepatocyte are disrupted following CCl_4, including the endoplasmic reticulum, mitochondria, lysosomes, and plasma membranes. The toxic lesions are thought to be mediated by a metabolite of CCl_4 and not CCl_4 itself (Slater, 1966). This has been suggested since 1) newborn animals possessing low capacity for drug metabolism are relatively resistant to CCl_4 toxicity (Dawkins, 1963) and 2) hepatotoxicity following CCl_4 may be enhanced following microsomal enzyme stimulation by pretreatment with phenobarbital and DDT (McLean and McLean, 1966). In 1966, Recknagel, Ghoshal and Slater proposed that homolytic cleavage

of the carbon-chloride bound of CCl_4 resulted in production of free radicals that interact with and disrupt membrane lipids. It was therefore suggested that the mechanism for CCl_4 toxicity was lipid peroxidation (Slater, 1966; Recknagel and Ghoshal, 1966). In support of this hypothesis were the earlier observations of the protective effect of Vitamin E, diphenyl-p-phenylenediamine (DPPD), and selenium on CCl_4 toxicity (Hove, 1948; Gallagher, 1962; DiLuzio and Costales, 1965). These substances act as lipid antioxidants and thus the protection afforded by these compounds against CCl_4 toxicity suggested destructive lipid peroxidation was involved in the toxic response. Further support of this hypothesis may be apparent in the functional changes resulting from exposure to CCl_4. CCl_4 affects cellular membranes containing lipid-rich material and thereby causes alterations in cellular and subcellular structure and function.

Some of the subcellular and biochemical effects of CCl_4 on the hepatic parenchyma cell hint at a possible influence on drug transport. A single dose of CCl_4 results in altered permeability of membranes of the endoplasmic reticulum followed by an increased permeability of the mitochondrial and cellular membranes (Rouiller, 1964; Villela, 1964; Zimmerman, 1968). CCl_4-induced uncoupling of oxidative phosphorylation that occurs in mitochondria of the liver (Dianzani, 1954; Dianzoni and Bahr, 1954) results in a depletion of cellular ATP levels (Dianzani, 1976). Depletion in energy supply may result in a decrease in available energy for sustaining active drug transport (Reuning and Schanker, 1971). Another mechanism by which CCl_4 might influence hepatic transport may be through the decrease in hepatic blood flow that has been reported following CCl_4 treatment (Rice et al., 1967).

Hepatic excretory function is depressed following CCl_4 poisoning as indicated by retention in plasma of drugs administered secondarily. The effect of CCl_4 on hepatic drug transport, however, is not specific. Altered transport of both anions (Brauer and Pessoti, 1949; Plaa and Hine, 1960; Klaassen and Plaa, 1968b; Paumgartner et al., 1970) and neutral compounds (Reuning and Schanker, 1971) by the liver has been demonstrated following CCl_4 although these compounds are transported by separate mechanisms (Schanker, 1968). Liver slices and isolated perfused livers from CCl_4treated adult rats showed no differences in BSP uptake but biliary excretion of BSP was decreased following CCl_4 (Brauer and Pessotti, 1949). Retention of BSP in plasma following CCl_4 was primarily due to decreased biliary excretion associated with decreased bile flow (Klaassen and Plaa, 1968b). Ouabain (neutral compound) retention in plasma was accompanied by accumulation of the drug in the liver (Reuning and Schanker, 1971). These results may be consistent in that CCl_4 appeared to have a specific effect on the transport of drugs from liver to bile and did not appear to alter the uptake of drugs from plasma into liver. However, CCl_4 treatment may result in a reduction in BSP uptake in isolated perfused rat livers when measured soon after poisoning (Plaa and Hine, 1960). Furthermore, a decrease in the maximal removal rate of indocyanine green (ICG) from plasma has been observed following CCl_4 where hepatic uptake was exclusively measured (Paumgartner et al., 1970). CCl_4 has also been demonstrated to reduce conjugation of BSP to glutathione in vitro (Klaassen and Plaa, 1968b). Thus, treatment with CCl_4 may result in alteration of all steps in hepatic drug transport. Since CCl_4 poisoning results in hepatic cell death (Recknagel, 1967), it may

be expected that the effect of CCl_4 on drug transport would be non-specific.

Polybrominated Biphenyls

Polybrominated biphenyls (PBBs) are used commercially as flame retardants. The adverse consequences of exposure to PBBs are not completely known. However, a similar class of compounds, the polychlorinated biphenyls (PCBs), has been extensively studied and PBBs may share many of the biological and toxic properties of PCBs. PCBs are known to cause chloracne (Meigs et al., 1954) and Yusho disease in humans (Karatsune et al., 1972). Rodents exposed to PCBs exhibit decreased reproductive function (Kihlstrom et al., 1975), hepatic and renal histopathological changes (Bruckner et al., 1973), hepatic porphyria, and stimulation of hepatic microsomal enzymes (Vainio, 1974; Goldstein et al., 1975). Because PCBs accumulate and persist in the environment (Risebrough et al., 1968; Koeman et al., 1969) and are distributed on a world wide scale (Risebrough et al., 1968; Hutzinger et al., 1974; Report, 1976), they are considered to be an environmental hazard.

The production, distribution, and usage of PBBs has not been as widespread as PCBs (Report, 1976) and perhaps for this reason the toxicity of PBBs has not been extensively studied. However, recent interest in the toxicity of PBBs was generated following recognition of accidental contamination of a commercial animal feed supplement with PBBs. In 1973, 500-1000 pounds of the flame retardant Firemaster BP-6 was accidentally mixed into feed that was widely sold and distributed to Michigan farms (Carter, 1976). Firemaster BP-6 is a mixture of PBBs containing approximately 70% hexabrominated biphenyl (Jacobs

et al., 1976; Rickert et al., 1977). Signs of toxicity in cattle receiving feed containing PBBs (at levels as high as 3000 ppm) included anorexia, decreased milk production, abnormal hoof growth, decreased growth rate in young animals, and aborted and malformed calves (Report, 1976). The contamination of PBBs to Michigan farm animals eventually lead to the destruction of 30,000 cattle, over 1,000 sheep and pigs, and about 1.5 million chickens (Carter, 1976). It has been estimated that between the onset of contamination in the fall of 1973 and the establishment of quarantine of contaminated livestock in the spring of 1974, over 10,000 Michigan residents were exposed to PBBs through consumption of contaminated milk and meat (Report, 1976). Human exposure has not been associated with any acute ill effects (Report, 1976; Kay, 1977).

An important feature of the pharmacodynamics of halogenated biphenyls is the biological persistance of these compounds (Hutzinger et al., 1972). This may be especially true for biphenyls having six or more halogen moieties. Cumulative seven day excretion of hexachlorinated biphenyl was less than 20% of the injected dose (Matthews and Anderson, 1975). Studies with decabromobiphenyl in rats and Firemaster BP-6 (containing mostly hexabrominated biphenyl) in farm animals suggest that brominated biphenyls are also slowly eliminated from the body (Fries and Marrow, 1975; Lee et al., 1975; Gutenmann and Lisk, 1975; Willett and Irving, 1976). Because of their high solubility in fat and low solubility in water, polychlorinated biphenyls (Matthews and Anderson, 1975) and polybrominated biphenyls (Fries and Marrow, 1975; Lee et al., 1975; Rickert et al., 1977) may accumulate and be stored in fat. In particular, halogenated biphenyls accumulate in

mammary tissue and are present in milk fat (Fries and Marrow, 1975; Willett and Irving, 1975; Takagi et al., 1976; Rickert et al., 1977). Human exposure to PBBs has been associated with detectable levels of PBBs in human breast milk (Report, 1976).

In laboratory animals, one of the most prominent effects of both PBBs and PCBs is induction of a large increase in liver weight and, in addition, stimulation of hepatic drug metabolizing capabilities (Johnstone et al., 1975; Goldstein et al., 1975; Dent et al., 1976a,b). PBBs are several times more potent than PCBs in stimulating microsomal enzyme activity (Farber and Baker, 1974). PBBs and PCBs represent a class of hepatic mixed function oxidase stimulators which exhibit characteristics of both phenobarbital and 3-methylcholanthrene (Alvarez et al., 1973; Stonard, 1975; Dent et al., 1976a), two agents which are distinct in their stimulating properties (Sladek and Mannering, 1969a,b). Following a single intraperitoneal injection of PBBs to adult rats, the pattern of hepatic microsomal enzyme stimulation changes from phenobarbital-like initially to 3-methylcholanthrene-like at later times after administration (Dent et al., 1976b). However, the same treatment of PBBs to young rats resulted in a different pattern of stimulation and appeared to more closely resemble 3-methylcholanthrene stimulation initially with a phenobarbital-like effect occurring at later times (McCormack et al., 1977). Following dietary exposure of pregnant and lactating rats to PBBs, hepatic and extrahepatic mixed function oxidase activity was stimulated in 15 day old offspring when neonates were exposed transplacentally and/or via the mothers milk (Dent et al., 1977). In summary, these studies demonstrate a number of important properties of PBBs as hepatic microsomal

enzyme stimulating agents: 1) the pattern of stimulation may change with time following exposure, 2) the characteristics of this pattern may be age dependent, and 3) stimulation may occur in developing rodents following pre- and postnatal exposure.

Experimental Rationale

Excretion of drugs from blood into bile requires hepatic uptake, intrahepatic metabolism, and biliary excretion. The capacity for drug elimination into bile in young rats is low when compared to adults and functional immaturity may be attributed, in part, to a deficiency in hepatic uptake (Klaassen, 1972,1973b). However, in adult rats, hepatic uptake is rapid and is not rate-limiting for overall drug transport. Therefore, if the maturation of hepatic excretory function is characterized by an increase in hepatic uptake capacity, uptake may be the rate-limiting step for drug transport in young animals, and with age, the rate-limiting step for drug elimination may change.

In adult animals, overall transport function may be altered following changes in the rates of 1) bile flow; 2) hepatic blood flow; and 3) drug biotransformation. In addition, hepatic drug elimination may be influenced by 4) hepatic content of ligandin, and 5) the presence of endogenous and specific exogenous competitive inhibitors.

The first objective of this investigation was to characterize the maturation of hepatic excretory function and to determine the relative importance of hepatic uptake for overall drug transport in developing rats. This was accomplished by comparing hepatic function in developing and adult rats in the control state, as well as in experimentally-induced situations that are known to influence drug transport in the adult.

This investigation was also concerned with chemical-induced alterations in hepatic function. The second objective of this research was to determine the effect of carbon tetrachloride and polybrominated biphenyls on hepatic excretory function in both developing and adult rats.

MATERIALS AND METHODS

Animals

All animals used were Sprague-Dawley rats purchased from Spartan Research Animals, Inc., Haslett, Michigan. Animals were maintained in clear solid bottomed polypropylene cages at 22°C with a 12 hour light cycle and were allowed free access to food (Wayne Lab Blocks; Anderson Mills, Maumee, Ohio) and water. Timed pregnant or lactating rats with litters of 8-10 offspring were received at least one day prior to experimentation. Adult rats (200-250 gm) were also used.

Hepatic Transport of Ouabain and Sulfobromophthalein in Developing Rats

Disposition of Ouabain

Rats of 15, 21, 25, 35, and 45 days of age were lightly anesthetized with ether and injected with [^{3}H]-ouabain (1 mg/kg) via the tail vein. The injection volume was 2.5 ml per kg body weight. Specific activity of ouabain was 116 µCi/mg and was prepared by mixing non-radioactive ouabain (Sigma Chemical Co., St. Louis, Mo.) with randomly labelled (^{3}H)-ouabain (New England Nuclear, Boston, Mass.) in normal saline. Following ouabain injection, rats were placed under a heat lamp to maintain normal body temperature. Three, 20, and 40 minutes following administration of ouabain, rats were anesthetized with ether, a blood sample was taken (with a heparinized syringe) by

cardiac puncture and the entire liver and small intestine were rapidly removed. The liver was blotted, weighed, coarsely chopped with a scissors and duplicate samples (100 mg) were placed in scintillation vials. Blood was centrifuged at 2500 rpm to obtain plasma and plasma samples (100-200 µl) were placed in scintillation vials. The quantity of radioactivity in the intestine was measured to estimate biliary excretion of ouabain (Klaassen, 1974b). The entire small intestine was homogenized in 5-10 ml distilled water with a Polytron^{R} homogenizer (Brinkman Instruments, Westbury, N.Y.) and aliquots (200 µl) of whole homogenate were placed in scintillation vials.

Samples of liver, plasma, and intestine were solubilized at 80°C in 1 ml of a mixture of water, methanol, and Triton X-405 (6:3:1 containing 2 moles of NaOH/liter) for 2-3 hours. When solubilization was complete, the samples were acidified by the addition of 0.5 ml 4.4 M HNO_3 and counted in 12 ml of toluene-Triton X-100 (2:1) scintillation cocktail [which contained 2.5 gm PPO; 2,5-diphenyloxazole, and 100 mg dimethyl POPOP; 1,4-bis(2-(4-methyl-5-phenyloxazolyl)benzene; per liter] (Dent and Johnson, 1974). Radioactivity in all samples was determined with a Packard Model 3380 liquid scintillation spectrometer equipped with automatic external standard for quench correction (Packard Instrument Company, Downers Grove, Ill.). Radioactivity in blanks for plasma liver, and intestine was negligible.

Disposition of Sulfobromophthalein

Eighteen day old and adult rats were lightly anesthetized with ether and injected with 120 mg/kg sulfobromophthalein (Hynson, Westcott, and Dunning, Inc., Baltimore, Md.) via the tail vein. Sulfobromophthalein (BSP) solutions were diluted in normal saline for injection

volumes of 10 ml/kg (18 day old animals) or 4 ml/kg (adult rats). Following injection of BSP, rats were placed under a heat lamp (for maintenance of normal body temperature). Three, 20, and 40 minutes following BSP injection, blood samples were taken by cardiac puncture with a heparainized syringe and the liver was rapidly removed. Concentration of BSP in plasma was determined by diluting plasma samples (20-100 µl) with water and 0.1 N NaOH and measuring absorbance at 580 mµ in a Beckman dual beam spectrophotometer (Beckman, Instruments, Fullerton, Calif.). Hepatic concentrations of BSP were determined by the method of Whelan et al. (1970b). Liver was blotted, weighed, and coarsely chopped with a scissors. Duplicate 1 gm samples were homogenized with 1 ml of distilled water in a Polytron homogenizer. BSP was extracted from liver by addition of 5 ml anhydrous acetone and centrifuged at 2500 rpm in an International Centrifuge, model PR-2 (International Equipment Co., Needham, Hts., Mass.). The resulting supernatant was diluted with 0.1 N NaOH and optical density recorded at 580 mµ and at 620 mµ in a Beckman Dual Beam Spectrophotometer. Readings were carried out at 620 mµ and subtracted from values at 580 mµ in liver extracts to correct for tissue turbidity. The extraction procedure was performed twice and hepatic BSP concentration for each sample was calculated by the sum of BSP levels in both extracts. Final calculations for BSP in plasma and liver were made from standard curves containing BSP in plasma and liver blanks.

In Vitro Conjugation of BSP to Glutathione in Developing Rats

Liver BSP conjugating activity was assayed in rats 8 to 70 days of age. Animals were decapitated and the livers were excised, blotted, and coarsely chopped into ice-cold 1.15% KCl. Soluble liver fractions

containing this enzyme (Goldstein and Combes, 1966) were prepared by homogenization (Polytron) of liver in 4 volumes of 1.15% KCl buffered to 7.4 with 20 mM Tris-HCl. The homogenate was centrifuged at 10,000 x g in a Sorvall model RC2-B centrifuge (Ivan Sorvall Inc., Newtown, Conn.) for 20 minutes and the supernatant was then recentrifuged for 60 minutes at 105,000 x g in a Beckman L3-50 ultracentrifuge. The supernatant was assayed for BSP conjugating activity by the method of Goldstein and Combes (1966) as described by Klaassen and Plaa (1967). Varying amounts of soluble supernatant were incubated at 37°C for 5 minutes in a Dubnoff incubator with 20.5 mM reduced glutathione (Sigma Chemical Co., St. Louis, Mo.), 227 μM BSP, and sufficient volume of 0.1 M sodium pyrophosphate buffer, pH 8.2, to bring the total incubation volume to 4.4 ml. The reaction was started by addition of BSP. BSP conjugating activity was measured by recording 5 minute change in optical density (O.D.) at 330 mμ from the time of addition of BSP. Increases in O.D. at this wavelength reflect production of conjugated BSP since BSP-GSH but not unconjugated BSP absorbs light at this wavelength (Goldstein and Combes, 1966). The conjugation of BSP to glutathione also occurs non-enzymatically and thus non-enzymatic activity (changes in O.D. measured in incubations containing substrates but not liver homogenate) were subtracted from values for the total reaction to obtain net enzymatic activity. Reactions were protein dependent for the 5 minute incubation period. Activity of the enzyme was expressed as nmoles BSP conjugate produced/min/gm liver and was calculated by the method of Goldstein and Combes (1966).

Plasma Disappearance of BSP and Conjugated BSP (BSP-GSH) in 15 Day Old and Adult Rats

Preparation of Conjugated BSP

Conjugated BSP (BSP-GSH) was prepared by the method of Whelan et al. (1970a,b). Reduced glutathione (736 mg) was dissolved in a 40 ml commercial solution containing concentrated BSP (50 mg/ml). The pH was brought to approximately 10.0 with 2 ml of concentrated ammonium hydroxide and the contents were covered and shaken slowly at 37°C in a Dubnoff incubator. Following 2 hours of incubation, 105 ml of anhydrous acetone was added and contents centrifuged at 2500 rpm for 10 minutes. The resulting precipitate was discarded and an equal volume of anhydrous acetone was added to the supernatant. Following recentrifugation, the supernatant was discarded and the precipitate was washed in 87.5% acetone, dissolved in distilled water, quick frozen in a dry ice-ethanol bath, and lypholized overnight (Virtis Freeze-Mobile, Gardiner, Mich.). Confirmation that the resulting powder was conjugated BSP (BSP-GSH) was made by the paper chromatography method of Combes (1959). Standard aqueous solutions of the powder (BSP-GSH) were spotted on Whatman No. 3 filter paper, dried with a hair dryer, and developed by descending chromatography in a solvent system consisting of n-propyl alcohol, distilled water, and glacial acetic acid, 10:5:1 (v/v). Following 12-14 hours, BSP on the chromatographs was identified by exposure to ammonia vapor and was then eluted in distilled water. R_f value for the newly synthesized powder (BSP-GSH) was compared to literature values for BSP-GSH (Whelan et al., 1970b), standard BSP solutions, and samples of bile from adult rats administered BSP. By this procedure, the resulting powder was determined to be approximately 95% conjugated BSP (BSP-GSH).

Elimination of BSP and BSP-GSH from Plasma of 15 Day Old Rats

Fifteen day old rats were lightly anesthetized with ether, injected via the tail vein with 150 μmoles/kg BSP or BSP-GSH (determined colorimetrically) and placed under a heat lamp. BSP and BSP-GSH were diluted in normal saline for an injection volume of 10 ml/kg. Three, 10 and 20 minutes following drug injection, blood samples were taken by cardiac punture with a heparinized syringe. Concentrations of BSP and BSP-GSH in plasma were determined by measuring absorption at 580 mμ after dilution of plasma samples (20-100 μl) with water and 0.1 N NaOH. The rate of elimination of BSP or BSP-GSH from plasma was determined by the method of least squares (Goldstein, 1971) and fiducial limits and correlation coefficients determined as described by Goldstein (1971).

Elimination of BSP and BSP-GSH-from Plasma of Adult Rats

Rats were anesthetized with 3 ml/kg of Equi-Thesin^{R} (Jensen-Salsbery, Inc., Kansas City, Mo.). This anesthetic preparation contained 44 ml propylene glycol, 0.97 gm pentobarbital, 4.25 gm chloral hydrate, 2.126 gm $MgSO_4$, and 11 ml ethanol, in 100 ml of distilled water. Anesthetized rats were placed on a 6"x12" board and the femoral artery and vein were cannulated with PE_{50} polyethylene tubing. The animals were placed under a heat lamp to maintain body temperature. BSP or BSP-GSH (100 μmoles/kg) was injected through the venous cannula and blood samples (0.3 ml) were drawn into a heparinized syringe through the artieral cannula at 3, 10 and 20 min. Plasma samples were analyzed for BSP or BSP-GSH as described above. The rate of elimination of BSP or BSP-GSH from plasma was determined by the method of least

squares (Goldstein, 1971) and fiducial limits and correlation coefficients determined as described by Goldstein (1971).

Influence of Experimentally-Induced Alterations in Bile Flow on Drug Transport in Adult and Young Rats

Effect of Bile Salt Administration on Plasma Elimination of BSP

The effect of simultaneous administration of the bile salt taurocholate (sodium) and 120 mg/kg sulfobromophthalein (BSP) on the plasma disappearance of BSP was examined in 15 day old and adult rats. Sodium taurocholate (100 mg/kg) was dissolved in normal saline for an injection volume of 3.2 ml/kg and BSP was appropriately dissolved in saline for injection volumes of either 2.4 ml/kg (adult rats) or 6.8 ml/kg (15 day old rats). The two solutions were mixed in a syringe immediately prior to intravenous injection and control rats (no bile salt) received an additional (3.2 ml/kg) volume of normal saline. Fifteen day old rats were injected with the drug(s) via the tail vein and plasma and liver concentrations of BSP were determined 3, 10, 20, 30 and 40 minutes following injection as described in "Disposition of Sulfobromophthalein". Adult rats were anesthetized with Equi-Thesin and administration of drug(s) and collection of serial (3-40 minute) blood samples taken from each rat as described in "Elimination of BSP and BSP-GSH from Plasma of Adult Rats".

Effect of Bile Duct Ligation on Ouabain and BSP Transport

Fifteen day old rats were lightly anesthetized with ether and the bile duct was exposed following a midline abdominal incision. Ligation of the common bile duct was made with silk sutures and the abdominal wound was closed by means of an autoclip applier (Clay-Adams, Inc., New York, N.Y.). Sham operated animals were used as

controls. Following the surgical procedure, animals were placed under a heat lamp for recovery and, after 1 hour, were administered a single bolus, by tail vein injection, of ^{3}H-ouabain (1 mg/kg; specific activity of 200 μCi/mg) or BSP (120 mg/kg). After drug administration, rats were placed under a heat lamp and, at various times following drug administration, blood samples were taken by cardiac puncture and concentration of drugs in plasma was determined as described in "Disposition of Ouabain" and "Disposition of Sulfobromophthalein". In experiments with ^{3}H-ouabain, hepatic and intestinal ouabain levels were also determined as previously described. In a separate experiment, the effect of bile duct ligation on ouabain transport was determined in 15 day old rats injected with ouabain immediately following the surgical procedure and the rats were not allowed a 1 hour recovery period.

The effect of bile duct ligation on (^{3}H)-ouabain (1 mg/kg; specific activity of 155 μCi/mg) and BSP (120 mg/kg) elimination from plasma was also determined in adult rats. Adult animals were anesthetized with Equi-Thesin and the common bile duct was ligated with silk sutures. After 1 hour, ouabain or BSP was injected into a PE50 femoral vein cannula. Sham operated animals were used as controls. Serial blood samples were drawn from an arterial cannula (in the femoral artery) at various times following drug administration. Plasma concentration of ouabain (tritium) and BSP were determined as described in "Disposition of Ouabain" and "Disposition of Sulfobromophthalein". After collection of the final blood sample (40 min), liver and intestine were immediately removed (from ouabain injected rats) and ouabain levels in these tissues were determined as previously described.

Effect of Pentobarbital-Induced Hypothermia on Ouabain Disposition

The effect of hypothermia on the disposition of intravenously administered ouabain was determined in 15 day old and adult rats. Hypothermia was induced in these animals with a single intraperitoneal injection of sodium pentobarbital (30 mg/kg) prepared in normal saline. Control animals received comparable doses of pentobarbital, but were placed under a heat lamp to maintain normal body temperature. Body temperature was continuously monitored by means of a rectal probe attached to a telethermometer (Yellow Springs Instrument Co., Yellow Spring, Ohio). When temperature decreased to about 30-33°C, rats were administered a single bolus injection of ^{3}H-ouabain (1 mg/kg; specific activity in adults, 307 μCi/mg; in 15 day old rats, 206 μCi/mg). Thirty minutes following ouabain injection, the amount of ouabain (tritium) in plasma, liver and intestine was determined as described in "Disposition of Ouabain".

Effect of Carbon Tetrachloride on Hepatic Transport of Ouabain in Developing Rats

Rats of 14, 20, 24, 32 and 45 days of age received a single ip injection of corn oil (5 ml/kg) or CCl_4 (1 ml/kg) prepared in corn oil. The administration of CCl_4 across litters was arranged so that both control and CCl_4-treated animals were obtained from each litter. Twenty-four hours following administration of CCl_4, animals were lightly anesthetized with ether and injected with (^{3}H)-ouabain (1 mg/kg) via the tail vein. The specific activity of (^{3}H)-ouabain was 250 μCi/mg. Thirty minutes following administration of ouabain, rats were anesthetized with ether, a blood sample was taken (with a heparinized syringe) by cardiac puncture, and the entire liver and small

intestine were rapidly removed. Radioactivity in duplicate samples of plasma, liver, and intestine was determined as described in "Disposition of Ouabain".

Digoxin-Mediated Inhibition of Ouabain Transport in Adult and Developing Rats

The effect of simultaneous administration of digoxin (nonradioactive) and ^{3}H-ouabain on hepatic transport of ^{3}H-ouabain was examined in 15 day old, 21 day old, and adult rats. Specific activity of ouabain was 200 μCi/kg (in 15 day old rats), 180 μCi/mg (in 21 day old rats) or 155 μCi/mg (in adult rats). ^{3}H-Ouabain (1 mg/kg) was prepared in normal saline for administration of 5 ml/kg injection volume. Digoxin (0.5 mg/kg) was mixed in 100% ethanol, sonicated for 10 minutes, and, following sonication, diluted further with normal saline for injection volume of 2.5 ml/kg which contained 20% ethanol. The two injection solutions were prepared separately and were mixed in a syringe immediately prior to tail vein injection. Thus, rats injected with ^{3}H-ouabain plus digoxin were administered a total injection volume of 7.5 ml/kg which contained 1 mg/kg ^{3}H-ouabain, 0.5 mg/kg digoxin (nonradioactive), and approximately 7% ethanol. Control rats (administered ^{3}H-ouabain but no digoxin) received 7.5 ml/kg injection solution which contained 7% ethanol. Rats were administered drug(s) as a single bolus injection and plasma, liver and intestinal ouabain (tritium) was measured in animals sacrificed 3, 20, and 40 minutes following injection as outlined in "Dispostion of Ouabain".

Toxicity of Polybrominated Biphenyls (PBBs) in Developing Rats

One day old Sprague-Dawley rats and lactating mothers were obtained from Spartan Research Animals, Inc. (Haslett, Michigan) on the morning following parturition. Lactating dams and litters of 10 offspring were housed in clear plastic shoe box cages and were immediately placed on diets containing 0 or 50 ppm PBBs (in the form of Firemaster BP-6, Michigan Chemical Co., which contains a mixture of PBBs of which 2,2',4,4',5,5'-hexobromobiphenyl comprises about 70%; Jacobs et al., 1976). The PBBs were dissolved in acetone or ether (10 ml/kg diet) and were thoroughly mixed with powdered food pellets (Wayne Lab Blocks) over a 10 minute period. The control diet was powdered food to which only solvent had been added. Analysis of diets for PBBs was made by the method of Rickert et al. (1977) following extraction of PBBs into petroleum ether (Fehringer, 1975). Control diet (0 ppm) contained less than 50 parts per billion PBBs and experimental diet (50 ppm) was determined to contain 40.7-50.1 ppm PBBs.

Total litter body weights and mortality were recorded at weekly or biweekly intervals from postnatal day 7 to termination of the experiment at postnatal day 49. Litters were weaned on postnatal day 28 and weanlings were continued on the same diet fed to their mothers.

In a separate experiment, timed pregnant Sprague-Dawley rats were obtained on day 5 of gestation from Spartan Research Animals and on day 8 of gestation were placed on diets containing 0 or 50 ppm PBBs. After birth lactating rats were continued on respective diets until postnatal day 15. At birth all litters were normalized to 10 pups. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal,

0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50) Total litter weight was recorded on postnatal day 8 and postnatal day 15.

Interaction of PBBs with Ouabain Lethality in 15 Day Old Rats

The lethality of ouabain was determined in 15 day old rats whose mothers received dietary PBBs (0, 50, or 100 ppm) beginning at birth and continued until the day of the experiment. Various doses of ouabain (octahydrate; Sigma Chemical Co., St. Louis, Mo.) were dissolved in distilled water and injected intraperitoneally. Mortality was recorded over a 24 hour period and calculation of ouabain LD50 was made by the method of Litchfield and Wilcoxon (1949). Potency ratios were calculated following affirmation that log-probability plots yielded curves that were parallel.

Effect of Polybrominated Biphenyls (PBBs) on Hepatic Excretory Function in Developing Rats

Effect of Exposure to PBBs on Ouabain Transport in Developing Rats

The effect of exposure to PBBs on ouabain elimination from plasma and hepatic and intestinal dispostion of ouabain was determined in 15, 21, 35 and 49 day old rats. The animals used in this experiment were exposed to PBBs by two treatment regimens. The first group received 0 or 50 ppm PBBs through the mothers diet from the day of birth to the day of the experiment. The mothers received dietary PBBs on postnatal day 1 and on postnatal day 28 the weanlings received the same dietary dose of PBBs. Hepatic transport of ouabain was determined when rats were 15, 21, 35, or 49 days old. Hepatic transport of ouabain was determined in the second group of animals on postnatal day 15. Rats

in this group received prenatal and/or postnatal PBBs through the mother's diet (at a dietary dose of 0 or 50 ppm) as described for the cross-fostering procedure in "Toxicity of Polybrominated Biphenyls (PBBs) in Developing Rats".

Hepatic transport of ^{3}H-ouabain (1 mg/kg) administered by a single tail vein injection was determined as described in "Disposition of Ouabain". The specific activity of ^{3}H-ouabain was 200 μCi/mg.

Effect of PBBs on Initial Rate of Elimination of Indocyanine Green (ICG) from Plasma in 21 Day Old Rats

Twenty-one day old rats whose mothers received dietary PBBs (0 or 50 ppm) beginning at parturition (and continued until postnatal day 21) were lightly anesthetized with ether, injected with a single bolus of ICG (Hynson, Westcott and Dunning, Inc., Baltimore, Md.; 40 mg/kg) via the tail vein, and were placed under a heat lamp. One, 5, 10 and 15 minutes following administration of ICG, blood samples were taken by cardiac puncture into a syringe rinsed in sodium oxalate (1.6 mg %). Sodium oxalate was used as the anticoagulant since the sodium bisulfide present in heparin preparations interferes with ICG absorbance (Cobb and Barnes, 1965). ICG in plasma was quantified by diluting the plasma sample in water and measuring absorbance at 805 mμ(Caesar et al., 1961). The rate of elimination of ICG from plasma was determined by the method of least squares (Goldstein, 1971) and fiducial limits and correlation coefficients were determined as described by Goldstein (1971).

Characteristics of Stimulation of Drug Transport in Young Rats

Digoxin-Mediated Inhibition of Ouabain Transport in Rats Exposed to Polybrominated Biphenyls (PBBs)

Control 15 day old rats, 15 day old rats exposed to PBBs during the prenatal period (50-0; from 8 of gestation to birth) and 15 day old rats exposed to PBBs during the postnatal period (0-50; from birth to postnatal day 15) were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein. In addition to ouabain, the rats were simultaneously injected with digoxin (0.5 mg/kg in 7% ethanol) or with a saline-ethanol mixture (7%). The specific activity of ^{3}H-ouabain was 200 μCi/mg and the experimental procedure was the same as outlined in "Digoxin-Mediated Inhibition of Ouabain Transport in Adult and Developing Rats".

Effect of Carbon Tetrachloride (CCl_4) on Tissue Distribution of Ouabain in 15 Day Old Rats Treated with Polybrominated Biphenyls (PBBs)

Fourteen day old rats whose mothers were fed diets containing 0, 50, or 100 ppm PBBs continuously from the day of birth were injected intraperitoneally with a single dose of CCl_4 (1 ml/kg). CCl_4 was prepared in corn oil for an injection volume of 5 ml/kg. Twenty-four hours after CCl_4, animals were injected with ^{3}H-ouabain (1 mg/kg; specific activity of 246 μCi/mg) via the tail vein and placed under a heat lamp. Thirty minutes following ouabain injection, blood was obtained by cardiac puncture, liver and small intestine were removed, and radioactivity in plasma, liver and intestinal homogenates was determined as outlined in "Disposition of Ouabain".

Statistics

Statistical evaluation of the data was made by Student's *t*-test (Steel and Torrie, 1960). The level of significance was chosen as $p<0.05$.

RESULTS

Hepatic Transport of Ouabain and Sulfobromophthalein in Developing Rats

The disappearance of ouabain from plasma of developing rats is depicted in Figure 1. Rats 15 and 21 days of age retained ouabain in plasma relative to the older animals. For animals older than 21 days of age, differences in the disappearance of ouabain from plasma are less conspicuous and it appears that the curves representing the plasma disappearance of ouabain in 35 and 45 day old rats are superimposable (Figure 1). In the 40 minute experimental period, the disappearance of ouabain from plasma may be represented by a single line on a logarithmic-concentration vs. time plot in 15 day old rats; however, in older animals, the curves appear to be biphasic (Figure 1).

Following a single intravenous injection of ouabain, cumulative hepatic excretion of the drug, estimated by 40 minute intestinal content, increased with age. Forty minute intestinal ouabain content in 15 day old rats was 116.6±23.0 μg/kg body weight and was 2.5-3 times less than values obtained from 35 and 45 day old rats (Table 1).

The concentration of ouabain in the liver changed with time following a single bolus injection of the drug (Figure 2). In 15 and 21 day old rats, hepatic ouabain concentration and total hepatic ouabain content (μg in total liver/kg body weight) reached maximum

Figure 1. Elimination of (^{3}H)ouabain from plasma of rats of ages ranging from 15-45 days. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20 and 40 minutes, blood was obtained by cardiac puncture and plasma samples analyzed for tritium. Each point represents the mean value for 4 rats obtained from 4 litters. Standard errors (not shown for clarity) were approximately 10% of the mean values.

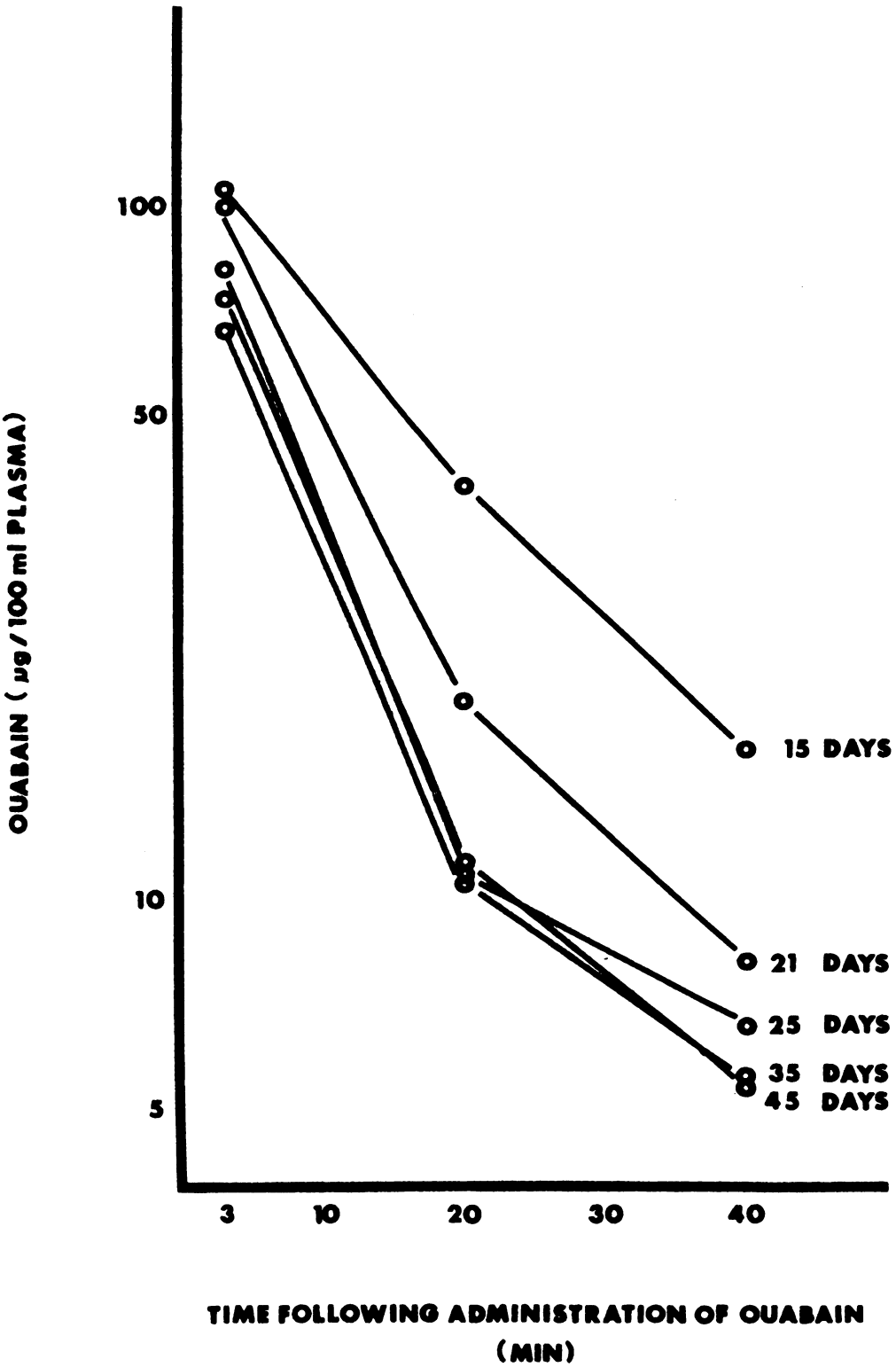
100
50
10
5
OUABAIN (µg / 100 ml PLASMA)
15 DAYS
21 DAYS
25 DAYS
35 DAYS
45 DAYS
3
10
20
30
40
TIME FOLLOWING ADMINISTRATION OF OUABAIN
(MIN)

Figure 1

TABLE 1

Cumulative Hepatic Excretion of Ouabain[a] in Developing Rats

Age (Days)	Ouabain Excretion (μg/kg body wt)
15	116.6±23.0[b]
21	168.0±47.7
25	222.2±29.3
35	350.9±16.0
45	300.0±13.6

[a]Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 40 min the intestine was analyzed for tritium.

[b]Values represent the mean ± S.E. for 4 rats obtained from 4 litters.

Figure 2. Hepatic ouabain concentration and total hepatic ouabain content with time following (^{3}H)ouabain administration in rats of ages ranging from 15-45 days. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 minutes, samples of liver were analyzed for tritium. Each point represents the mean ± S.E. for 4 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point.

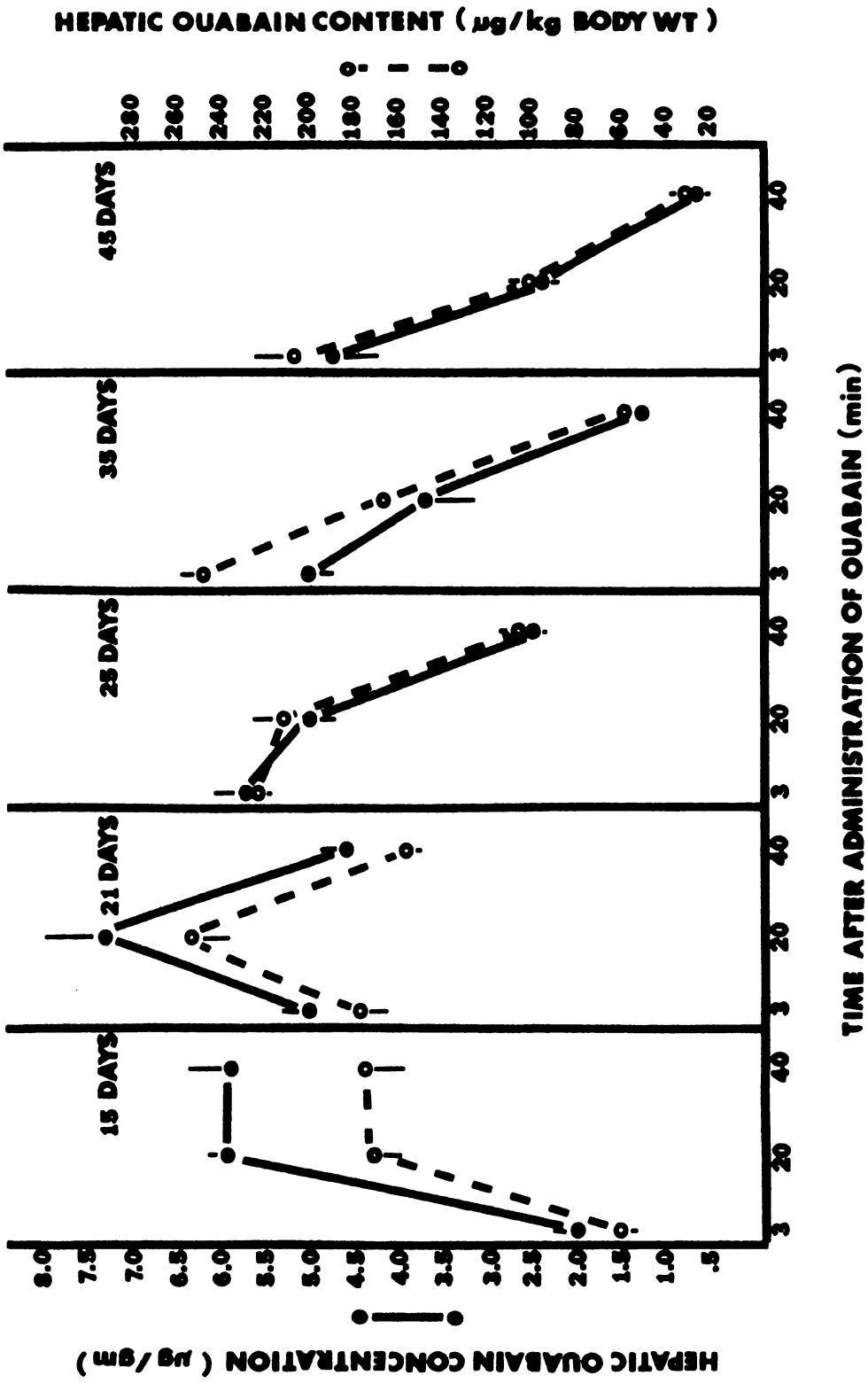
HEPATIC OUABAIN CONTENT (µg/kg BODY WT)
45 DAYS
35 DAYS
25 DAYS
21 DAYS
15 DAYS
TIME AFTER ADMINISTRATION OF OUABAIN (min)
HEPATIC OUABAIN CONCENTRATION (µg /gm)

Figure 2

values 20 minutes following drug administration. In 15 day old rats, 20 minute ouabain concentration in liver was 6.02±0.23 μg/g wet weight tissue which was almost three times the concentration at the 3 minute time point but essentially the same as the 40 minute value. In 21 day old rats, 20 minute hepatic ouabain concentration was 7.40±0.28 μg/g and was higher than hepatic ouabain concentrations at both the 3 and 40 minute intervals. Hepatic ouabain concentration and total hepatic ouabain content in 25, 35, and 45 day old rats were maximum at the earliest (3 minute) time point. The time dependent pattern of appearance and disappearance of hepatic ouabain was similar when calculated on a per gram tissue or on a body weight basis for rats of all ages. Liver to body weight ratios, however, increased with age (Table 2).

Plasma concentrations of sulfobromophthalein (BSP) in adult rats were significantly different from values obtained from 18 day old rats. When compared to adult values, significantly higher plasma BSP concentrations were detected in the 18 day old rats 10, 20, 30 and 40 minutes following a single bolus injection of the dye (Figure 3). The time dependent pattern for hepatic disposition of BSP was also age dependent. In the young animals, hepatic BSP content appeared to be maximum 20 minutes following dye administration; whereas, in the adult, maximum values were apparent at the 3 minute time interval (Figure 4). Hepatic BSP content in 18 day old rats was significantly lower than adult values at the early (3 minute) time point, but significantly higher than adult levels 40 minutes following injection of BSP (Figure 4).

TABLE 2

Liver to Body Weight Ratio in Developing Rats

Age (Days)	Liver Wt/Body Wt (%)
15	2.97±0.10[a]
21	3.50±0.06
25	4.15±0.06
35	4.64±0.10
45	4.50±0.13

[a]Each point represents the mean ± S.E. for 11 rats obtained from 4 litters.

Figure 2. Hepatic ouabain concentration and total hepatic ouabain content with time following (^{3}H)ouabain administration in rats of ages ranging from 15-45 days. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 minutes, samples of liver were analyzed for tritium. Each point represents the mean ± S.E. for 4 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point.

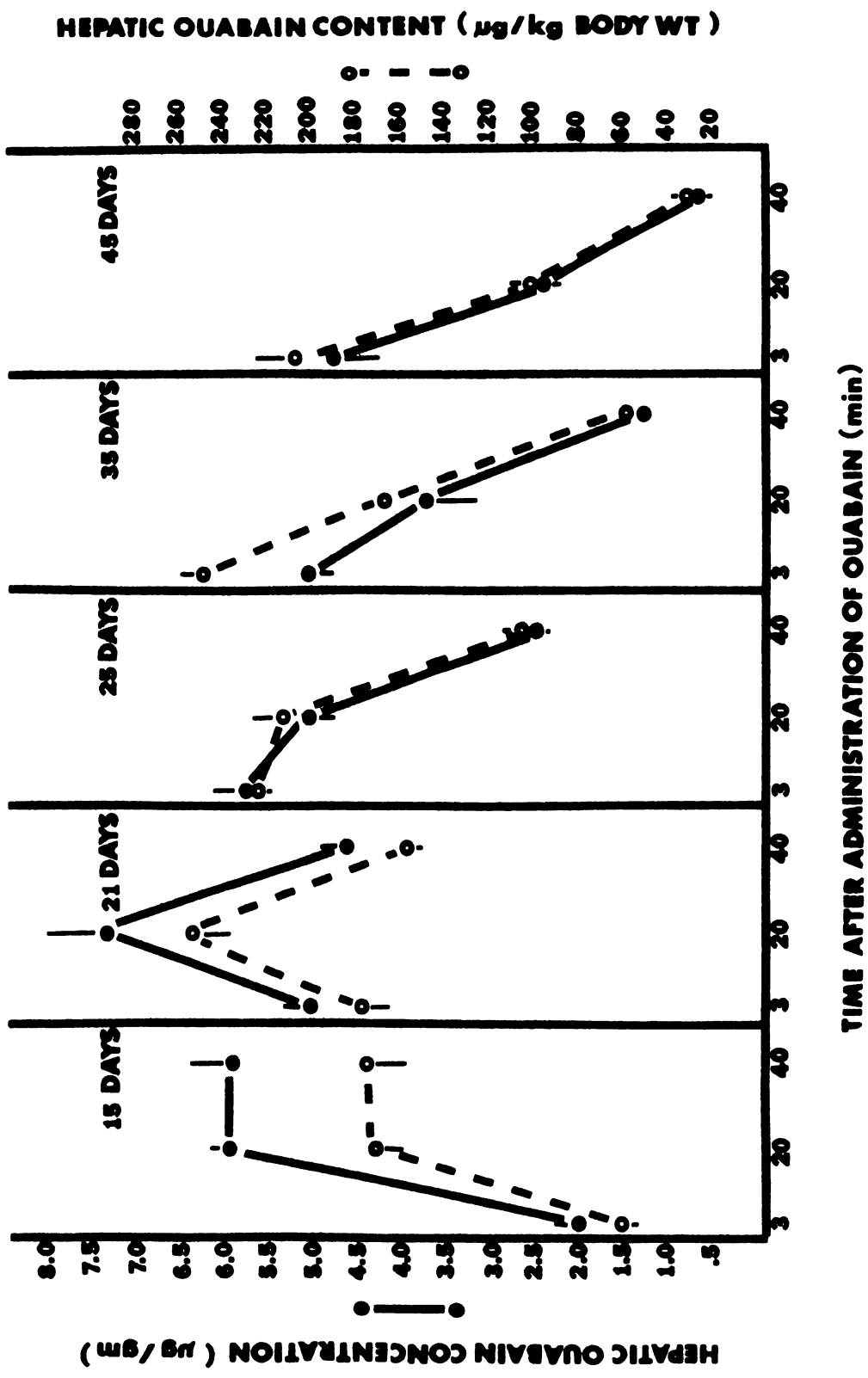
HEPATIC OUABAIN CONTENT (µg/kg BODY WT)
45 DAYS
35 DAYS
25 DAYS
21 DAYS
15 DAYS
TIME AFTER ADMINISTRATION OF OUABAIN (min)
HEPATIC OUABAIN CONCENTRATION (µg /gm)

Figure 2

Figure 3. Elimination of sulfobromophthalein (BSP) from plasma of 18 day old and adult rats. Animals were administered BSP (120 mg/kg) via the tail vein and following various times, blood was obtained by cardiac puncture and BSP concentration was determined in plasma. Each point represents the mean ± S.E. for 3 rats. Eighteen day old animals were obtained from 3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma BSP concentration significantly different from values obtained from adults ($p<0.05$).

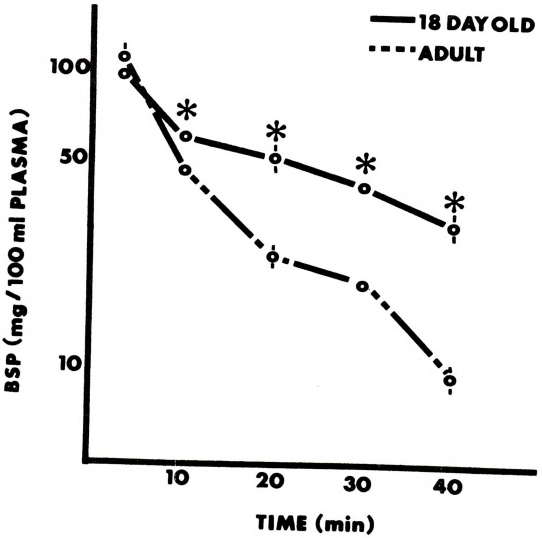
18 DAY OLD
ADULT
100
50
10
BSP (mg/100 ml PLASMA)
10
20
30
40
TIME (min)

Figure 3

Figure 4. Hepatic content of sulfobromophthalein (BSP) with time following BSP administration in 18 day old and adult rats. Rats were administered BSP (120 mg/kg) via the tail vein and following 3, 20 and 40 minutes, samples of liver were analyzed for BSP. Each point represents the mean ± S.E. for 3 rats. Eighteen day old animals were obtained from 3 litters. Asterisk indicates hepatic BSP content significantly different from values obtained from adults ($p<0.05$).

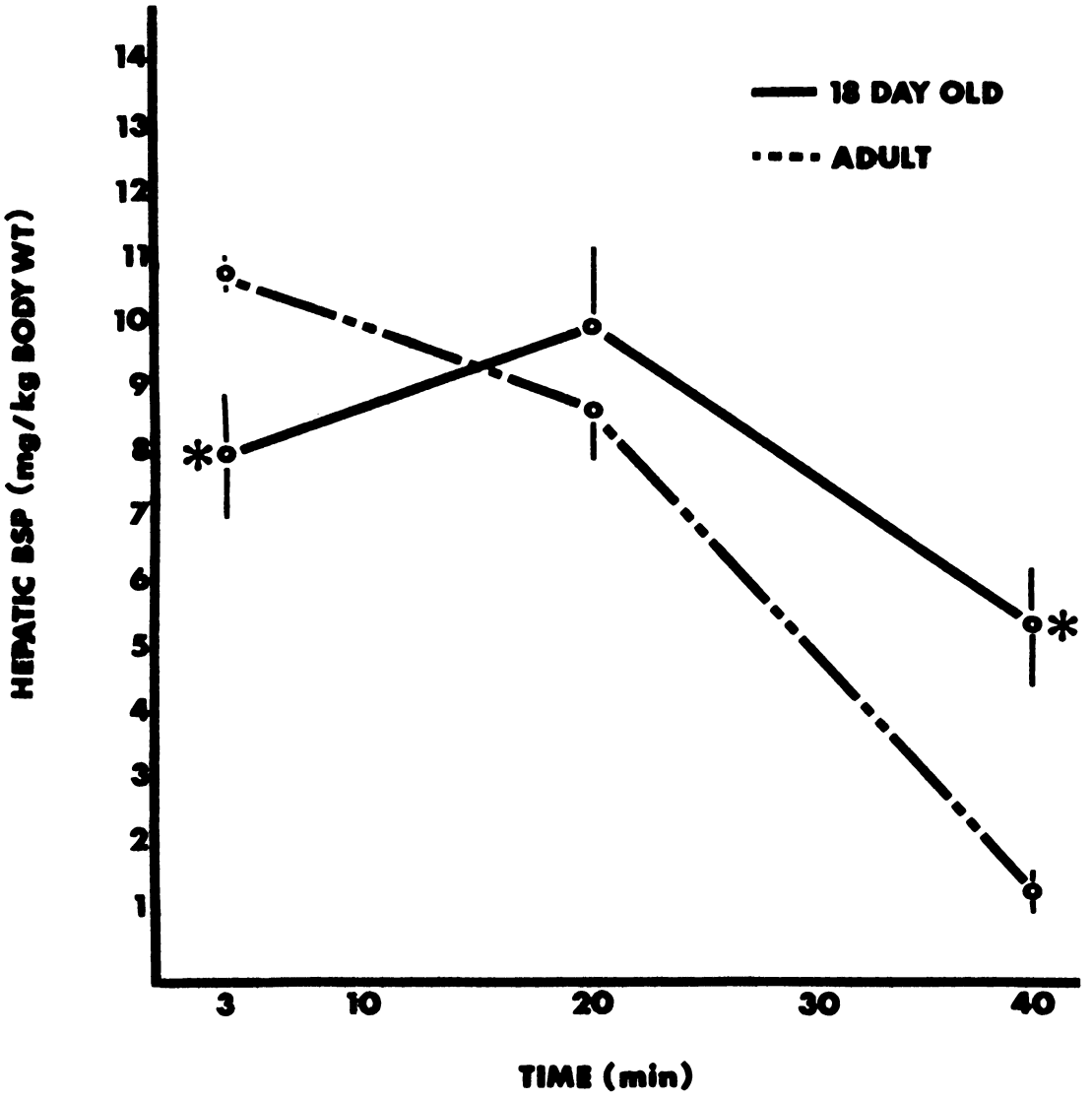
18 DAY OLD
ADULT
HEPATIC BSP (mg/kg BODY WT)
14
13
12
11
10
9
8
7
6
5
4
3
2
1
3
10
20
30
40
TIME (min)

Figure 4

In Vitro Conjugation of BSP to Glutathione in Developing Rats

The activity of hepatic glutathione S-aryl transferase (using BSP as substrate) was low in young rats when compared to adults (Table 3). BSP conjugating activity in 21 day old rats was approximately 2 times higher than activity in 1-2 week old animals and appeared to reach maximum levels in 35 day old animals (Table 3).

Plasma Disappearance of BSP and Conjugated BSP in 15 Day Old and Adult Rats

The disappearance of BSP and conjugated BSP (BSP-GSH) from plasma of 15 day old and adult rats is depicted on Table 4 by the rate of elimination of these drugs from plasma. Each calculated regression line (slope) for plasma drug concentration vs. time demonstrated a significant correlation (Table 4). The rate of elimination of both BSP and BSP-GSH from plasma was significantly lower in 15 day old animals than in adults. In adult rats, plasma half-lives ($t_{\frac{1}{2}}$) for BSP or BSP-GSH were not different from each other (7 min vs 6 min, respectively) but were 2-4 times lower, respectively, than values from 15 day old animals. The rate of elimination of BSP from plasma was almost 2 times greater than removal rate for conjugated BSP in the young animals.

Influence of Experimentally-Induced Alterations in Bile Flow on Drug Transport in Adult and Young Rats

Effect of Bile Salt Administration on Plasma Elimination of BSP

The disappearance of BSP from plasma was enhanced in adult and, to a lesser extent, 15 day old rats by simultaneous administration of the bile salt taurocholate. Adult rats administered taurocholate plus BSP had significantly lower plasma BSP concentrations 30 and 40 minutes following BSP injection with values of 54% and 45% of control at these

TABLE 3

Hepatic Glutathione S-Aryl Transferase Activity in Developing Rats

Age (Days)	Glutathione Transferase[a]
8	432.8± 35.5[b]
9	228.7± 8.3
10	254.2± 14.2
14	363.7± 8.7
21	772.3± 38.3
35	1385.7±183.3
70	718.8± 17.3

[a]Activity, designated as nmoles BSP conjugate produced/min/gm liver, determined in rat liver 100,000 x g supernatant fraction. The reaction mixture contained 227 μM sulfobromophthalein and 20.5 mM reduced glutathione in 0.1 M sodium pyrophosphate buffer, pH 8.2.

[b]Mean ± S.E. for 6 rats obtained from 3 litters.

TABLE 4

Disappearance Rate[a] of BSP or Conjugated BSP (BSP-GSH) from Plasma of 15 Day Old and Adult Rats

Drug	Rate of Elimination (Slope)[b]	Estimated $t_{\frac{1}{2}}$ (min)	Correlation Coefficient
15 Day Old Rats			
BSP	0.019±0.004[d]	15	.92[c]
BSP-GSH	0.010±0.007[d,e]	30	.76[c]
Adults			
BSP	0.046±0.008	7	.96[c]
BSP-GSH	0.053±0.001	6	.99[c]

[a]Disappearance rates determined by the method of least squares from plasma samples taken 3, 10 and 20 min following injection of 150 μmoles/kg (15 day old rats) or 100 μmoles/kg (adult rats) of BSP or BSP-GSH.

[b]Elimination rate ± fiducial limits for 3 (adult group) or 9 (15 day old rats) animals. Fifteen day old animals were obtained from 3 litters.

[c]Significant correlation ($p<0.05$).

[d]Significantly different from rate in adult rats ($p<0.05$).

[e]Significantly different from rate of BSP removal ($p<0.05$).

time points, respectively (Figure 5). In 15 day old rat neonates injected with taurocholate, significantly lower plasma BSP concentration was detected at only the 30 minute time point and represented a 19% decrease when compared to neonates injected with BSP alone (Figure 5). Hepatic concentrations of BSP in rat neonates injected simultaneously with taurocholate were significantly lower than BSP concentrations in the control group 3 and 10 minutes following bolus injection of BSP (Figure 6).

Effect of Bile Duct Ligation on Ouabain and BSP Transport

Adult and 15 day old rats whose bile ducts had been ligated 1 hour prior to bolus injection of BSP and ouabain significantly retained these compounds in plasma (Figure 7). When compared to sham operated controls, plasma drug concentrations were significantly higher in bile duct ligated adult rats at all times following BSP injection and at all time points except the earliest (3 minutes) in rats injected with ouabain. Acute bile duct ligation in 15 day old rats resulted in significantly higher plasma concentrations of BSP 10 and 40 minutes following BSP injection when compared to sham operated rat neonates; whereas ouabain concentrations in plasma were significantly higher than control values in bile duct ligated rat neonates 3, 20 and 40 minutes following ouabain injection (Figure 7). Retention of ouabain and BSP in plasma was more pronounced in bile duct ligated adult rats than in similarly treated 15 day old animals. Forty minutes following intravenous injection of BSP and ouabain, plasma concentrations of these drugs in bile duct ligated adult rats were 513 and 780% of sham operated controls, respectively. In bile duct ligated 15 day old rats, plasma

Figure 5. Effect of sodium taurocholate (bile salt) on elimination of sulfobromophthalein (BSP) from plasma of 15 day old and adult rats. Rats were intravenously administered BSP (120 mg/kg) or BSP (120 mg/kg) plus taurocholate (100 mg/kg) and, at various times, BSP concentration was determined in plasma. Each point represents the mean ± S.E. for 3-4 rats. Fifteen day old animals were obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma BSP concentration significantly different from values obtained from rats administered BSP alone ($p<0.05$).

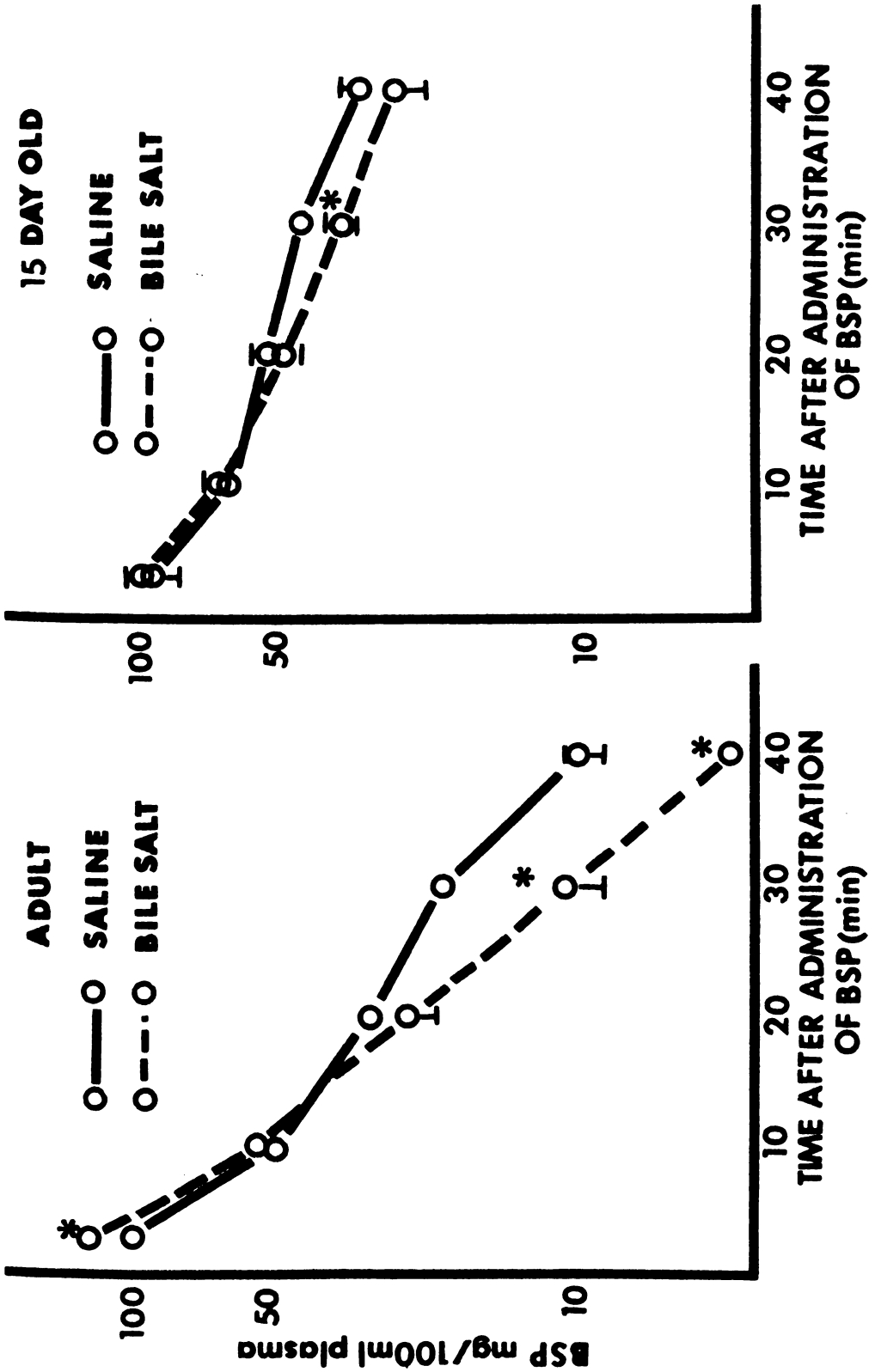
ADULT
SALINE
BILE SALT
15 DAY OLD
SALINE
BILE SALT
BSP mg/100ml plasma
100
50
10
10
20
30
40
TIME AFTER ADMINISTRATION OF BSP(min)

Figure 5

Figure 6. Effect of sodium taurocholate on sulfobromophthalein (BSP) concentration in liver of 15 day old rats. Rats were administered BSP (120 mg/kg) or BSP (120 mg/kg) plus taurocholate (100 mg/kg) via the tail vein and at various times, samples of liver were analyzed for BSP. Each point represents the mean ± S.E. for 4 rats obtained from 4 litters. Asterisk indicates hepatic BSP concentration significantly different from values obtained from rats administered BSP alone ($p<0.05$).

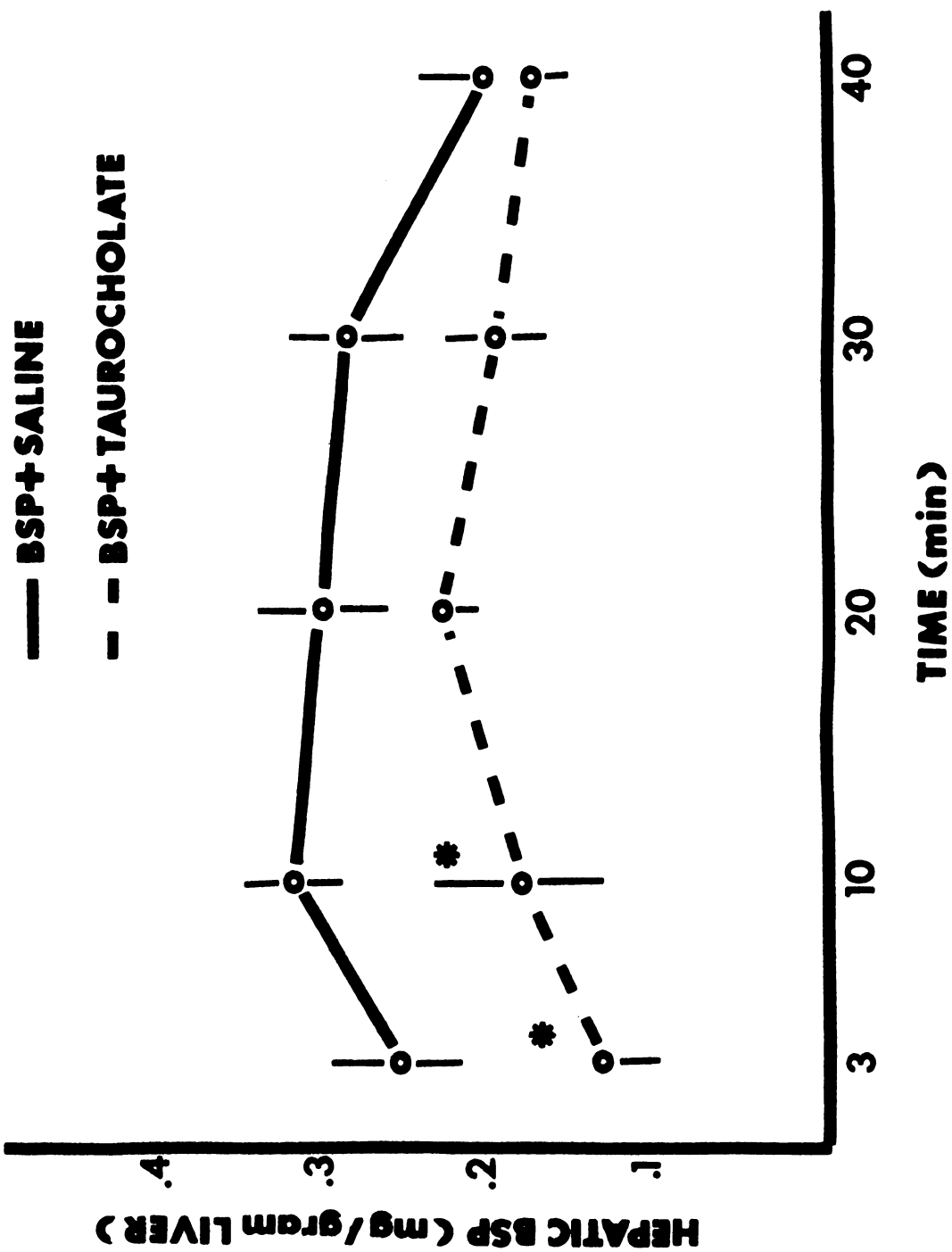
BSP+SALINE
BSP+TAUROCHOLATE
HEPATIC BSP (mg/gram LIVER)
.4
.3
.2
.1
3
10
20
30
40
TIME (min)
*
*

Figure 6

Figure 7. Effect of bile duct ligation on elimination of sulfobromophthalein (BSP) and (^{3}H) ouabain from plasma of adult and 15 day old rats. Rats were intravenously administered BSP (120 mg/kg) or (^{3}H)ouabain (1 mg/kg) 1 hour following bile duct ligation. At various times following drug administration, the concentration of BSP and ouabain (tritium) was determined in plasma. Broken line (---) represents values obtained from bile duct ligated rats and solid line (——) represents values from sham operated controls. Each point represents the mean ± S.E. for 3-4 rats. Fifteen day old animals were obtained from 3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma concentration significantly different from respective sham operated control ($p<0.05$).

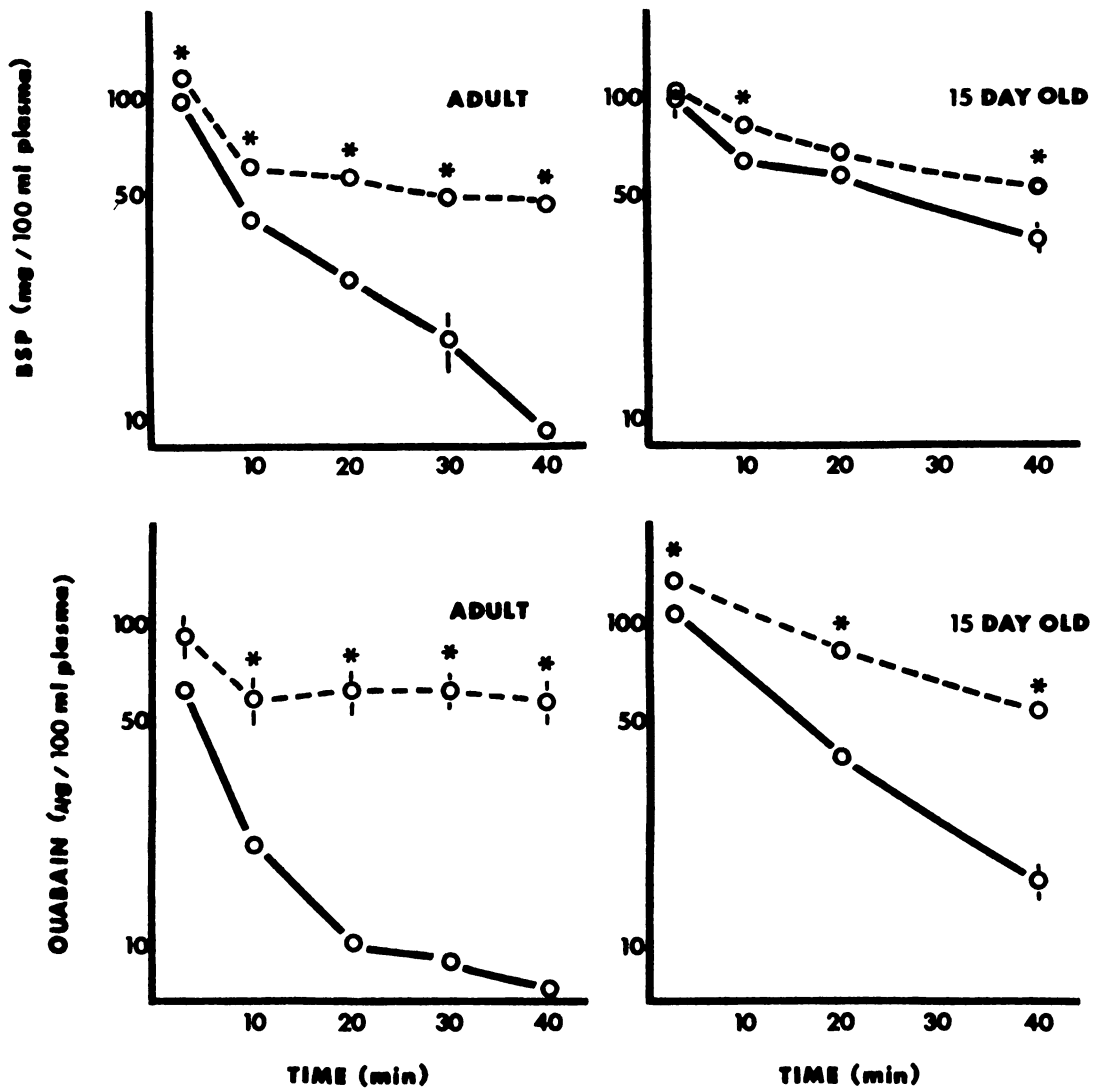
BSP (mg / 100 ml plasma)
ADULT
15 DAY OLD
100
50
10
10
20
30
40
OUABAIN (μg / 100 ml plasma)
TIME (min)
TIME (min)

Figure 7

concentrations of BSP and ouabain were 145 and 340% of sham operated controls at the 40 minute time point (Figure 7). When bile ducts were ligated in 15 day old rats immediately (approximately 1 min) prior to drug administration, plasma concentration of ouabain was significantly higher than the control level at only the 40 minute time point (Figure 8). Effectiveness of bile duct ligation in reducing hepatic excretion of ouabain is shown in Table 5. Cumulative (40 minute) intestinal content of ouabain in bile duct ligated rats was 3 to 19% of sham operated controls (Table 5).

When compared to sham operated controls, hepatic concentrations of ouabain were significantly lower in bile duct ligated 15 day old rats 3, 20, and 40 minutes following drug injection when ouabain was administered 1 hour following the surgical procedure, and 40 minutes following ouabain injection if administered immediately (approximately 1 min) following bile duct ligation (Table 6). In contrast, hepatic concentration of ouabain in bile duct ligated adult rats was almost 10 times greater than hepatic levels in sham operated adults (Table 6).

Effect of Pentobarbital-Induced Hypothermia and Ouabain Disposition

Intraperitoneal administration of pentobarbital (30 mg/kg) resulted in a significant reduction in body temperature to 30.6±0.3°C in 15 day old rats and 33.3±0.1°C in adult rats (Table 7). Low body temperature in these rats was associated with decreased hepatic transport of ouabain then compared to pentobarbital treated rats whose body temperatures were regulated to approximately 38°C by means of a heat lamp. Low body temperature resulted in significant retention of ouabain in plasma of 15 day and adult rats when measured 30 minutes following tail vein injection of the glycoside. In 15 day old rats,

Figure 8. Effect of bile duct ligation on elimination of (^{3}H)ouabain from plasma of 15 day old rats. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein immediately (1 minute) or 1 hour following bile duct ligation. At various times following administration of (^{3}H)ouabain, blood was obtained by cardiac puncture and plasma samples analyzed for tritium. Each point represents the mean ± S.E. for 3 rats obtained from 3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma ouabain concentration significantly different from sham operated control ($p<0.05$).

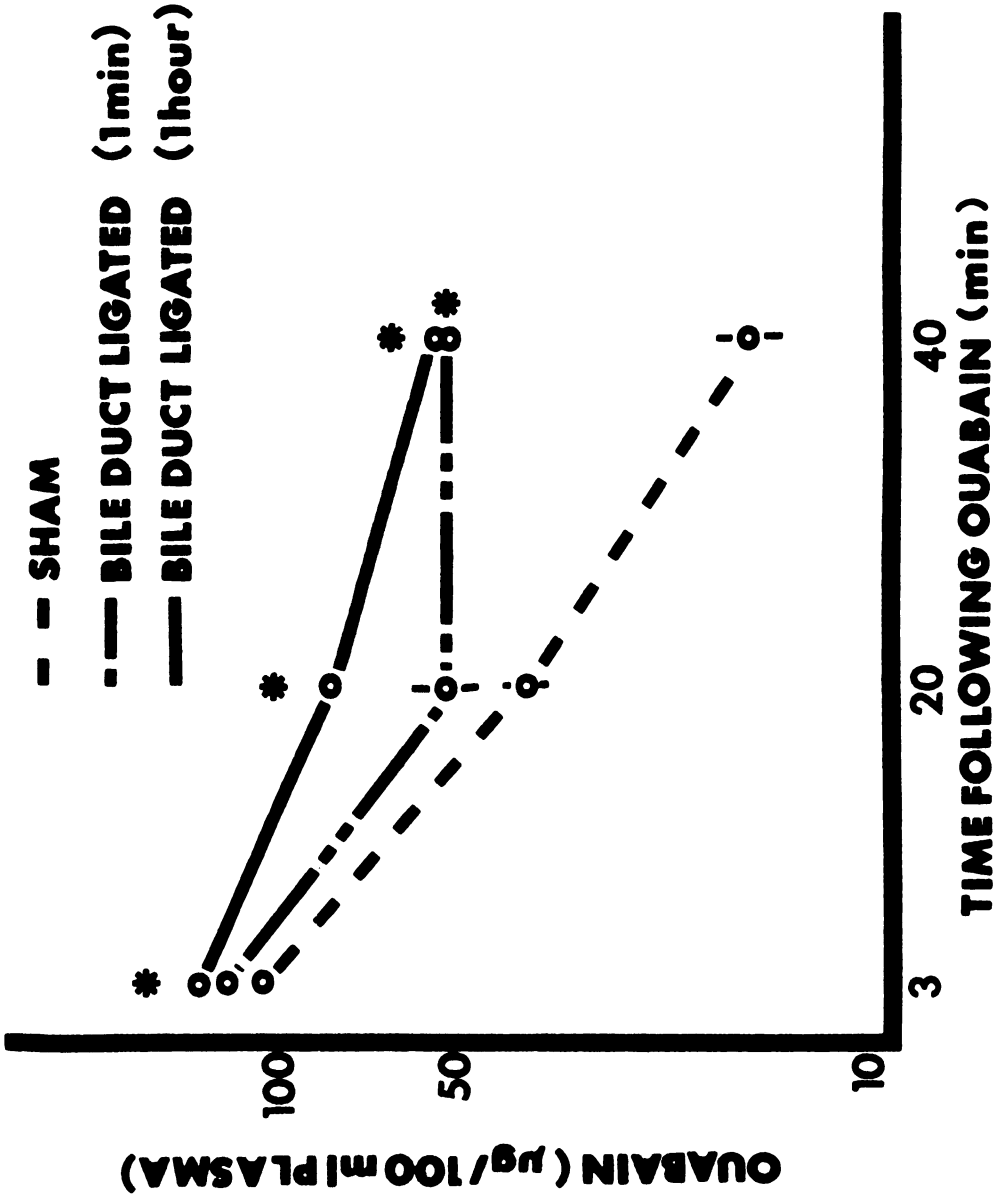
SHAM
BILE DUCT LIGATED (1min)
BILE DUCT LIGATED (1hour)
OUABAIN (μg/100 ml PLASMA)
100
50
10
3
20
40
TIME FOLLOWING OUABAIN (min)

Figure 8

TABLE 5

Cumulative Intestinal Ouabain[a] in Sham or Bile Duct Ligated 15 Day Old and Adult Rats

Age	Treatment[b]	Intestinal Ouabain (µg/kg B.W.)
15 day old	Sham	116.6±23.0[c]
15 day old	Bile Duct Ligated (1 hour)	20.7± 1.9[d]
15 day old	Bile Duct Ligated (1 min)	22.7±10.6[d]
Adult	Sham	236.6±63.1
Adult	Bile Duct Ligated (1 hour)	6.8± 0.1[d]

[a]Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 40 minutes the intestine was analyzed for tritium.

[b]Common bile duct was isolated and ligated following a midline abdominal incision. Animals were injected with ouabain either 1 minute or 1 hour following surgical procedure.

[c]Values represent the mean ± S.E. for 3-4 rats. Fifteen day old animals were obtained from 3 litters.

[d]Significantly different from sham at appropriate age ($p<0.05$).

TABLE 6

Hepatic Concentration of Ouabain[a] Following Intravenous Administration in Sham or Bile Duct Ligated (BDL) Adult and 15 Day Old Rats

Age (Days)	Treatment[b]	Hepatic Ouabain (μg/gm)		
		3 min	20 min	40 min
15	Sham	2.1±0.1[c]	6.0±0.2	6.0±0.5
15	BDL (1 hr)	1.0±0.3[d]	1.5±0.2[d]	1.5±0.5[d]
15	BDL (1 min)	2.2±0.1	7.4±0.7	5.0±0.3[d]
Adult	Sham	-------	-------	0.5±0.0
Adult	BDL (1 hr)	-------	-------	4.6±0.7[d]

[a] Rats were injected with ^{3}H-ouabain (1 mg/kg) and at designated times the liver was analyzed for tritium.

[b] Common bile duct was isolated and ligated following a midline incision. Animals were injected with ouabain either 1 minute or 1 hour following surgical procedure.

[c] Each value represents the mean ± S.E. for 3-4 rats. Fifteen day old animals were obtained from 3 litters.

[d] Significantly different from sham of the appropriate age ($p<0.05$).

TABLE 7

Effect of Anesthesia Induced Hypothermia on Ouabain[a] Tissue Distribution in 15 Day Old and Adult Rats

Treatment[b]	Mean Body Temperature (°C)	Plasma (μg/100 ml)	Liver (μg/kg BW)	Intestine (μg/kg BW)
		15 Day Old		
		Ouabain Distribution		
Pentobarbital	30.6±0.3[c,d]	38.3±5.7[d]	120.6±19.5	23.3± 2.7[d]
Pentobarbital + Heat Lamp	38.0±0.0	19.5±2.4	122.4±14.2	91.8±16.5
		Adult		
		Ouabain Distribution		
Pentobarbital	33.3±0.1[d]	4.7±0.3[d]	37.3± 6.3[d]	147.4±13.1
Pentobarbital + Heat Lamp	37.9±0.1	3.1±0.4	16.6± 3.5	179.2±13.9

[a]Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 30 min plasma, liver and intestine were analyzed for tritium.

[b]Animals were injected intraperitoneally with pentobarbital (30 mg/kg) and were either placed under a heat lamp to maintain normal body temperature or were placed in cages without heat lamp.

[c]Each value represents the mean ± S.E. for 4 rats. Fifteen day old animals were obtained from 3 litters.

[d]Significantly different from pentobarbital + Heat Lamp ($p<0.05$).

retention of ouabain in plasma in the non-heat regulated group correlated to a 75% reduction in hepatic excretion (intestinal) of ouabain. In adults, the slight (19%) depression of cumulative (30 minutes) intestinal ouabain in non-heat regulated adult rats was not statistically significant (Table 7). Hepatic ouabain content was unaffected by low body temperature in the 15 day old rats, whereas in adults, hepatic ouabain content was more than doubled in animals not placed under a heat lamp (Table 7).

Effect of Carbon Tetrachloride (CCl_4) on Hepatic Transport of Ouabain in Developing Rats

The dose of CCl_4 used in this study (1 ml/kg) did not result in the death of animals at any age (data not shown). The effect of CCl_4 on hepatic transport of ouabain in rats of various ages is shown in Figure 9. CCl_4 treatment resulted in significantly higher plasma concentrations of ouabain at every age tested. Coincident with the retention of ouabain in plasma, CCl_4 treated animals excreted less ouabain into bile. The lower amount of intestinal (biliary) ouabain was significant except in 15 day old animals (Figure 9).

Of particular interest is the effect of CCl_4 on hepatic content of ouabain. In young animals (less than 25 days of age), CCl_4 treatment resulted in significantly less ouabain in the liver. In contrast, 46 day old CCl_4-treated animals had significantly more ouabain in the liver. Although ouabain was retained in plasma of 25 and 33 day old treated rats and less intestinal ouabain was detected in these animals, no significant differences were observed in the hepatic content of ouabain (Figure 9).

Figure 9. Effect of carbon tetrachloride (CCl_4, 1 ml/kg) on the concentration of ouabain in plasma, the amount of ouabain in the liver, and the amount of ouabain excreted into the intestine 30 minutes after (^3H)ouabain (1 mg/kg, i.v.) administration in rats of various ages. Each point represents the mean ± S.E. for 5-8 rats obtained from 2-3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates values significantly different from corresponding control ($p<0.05$).

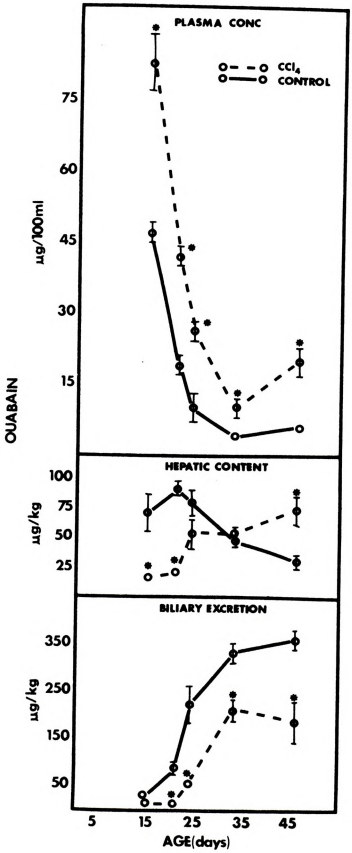
PLASMA CONC
CCl4
CONTROL
OUABAIN
µg/100ml
75
60
45
30
15
HEPATIC CONTENT
µg/kg
100
75
50
25
BILIARY EXCRETION
µg/kg
350
250
150
50
5
15
25
35
45
AGE(days)

Figure 9

Digoxin-Mediated Inhibition of Ouabain Transport in Adult and Developing Rats

Simultaneous tail vein injection of digoxin and ouabain resulted in retention of ouabain in plasma in 15 day old, 21 day old, and adult rats when compared to rats administered ouabain alone (Figure 10). Statistically significant increases in plasma ouabain concentration were detected in rats administered digoxin plus ouabain 20 and 40 minutes following injection in 15 day old rats, and 3 and 20 minutes following injection in 21 day old and adult rats when compared to the appropriate control (Figure 10). Digoxin treatment resulted in significantly lower cumulative hepatic excretion of ouabain only in 15 day old rats (Table 8). In 15 day old rats, digoxin-mediated transport inhibition resulted in significantly lower hepatic ouabain content 20 and 40 minutes following drug administration (Figure 11). Similarly, hepatic ouabain content in 21 day old rats was also less in rats administered digoxin plus ouabain when compared to animals injected with ouabain alone (Figure 11). In adult rats, 3 minutes following administration of the drugs, hepatic ouabain content was significantly lower in the digoxin plus ouabain group; however, this relationship changed with time and 20 and 40 minutes following injection, hepatic ouabain content was higher in rats administered both drugs (Figure 11).

Toxicity of Polybrominated Biphenyls (PBBs) in Developing Rats

Exposure to 50 ppm PBBs from birth to postnatal day 49 did not affect growth or mortality rates in rats. Initial treatment in these animals began at birth through the mother's diet and was continued at postnatal day 28 in the weanlings diet (Figures 12, 13). However,

Figure 10. Effect of digoxin on elimination of (^{3}H) ouabain from plasma of 15 day old, 21 day old, and adult rats. Rats were administered (^{3}H) ouabain (1 mg/kg) or (^{3}H)ouabain (1 mg/kg) plus digoxin (0.5 mg/kg) via the tail vein and following 3, 20, and 40 minutes, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 3-4 rats. Fifteen day old and 21 day old rats were obtained from 3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma ouabain concentration significantly different from values obtained from rats administered ouabain alone ($p<0.05$).

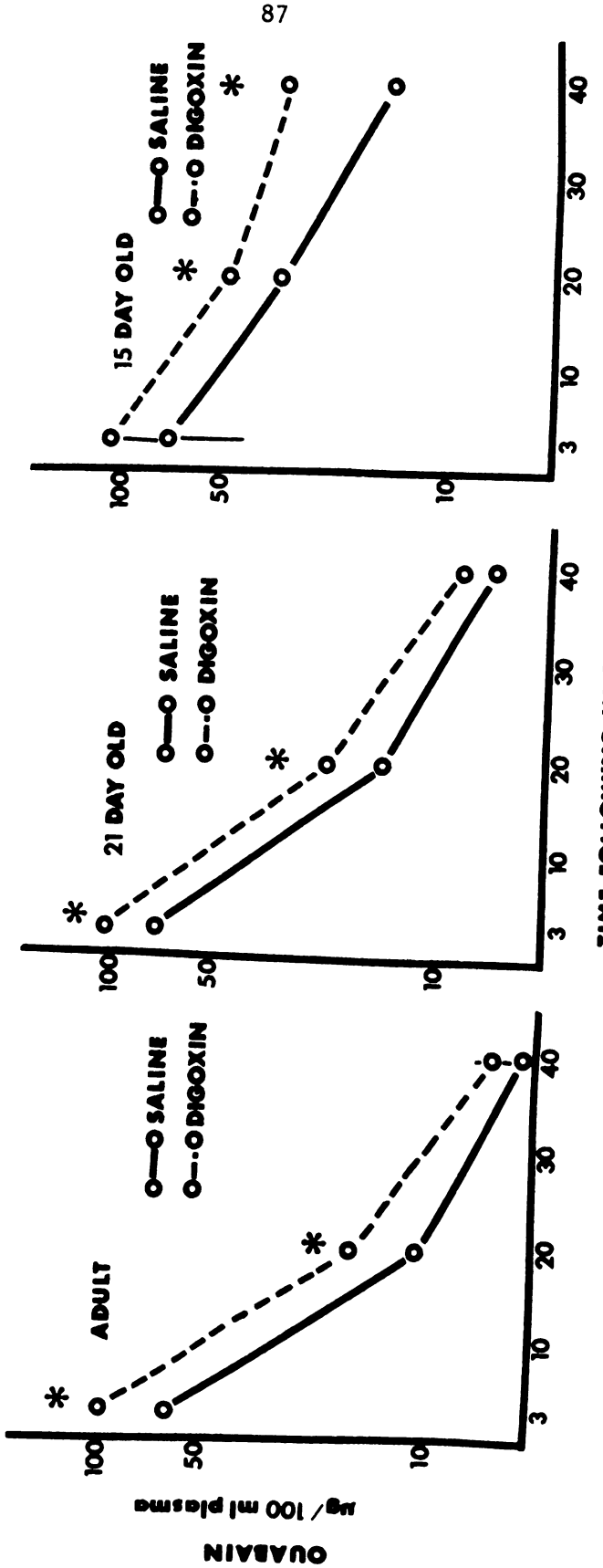
OUABAIN
mg/100 ml plasma
ADULT
21 DAY OLD
15 DAY OLD
O—O SALINE
O--O DIGOXIN
TIME FOLLOWING INJECTION
(min)
100
50
10
3
10
20
30
40
*

Figure 10

TABLE 8

Effect of Digoxin on Cumulative Hepatic Excretion of Ouabain in Developing and Adult rats

Age	Ouabain Excretion (µg/kg Body Wt)[a] Ouabain + Saline	Ouabain + Digoxin[b]
15 days	107.6±11.6[c]	48.9± 5.6[d]
21 days	209.8±37.7	119.3±50.7
Adult	288.2±27.6	285.2±39.9

[a]Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 40 minutes the intestine was analyzed for tritium.

[b]Digoxin (0.5 mg/kg) was administered simultaneously with ^{3}H-ouabain (1 mg/kg).

[c]Each value represents the mean ± S.E. for 3-4 rats. Fifteen and 21 day old animals were obtained from 3 litters.

[d]Significantly different from rats injected with ^{3}H-ouabain alone ($p<0.05$).

Figure 11. Effect of digoxin on hepatic content of (^{3}H)ouabain in 15 day old, 21 day old, and adult rats. Rats were administered (^{3}H)ouabain (1 mg/kg) or (^{3}H)ouabain (1 mg/kg) plus digoxin (0.5 mg/kg) via the tail vein and following 3, 20, and 40 minutes, samples of liver were analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 3-4 rats. Fifteen day old and 21 day old rats were obtained from 3 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates hepatic ouabain content significantly different from values obtained from rats administered ouabain alone ($p<0.05$).

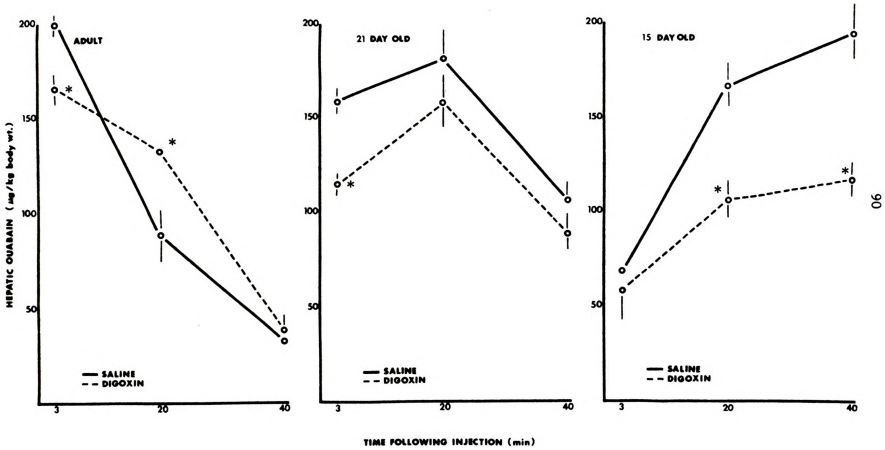
ADULT
21 DAY OLD
15 DAY OLD
HEPATIC OUABAIN (ug/kg body wt.)
200
150
100
50
3
20
40
SALINE
DIGOXIN
TIME FOLLOWING INJECTION (min)

Figure 11

Figure 12. Effect of exposure to polybrominated biphenyls (PBBs) from birth to 49 days after birth on the postnatal growth of rats. Young rats were exposed to PBBs by placement of 50 ppm PBBs in diet of lactating mother. At weaning, postnatal day 28, rats were weaned onto the same diet fed to mother. Each point is the mean rat weight within litters for 10-16 litters. There were 10 rats per litter on day 1.

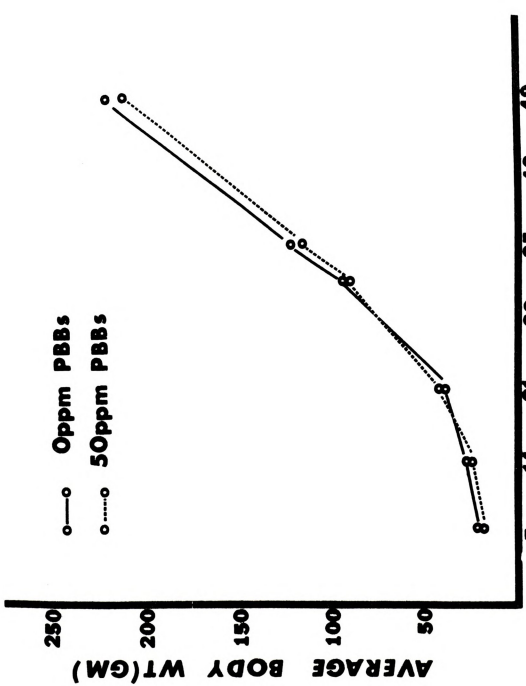
AVERAGE BODY WT(GM)
250
200
150
100
50
0ppm PBBs
50ppm PBBs
7
14
21
28
35
42
49
DAYS AFTER BIRTH

Figure 12

Figure 13. Effect of exposure to polybrominated biphenyls (PBBs) from birth to 49 days after birth on postnatal mortality of rats. Young rats were exposed to PBBs by placement of 50 ppm PBBs in diet of lactating mother. At weaning, postnatal day 28, rats were weaned onto the same diet fed to mother. Each point is the mean percent mortality within litters for 10-16 litters. There were 10 rats per litter on day 1.

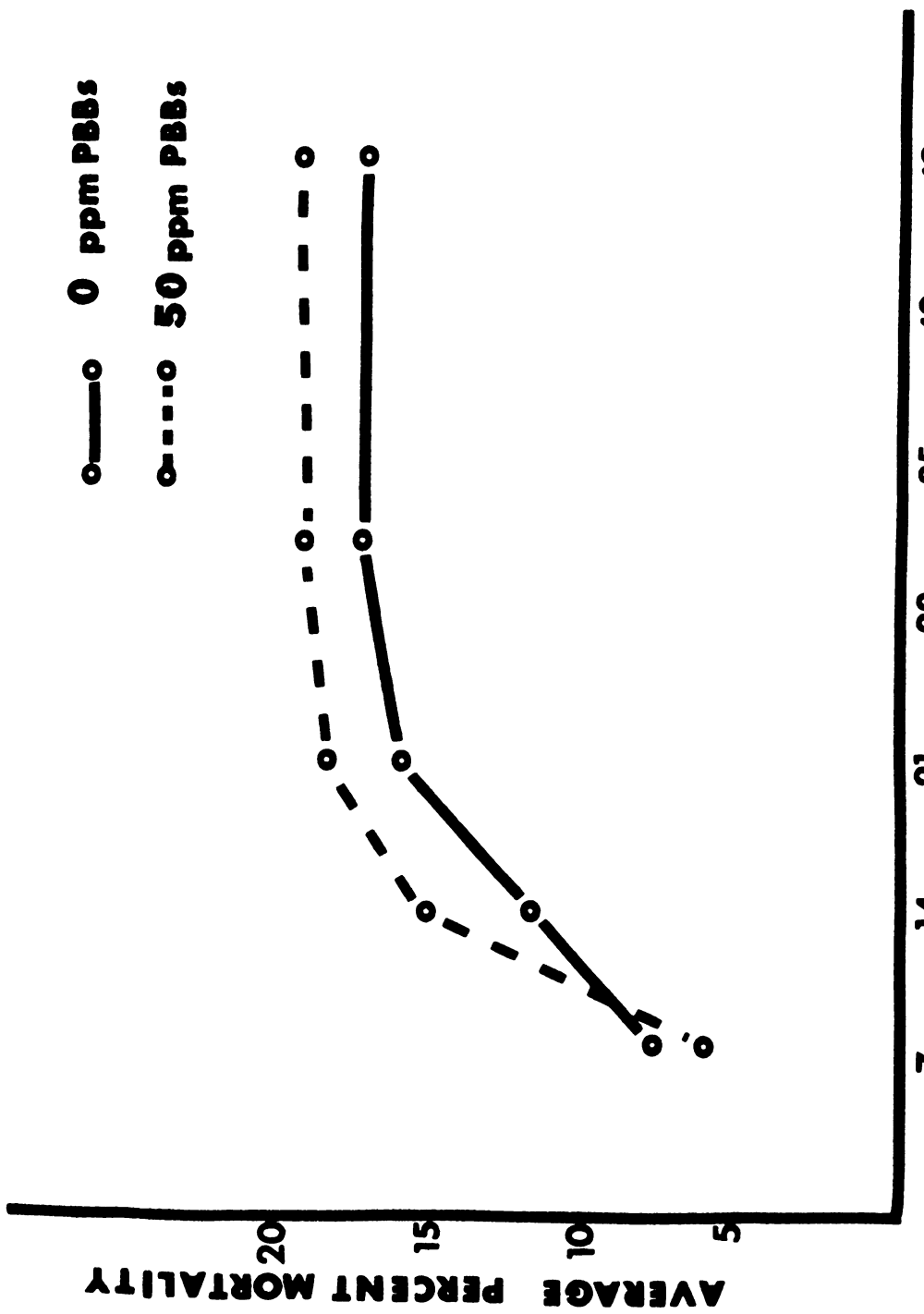
0 ppm PBBs
50 ppm PBBs
AVERAGE PERCENT MORTALITY
5
10
15
20
7
14
21
28
35
42
49
DAYS AFTER BIRTH

Figure 13

exposure to PBBs resulted in significant increases in liver weight to body weight ratios when compared to controls on postnatal days 15, 21, 35 and 49 (Table 9). In a separate cross-fostering study, one and two week body weight gain was unaffected by prenatal (from day 8 of gestation to birth) and/or postnatal (from birth to postantal day 15) exposure to PBBs (Table 10). However, when compared to 15 day old rat neonates whose natural and foster mother received no dietary PBBs (0-0), a significant elevation of liver to body weight ratio was detected in 15 day old rats exposed to PBBs prenatally (50-0), postnatally (0-50) and when combined pre- and postnatally (50-50) (Table 10).

Effect of PBBs on Ouabain Lethality in 15 Day Old Rats

When compared to ouabain LD_{50} in untreated rat neonates, ouabain LD_{50} values were significantly higher in 15 day old rats whose mothers had been exposed to dietary PBBs (Table 11). The ouabain LD_{50} in control rats was 20 mg/kg and was increased to 24.5 mg/kg in rats whose mothers were fed 50 ppm PBBs and to 35 mg/kg in rats whose mothers were fed 100 ppm PBBs (Table 11). The log-probability plots of the dose-lethality data from PBBs treated rats yielded curves that parallel to that from control rats.

Effect of Polybrominated Biphenyls (PBBs) on Hepatic Excretory Function in Developing Rats

Effect of Postnatal Exposure to PBBs on Ouabain Transport in Developing Rats

The effect of PBBs on elimination of ouabain from plasma of 15, 21, 35, and 49 day old rats is depicted in Figure 14. Exposure to PBBs began at birth by addition of 50 ppm PBBs to the mother's diet. Exposure was continued at postnatal day 28 through the weanling's

TABLE 9

Effect of Continuous Exposure to Polybrominated Biphenyls (PBBs) on Liver to Body Weight Ratio in Developing Rats

Age (Days)	Dietary PBBs (ppm)[a]	% Liver Wt/Body Wt	Percent Change
15	0	3.15±0.06[b]	147
15	50	4.62±0.05[c]	
21	0	3.98±0.10	161
21	50	6.41±0.12[c]	
35	0	3.98±0.07	155
35	50	6.18±0.16[c]	
49	0	4.73±0.08	139
49	50	6.59±0.10[c]	

[a] Concentration of PBBs in diet from day of birth until day of sacrifice (rats younger than 28 days, weaning age, received PBBs through mother's milk).

[b] Each value represents the mean ± S.E. for at least 4 rats obtained from 4 litters.

[c] Significantly different from 0 ppm PBBs at corresponding age ($p<0.05$).

TABLE 10

Effect of Prenatal, Postnatal, and Combined Pre- and Postnatal Exposure to Polybrominated Biphenyls (PBBs) on Body Weight Gain and % Liver Wt/Body Wt in 15 Day Old Rats

Parameter		Treatment[a]			
		0-0	50-0	0-50	50-50
Average Body Weight Gain (gms)	1 wk[b]	8.23 ± 0.17[d]	8.55 ± 0.20	8.24 ± 0.45	8.14 ± 0.74
	2 wk	17.12 ± 0.09	17.41 ± 0.52	17.6 ± 1.75	14.52 ± 3.81
Liver Wt/Body Wt (%)[c]		3.15 ± 0.06	3.47 ± 0.05[e]	5.21 ± 0.08[e]	4.96 ± 0.11[e]

[a]Treatments: Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50).

[b]Average body weight gained in grams during 1 and 2 weeks after birth. Rats were weighed on a whole litter basis and values represent average weight per rat.

[c]Liver to body weight ratios determined on postnatal Day 15.

[d]Each value represents the mean ± S.E. for at least 8 rats obtained from 4-8 litters.

[e]Significantly different from 0-0 ($p<0.05$).

TABLE 11

Effect of Dietary Polybrominated Biphenyls (PBBs) on Ouabain LD_{50} in 15 Day Old Rats

PBBs[a]	Ouabain LD_{50} (mg/kg)[b]	95% Confidence Limits	Potency Ratio
0 ppm	20.0[c]	17.9-22.4	----
50 ppm	24.5	22.6-26.6	1.23[d]
100 ppm	35.0	31.3-39.2	1.75[d]

[a] Concentration of PBBs in mother's diet from day of birth until day of the experiment.

[b] Twenty-four hour LD_{50} following injection (i.p.) of aqueous solutions of ouabain.

[c] Values obtained from at least 36 rats taken from 4-6 litters.

[d] Significantly different from 0 ppm ($p<0.05$).

Figure 14. Effect of exposure to polybrominated biphenyls (PBBs) on elimination of (^{3}H)ouabain from plasma of rats of ages ranging from 15-49 days. Young rats were exposed to PBBs by placement of 50 ppm PBBs in diet of lactating mother. At weaning, postnatal day 28, rats were weaned onto the same diet fed to mother. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 minutes, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 4 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma ouabain concentrations significantly different from values obtained from rats exposed to 0 ppm PBBs ($p<0.05$).

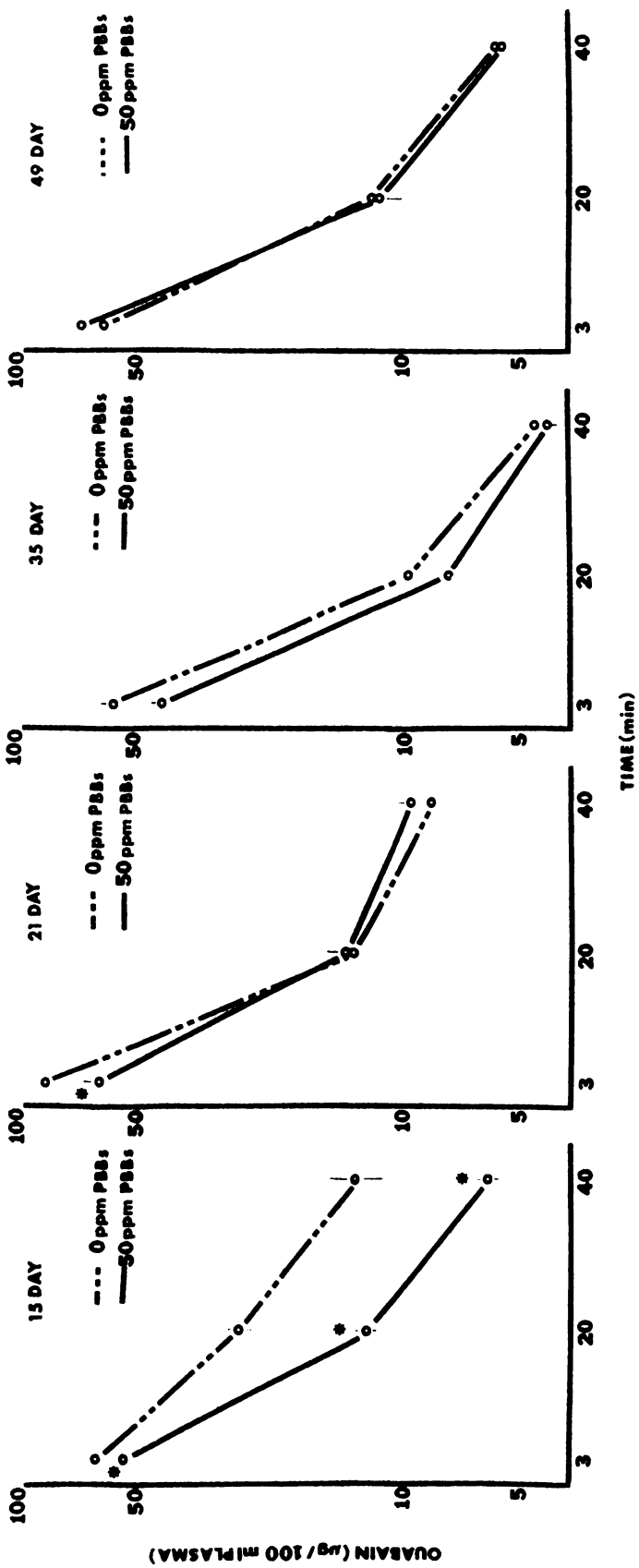
15 DAY
21 DAY
35 DAY
49 DAY
0 ppm PBBs
50 ppm PBBs
OUABAIN (μg/100 ml PLASMA)
TIME (min)
100
50
10
5
3
20
40

Figure 14

diets. Plasma concentrations of ouabain were significantly lower than control values in PBBs-exposed 15 day old rats 3, 20, and 40 minutes following ouabain injection and in PBBs-treated 21 day old rats 3 minutes following ouabain injection. In contrast, plasma concentrations of ouabain in 35 and 49 day old PBBs-exposed rats were not significantly different from plasma ouabain concentrations in the controls (Figure 14).

Total hepatic ouabain content was significantly higher than control values 3 minutes following ouabain injection in 15 day old and 21 day old PBBs-exposed rats, however, ouabain content in liver was significantly lower with respect to control values in PBBs-exposed 15 day old rats 40 minutes following ouabain administration and 20 minutes following ouabain in 21 day old treated rats (Figure 15). Hepatic content of ouabain was the same in control and PBBs treated 35 and 49 day old rats (Figure 15). The increases in hepatic ouabain content (3 min following ouabain in 15 and 21 day old treated animals) reflected the effect of PBBs on liver weight (Table 9). When compared to control rats of the same age cumulative 40 minute intestinal ouabain content was significantly elevated in PBBs exposed 15 day old rats, but not in 21, 35 and 49 day old PBBs-exposed animals (Table 12).

Effect of Prenatal and/or Postnatal Exposure to PBBs on Ouabain Transport in 15 Day Old Rats

Following a single bolus ouabain injection, plasma concentrations of ouabain were significantly lower in fifteen day old rats exposed to PBBs prenatally (from day 8 of gestation to birth) and/or postnatally (from birth to postnatal day 15) when compared to 15 day old controls (0-0) (Figure 16). Among all of the dietary treatment regimens,

Figure 15. Effect of exposure to polybrominated biphenyls (PBBs) on hepatic content of (^{3}H)ouabain in rats of ages ranging from 15-49 days. Young rats were exposed to PBBs by placement of 50 ppm PBBs in diet of lactating mother. At weaning, postnatal day 28, rats were weaned onto the same diet fed to the mother. Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20 and 40 minutes, samples of liver were analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 4 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates hepatic content of ouabain significantly different from values obtained from rats exposed to 0 ppm PBBs ($p<0.05$).

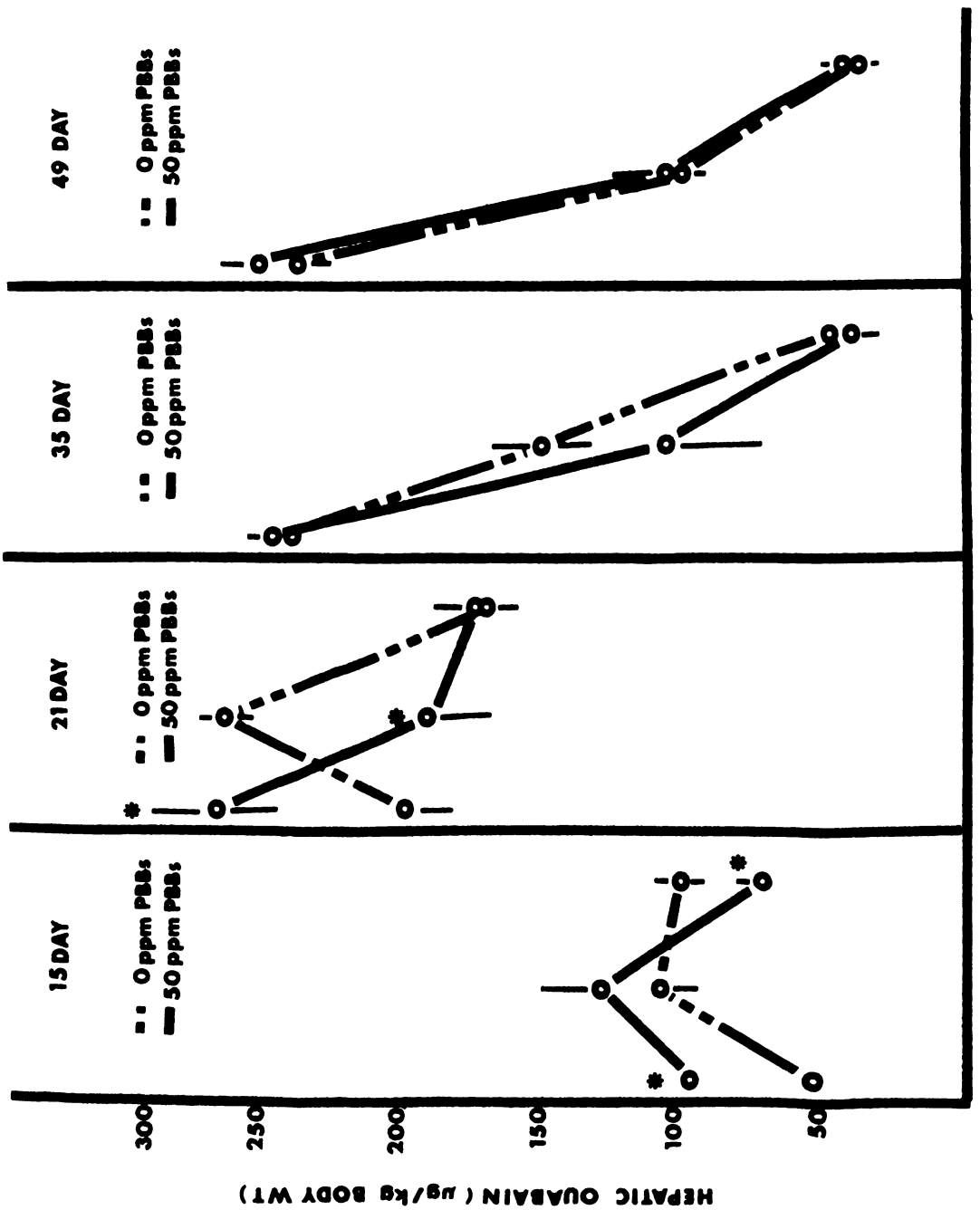
15 DAY
21 DAY
35 DAY
49 DAY
0 ppm PBBs
50 ppm PBBs
HEPATIC OUABAIN (µg/kg BODY WT)
300
250
200
150
100
50
3
20
40
TIME (min)

Figure 15

TABLE 12

Effect of Exposure to Polybrominated Biphenyls (PBBs) on Cumulative Hepatic Excretion of Ouabain[a] in Developing Rats

Age (Days)	Dietary PBBs (ppm)	Ouabain Excretion (µg/kg Body Weight)
15	0[b]	122.3 ± 10.7[d]
15	50[b]	289.5 ± 16.0[e]
15	50-0[c]	174.6 ± 14.3[e]
15	0-50[c]	274.1 ± 10.8[e]
15	50-50[c]	277.3 ± 28.4[e]
21	0[b]	178.4 ± 5.7
21	50[b]	202.1 ± 25.8
35	0[b]	211.6 ± 53.5
35	50[b]	206.0 ± 31.6
49	0[b]	343.1 ± 15.2
49	50[b]	398.0 ± 19.3

[a] Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 40 min the intestine was analyzed for tritium.

[b] Concentration of PBBs in diet from day of birth until day of sacrifice (rats younger than 28 days, weaning age, received PBBs through mother's diet).

[c] Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal Day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50).

[d] Each value represents the mean ± S.E. for at least 4 rats obtained from 4 litters.

[e] Significantly different from 0 ppm PBBs at corresponding age ($p<0.05$).

Figure 16. Effect of pre- and/or postnatal exposure to polybrominated biphenyls (PBBs) on elimination of (^{3}H)ouabain from plasma of 15 day old rats. Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50). Rats were administered (^{3}H) ouabain (1 mg/kg) via the tail vein and following 3, 20, and 40 minutes, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean for 6-8 rats obtained from 4 litters. Standard error (not shown for clarity) was approximately 10% of mean value. Asterisk indicates plasma ouabain concentration significantly different from values obtained from 0-0 ($p<0.05$).

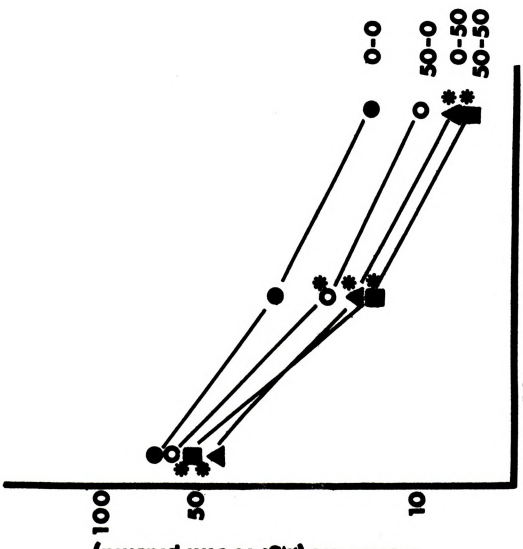
OUABAIN (µg/100ml plasma)
100
50
10
10
20
30
40
TIME (min)
0-0
50-0
0-50
50-50

Figure 16

prenatal exposure to PBBs (50-0) was least effective in enhancing ouabain elimination from plasma. When compared to plasma concentrations in control rats (0-0), rats prenatally exposed to PBBs (50-0) had significantly lower plasma concentration of ouabain at only the 20 minute time point; whereas, postnatal (0-50) and pre- and postnatal (50-50) exposure resulted in significantly lower plasma ouabain concentration 3, 20 and 40 minutes following administration (Figure 16). Similarly, cumulative 40 minute intestinal ouabain content, an estimate of biliary excretion of ouabain, was highest in rats exposed to PBBs postnatally, and was affected by prenatal PBBs exposure to a lesser, but statistically significant, extent when compared to controls (Table 12). Total hepatic ouabain content in these animals is depicted in Figure 17. When compared to values from control (0-0) rats, 15 day old animals exposed to PBBs postnatally (0-50) or pre- and postnatally (50-50) had significantly higher hepatic ouabain content 3 minutes following ouabain injection (Figure 17). These increases reflect the effect of PBBs on liver weight (Table 10). Rats treated with PBBs during the prenatal period (50-0) had higher hepatic ouabain content 3, 20, and 40 minutes following ouabain injection, however, these increases were not statistically significant.

Effect of PBBs on Initial Elimination Rate of Indocyanine Green (ICG) from Plasma in 21 Day Old Rats

The disappearance of ICG from plasma of 21 day old control rats and rats exposed to PBBs is depicted in Table 13 by the rate of ICG elimination from plasma. Correlation coefficient (r^2) for the rate of elimination (slope) of ICG from plasma of treated (.86) and control (.75) rats was significant. Elimination of ICG from plasma was

Figure 17. Effect of pre- and/or postnatal exposure to polybrominated biphenyls (PBBs) on hepatic content of (^{3}H)ouabain in 15 day old rats. Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50); 50 ppm prenatal, 50 ppm postnatal (50-50). Rats were administered (^{3}H)ouabain (1 mg/kg) via the tail vein and following 3, 20 and 40 minutes, samples of liver were analyzed for tritium (ouabain). Each point represents the mean for 6-8 rats obtained from 4 litters. Standard error (not shown for clarity) was approximately 10% of mean value. Asterisk indicates hepatic content of ouabain significantly different from values obtained from 0-0 ($p<0.05$).

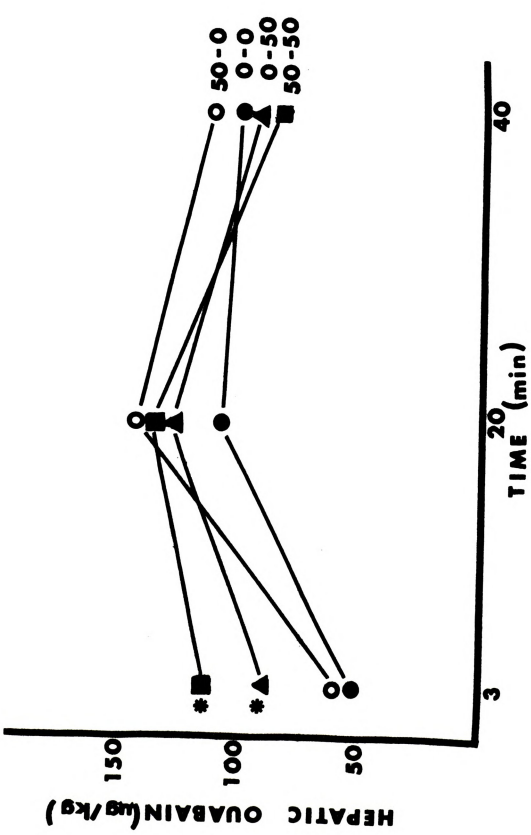
HEPATIC OUABAIN(μg/kg)
150
100
50
3
20
40
TIME (min)
50-0
0-0
0-50
50-50

Figure 17

TABLE 13

Initial Rate of Elimination of Indocyanine green (ICG) from Plasma in 21 Day Old Rats Exposed to Dietary Polybrominated Biphenyls (PBBs)

PBBs (ppm)[a]	Rate of Elimination (Slope)[b]	(±)Fiducial Limits	Estimated $t_{\frac{1}{2}}$ (min)
0	0.025[c]	0.010	10.5
50	0.042[d]	0.013	7.0

[a]Concentration of PBBs in mothers diet from day of birth until day of experiment.

[b]ICG (40 mg/kg) was injected via the tail vein and the rate of elimination of ICG from plasma was determined from plasma ICG concentrations at 1, 5, 10, and 15 min following injection by the method of least squares.

[c]Rate of elimination ± fiducial limits for 12 rats obtained from 3 litters.

[d]Significantly different from 0 ppm PBBs ($p<0.05$).

significantly greater in 21 day animals whose mothers were fed 50 ppm PBBs (Table 13). Animals (21 day old) used in this experiment were exposed with the same dose and treatment regimen of PBBs as were 21 day old animals used in the experiment depicted on Figure 14 for hepatic transport of ouabain. The initial rate (from 1 to 15 minutes following a single ICG injection) of ICG removal from plasma was 0.025±0.010 (mg/min) in control rats ($t_{\frac{1}{2}}$ = 10.5 min) and was 0.042±0.013 (mg/min) in rats exposed to PBBs ($t_{\frac{1}{2}}$ = 7 min) (Table 13).

Characteristics of Stimulation of Drug Transport in Young Rats

Digoxin-Mediated Inhibition of Ouabain Transport in Rats Exposed to Polybrominated Biphenyls (PBBs)

In comparison with plasma ouabain concentrations in rats injected with only ouabain, simultaneous tail vein injection of digoxin plus ouabain resulted in retention of ouabain in plasma of 15 day old control rats (0-0), and in plasma of rats exposed to PBBs (Figure 18). Plasma ouabain concentrations were significantly higher in digoxin treated rats than in controls (no digoxin): 3 and 40 min following drug administration in rats whose natural and foster mothers received no dietary PBBs (0-0); 3, 20, and 40 min following drug administration in 15 day old rats exposed to PBBs prenatally (50-0); and 3 and 20 min following drug administration in animals postnatally exposed to PBBs (0-50) (Figure 18). In 15 day old rats treated with all three exposure regimens to PBBs, digoxin-mediated transport inhibition resulted in a decrease in hepatic ouabain content for the entire 40 min experimental period (Figure 19). Significantly lower hepatic content of ouabain was detected in digoxin treated rats 3 min following administration of

Figure 18. Effect of digoxin on elimination of (^{3}H)ouabain from plasma of control 15 day old rats and 15 day old rats pre- or postnatally exposed to polybrominated biphenyls (PBBs). Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50). Rats were administered (^{3}H)ouabain (1 mg/kg) or (^{3}H)ouabain (1 mg/kg) plus digoxin (0.5 mg/kg) via the tail vein and following 3, 20, and 40 minutes, blood was obtained by cardiac puncture and plasma samples analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 3-5 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates plasma ouabain concentration significantly different from values obtained from rats administered ouabain alone ($p<0.05$).

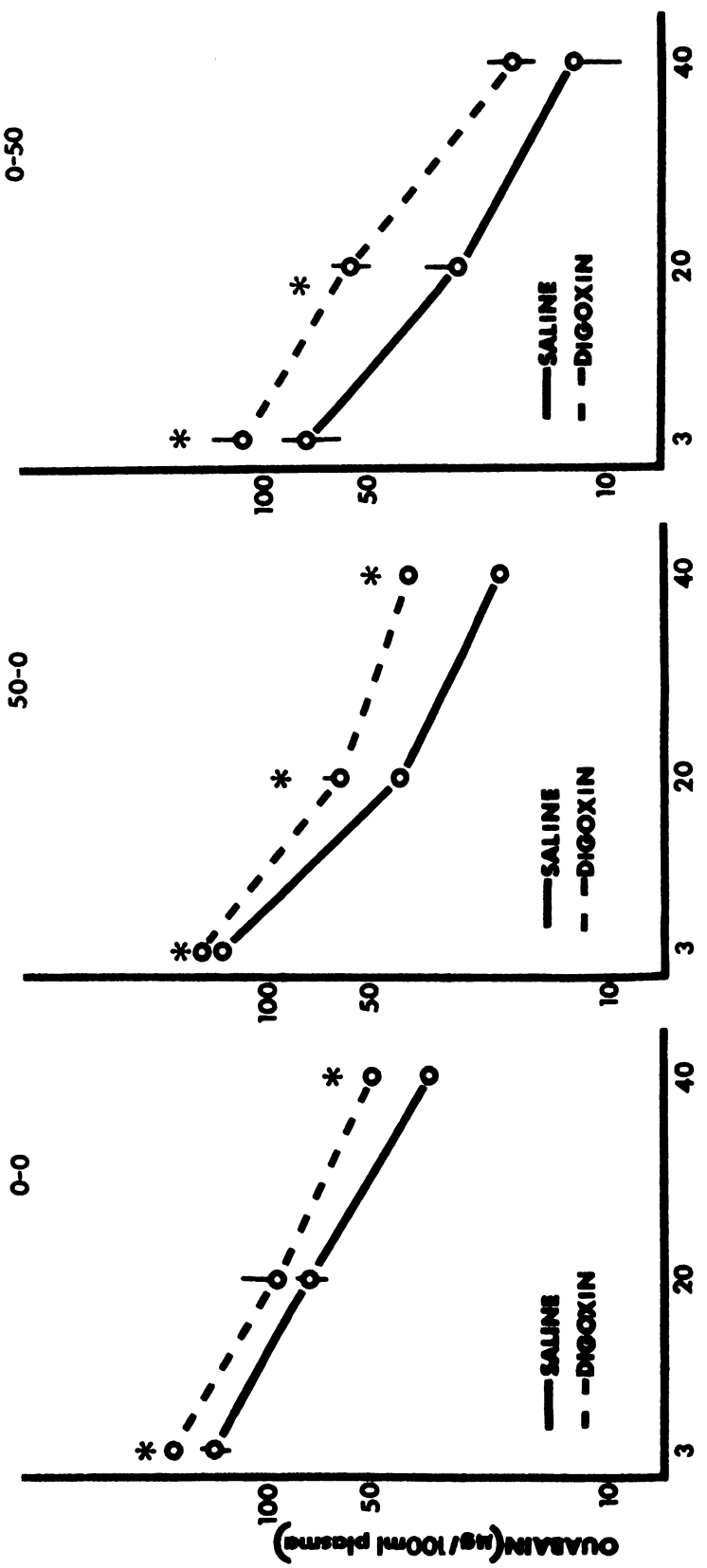
0-0
50-0
0-50
SALINE
DIGOXIN
OUABAIN(ng/100ml plasma)
TIME(min)
100
50
10
3
20
40
*

Figure 18

Figure 19. Effect of digoxin on hepatic content of ouabain in 15 day old control rats and 15 day old rats pre- or postnatally exposed to polybrominated biphenyls (PBBs). Pregnant rats were fed 0 or 50 ppm PBBs from day 8 of gestation to postnatal day 15. All litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50). Rats were administered (^{3}H)ouabain (1 mg/kg) or (^{3}H)ouabain (1 mg/kg) plus digoxin (0.5 mg/kg) via the tail vein and following 3, 20, and 40 minutes, liver samples were analyzed for tritium (ouabain). Each point represents the mean ± S.E. for 3-5 rats obtained from 4 litters. When standard error bars are not shown, standard error was smaller than the diameter of the point. Asterisk indicates hepatic ouabain content significantly different from values obtained from rats administered ouabain alone ($p<0.05$).

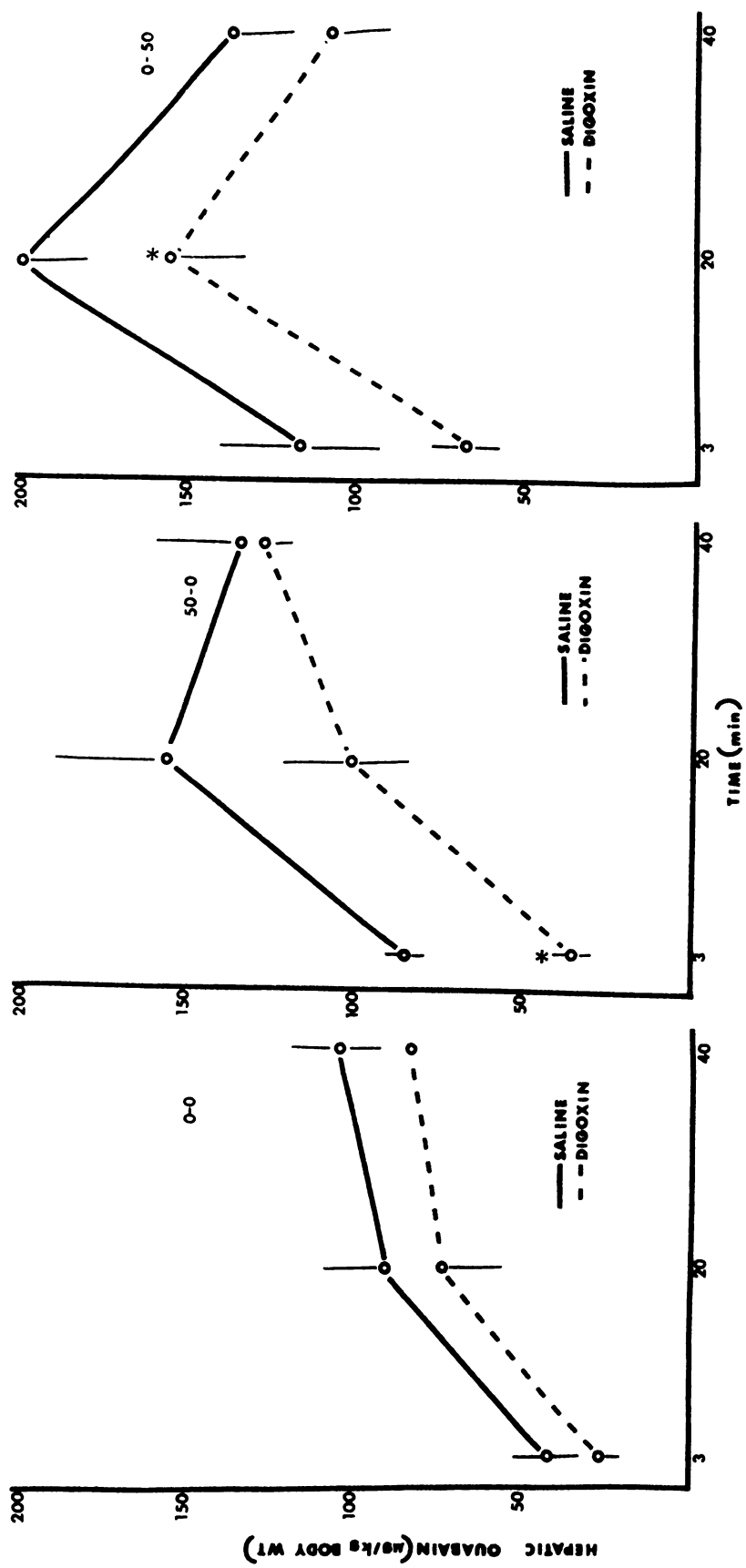
HEPATIC OUABAIN (ng/kg BODY WT)
200
150
100
50
3
20
40
TIME (min)
0-0
50-0
0-50
SALINE
DIGOXIN

Figure 19

drugs in the 50-0 (prenatal PBBs) group and 20 min following digoxin injection in the 0-50 (postnatal PBBs) group (Figure 19). When compared to rats administered ouabain alone, cumulative 40 min intestinal ouabain content, an estimate of biliary excretion of ouabain, was significantly lower in digoxin-treated control rats and in digoxin-treated rats that were exposed to PBBs postnatally (Table 14).

Effect of Carbon Tetrachloride (CCl_4) on Tissue Distribution of Ouabain in 15 Day Old Rats Treated with Polybrominated Biphenyls (PBBs)

The effect of CCl_4 on hepatic transport of ouabain in control and PBBs treated 15 day old rats is shown in Table 15. CCl_4 treatment resulted in significantly higher plasma ouabain concentration in control (0 ppm) rats and in 15 day old rats whose mothers were fed 50 and 100 ppm PBBs. Similarly, CCl_4 treated rats excreted less ouabain into intestine than rats injected only with corn oil. The retention of ouabain in plasma and depressed hepatic excretion of ouabain (intestinal) following CCl_4 correlated to a decrease in total hepatic ouabain content in 15 day old rats exposed to all doses of PBBs (Table 15).

TABLE 14

Effect of Digoxin on Cumulative Hepatic Excretion of Ouabain in 15 Day Old Rats Exposed to Polybrominated Biphenyls (PBBs)

Treatment	Ouabain Excretion (µg/kg Body Wt)[a] Ouabain + Saline	Ouabain + Digoxin[b]
Control (0-0)[c]	70.3± 8.5[d]	40.2± 6.4[e]
Prenatal PBBs (50-0)[c]	115.4±30.1	72.8±17.1
Postnatal PBBs (0-50)[c]	140.4± 6.4	113.3± 6.6[e]

[a] Rats were injected with ^{3}H-ouabain (1 mg/kg) via the tail vein and after 40 minutes the intestine was analyzed for tritium.

[b] Digoxin (0.5 mg/kg) was administered simultaneously with ^{3}H-ouabain (1 mg/kg).

[c] Pregnant rats were fed 0 or 50 ppm PBBs from Day 8 of gestation to postnatal Day 15. Litters were cross-fostered at birth to give litters born to and nursed by mothers with the following dietary exposures: 0 ppm prenatal, 0 ppm postnatal (0-0); 50 ppm prenatal, 0 ppm postnatal (50-0); 0 ppm prenatal, 50 ppm postnatal (0-50).

[d] Each value represents the mean ± S.E. for 4-5 rats obtained from 4 litters.

[e] Significantly different from rats injected with ^{3}H-ouabain alone ($p<0.05$).

TABLE 15

Effect of Carbon Tetrachloride (CCl_4) on Tissue Distribution of Ouabain[a] in 15 Day Old Rats Treated With Polybrominated Biphenyls (PBBs)

Treatments		Ouabain		
PBBs[b]	CCl_4 (ml/kg)[c]	Plasma (µg/100 ml)	Liver (µg/kg body wt)	Intestine (µg/kg body wt)
0 ppm	0	21.5± 3.7[d]	149.5±14.4	118.3±24.3
0 ppm	0.5	58.5± 4.1[e]	35.1± 8.2[e]	29.2± 3.8[e]
50 ppm	0	12.9± 1.6	125.9±14.5	262.3±17.0
50 ppm	0.5	65.8± 0.9[e]	29.6± 3.9[e]	18.5± 0.6[e]
100 ppm	0	9.1± 1.1	122.5±12.5	289.4± 9.2
100 ppm	0.5	76.7±31.1[e]	21.0± 5.3[e]	16.0± 1.5[e]

[a] Rats were injected with 1 mg/kg ouabain (H^3) via the tail vein and after 30 min plasma, liver, and intestine were analyzed for tritium.

[b] Concentration of PBBs in mother's diet from day of birth until day of experiment.

[c] CCl_4 was injected (i.p.) 24 hr before ouabain.

[d] Each value represents the mean ± S.E. for 3-6 rats obtained from 3 litters.

[e] Significantly different from corresponding 0 ml/kg CCl_4 group ($p<0.05$).

DISCUSSION

Hepatic Transport of Ouabain and Sulfobromophthalein in Developing Rats

In a series of experiments, Klaassen (1972,1973b) demonstrated the low capacity of young rats for biliary excretion of drugs. Plasma concentrations of sulfobromophthalein (BSP) were five times higher in newborns than in adults when measured 30 minutes following a single intravenous dose of BSP, and did not reach adult levels until the rats were one month of age. Similarly, young rats were unable to effectively eliminate ouabain from plasma when compared to adults. The contention that plasma retention of these drugs in the young was due to decreased biliary excretion was tested directly and young rats excreted significantly less ouabain (Klaassen, 1972) and BSP (Klaassen, 1973b) into bile (intestine) than adults.

Maturation of hepatic excretory function was also observed in the present investigation. Plasma concentrations of ouabain were higher in 15 day old rats than in older animals and appeared to reach adult levels when rats were 35 days old (Figure 1). Coincident with retention of ouabain in plasma, young rats also excreted less ouabain into intestine (Table 1). Retention of BSP in plasma was observed in eighteen day old rats, when compared to plasma levels in adults, which again demonstrates that rats less than one month of age are immature in their ability to eliminate drugs from plasma (Figure 3).

Elimination of xenobiotics from plasma into bile occurs in a stepwise manner. Drug transport into bile requires specific uptake into liver, intrahepatic metabolism and storage, and finally secretion from liver into bile. The evidence supporting specific transport at both the sinusoidal (uptake) and canalicular (secretion) membranes is, for the most part, descriptive. Drugs concentrate in liver many times above that in plasma and concentration in bile is higher than that in liver (Kupferberg and Schanker, 1968; Wheeler, 1969). The two-step transport process is often reflected in the shape of the curve representing the elimination of drugs from plasma. When plasma concentrations of drugs are plotted on a logarithmic scale as a function of time following drug administration, the exponential plot takes the shape of 2 straight lines (Serge, 1971). An initial rapid slope represents hepatic uptake and is followed by a more prolonged second line representing biliary excretion. In adult rats, the initial rapid phase for the plasma disappearance of ouabain has a half-life ($t_{\frac{1}{2}}$) of 1.5 minutes and the $t_{\frac{1}{2}}$ for the slow components is 26 minutes (Meijer et al., 1976). Although plasma concentrations of ouabain were determined only three times following ouabain administration, the plasma disappearance of ouabain in 25, 35, 45, and, to a lesser extent 21 day old rats, appeared to be biphasic (Figure 1). The initial slope (fast component) was between the 3 and 20 minute time points and the more prolonged curve was between the 20 and 40 minute intervals. In contrast, the elimination of ouabain from plasma in 15 day old rats appeared to take the shape of a single line for the entire (3 to 40 minute) experimental period (Figure 1). This may suggest that elimination of ouabain from plasma in 15 day old rats is different from that in older animals and

that the removal of ouabain from plasma may be dependent on only one transport step (Figure 1).

Hepatic concentration and total hepatic ouabain content (μg in liver per kg body weight) changes with time following a single bolus injection of ouabain (Figure 2). At any given time following administration, the hepatic concentration of ouabain is probably the net result of a number of events: 1) Ouabain is actively accumulated into rat liver slices (Kupferberg and Schanker, 1968) and thus hepatic accumulation of the glycoside may be due to an active uptake process. 2) However, accumulation of the drug into liver creates a concentration gradient across the sinusoidal membrane and thus, efflux of ouabain from liver may occur as ouabain diffuses back into plasma. 3) Ouabain efflux from liver is also the result of transport across the canalicular membrane into bile. Biliary excretion of ouabain from liver into bile may be carrier mediated and thus ouabain is concentrated in bile across the canalicular membrane (Kupferberg and Schanker, 1968). 4) This may result in passive diffusion of the drug back into liver.

In rats 25 days old and older, both hepatic ouabain concentration and total hepatic ouabain content were maximal 3 minutes following ouabain injection (Figure 2). Based on the injected dose of ouabain (1 mg/kg), approximately 20-25% (200 μg/kg body wt) of the dose was in the liver at the early time point in these animals (Figure 2). These results are similar to observations of Meijer et al. (1976) when 24% of the dose of ouabain was in liver of adult rats 5 min after injection and decreased with time after administration. From their observations, and the assumption that ouabain is excreted into bile unchanged (Kupferberg and Schanker, 1968), the investigators concluded that biliary

excretion of ouabain is rate-limiting in the overall transport from plasma into bile (Meijer et al., 1976). Thus, active uptake of ouabain that had been originally characterized in adult rat liver slices, is rapid in vivo and is not rate-limiting in the adult. The net decrease in hepatic ouabain levels with time following administration, therefore, reflects biliary excretion as well as diffusion of the glycoside back into plasma (Figure 2).

In contrast to the pattern of hepatic ouabain disposition in older animals, hepatic ouabain concentration was initially (3 min) low and then increased with time following administration in 15 day old rats (Figure 2). At the 3 minute time interval, hepatic ouabain content in 15 day old rats was approximately 80 μg/kg body weight which represents about one third that detected in liver of 21, 25, 35, and 45 day old rats (at 3 min; Figure 2). These results suggest that uptake of ouabain into liver is low in 15 day old rats, when compared to adults, and supports the previous suggestion that decreased uptake is one mechanism for immaturity of hepatic function in young rats (Klaassen, 1972,1975). The time dependent accumulation of ouabain in liver may also reflect the low capacity for hepatic uptake in the young. Thus, the rate of uptake in older rats is rapid ($t_{\frac{1}{2}}$ = 1.5 min; Meijer et al., 1976) and cannot be detected from the pattern of hepatic ouabain disposition since maximum hepatic ouabain levels were apparent at the earliest (3 min) time point. The rate of hepatic accumulation of ouabain in 15 day old rats is slower and thus detectable (Figure 2).

The pattern of disposition of ouabain in 21 day old rats is different from that observed in either 15 day old animals or in rats older than 25 days (Figure 2). Since hepatic ouabain levels at the 3 minute time point are higher in 21 day old rats than in the 15 day old animals, the mechanism(s) for hepatic uptake are apparently increasing during the third postnatal week. However, ouabain concentrations in liver increased with time following ouabain administration in 21 day old rats and were not maximum at the earliest (3 min) time following ouabain administration. Thus, since uptake is detectable, the rate of uptake may be lower in 21 day old rats than in 25, 35, and 45 day old animals (Figure 2). A similar conclusion may be made with respect to differences in hepatic content of BSP in 18 day old and adult rats (Figure 4). Hepatic uptake of BSP may be low in 18 day old rats when compared to adults and thus: 1) hepatic BSP content at the 3 minute time interval is significantly lower in the young animals, when compared to the adult, and 2) a rate of uptake is detectable in 18 day old rats; whereas in adults, maximum BSP content occurs at the earliest time (3 minutes) following BSP injection (Figure 4).

The pattern of ouabain and BSP disposition in liver in developing rats may therefore reflect the mechanism for the maturation of hepatic function. Since liver weight increases with age (Table 2; designated as liver to body weight ratio), functional development for BSP and ouabain uptake may be partially attributed to increases in liver mass and possibly higher rates of hepatic blood flow. The relative importance of these factors cannot be determined from the data presented in this investigation, however, the patterns of disposition of hepatic

ouabain content is the same as that for hepatic ouabain concentration (Figure 2). Thus, it is unlikely that increase in liver mass is the only factor responsible for the development of hepatic uptake capacity.

Based on the assumption that uptake into liver in adult (25-45 day old) rats is rapid and is not rate-limiting for overall drug transport, a time dependent efflux of ouabain from liver would be associated with adult-like function. With this interpretation, the mechanisms for hepatic ouabain uptake are low in young rats and the age related maturation of uptake may be the major contributing factor to the maturation of overall hepatic function. This hypothesis is consistent with the characteristics of the morphological development of rat liver (DeWolf-Peeters et al., 1972). Canalicular structure, although underdeveloped in fetal and early neonatal life, appeared adult-like in 10 day old rats (DeWolf-Peeters et al., 1972). Thus, with the assumption that canalicular structure reflects hepatic secretory function, even the youngest group of rats in the present investigation (15 day old) may already be mature in their ability to excrete ouabain from liver. However, this function may be limited by the amount of ouabain available to it.

Although maturation of the hepatic excretory system may be attributed, to a large extent, to development of hepatic uptake mechanisms, decreased function of the remaining components of the excretory system may also contribute to overall functional immaturity. This may be suggested from the fact that ouabain accumulates in liver of 21 day old rats to approximately 30% of injected dose (at the 20 minute time interval; Figure 2) and thus, transport from liver into bile may be depressed in these animals. Similarly, accumulation of BSP in liver of

18 day old animals may be attributed, in part, to decreased hepatic excretion (Figure 4). In regard to BSP, decreased hepatic excretion may be related to low intrinsic capacity to excrete conjugated BSP or low in vivo metabolism of free BSP to the conjugated form. In vitro, hepatic activity of glutathione-S-aryltransferase, the enzyme that mediates conjugation of BSP to glutathione in liver, is low in young rats and increases with age (Combes and Stakelum, 1962; Krasner and Yaffe, 1968; Table 3). Since unconjugated BSP is transported from liver into bile at a slower rate than the conjugated form (Whelan et al., 1970b), accumulation of BSP in liver of 18 day old rats may be partially attributed to low conjugation capacity (Table 3). Thus, the low capacity for hepatic uptake of ouabain and BSP in young rats might not be the only mechanism for retention of these drugs in plasma.

In the present study, immaturity of hepatic uptake was most evident in 15 day old rats, the youngest animals studied. Klaassen (1972, 1973b,1974b,1975) detected low hepatic uptake of ouabain, BSP-GSH, BSP, and indocyanine green (ICG) in rats as young as 5 days old, and uptake capacity was depressed to the greatest extent in the youngest animals. Thus, relative to the adult, where hepatic uptake mechanisms are extremely rapid and not rate-limiting, the ability of young rats to accumulate drugs in liver from plasma may be markedly reduced. It is noteworthy therefore, that the time dependent pattern of hepatic disposition of ouabain was exactly opposite in 15 day old rats when compared to adult rats (25-45 days old; Figure 2) even though plasma concentrations of ouabain were decreasing with time following ouabain administration in both groups (Figure 1). This suggests that the transport

step influencing the elimination of ouabain from plasma in 15 day old rats is different from that in the adult. In young rats, elimination of ouabain from plasma correlates with ouabain uptake into liver. Thus, uptake may be the primary transport step for the elimination of ouabain from plasma of young rats (Figures 1,2). Whereas in the adult (25-45 day old), elimination of ouabain from plasma parallels elimination of the drug from liver (Figures 1,2) and suggests biliary excretion of ouabain is the essential transport step (Meijer et al., 1976). Conceptually, this may indicate that, in contrast to the situation in adult rats, ouabain uptake into liver of young rats may limit overall ouabain transport. This hypothesis would be consistent with the single, as opposed to biphasic, exponential plot that may be drawn for the plasma disappearance of ouabain in 15 day old rats (Figure 1).

Klaassen (1973b) demonstrated that decreased biliary excretion of BSP in 7 day old rats was primarily due to a slow rate of BSP uptake. The conclusion was based on the observation that both BSP and conjugated BSP (BSP-GSH) were retained in plasma of seven day old rats when compared to plasma concentrations in the adult (Klaassen, 1973b). Since the deficient BSP conjugating system in young rats (Combes and Stakelum, 1962; Krasner and Yaffe, 1968; Table 3) would not account for decreased biliary excretion of BSP-GSH, it was suggested that retention of BSP in plasma of newborn rats was primarily due to low hepatic uptake mechanisms (Klaassen, 1973b). In agreement with this hypothesis are the results depicted in Table 4. The rate of elimination of BSP and BSP-GSH from plasma of 15 day old rats was slower than the respective rate in adult rats (Table 4). It is noteworthy, however, that BSP-GSH ($t_{\frac{1}{2}}$ = 30 min) was removed from plasma at a slower rate than was

BSP ($t_{\frac{1}{2}}$ = 15 min) in 15 day old rats, whereas in adult rats, the rate of elimination of BSP ($t_{\frac{1}{2}}$ = 7 min) and BSP-GSH ($t_{\frac{1}{2}}$ = 6 min) from plasma was the same (Table 4). The slower rate of removal of BSP-GSH from plasma of 15 day old rats may reflect the importance of hepatic uptake in these animals. Although BSP-GSH is excreted from liver into bile more readily than is BSP (Whelan et al., 1970b), BSP uptake into liver is faster than uptake for BSP-GSH (Krebs, 1959; Meltzer et al., 1959; Whelan et al., 1970a). Since the rate of elimination of BSP and BSP-GSH from plasma of adult rats was the same, the lower affinity of BSP-GSH for hepatic uptake was not important in these animals during the 20 minute experimental period (Table 4). These studies therefore support the contention that hepatic uptake may be the rate-limiting step for overall drug transport in the newborn rat. Since uptake is not rate-limiting for transport in adults, these results provide a basis for further investigations for the possibility that biliary function in the newborn is not only quantitatively but also qualitatively different from function in the adult.

Differential Effects of Bile Salt Administration, Bile Duct Ligation and Hypothermia on Biliary Function in Adult and Developing Rats

Administration of the bile salt taurocholate significantly enhanced the elimination of BSP from plasma of adult rats (Figure 5). Although the same treatment produced a slight reduction in plasma BSP concentrations in 15 day old rats, this effect was negligible when compared to the effect in the adult (Figure 5). Bile salts (acids), secreted into the canalicular lumen, may provide an osmotic driving force for water and electrolytes and thus initiate bile flow (Sperber, 1959). The rate of bile flow was not determined in these rats, however,

it is likely that an exogenous load of taurocholate produced a stimulation of canalicular bile flow in the adult rats.

By reducing the BSP concentration gradient across the canalicular membrane and thus diminishing a passive back diffusion of BSP from the canaliculus to the hepatocyte, increased bile flow may result in enhanced excretion of BSP from liver into bile (O'Maille, 1966). Alternatively, taurocholate itself may exert a specific, as yet undefined, effect on the BSP excretory mechanism which may be independent of the effect of taurocholate on bile flow (Forker and Gibson, 1973; Gibson and Forker, 1974). Nonetheless, stimulation of BSP transport by taurocholate would be mediated at the canalicular and not sinusoidal membrane of the hepatocyte. The significant reduction of plasma BSP concentration in taurocholate treated adult rats confirms the idea that elimination of BSP from plasma of the adult is, at least at later time intervals, dependent on transport of BSP from liver into bile (Figure 5).

The lack of appreciable stimulation of BSP removal from plasma following taurocholate in young rats may be attributed to the inability of young rats to transport taurocholate into bile. Thus, relatively low taurocholate concentration in bile of 15 day old rats would not provide significant stimulation of BSP secretion. However, hepatic concentration of BSP was lower in taurocholate treated 15 day old rats when compared to 15 day old controls (Figure 6). Since it has been demonstrated that accumulation of BSP into liver is independent of taurocholate uptake (Paumgartner and Reichen, 1976), decreased hepatic concentration of BSP in taurocholate treated animals suggests enhanced

transport of BSP from liver into bile (Figure 6). These data are therefore inconsistent with the hypothesis that limited taurocholate transport into the bile canaliculus in 15 day old rats was the reason for the lack of effect of taurocholate on BSP elimination from plasma. Alternatively, biliary excretion of BSP in 15 day old rats might not play a significant role in the removal of BSP from plasma and thus, specific stimulation of BSP secretion in these animals would not be important. Moreover, since enhanced BSP secretion did not alter BSP removal from plasma, low hepatic secretion of BSP in young rats might not be the mechanism for the immaturity of overall transport function. These results support the hypothesis that hepatic elimination of drugs from plasma of young rats is essentially a function of hepatic uptake.

Cholestasis produced by acute bile duct ligation resulted in retention of BSP and ouabain in plasma of both adult and 15 day old rats (Figure 7). Bile duct ligation was confirmed to be effective in disrupting biliary excretion of ouabain in these animals by the significant reduction in cumulative intestinal ouabain content (Table 5). Although bile duct ligation appeared to produce a greater reduction in intestinal ouabain content in adult rats, calculation of ouabain excretion is made on a body weight basis and thus equal ouabain leakage through the ligature (or contamination) in adult and 15 day old rats would be greater per kg body weight in the younger rats (Table 5).

The magnitude of retention of ouabain and BSP in plasma of bile duct ligated adult rats was greater than that in bile duct ligated 15 day old rats (Figure 7). This may be especially obvious for the elimination of BSP from plasma where plasma concentration of BSP (at 40

minute time point) in bile duct ligated adult and 15 day old rats was 513 and 145% of sham operated (respective) control (Figure 7). These results suggest that drug secretion from liver into bile has a primary role in the elimination of compounds from plasma in the adult, whereas biliary excretion plays only a minor role in young rats. However, the rate of elimination of BSP and ouabain from plasma of adult rats is greater than that in newborns, and thus bile duct ligation may only appear to alter transport to a greater extent in the adult because of the higher (control) transport capacity. This being the case, significant retention of ouabain, and to a lesser extent BSP, in plasma of bile duct ligated 15 day old rats may demonstrate the importance of biliary excretion for elimination of these compounds from plasma (Figure 7).

Since bile duct ligation has recently been shown to impair net hepatic uptake capacity as well as biliary excretion (Yam and Roberts, 1977), the relative importance of biliary excretion in drug elimination from the plasma of adult and young rats cannot be accurately made by use of this technique. However, the influence of bile duct ligation on hepatic uptake has been demonstrated to be dependent on time of biliary obstruction. Yam and Roberts (1977) detected decreased hepatic uptake of BSP as early as 2 hours following ligation of the common bile duct. In the present investigation, decreased hepatic concentration of ouabain was observed in 15 day old rats administered ouabain 1 hour following bile duct ligation (Table 6). Thus, decreased uptake may occur as early as 1 hour following biliary obstruction.

Hepatic concentration of ouabain in 15 day old rats administered ouabain immediately (1 minute) following bile duct ligation, however, was not different from control concentration until 40 minutes following ouabain administration (Table 6). It may be noteworthy that retention of ouabain in plasma of 15 day old bile duct ligated rats coincided with the effect of bile duct ligation on hepatic uptake (Figure 8). Thus, the effect of bile duct ligation on ouabain transport in 15 day old rats was directly associated with impaired uptake and not decreased biliary excretion.

Hepatic concentration of ouabain in adult bile duct ligated (1 hour) rats was almost 10 times greater than the concentration in control rats, suggesting a marked depression of ouabain excretion from liver (Table 6). Moreover, Yam and Roberts (1977) detected comparable plasma retention of BSP in adult rats whose bile ducts were ligated 30 minutes and 2 hours prior to BSP administration even though decreased hepatic uptake was apparent only in the 2 hour group. Thus, in adult rats, the effect of bile duct ligation on hepatic uptake does not coincide with retention of drugs in plasma and decreased biliary excretion following bile duct ligation may be the most important effect in the adult.

Low body temperature following treatment with pentobarbital resulted in decreased ability of 15 day old and adult rats to transport ouabain from blood to bile. Relative to pentobarbital treated rats that were placed under a heat lamp to maintain normal body temperature, animals with rectal temperature of approximately 30-33°C excreted less ouabain into bile (intestine) and consequently retained ouabain in plasma (Table 7).

Hypothermia may influence both hepatic uptake and biliary excretion of ouabain. Decreased hepatic uptake of BSP was demonstrated in dogs with low body temperature (Brokaw and Penrod, 1949). This effect was attributed to reduction in hepatic blood flow that occurs during hypothermia (Brokaw and Penrod, 1949). Bile flow was reduced in rats with low body temperature and was assoicated with a decrease in the maximal excretion rate (T_m) of BSP and bilirubin (Roberts et al., 1967).

Although decreased ouabain transport in adult rats with low body temperature was associated with significant accumulation of ouabain in liver, this was not apparent in young rats and ouabain content in hypotherm and normotherm 15 day old rats was the same (Table 7). Even though hypothermia may result in nonspecific disruption of hepatic drug transport, these results demonstrate an age dependent response to this experimental treatment. Since hepatic ouabain content in adult rats was more than two times higher in animals with low body temperature, hypothermia appeared to preferentially alter drug transport from liver into bile. These results would be consistent with the hypothesis that hepatic secretion of ouabain limits overall ouabain transport in these animals. Inasmuch as hepatic ouabain content was not changed following hypothermia in 15 day old rats, it is difficult to ascertain the mechanism by which hypothermia depressed ouabain transport in these animals. Neverthless, these results 1) reaffirm the importance of hepatic secretion in limiting overall drug transport in adult rats and 2) suggest this is not necessarily the situation for drug transport in young rats.

The differential effects of bile salt administration, bile duct ligation, and hypothermia on drug transport in adult and young rats suggest, but do not necessarily prove, that the rate-limiting step for overall drug transport changes with age. It is clear, however, that experimental alterations in hepatic uptake may have occurred in adult rats following bile duct ligation and hypothermia, but were overshadowed by the effects on hepatic secretion, the rate-limiting step. Disruption in hepatic uptake may have been more obvious in 15 day old animals because of the relative importance of hepatic uptake for overall drug transport.

Effect of Carbon Tetrachloride (CCl_4) on Hepatic Transport of Ouabain in Developing Rats

The major effect of CCl_4 on hepatic transport of ouabain in adult rats was a decrease in the transport of ouabain from liver to bile (Reuning and Schanker, 1971). The data depicted in Figure 9 are consistent with this conclusion in that CCl_4-induced retention of ouabain in plasma was accompanied by accumulation of the drug in liver in 46 day old rats (Figure 9). This effect, however, appeared to be age dependent. Treated rats younger than 25 days of age could not effectively accumulate ouabain from plasma into liver (Figure 9). Since the effect of CCl_4 on hepatic concentration of ouabain is exactly opposite in rats of different ages, CCl_4 may act at a different "site" on the parenchymal cell in young rats than in adults.

Since it is believed that CCl_4 toxicity is mediated through a toxic metabolite (Slater, 1966; McLean and McLean, 1966; Plaa, 1975) and young animals are immature in their ability to metabolitze drugs (Rane et al., 1973; Fouts, 1973), the toxicity of CCl_4 may be expected

to be age dependent. From this explanation, however, one might expect a difference in the dose of CCl_4 required to elicit toxicity but not in the "site" of damage. It is important to note that the dose of CCl_4 (1 ml/kg) was enough to cause retention of ouabain in plasma but did not result in the death of animals at any age tested. Furthermore, although newborn (1 day old) rats are resistant to CCl_4 toxicity, Dawkins (1963) observed that 7 day old rats appeared to be as sensitive as adults to the hepatotoxic action of CCl_4. Since the youngest rat used in this study was 15 days old, the age difference in the qualitative effect of CCl_4 on ouabain transport was probably not due to differences in the extent of CCl_4 toxicity.

In adults, CCl_4-induced drug retention in plasma may be attributed, in part, to decreased hepatic uptake. Thus, depressed uptake of organic anions (e.g., BSP and ICG), has been demonstrated following CCl_4 under experimental conditions designed to measure only uptake (Plaa and Hine, 1960; Paumgartner et al., 1970). BSP and ouabain retention in plasma (in adults), however, is thought to be mainly due to decreased hepatic secretion into bile (Klaassen and Plaa, 1968b; Reuning and Schanker, 1971). Nevertheless, CCl_4-induced hepatic damage may not be specific and thus affects both sinusoidal and canalicular transport.

The effect of CCl_4 in young rats was qualitatively different from the effect observed in adults (Figure 9) and these results may be similar to observations made by Klaassen (1974b) on the effect of hepatic stimulators on ouabain transport in developing rats. Stimulation of biliary function in adult rats following phenobarbital,

spironolactone, and pregnenolone-16α-carbonitrile involved increases in bile flow and transport of drugs (such as ouabain) from liver to bile. Thus, stimulation with these agents resulted in reduced concentrations of ouabain in plasma and liver. In contrast, the principle effect of these microsomal enzyme stimulators in young rats was to increase the transport of ouabain from plasma into liver such that young animals have less ouabain in plasma but higher concentrations in liver (Klaassen, 1974b). Since the biological responses (liver damage with CCl_4 vs. hepatic microsomal enzyme stimulation with phenobarbital) induced with these chemicals are markedly different, age may be an important variable in characterizing the effect of drugs on biliary function. CCl_4 and phenobarbital may affect the molecular correlate for both hepatic uptake and biliary secretion of drugs in rats of all ages, but the developmental status of the animal is important in determining the in vivo characterization of the response. These results, therefore, are consistent with the hypothesis that the rate-limiting step in drug transport changes with age and that experimental treatments may simply act to exaggerate age differences that already exist.

This conclusion is supported by the existence of an age dependent response to digoxin mediated alteration of ouabain transport (Figures 10,11). When compared to rats administered ouabain alone, retention of ouabain in plasma was observed in 15 day old, 21 day old, and adult rats simultaneously administered digoxin (plus ouabain; Figure 10). Since digoxin inhibits ouabain uptake into rat liver slices (Kupferberg and Schanker, 1968), cardiac glycosides may be transported in rat liver by a similar mechanism. Thus, retention of ouabain in plasma of rats administered digoxin may be attributed to digoxin occupation of ouabain transport sites.

In 15 day old rats, and to a lesser extent 21 day old rats, digoxin mediated transport inhibition was associated with a decrease in hepatic ouabain content (Figure 11). Thus, in these rats, digoxin appeared to inhibit ouabain uptake (Figure 11). However, lower hepatic ouabain content in adult rats was apparent at only the 3 minute time point. At the 20 minute interval, adult rats administered digoxin accumulated ouabain in liver to a greater extent than did controls (Figure 11). Thus, in adult rats, digoxin appears to inhibit both hepatic ouabain uptake into liver and excretion from liver. The effect of digoxin on ouabain transport in developing rats may be similar to the effect of CCl_4. That is, digoxin, like CCl_4, may disrupt both hepatic uptake and biliary excretion, but the *in vivo* response is age dependent in accordance with existing age differences in transport function. However, experimental proof of this interpretation will require kinetic analysis based on more than one dose of ouabain and digoxin.

The basic assumptions that allow the interpretation that digoxin inhibits both ouabain uptake and secretion are: 1) digoxin inhibition of ouabain accumulation into liver slices reflects *in vivo* inhibition of hepatic uptake, and 2) digoxin and ouabain are excreted from liver into bile by the same mechanism. Since digoxin treatment results in a decrease in hepatic content of ouabain, hepatic uptake of ouabain would appear to be inhibited following digoxin (Figure 11). Thus, the hepatic slice technique may be an adequate estimate for hepatic uptake of glycosides (Kufperberg and Schanker, 1968). There is, however, no experimental evidence suggesting that digoxin and ouabain are excreted from liver into bile by the same mechanism. In fact, digoxin but not

ouabain is metabolized in the liver before excretion into bile (Cox and Wright, 1959; Russell and Klaassen, 1973; Klaassen, 1974a). Thus, digoxin may be secreted into bile by transport systems other than the neutral transport system that excretes ouabain. Because of the limited information available concerning the mechanism and specificity of canalicular transport, it is difficult to predict the extent to which digoxin competes for ouabain excretory sites.

The characteristics of digoxin mediated inhibition of ouabain transport, however, were age dependent. In addition to the differences in hepatic disposition of ouabain among animals from the three age groups, transport inhibition appeared to be less dramatic in adult rats than in the younger animals. Plasma concentrations of ouabain in 15 day old digoxin treated rats were significantly higher than control values at all three time points; whereas, in adult and 21 day old rats, plasma ouabain concentration was the same in digoxin treated and control rats at the latest (40 min) time point (Figure 10). Similarly, cumulative intestinal content of ouabain, an estimate of biliary excretion, was significantly decreased, following digoxin, only in 15 day old rats (Table 8). Although the mean values for ouabain excretion in digoxin treated and control 21 day old rats were not significantly different, rats injected with digoxin excreted only about half as much ouabain as the control (Table 8). Forty minute cumulative intestinal excretion of ouabain in adult rats, however, was not affected by simultaneous injection of digoxin (Table 8). Older animals with a greater capacity to excrete ouabain may also be capable of overcoming transport inhibition. Although it may not be clear to what extent digoxin inhibits canalicular transport of ouabain, the age-dependent

characteristics of digoxin inhibition of ouabain transport may reflect age differences in transport capacity.

Effect of Polybrominated Biphenyls (PBBs) on Hepatic Drug Transport in Developing Rats

Repeated exposure of 50 ppm PBBs in the diet of lactating and weanling rats did not appear to alter growth as reflected in average body weight (Figure 12) and in average mortality rate (Figure 13). Treated rats reaching 49 days of age weighed the same as controls, and did not exhibit any signs of overt toxicity.

Although animals less than 28 days of age were exposed to PBBs through the mother's diet, there may be little doubt that the developing animals gained considerable exposure. PBBs accumulate in tissues with high lipid content including mammary tissue and have been reported to be present in milk fat (Fries and Marrow, 1975; Willet and Irving, 1975). Although concentrations of PBBs were not determined in tissues from animals used in the present investigation, the dose of PBBs in the milk may have been even greater than the 50 ppm to which their mothers were exposed. In similar experiments, hepatic levels of PBBs in the nursing rat were higher than maternal levels (Rickert et al., 1977). In the present investigation, significant exposure of developing rats to PBBs may be evident from the elevated liver to body weight ratios in the treated animals (Table 9). Increased liver mass is invariably associated with hepatic microsomal enzyme stimulation that is produced following exposure to PBBs (Dent et al., 1976a,b, 1977). Since continuous treatment with PBBs did not seem to grossly affect normal development (Figures 12, 13), the 40-60% increase in liver weight produced by PBBs (Table 9) may not be detrimental to normal growth.

Similar results were obtained in an additional cross-fostering experiment. Prenatal and/or postnatal exposure to PBBs did not alter postnatal body weight gain, but resulted in significant increases in liver mass in 15 day old animals (Table 10). The observation that prenatal exposure to PBBs (50-0) was least effective in increasing liver mass may be consistent with the suggestion that the most important route of transfer of PBBs from mother to young is via the milk and not via the placenta (Rickert et al., 1977).

Prenatal and/or postnatal exposure to PBBs resulted in stimulation of hepatic excretory function in 15 day old rats (Figures 14,16; Table 12). This was demonstrated by 1) enhanced rate of elimination of ouabain from plasma (Figures 14,16), and 2) increased cumulative intestinal ouabain content (Table 12) in 15 day old treated animals, when compared to 15 day old control rats. In addition, 15 day old rats whose mothers were exposed to 50 and 100 ppm PBBs were protected against ouabain toxicity and when compared to controls, ouabain LD_{50} values were significantly higher in rats exposed to PBBs (Table 11). This provides additional evidence for enhanced ouabain elimination from plasma following PBBs, and also demonstrates that exposure to PBBs can result in a significant drug interaction in rats. Since many drugs are excreted by the liver into bile, the potential exists for additional drug interactions following exposure to PBBs.

The effect of PBBs on ouabain transport in 15 day old rats appeared to parallel the effect on liver mass. Stimulation of ouabain transport was most evident in rats receiving postnatal exposure to PBBs

and this effect coincided with the greater elevation in liver weight in these animals when compared to rats receiving prenatal (50-0) exposure (Figures 14,16; Table 12). However, the large increase in liver mass in 21, 35, and 49 day old rats exposed to PBBs (Table 9) was not associated with enhanced elimination of ouabain from plasma or increased ouabain excretion (Figure 14, Table 12). Thus, two factors that may be important in stimulation of drug transport following PBBs are 1) liver mass, and 2) age.

It has become apparent that the characteristics of stimulation of microsomal mixed function oxidase activities following PBBs are dependent on duration of exposure. Following a single intraperitoneal injection of PBBs to adult rats, the pattern of hepatic microsomal enzyme stimulation changes from phenobarbital-like initially to 3-methylcholanthrene-like at later times after administration (Dent et al., 1976b). The characteristics of hepatic stimulation during continuous dietary exposure may also change with time and thus, an additional factor that may influence the effect of PBBs in hepatic excretory function is 3) duration of exposure.

Treatment with PBBs resulted in a significant increase in hepatic ouabain content (μg/kg body weight) 3 minutes following ouabain administration in 15 day old rats (Figures 15,17). The increase in hepatic ouabain content was due, to some extent, to increased ouabain concentration (data not shown) but the magnitude of this effect mainly reflected the effect of PBBs on liver weight (Table 9,10). Nonetheless, these data suggest that stimulation of ouabain transport in 15 day old rats may be attributed to enhanced ouabain uptake into liver.

The mechanisms for hepatic uptake may also be stimulated following PBBs in 21 day old rats since hepatic ouabain content was significantly elevated in treated animals 3 minutes following ouabain administration (Figure 15). In addition, the initial rate of removal of indocyanine green (ICG) from plasma was enhanced following exposure to PBBs in 21 day old rats (Table 13). Since the initial rate of elimination of drugs from plasma represents hepatic uptake (Paumgartner et al., 1970), these data provide additional evidence for enhanced uptake capacity in 21 day old PBBs exposed rats. Following PBBs, stimulation of hepatic uptake in 21 day old rats was not associated with enhanced ouabain excretion (Table 12) and was associated with significantly lower plasma ouabain concentration at only the earliest (3 minute) time interval (Figure 14).

These results support the hypothesis that the rate-limiting step in drug transport changes with age. Hepatic uptake may be enhanced following exposure to PBBs in rats of all ages. Enhanced elimination of ouabain from plasma (Figures 14,16), and increased ouabain excretion (Table 12) following PBBs was apparent only in the youngest animals (15 day old) since uptake may be limiting for drug transport in 15 day old rats. Stimulation of ouabain transport following PBBs may be attenuated in 21 day old rats and, to a greater extent, in 35 and 49 day old animals, because the relative importance of hepatic uptake for overall drug transport may be diminished.

Although enhanced ouabain transport following PBBs in only 15 day old rats may be interpreted as a reflection of the importance of hepatic ouabain uptake for overall ouabain transport in these animals, this interpretation is dependent on the assumption that stimulation of ouabain transport following PBBs is due only to enhanced capacity for

hepatic uptake. However, stimulation of hepatic transport following other microsomal enzyme stimulators results in enhanced uptake and enhanced biliary excretion (Reyes et al., 1971; Klaassen, 1970,1974b). Treatment of developing and adult rats with phenobarbital may result in stimulation of all steps involved in transport of drugs from blood into bile, but because of the importance of hepatic uptake for drug excretion in young rats, stimulation of drug transport following phenobarbital is associated only with enhanced uptake (Klaassen, 1974b). Similarly, exposure to PBBs in 15 day old rats may also result in enhanced hepatic uptake and biliary excretion of ouabain. Since treatment with PBBs did not result in stimulation of ouabain transport in 35 and 49 day old rats (Figure 14; Table 12) and enhanced overall drug excretion in adult rats would be associated with increased drug excretion from the liver, it is unlikely that PBBs enhanced both uptake and biliary excretion.

Stimulation of bile flow and biliary excretion of drugs in adult rats is associated with treatment with phenobarbital-like agents but not with chemicals such as 3-methylcholanthrene (Klaassen, 1970). The characteristics of microsomal enzyme stimulation following PBBs resembles stimulation produced with phenobarbital and 3-methylcholanthrene (Dent et al., 1976a,b). Characteristics of stimulation of hepatic enzymes following PBBs may change with duration of exposure (Dent et al., 1976b), and moreover, the pattern and time course of this change may be age dependent (McCormack et al., 1977). Thus, stimulation of ouabain transport in 15 day old rats may have been due to enhanced hepatic uptake and biliary excretion, but ouabain transport in 35 and 49 day old rats may not be enhanced following PBBs because the effect of PBBs on hepatic function changed with duration of exposure.

Duration of exposure alone would not account for stimulation observed in 15 day old and not in older rats (Figure 14; Table 12). Enhanced ouabain transport was observed in 15 day old rats exposed to PBBs during the prenatal (50-0), postnatal (0-50) and pre- and postnatal (50-50) period (Figures 14,16; Table 12). Rats exposed to PBBs pre- and postnatally (50-50) would have a total duration of exposure of approximately 29 days (day 8 of gestation to postnatal day 15). Thus, extended duration of exposure per se may not account for lack of stimulation in 35 and 49 day old rats. However, since 1) prenatal (via the placenta) exposure to PBBs is minor in comparison to postnatal (via the milk) exposure (Rickert et al., 1977), and 2) hepatic stimulation following PBBs may be age dependent (McCormack et al., 1977), it may be difficult to maintain that the 29 day exposure time to PBBs in young rats would produce the same effects on the liver as 29 day exposure beginning at birth. Nonetheless, these results are consistent with the hypothesis that age (and thus rate-limiting step) rather than duration of exposure is the primary factor function that determines whether PBBs will stimulate hepatic excretory function.

Therefore, treatment with PBBs may specifically enhance hepatic uptake of ouabain and thereby increase ouabain transport in only young rats. The mechanism for the stimulation is not known but is probably related, in part, to increased liver mass (Tables 9,10).

Characteristics of Stimulation of Drug Transport in Young Rats

An age related increase in hepatic uptake capacity may represent the major, and for some compounds, only mechanism for the maturation of hepatic excretory function. This was suggested by the time dependent

patterns of hepatic disposition of ouabain in developing rats (Figure 2). Consistent with the hypothesis is the pattern of ouabain disposition in liver of rats that were exposed to PBBs (Figure 15). Hepatic content of ouabain in 15 day old rats that were exposed to PBBs was maximal at the 20 minute time interval which is a pattern similar to that observed in control 21 day old rats (Figure 15). Enhanced uptake capacity for ouabain in 21 day old rats exposed to PBBs resulted in a pattern of hepatic ouabain disposition similar to that observed in older rats, and maximal hepatic ouabain content was apparent at the earliest (3 min) time interval (Figure 15). Thus, treatment with PBBs resulted in enhanced uptake capacity and thereby may have stimulated the development of hepatic excretory function.

Adult-like function may be defined by the existence of maximal hepatic drug content at early times following drug administration. Accordingly, enhanced excretory function in 15 day old PBBs treated rats would not be adult-like. The maturation of hepatic function may also be defined in accordance to specific responses to certain experimental situations. Thus, the effect of CCl_4 on hepatic excretory function in adult rats was qualitatively different from that observed in younger animals and adult-like function may be defined by increased accumulation of ouabain in liver following CCl_4 (Figure 9). CCl_4-induced reduction in hepatic transport of ouabain in 15 day old PBBs-treated rats was associated with a decrease in hepatic ouabain content (Table 15). Since this specific response occurred in control rats less than 25 days of age (Figure 9), these data confirm the suggestion that enhanced function in 15 day old PBBs-treated rats was not adult-like.

Inhibition of ouabain transport following simultaneous administration of digoxin also produced age dependent responses (Figures 10, 11; Table 8). Although the exact mechanism for digoxin inhibition of ouabain transport is not precisely known, adult-like function was associated with 1) increased accumulation of ouabain in liver at later time intervals (20 min) and 2) the absence of reduction in cumulative ouabain excretion (intestinal content) following digoxin (Figure 11; Table 8). Digoxin inhibition of ouabain transport in fifteen day old rats that had been exposed to PBBs prenatally (50-0) or postnatally (0-50) (Figures 18,19; Table 14) resulted in a response similar to that observed in 15 or 21 day old control rats (Figures 10,11; Table 8). Thus, hepatic ouabain content in PBBs-exposed digoxin treated rats did not exceed ouabain content in liver of rats administered ouabain alone (Figure 19); and cumulative ouabain excretion into intestine was decreased following digoxin in 15 day old control rats and rats that had been exposed to PBBs (Table 14). These results provide additional evidence that the enhanced function in 15 day old PBBs-exposed rats is qualitatively different from adult-like function.

The specific response of adult rats to CCl_4-induced and digoxin-mediated inhibition of ouabain transport may be attributed to the high capacity for hepatic uptake of ouabain in adult rats. Since uptake capacity is low in young rats when compared to adults, differential effects of CCl_4 and digoxin on ouabain transport in adult and developing animals may be a reflection of existing age differences for transport capacity. Moreover, these experimental procedures may exaggerate age differences and thus provide a useful model for determining the relative importance of hepatic uptake for overall drug transport.

SUMMARY AND CONCLUSIONS

Maturation of the hepatic excretory system was determined in developing rats by measuring the plasma disappearance and hepatic and intestinal (biliary) appearance of intravenously administered ouabain. Cumulative (40 minute) intestinal ouabain content was lower in 15 day old rats than in 21, 25, 35, and 45 day old animals and reached adult levels when rats were 35 days old. Decreased ouabain excretion in young rats resulted in retention of ouabain in plasma when compared to plasma ouabain concentrations in older animals. Hepatic ouabain concentration was initially low and then increased with time following administration in 15 day old rats. Adult-like function was associated with high hepatic concentration of ouabain immediately following ouabain administration and was observed in animals 25 days old and older. These results suggested that low uptake of ouabain from plasma into liver is an important mechanism for decreased ouabain excretion in young rats.

The organic anion sulfobromophthalein (BSP) is excreted into bile following hepatic uptake and intrahepatic conjugation to glutathione (GSH). Relative to plasma concentrations of BSP in adult rats, BSP was retained in plasma of 18 day old rats and was associated with lower hepatic content of BSP in the young immediately following BSP administration. Enzymatic conjugation of BSP to glutathione *in vitro*

was low in young rats, however, relative to adults, elimination of conjugated BSP (BSP-GSH) from plasma was also slower in young rats. It was concluded that low hepatic uptake of BSP in young rats is the most important determinant for functional insufficiency.

Hepatic uptake of BSP is greater than uptake for BSP-GSH and intrahepatic conjugation augments BSP excretion from liver into bile. The rate of disappearance of BSP from plasma of 15 day old rats was twice as fast as the rate of elimination of BSP-GSH in these animals. In adult rats, however, BSP and BSP-GSH were eliminated from plasma at the same rate. These results support the contention that hepatic uptake is rapid and does not limit transport in adult rats. Overall transport function in 15 day old rats, however, may be limited by a slower rate of hepatic uptake.

Bile duct ligation and bile salt infusion, treatments that primarily depress and enhance (respectively) excretion of BSP from liver into bile, markedly altered the disappearance of BSP from plasma of adult rats but did not appreciably affect BSP disappearance from blood of 15 day old rats. The effect of bile duct ligation on ouabain transport in 15 day old rats was also not as dramatic as the effect produced in adult rats. Moreover, the slight effect of bile duct ligation on ouabain transport in 15 day old rats was associated with impaired uptake rather than decreased biliary excretion. Bile duct ligation in adult rats resulted in concentration of ouabain in liver ten times greater than hepatic ouabain concentration in sham operated controls (decreased excretion from liver into bile). The differential effects of bile salt administration

and bile duct ligation on hepatic drug transport in adult and young rats support the hypothesis that the rate limiting step for drug transport changes with age.

Carbon tetrachloride (CCl_4) depressed hepatic excretory function in adult and developing rats. Plasma ouabain concentrations were significantly higher and biliary (intestinal) ouabain content significantly lower than control levels in treated rats of 15 to 45 days of age. Hepatic ouabain content was significantly lower than control levels in CCl_4 treated young rats (decreased uptake into liver), but significantly higher than control values in treated older rats (decreased ouabain excretion from liver into bile). These results suggested that the "site" of altered biliary function following CCl_4-induced liver damage was age dependent. Treatment with CCl_4 may result in alteration of all mechanisms in the drug elimination process. Since the rate-limiting step in drug transport changes with age, it was concluded that treatment with CCl_4 results in exaggeration of existing age differences in transport function. This contention was supported by the existence of an age dependent response to digoxin-mediated alteration of ouabain transport. When compared to rats administered ouabain alone, simultaneous administration of digoxin plus ouabain resulted in retention of ouabain in plasma of 15 day old, 21 day old and adult rats. In 15 day old and 21 day old rats, digoxin-mediated transport inhibition was associated with a decrease in hepatic ouabain content (decreased ouabain uptake). Digoxin treatment to adult rats resulted in accumulation of ouabain in liver to a greater extent than in controls (decreased ouabain excretion from liver into bile). Administration of digoxin was suggested to simulate the

effect of CCl_4 on hepatic excretory function. Digoxin inhibits ouabain uptake and excretion and the age dependent responses to digoxin-mediated transport inhibition reflects age differences in the rate-limiting step for overall ouabain transport.

Exposure of developing rats to polybrominated biphenyls (PBBs) did not cause any significant alteration in body weight and did not produce overt toxicity when compared to controls over a 49 day postnatal period. However, prenatal and postnatal dietary exposure to PBBs (50 ppm in diet of pregnant or lactating mother or in diet of rat weanlings) resulted in elevated liver weight. In 15 day old rats that had been treated with PBBs, increased liver weight correlated to enhanced ouabain transport from plasma into bile. Liver weight was also elevated in 21, 35, and 49 day old rats treated with PBBs but this effect was not associated with stimulation of ouabain transport in these animals. The mechanism for stimulation of ouabain transport following PBBs in 15 day old rats was increased hepatic uptake of ouabain. The selective stimulation in only young rats was attributed to the relative importance of uptake for overall transport in 15 day old rats.

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