Pharmacokinetic Models Proposed for The Biliary Excretion of Dyes.

A thesis submitted for the partial fulfilment of the requirement for the degree of Master of Science in Pharmacy.

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Abstract

Pharmacokinetics is the study of the rate processes involved in the absorption, distribution and elimination of drugs in the animal body. The rate at which a compound is eliminated from the body is an important factor in regulating pharmacological response. However, animals are complex and to treat events taking place in intact organisms, some simplification of reality is required, which is the basis of pharmacokinetic modelling. Pharmacokinetic models are developed to trace the elimination of the compound. The "proof" for such a model is generally considered to be accurate fit of the experimental results. Model parameters are evaluated and examined for their biological significance.

In this work amaranth and rose bengal were adminstered intravenously to rats and the levels of dyes followed in blood and bile. For amaranth at doses up to twenty micro-moles per rat, the dye kinetics were found to obey the superposition rule and appeared to fit first-order linear relationships. Pharmacokinetic modelling using digital and analog curve fitting techniques shows that the data at low doses may be fitted accurately by Model I, involving first order uptake to the liver from blood, a reversible storage compartment in the liver probably associated with tissue binding of dye and first order excretion into the bile. The data for rose bengal at ten micro-moles per rat may be fitted to a model similar to I, to which the reflux of dye from liver to blood is

introduced. For the azo dyes geranine, lissamine, and dechlorolissamine, bile level data was available for examination of Model I. Model parameters obtained were correlated with the percentage of protein bound dye in the liver and with the liver:blood protein binding ratio. Excellent linear correlation was obtained for k_{12} (rate constant for hepatic uptake of dye from blood) versus percentage protein bound in liver, which suggests that hepatic protein binding of dyes plays an important part in the uptake of dyes in the liver.

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Chapter I

Introduction

Biliary excretion is an important route for the elimination of foreign compounds. The biliary excretion system is closely related to the fecal excretion, the enterohepatic circulation (Williams et al., 1965), and the intestinal metabolism of compounds. Interest in the hepatic uptake and biliary excretion of dyes and foreign compounds has centered around several problems:

1. The importance on therapeutics and toxicology of enterohepatic circulation as a factor in the duration of drug action (Williams et al., 1965).

2. The possible toxicology of biliary excretion of food dyes and other compounds in relation to liver damage (Smith, 1966).

3. The diagnostic utility of some compounds in the evaluation of liver function and biliary diseases (Brauer, 1959).

The physiochemical properties, which determine if a compound is to be excreted via the bile, have been extensively investigated. Anionic compounds with a sufficiently large molecular weight will pass into the bile, often as the exclusive means of elimination (Millburn, 1970). Numerous pharmacokinetic models have been proposed for biliary excretion (Winkler, 1965). In particular, studies have been made on sulfobromophthalein (BSP) (Winkler, 1965; Richards, 1965; Priestly, 1967; Quarfordt, 1971). Richards (1965) and Quarfordt (1971) proposed a model with reflux of dye from liver to blood which can satisfactorily fit

the blood curve for BSP in man. O'Reilly et al. (1971) used biliary measurements and a simple model to determine the rate constants for demethylation of methyl orange and its biliary excretion in the rat. Pharmacokinetic models for the excretion of amaranth have been proposed (O'Reilly et al., 1972), the study of which is further expanded in this thesis.

As a general introduction to the biliary excretion of dyes and other compounds, the following topics are discussed in this chapter:

1. Structure of biliary secretion in the liver.
2. Composition of normal biliary output and possible micellar involvement.
3. Types of foreign compounds excreted in the bile and the requirements for the secretory mechanism.

Section 1. Structures related to biliary excretion in liver

Bile is elaborated in the liver. To illustrate the formation of bile, the hepatic cellular structures are described.

The parenchyma

The liver is a continuous mass of parenchymal cells tunneled by the portal venous sinusoids. The parenchymal partitions between those vessels form a system of walls, the muralium. These walls are continuous with each other through the perforations in the parenchymal walls. Electron microscopy has shown that the liver cells contain an endoplasmic reticulum which consists of piles of band-shaped double membranes (Elias and Cohen, 1954). Toward the lacunae, the liver cells are studded with broad

microvilli. The hepatic cells are held together by special fastening structures (Fawcett, 1955; Ruttner and Vogel, 1957).

The sinusoids

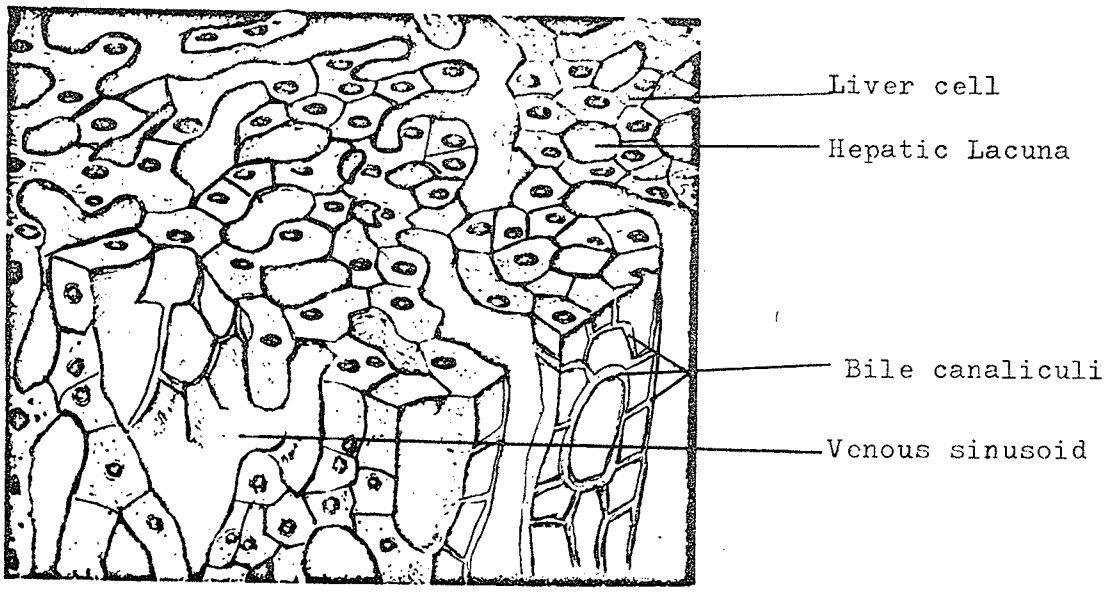
The sinusoids are specialized capillaries suspended in the lacunae of the liver cells. They form an uninterrupted, three dimensional network in the labyrinth of lacunae. They are lined with the Kupffer cells which are flat and overlap loosely (Holle, 1961). Between the Kupffer cells and the liver cells is the space of Disse which is normally narrow and contains a network of argyrophile fibres. The Kupffer cells are potential phagocytes which can control the blood flow by bulging into the lumen of sinusoids (Ruttner and Vogel, 1957).

The parenchyma and the sinusoids are illustrated in figures 1 & 2.

The liver lobule

Polyhedral lobular units appear in the histological sections of the hepatic laminae. Each lobule is about one millimeter in diameter, having a small central vein as its central axis, surrounded at its edges by portal canals. The central vein is a tributary of the hepatic vein. The portal canal contains a branch of the portal vein, a branch of the hepatic artery and an interlobular bile ductule. The triad is sheathed by connective tissue. It has been customary to consider the hepatic lobular as consisting of 'cords' of liver cells radiating from a central vein, each 'cord' consisting of two rows of liver cells with a bile canaliculus between them, and radiating venous sinusoids lying between adjacent 'cords' such that the bile

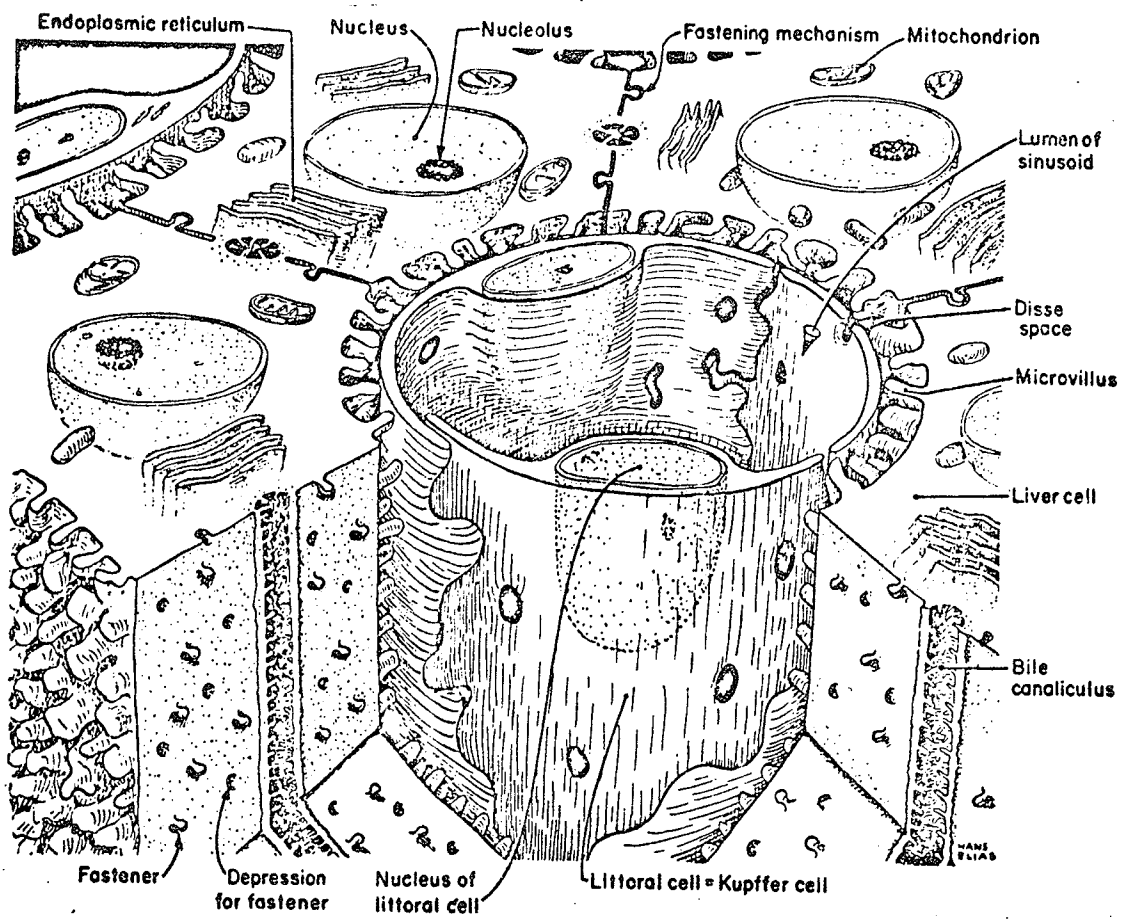
Fig. 1



Human liver parenchyma, reconstruction from serial sections showing that the liver is a muralium simplex.

(Reprint from Res. Serv. Med. 37, 1-25, G.D. Searle & Co., Stokie, Illinois, 1953.)

Fig. 2



Ultrastructure of the mammalian liver.

From Elias and Pauly, The Biliary System, Chapter 1, page 4, ed. W. Taylor, Blackwell Scientific Publications, 1965.

canaliculus is separated from the sinusoids by the thickness of a liver cell (Gray, 1966), as shown in figures 2 & 3.

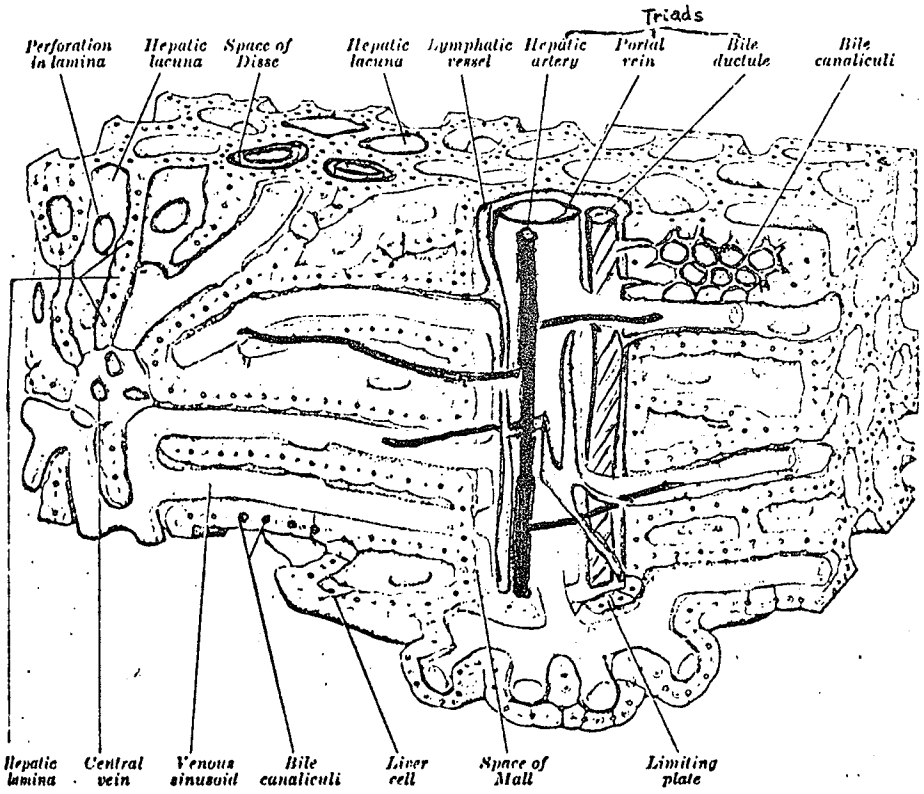
The bile canaliculus and bile ductule

Minute bile capillaries, the bile canaliculi, form networks of polygonal meshes, each mesh surrounding an individual liver cell except on the surface of the cell opposite to the venous sinusoids. An individual bile canaliculus lies between the walls of adjoining liver cells. Electron microscopy has revealed that the canaliculi walls are formed by a condensation of cellular exoplasm from the liver cells. At the periphery of the hepatic lobules the bile canaliculi join to form very thin intralobular ductules which enter the interlobular ductule in the portal canals. The bile ductules in the portal canals are lined with columnar cells. The epithelium of the ductules is continuous with that of the gall-bladder, but the columnar cells here have a convex free surface and microvilli (Eglitis and Hayes, 1957). Each microvillus has a thickened end, the capitulum, which is studded with extremely fine hair like extensions, the antennulae.

The bile and the gall-bladder

The bile duct enters the superior part of the duodenum. It may be joined with the pancreatic duct before the duodenum as in man, or may be separated from the pancreatic duct as in the rabbit. It is innervated by the parasympathetic vagus and the sympathetic system. A plexus formed of both kinds of fibres joined to surround the biliary passages, the hepatic

Fig. 3



A diagramatic illustration of the structure of the liver.

Based on H. Elias (Biol. Rev. 30, 1955).

artery and the portal vein (Bensley, 1948; Walker, 1965).

The gall-bladder is an extension structure of the bile duct where emptying of bile into the duodenum may be regulated after the bile content has been concentrated. The gall-bladder and the choledoduodenal junction cooperate with each other in that, when the gall-bladder contracts, the choledoduodenal junction is relaxed (Melzer, 1917). According to Magee (1965), the contraction of the gall-bladder and the relaxation of the choledoduodenal junction may be subjected to hormonal control. Possibly the role of extrinsic nerves is to facilitate or depress the response of these organs to hormones. The gall-bladder has its own autonomous function, with spontaneous, rhythmical contractions. Cholecystokinin has a gall-bladder contracting effect which can be augmented by secretin. The vagus stimulation increases while the sympathetic decreases the tone of the gall-bladder (Pallin and Skolund, 1961).

The above mentioned structures are closely related to the biliary secretion function of the liver. The hepatic cells are the site of bile elaboration and organic ions secretion (Krebs and Brauer, 1958; Hanzon, 1952; Novikoff and Essner, 1964; Schaffer et al., 1960). It appears likely that the hepatic secretion of osmotically active organic anions into the bile canaliculi is a major determinant of bile flow. Sperber (1959) has published a detailed review on this subject. According to this view, various organic anions are actively secreted in high concentration into the lumen of the biliary tract.

Appropriate concentrations of inorganic cations are delivered simultaneously into bile in order to maintain electrochemical neutrality. The relatively non-diffusible and osmotically active anions initiate the passive movement of water into bile. Additional solute diffuses passively into bile along concentration gradients that have been established. Such substances cause an inflow of water to maintain isotonicity. The resultant bile is a fluid isotonic with blood but varies widely in composition (Wheeler and Ramos, 1960). Klassen (1971) suggests that the canalicular bile production in rats is not solely determined by the rate of bile acid secretion. He examined the correlation between bile acid secretion and biliary flow in a number of experimental conditions which would be expected to alter biliary flow and/or bile acid secretion. A summary of his result is presented in the following table:

Experimental conditions	Bile flow rate in rats	Bile acids level
1. Interruption of entero-hepatic circulation of bile acids.	Decrease	Marked increase
2. Exogenous bile salts loading.	Normal	Marked increase
3. Phenobarbital pre-treatment.	Increase	Marked decrease
4. D-Thyroxine pre-treatment.	Normal	Marked decrease
5. Hypophysectomy.	Decrease	Marked decrease

If the active secretion of bile acids is the primary step in the formation of canalicular bile, and the volume is due to the osmotic addition of water and electrolytes in the presence of bile salts in the bile canaliculi, then the level of bile acids should remain constant at all times. Since the biliary bile acids do not remain constant in concentration, it would appear that the bile acid secretion is not the 'prime mover' in the initiation of canalicular bile flow in rats despite its choleretic effect. Other osmotically active compounds may be important in bile production. It is possible that normal bile volume is not produced by an osmotic response to substances secreted into the canaliculi. Other mechanisms may regulate bile production, such as the hypothetical active transport of water into the canaliculi or that bile may be 'prepackaged' in the Golgi complex and then transported into the canaliculi (Klassen, 1971). Recently, it has been suggested that there is a bile salt independent fraction of canalicular bile production, the physiological importance of which is still not clear (Nahrwold and Grossman, 1967; Wheeler et al., 1968; Erlinger et al., 1969).

Section 2. Composition of normal biliary output.

The normal constituents of bile include bile salts e.g. the taurine and the glycine conjugates of bile acids; bile pigments e.g. bilirubin diglucuronide and the free bilirubin; cholesterol, lecithin, mucin, fatty acids, inorganic electro-

lytes and water (Merck Manual, 1972). Brauer (1959) has classified the main constituents in bile into three groups, according to their bile/plasma concentration ratios.

Class A, ratio is 1:1--the examples include glucose, Na^+, K^+, Cl^-; the rate of excretion is dependent in the bile flow rate but independent of the plasma concentration.

Class B, ratio is 100:1 or greater--the examples include organic acids, organic cations, glucuronide metabolites of anionic compounds.

Class C, ratio is much less than one--the examples include sulfanilic acid and the biphenyls. These compounds are either not secreted into the bile or come out in a minute amount.

The glycocholates, taurocholates and bilirubin are concentrated in bile at a higher level than that in the plasma, which may be explained by an active uptake or secretion by the liver cells. The bile/plasma concentration ratio is maximal at a low plasma concentration and decreases as the plasma concentration increases. Hence they are characterized by a maximal transport ratio. This indicates that the active transport mechanism may be saturated by a high substrate concentration.

Bilirubin is the principal bile pigment derived from erythrocyte breakdown in the reticuloendothelial system. The porphyrin ring released is metabolized to form biliverdin which is reduced immediately to bilirubin (Rafelson and

Binkley, 1965). Bilirubin normally found in plasma appears to be the unconjugated form, but most of the biliary bilirubin is conjugated, primarily with glucuronic acid or to a less extent with the sulfates or other compounds (Lathe and Walker, 1958). Conjugation occurs in the liver microsomal fraction. Uridine diphosphate glucuronic acid is the major source of glucuronic acid and the reaction is catalysed by glucuronyl transferase (Schmid et al., 1957; Lathe and Walker, 1958). Bilirubin sulfate may be formed in the presence of an ammonium sulfate fraction of the liver (Isselbacher and McCarthy, 1959). Adenosine triphosphate is required, suggesting that high energy transfer occurs, and that active sulfate is an intermediate. Little information is available about the characteristics of hepatic uptake of bilirubin or about the mechanism responsible for the delivery of the conjugated form into the bile. It appears that conjugation of bilirubin is a prerequisite for biliary excretion (Combes, 1964). However, a small amount of unconjugated bilirubin does appear.

Bile acids are an important compound in bile. The structural similarity between cholesterol and bile acids leads to the hypothesis of a metabolic relationship (Block et al., 1943). The mechanism and the reaction sequence of bile acids formation from cholesterol have been studied extensively. Excellent reviews in this field have been published (Bergstrom and Danielsson, 1958; Bergstrom et al., 1960; Danielsson and Tchen, 1968; van Belle, 1965). Some steps in the sequence of

cholesterol conversion to bile acid conjugates are outlined in figure 4.

The two primary bile acids in mammalian species are cholic acid and chenodeoxycholic acid which are conjugated with taurine or glycine and are excreted in bile. Cholesterol catabolism occurs in the microsomal region (Schersten, 1967a). Bremer (1956) suggested that there are two transferases involved in the conjugation, one for taurine and one for glycine. The reaction may be represented as follows:

1. Cholic acid + ATP + CoA-S-H

Bile acid activating enzyme ⇌ (Mg^{++} or Mn^{++})

Cholyl-S-CoA + AMP + Pyrophosphate

2.

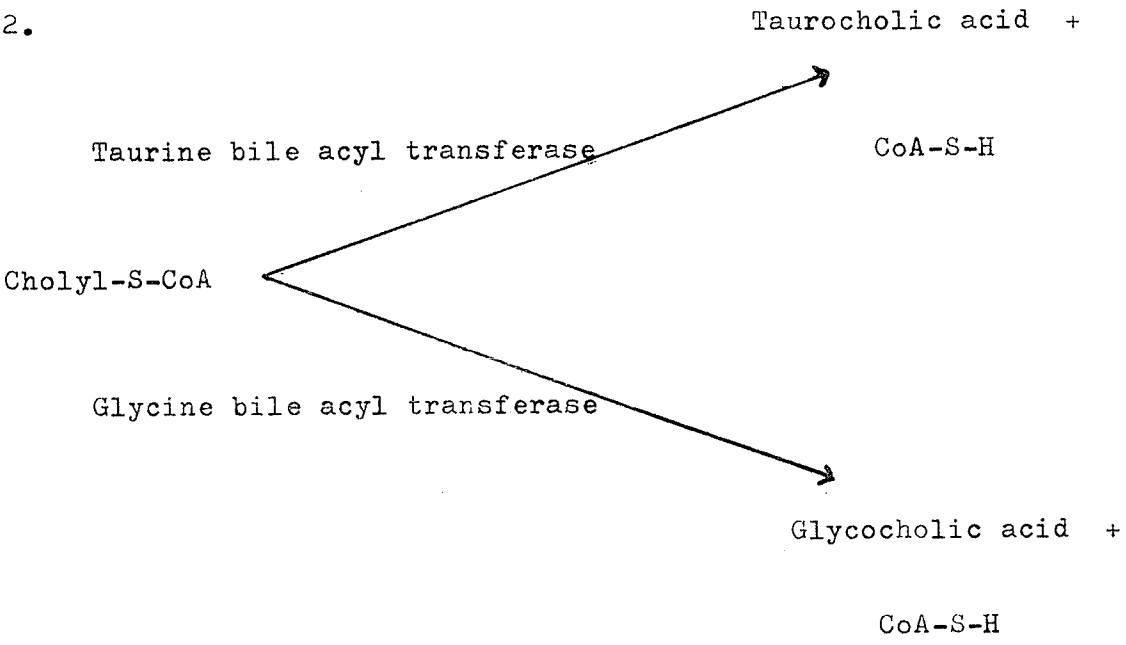
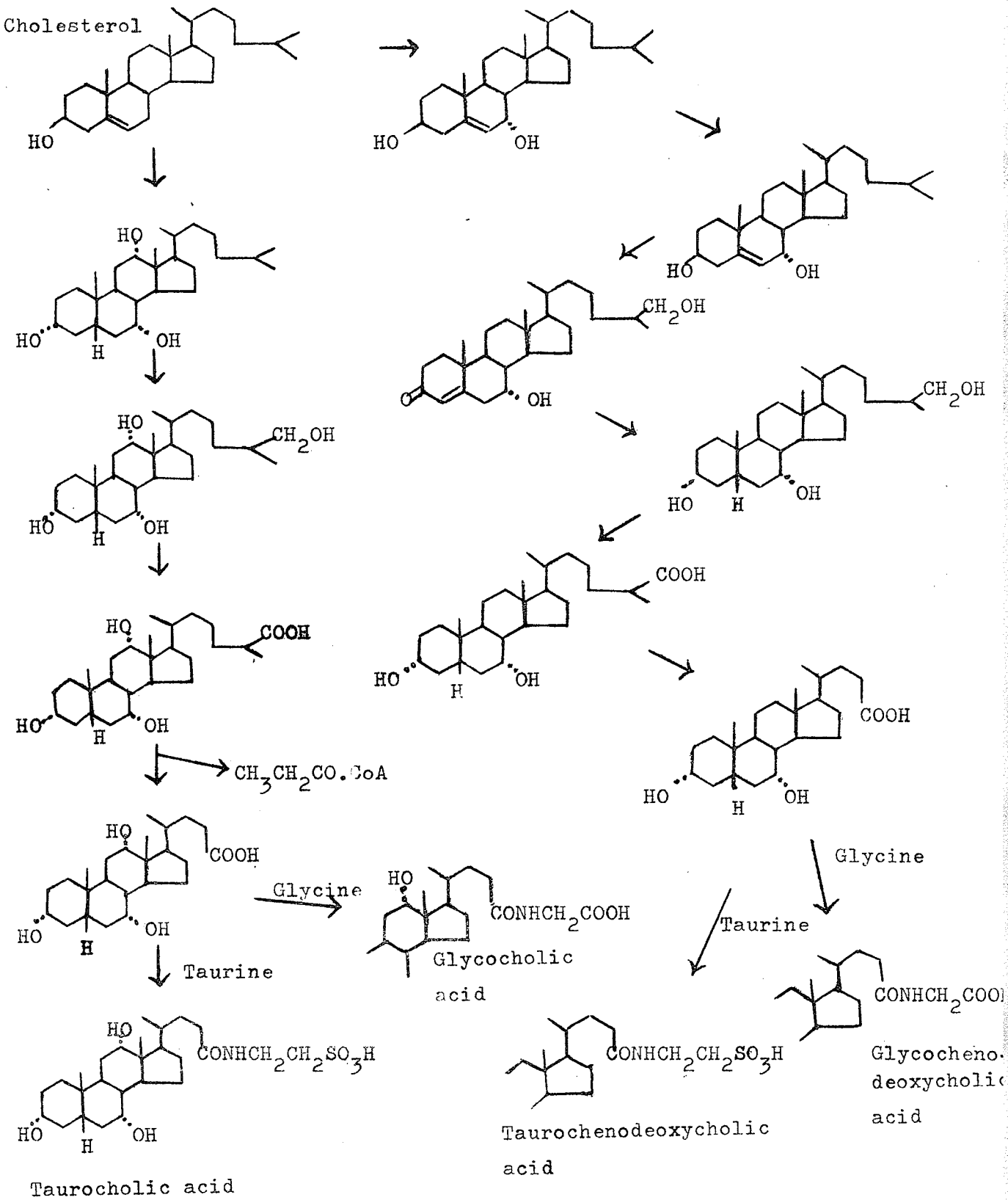


Fig. 4 Cholic acids formation from cholesterol (Millburn, 1970)



Schersten (1967b) has studied the subcellular localization of the bile acid conjugating enzymes. He suggests that the microsomes are bearers of the bile acid activating enzymes, and the L-fraction (where lysosomes are concentrated) contains the two kinds of bile acyl transferase. A special kind of transport mechanism has been postulated to explain the direction of bile acids channeling through the liver cell where enzymes are located, and to account for the lack of interference on the cellular function by the detergent effect of bile acids. In the enterohepatic circulation bile acids are transported from the portal blood to the bile. A direct uptake of bile acids from the extracellular space to the endoplasmic reticulum has been discussed by Mirsky and Osawa (1961) and Porter (1961). A connection between the endoplasmic reticulum where bile acid activation occurs and the primary lysosomes where transference of enzymes takes place, has been suggested by Novikoff and co-workers (1960, 1964), and Cohn and Benson (1965a,b,c,d). The further transport of bile acid conjugates through out the liver cell may be considered to take place in the lysosomes before final secretion into the bile. The peri-biliary localization of liver lysosomes, the existence of lysosome content in the biliary capillaries (Bruni and Porter, 1965) as well as the localization of lysosome enzymes in the rat bile (DeDuve and Wattiaux, 1966), indicate that liver lysosomes may empty their contents into bile. Hence bile acid conjugates may possibly be secreted by the hepatic lysosomes. The bile

acid conjugates seem to have some physiological importance in the biliary transport of cholesterol and lecithin in the form of micelles, and in other parts of the enterohepatic circulation (Schersten, 1967b).

Cholesterol and lecithin (the main phospholipid) depend on bile for their biliary elimination. Cholesterol synthesis takes place in many types of tissues but the liver and the gastro-intestinal tract handle about ninety percent of the total demonstrable cholesterogenesis (Dietschy and Wilson, 1968). Bile acids control the cholesterogenic activity in the intestine, but hepatic cholesterogenesis is dependent on the amount of exogenous cholesterol absorbed by the intestine. Whether bile acids would affect the level of cholesterol in the liver or not is still unclear (Danielsson et al., 1967).

Evidence has recently been presented that there is a special, functionally compartmented pool of phospholipids in the liver with a high turn over rate (Schersten et al., 1967). The secretion from this pool seems to be directed exclusively into the bile.

Since cholesterol and phospholipids are essentially insoluble in water, it is postulated that the lipids are excreted from the cell together with the bile acid conjugates forming micelles in the bile. The important quaternary system of conjugated bile acids-water-lecithin-cholesterol has been studied by Small and Bourges (1966) and Small et al. (1966a,b). They clarified the fact that only a certain amount of cholesterol

can be dissolved in a mixed micellar phase of conjugated bile acids-lecithin-water. At a low lecithin-bile acids ratio, excess of cholesterol will appear in a crystalline form. At a high lecithin-bile acids ratio, cholesterol will appear in a liquid crystalline phase.

Electrolytes like Na^+, K^+, Cl^-, HCO_3^-, are similar in concentration in both the bile and the plasma (Combes, 1964). The cations are transported with the organic ions to maintain electrochemical neutrality. Additional electrolytes diffuse into the bile along the concentration gradients established during the passive diffusion of water (Wheeler and Ramos, 1960; Preisig et al., 1961). Wheeler and his associate have accumulated convincing evidence for another mechanism for electrolyte secretion into the bile. He suggests that the passive diffusion into the bile of a protein-free electrolyte solution takes place in the narrow space between adjacent cells which form a continuous channel from the sinusoids through the space of Disse to the canaliculi. Some bicarbonates and chlorides are transported via this route (Asworth and Sanders, 1960). Rapid appearance and equilibration of radioactive potassium and sodium in the bile following intravenous injection, with slow equilibration of potassium in hepatic cells, also suggests a more distal site for the addition of electrolyte solutions (Leong et al., 1957).

The sum of anions and cations in bile exceeds the determined osmolality (Wheeler and Ramos, 1960). The greatest discrepancy

occurs in bile with high bile acid concentration. The osmotic inactivity of bile acids is explained by their tendency to form micelles at physiological concentrations (Wheeler and Ramos, 1960; Ekwall et al., 1957a,b).

Compounds like alkaline phosphatase, inulin, sucrose, glucose and others appear in bile at a lower concentration than that of plasma (Brauer, 1959). Diffusion from blood probably accounts for their appearance in the bile.

Section 3. Biliary secretion of foreign compounds.

Biliary secretion is an important route in the fecal elimination of foreign compounds. Reviews on this topic have been presented by Stowe and Plaa (1968), Smith (1966), Taylor (1965) and Sperber (1959). The types of compounds may be discussed from the point of view of:

1. Chemical structure relating to biliary excretion.

2. Metabolic modification for extensive biliary excretion.

3. Species difference in biliary excretion of compounds.

Compounds secreted into bile may be in the forms of anions, cations, or uncharged molecules. Their chemical structures may be widely different but their molecular weights are in the region of 400-900 for any extensive biliary secretion, and the presence of a polar group is another contributing factor (Millburn, 1970). The types of compounds are discussed according to the following classification:

1a. Organic anions of low molecular weight (less than 300).

1b. Organic anions of molecular weight from 300-500.

1c. Organic anions of molecular weight above 500.

2. Organic cations.

3. Uncharged molecules.

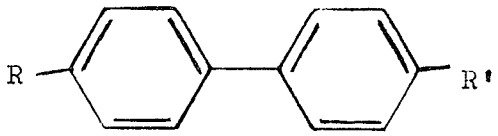
Class 1a. Organic anions of low molecular weight.

Benzene derivatives of molecular weight less than 300 eliminated in bile have been studied by Abou-El-Makarem et al. (1967). Their results are shown in figure 5.

The benzene derivatives are poorly excreted in the bile. Most of the compounds are anions at pH 7.4, and form acid conjugates which are too polar to come out in bile and are excreted preferentially into urine. Some of the polar conjugates have a molecular weight of 300 or more, examples of which include the biphenyls and some sulfonamides. The biphenyl glucuronides have a molecular weight of about 350 and exist in the alkaline pH of the bile as anions (Millburn, 1967a). The glucuronides of sulfapyridine and sulfamethoxypyridazine meet such a requirement and about ten percent of the administered dose is excreted in the bile (Millburn et al., 1967b).

Phenolic compounds including stilbestrol and phenolphthalein are conjugated in the liver to yield monoglucuronides of molecular weight about 500. The metabolites are excreted in large amounts (75-100% of the given dose) in bile. The disulfate conjugates of these drugs also appear as biliary metabolites.

The low biliary output of low molecular weight compounds may be due to two reasons:



Biphenyl

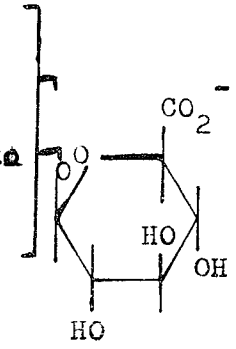
Compound	R	R'	M.W.	Dose mg/Kg	Percent dose in bile in 24 hours.
1. Biphenyl	H	H	154	31	12
2. 4-hydroxybiphenyl	OH	OH	170	35	37
3. 4,4'-dihydroxy-biphenyl	OH	OH	186	38	65
4. 4-glucuronosido-biphenyl		H	346	12	59
5. 4,4'-glucuronosido-hydroxybiphenyl		OH	362	9 - 45	92

Fig. 5 Biliary excretion of biphenyl and some of its derivatives.

1. Rapid clearance of such compounds by kidneys. But it is not so simple as renal pedicle ligation does not increase the biliary output.

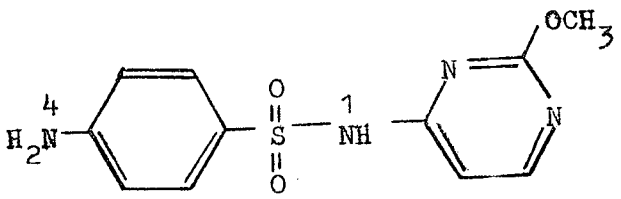
2. Low hepatic uptake due to a low permeability of hepatic cell membrane to these compounds. Alternatively, the compounds may be secreted from the hepatic cells to plasma against a concentration gradient. Smith and William (1970) have shown that small organic anions like 3-aminophenyl sulfate and 4-aminophenyl glucuronide, following their retrograde injection into the bile duct of rat, are readily absorbed from the biliary system and eliminated in the urine.

Class 1b. Organic anions of molecular weight 300-500.

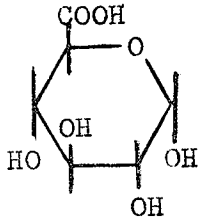
Molecular weight and polarity are the two important determinants of the amount of the compounds secreted into the bile. For instance, sulfadimethoxine is excreted unchanged in bile at a low level while its N^4- and N^1- glucuronides are excreted extensively in bile, hence the addition of a polar glucuronyl radical facilitates biliary excretion (Bridges et al., 1968). The structure of sulfadimethoxine and the metabolites are presented in figure 6.

Among the miscellaneous group of compounds within this range of molecular weight, some are of practical importance. Their biliary excretion has been studied and the results are outlined in figure 7.

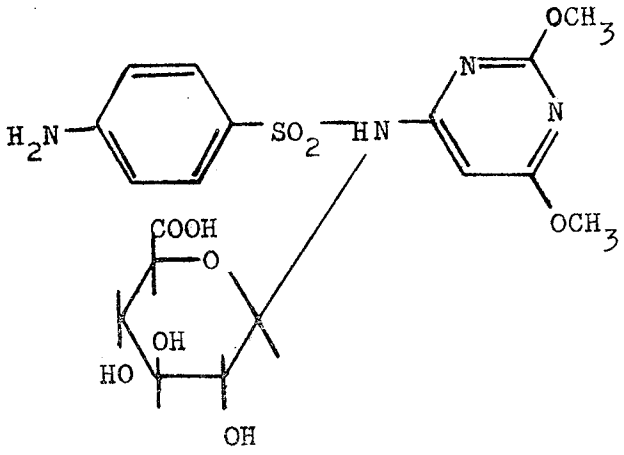
Fig. 6 Structures of sulfadimethoxine and metabolites.



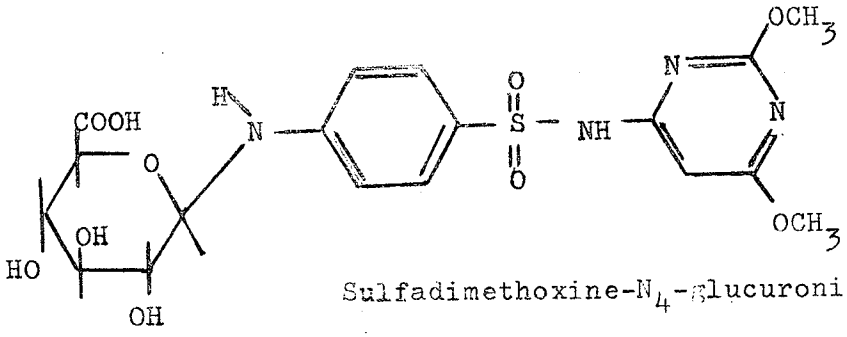
Sulfadimethoxine



α-D-glucuronic acid



Sulfadimethoxine-N_1-glucuronide



Sulfadimethoxine-N_4-glucuronide

Fig. 7 Biliary excretion of some miscellaneous compounds by rats (Millburn, 1970).

Structure of compound	M.W.	Dose mg/Kg ip	% in bile in 24 hrs.
1. 5,5'-Methylenedisalicylic acid	288	10	54
2. Thiambutosine	344	50	33
3. Dieldrin	381	5	less than 1
4. β-Glycyrrhetic acid	471	25	100
5. Neoprontosil	544	100	60

Class 1c. Organic anions of molecular weight greater than 500.

Most organic anions in this group are dyes and cholecystographic agents. The dyes are listed as follows:

a. Tricarbocyanine dye, e.g. indocyanine green, molecular weight 752.

b. Sulfonic acid dye, e.g. sulfobromophthalein, molecular weight 792.

c. Halogenated fluorescein derivatives, e.g. rose bengal, molecular weight 974, and rhodamine B, molecular weight 479.

d. Azo dyes, e.g. amaranth, molecular weight 604.5.

The cholecystographic agents include:

a. Tetrabromophenolphthalein, molecular weight 634.

b. Iodophthalein, molecular weight 822.

c. Pheniodol, molecular weight 494.

d. Iopanoic acid, molecular weight 571.

e. Iophenoxic acid, molecular weight 572.

f. Iodipamide, molecular weight 1140.

Anionic dyes

Indocyanine green is rapidly cleared by the liver to the bile in an unchanged form (Millburn, 1970). About 60-100% of the administered dose has been observed in the bile of the rat, dog, rabbit, and man (Wheeler et al., 1958; Caesar et al., 1961; Cherrick et al., 1960).

Sulfobromophthalein (BSP) is excreted in bile in the form of free dye and as metabolites. Krebs and Brauer (1958) injected

the dye into rat, cat, sheep and chicken and obtained several metabolites plus the unchanged form on paper chromatography. The number of metabolites, their relative amounts and the quantity of unchanged dye in the bile vary with the species, e.g. there is no metabolite in the cat's bile. The biliary form of BSP is a glutathione conjugate, in which BSP is joined to the sulfhydryl group of cysteine by a thioether link (Combes and Stakelum, 1960; Javitt et al., 1960). There are four possible isomers of the conjugate having molecular weight of about 1020. Other metabolites of the dye may be partial breakdown products of the glutathione conjugate. The dye can be bound by liver protein as well as plasma protein. Their relative degrees of binding will determine the amount taken by the liver and eventually secreted into the bile (Priestly, 1967).

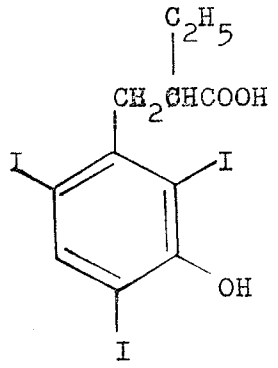
Amaranth is known to be excreted extensively in an unchanged form into the bile of rats (Ryan and Wright, 1961). Earlier work indicated that the relative degree of binding to the liver tissue was an important factor in determining the rate of biliary excretion of several azo dyes (Priestly and O'Reilly, 1966). The liver : blood protein binding ratio is larger than one which indicates an extensive and rapid clearance into the bile. The biliary clearance is inhibited by probenecid which suggests that the excretory mechanism resembles that of hippurates and sulfonamides (Despopoulos, 1966). Since amaranth is structurally related to the sulfonic acid dyes, it

may be expected to participate in the active secretory process in the liver and the kidneys (Despopoulos, 1968). Orally administered amaranth and other azo dyes are reduced by azo reductase in the gut of rats and the products are excreted in urine. The relation between the chemical structures of some azo dyes and the biliary excretion have been studied (Ryan and Wright, 1961). It was found that amaranth was the most extensively excreted dye among azorubin S, amaranth and new coccine in the bile of rats. The number of sulfonate groups and their position in the molecule appear to play an important role in enhancing biliary excretion. In this study, amaranth has been used as a test compound to evaluate the utility of several pharmacokinetic models designed to describe the blood level and biliary excretion rate of anionic compounds in the rat.

Rose bengal is an iodinated fluorescein derivative which has been tested for hepatic uptake in rat, rabbit and man (Nosslin and Morgan, 1965; Jirsa and Raban, 1962). About 50-70% of an intravenous dose of the radio-iodinated dye was excreted in the bile of rat and rabbit. Rose bengal does not appear to be metabolized in the mammalian species and is eliminated unchanged in bile (Kubin et al., 1960). Turco et al., (1966) have studied the hepatogram and the blood kinetics of I^{131}- labelled rose bengal. Their model will be further discussed in this work.

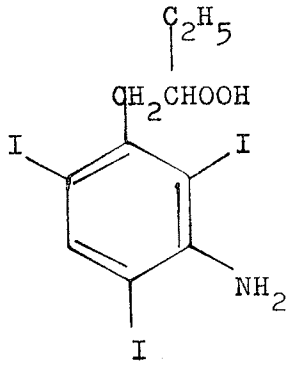
Cholecystographic agents (Fig. 8) are all high molecular weight, halogenated, aromatic compounds used to render the

Fig. 8 Structures of cholecystographic agents.



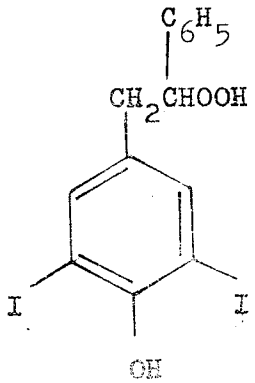
Iophenoxic acid

M.W. 572



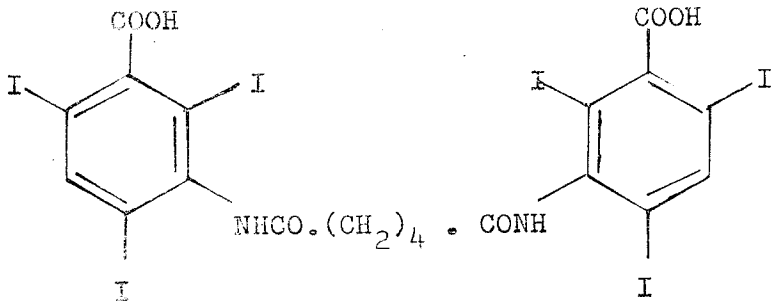
Iopanoic acid

M.W. 571



Ioalphionic acid (Pheniodol)

M.W. 494



Iodipamide

M.W. 1530

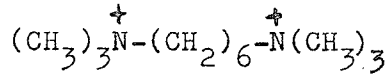
gall-bladder opaque to X-rays. They are secreted by the liver into the bile and pass to the gall-bladder. Iodophthalein has been banned as a cholecystographic agent due to its toxicity (Paul et al., 1944; Unfug, 1946). Pheniodol has been introduced by Dohrn and Diedrich in 1940 for the examination of the biliary tract (Millburn, 1970). It is excreted in the bile of rats and cats in an appreciable amount (Free et al., 1951). Iopanoic acid can be excreted by the kidney and in the bile, and the latter is the major pathway in man, cats, and dogs (McChesney and Hoppe, 1956). It is excreted in the form of an ester glucuronide. Iophenoxic acid and Iodipamide are rapidly removed from the liver and concentrated in the biliary tract of man and other species (Seedorf and Powell, 1955; Link et al., 1955; Sutton and Tillett, 1954).

Class 2. Organic cations.

There is relatively little information on this topic except for a number of triphenyl methane dyes and some quaternary ammonium compounds (Fig. 9) which are secreted in the bile of rats to the extent of ten percent or more of the given dose (Schanker, 1965). These compounds have common characteristics in the structure, a polar quaternary amine group at one end of the molecule and one or more nonpolar rings at the opposite end. The polar group appears to be essential for extensive biliary excretion (Levine and Clark, 1955). Divalent organic cations e.g. hexamethonium and decamethonium are poorly eliminated

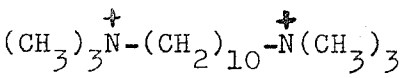
in bile (Levine, 1960; Schanker, 1962).

Structures of Hexamethonium



M.W. 202

Decamethonium



M.W. 258

The quaternary amines are excreted in relatively small portions of the dose. Schanker (1965) suggested that the lack of a phenyl ring at one end of the molecule is involved. No marked difference exists between the relative amounts excreted by rabbits, rats and man (Millburn, 1970).

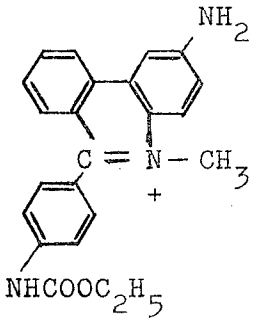
Iga et al., (1971b) investigated the factors affecting the biliary excretion of triphenylmethane dyes. They found that the number of sulfonate groups and their position of substitution on the molecule had a remarkable effect on the percentage excreted and the excretion pattern. Dose dependency was shown in the excretion behavior.

Class 3. Uncharged molecules.

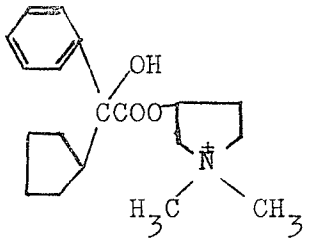
The cardiac glycosides, steroids and some lipophilic compounds are examples of uncharged molecules eliminated in bile (Schanker, 1968).

Cardiac glycosides consist of a steroid ring system with a sugar residue substituted at C-3 and an unsaturated lactone ring at C-17. Their structures are shown in figure 10 (Millburn, 1970). The sugar moieties in the glycosides confer polarity on the molecules. Glucose and rhamnose render the compounds more polar than digitoxose. Cox and Wright (1959) suggest

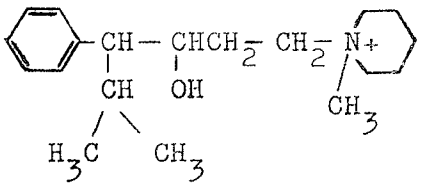
Fig. 9 Structures of cationic compounds (Millburn, 1970)



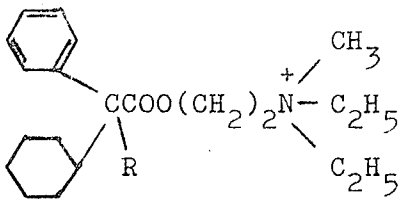
Carbidium



Glycopyrrolate

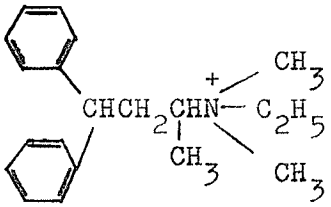


Darstine

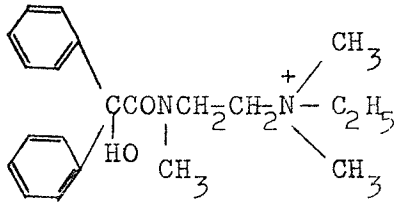


Oxyphenonium

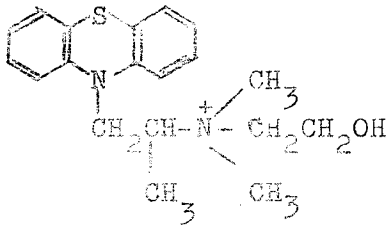
R=OH



Cetiprin



Benzomethamine



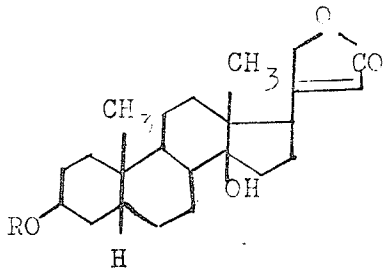
Aprobit

Fig.10 Chemical structures of some cardiac glycosides.

Digitoxigenin, R= H

Digitoxin, R= (digitose)$_3$

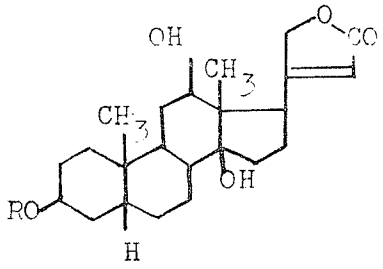
Lanatoside A, R= (digitose)$_3$-glucose
|
Acetyl



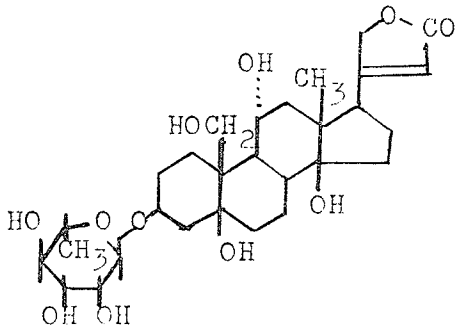
Digoxigenin, R= H

Digoxin, R= (digitose)$_3$

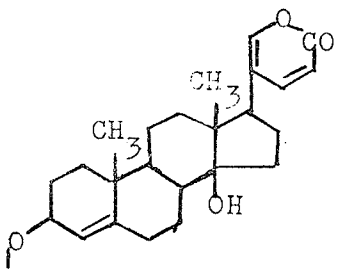
Lanatoside C, R= (digitose)$_3$-glucose
|
Acetyl



Ouabain, rhamnoside of the aglycone ouabagenin.



Scillaren A, the aglycone scillaridin A linked to glucose and rhamnose.



glucose
|
rhamnose

that there is a relationship between the relative polarity of these compounds and the extent of their biliary excretion. Lanatosides A and C contain glucose in their sugar residues and are the more highly excreted compounds in the bile. Ouabain (Cox et al., 1959) and Scillaren A (Simon and Wright, 1960) behave similarly.

Many steroids are eliminated via the bile of the different species. They are excreted as conjugates of glucuronides and to a small extent, as sulfate conjugates. Some steroids undergo an extensively enterohepatic circulation (Smith, 1966). The availability of ^{14}C-labelled steroids has made possible quantitative study of steroid metabolism. Sandberg and Slaunwhite (1956) have divided the compounds into three groups based on their biliary excretion in man:

1. Estrone and progesterone, 30-42% of dose in bile.
2. Testosterone and corticosterone, 12-25% of dose in bile.
3. Cortisol and cortisone, 1-5% of dose in bile.

Plasma protein binding is high for group one and low for group three which reflect that biliary excretion of the steroids may be related to the intrahepatic protein binding or to the metabolism of plasma protein to which steroids are bound.

Uncharged lipophilic drugs are assumed not to be excreted extensively in bile since these molecules are expected to diffuse passively across the canalicular membranes of hepatocytes. A few exceptions to this rule have been reported by Haddock et al. (1965). They found that within a series of iron chelates only

the more lipophilic members were excreted into bile in rabbits. Meyer-Brunot and Keberle (1968) reported that ferrioxamines are excreted into the bile of rats. The derivatives tested were neutral molecules which were not metabolized. Their rates of biliary excretion followed the lipophilic property of the compounds, the most lipophilic reached a bile : plasma ratio of 100:1. Bickel and Weder (1968) found that after parenteral administration of imipramine to rats, more than half of the dose can be localized in the intestinal contents. Considerable amounts of imipramine and desmethylimipramine were also present among the glucuronides and other polar conjugates, which indicated a biliary excretion of lipophilic molecules. Bickel and Minder (1970) demonstrated by equilibrium dialysis and ultracentrifuge sedimentation that the bile salt-phospholipid micelles would solubilize imipramine and desmethylimipramine but not their hydrophilic metabolites. Protein in bile and small molecules which are not surface active are not taken up by the micelles. A molecular composition of about 60:10:7 for the bile salt:lecithin:imipramine micelles has been calculated from experiments, which corresponds to that obtained for the bile salt:phospholipid:cholesterol micelles of human bile by Nakayama (1966). The site of micelle formation is unknown. It has been suggested by Desai et al. (1965) and Swell et al. (1967) that bile micelles may be formed in the hepatocytes. This may be explained by the fact that only micelle-forming lipids are excreted in the bile, and also by

the morphological relationship of canalicular villi to the Golgi complexes (Sherlock, 1965). However the question must remain open since canalicular bile has not been collected and the bile may concentrate during secretion.

Biliary secretion of compounds with colloidal properties

Brauer (1959) has noted that compounds with high molecular weights do not appear in bile e.g. inulin and plasma protein with molecular weight greater than 5000. Millburn (1970) stated that azo dyes with colloidal properties do not appear in rat bile. Examples of such dyes include Evan's blue and Trypan blue. The anions of these dyes have a molecular weight of about 800 and would be expected to have a high biliary excretion. From dialysis and molecular weight measurements the molecular weight of these dyes are highly associated to form colloids (Fieser and Fieser, 1950) which surpass the upper limit of molecular size for extensive biliary excretion (Millburn, 1970).

Mechanism for the secretion of foreign compounds in bile

There seems to be separate mechanisms for the hepatic secretion of organic anions, cations and uncharged molecules (Schanker and Solomon, 1963; Schanker, 1968). Several chemical and biological factors appear to determine the biliary elimination of compounds. The chemical factors include:

1. Molecular weight of at least 325-350.
2. A polar anionic, cationic, or non-ionic group in the structure.

3. The number of substituents and their position in the molecule.

The main biological factors include:

1. Metabolism and the influence of species difference and sex difference on metabolism.
2. Functions of the hepatic cells in taking and secreting the compounds against a concentration gradient.
3. Enterohepatic circulation and its effect on the clearance of a substance from the body.

The chemical factors play an important role in the biliary excretion of foreign compounds by the different species. Factors 1 and 2 facilitate the transfer of substances across the hepatic cell membrane. Some organic anions with high biliary excretion generally form complexes with plasma proteins. This is not a necessary prerequisite since some sulfonamides e.g. succinylsulfathiazole and sulfadimethoxine-N'-glucuronide are not bound to plasma protein but are excreted in the bile at a high level (Millburn, 1970). The binding of organic anions to the hepatic celluclar proteins may possibly be a factor in the extent of their biliary elimination. The cytoplasmic proteins have been isolated from the rat liver (Levi et al., 1968) which have a high affinity for BSP and bilirubin. It has been suggested that these proteins may be involved in the hepatic uptake of organic anions. Sandberg and Slaunwhite (1956) have pointed out that such binding may be important in the biliary excretion of uncharged molecules e.g. steroids. Priestly and O'Reilly (1966) have worked out the liver : blood protein

binding ratios for amaranth, geranine, lissamine and dechlorolissamine, and compared them to the $t_{\frac{1}{2}}$ (half life in minutes) for the primary biliary excretion rate of the drugs (Table 1). A higher ratio indicates a more rapid clearance of the dye in the bile.

Organic cations excreted in bile have one characteristic feature: a polar quaternary amine at one end of the molecule and one or more phenyl rings at the opposite end. Their importance in determining biliary secretion may be similar to that for anions, but a separate mechanism is believed to handle cation elimination in the bile (Millburn, 1970).

Schanker (1968) considers that uncharged molecules are excreted in bile involving a transport mechanism different from those for the anions or cations. Most of the compounds have a high molecular weight from 400-500 and possess a polar residue, which renders the molecule hydrophilic and facilitates the biliary excretion.

Modification of a compound via metabolism is an important factor e.g. conjugation with glucuronic acid which would increase the size and polarity in a molecule. Both molecular size and polarity are important for an extensive secretion into the bile.

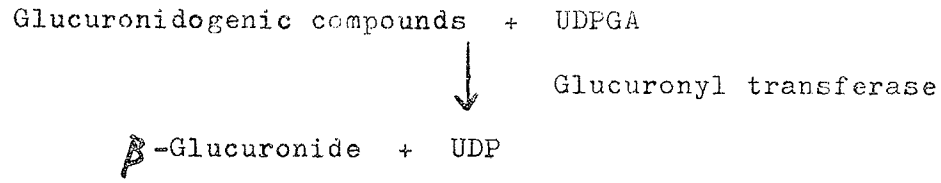
Metabolism of most organic compounds usually occur in two consecutive phases (Williams, 1959). Phase I may be oxidation, reduction, hydrolysis or deamination. Phase II may involve synthetic reactions such as conjugation with glucuronic acid,

Table I Protein binding and biliary secretion. (Priestly and O'Reilly, 1966)

Dye	Amaranth	Geranine	Lissamine	Dechlorolissamine
% excretion in bile in 6 hours.	53	46	96	80
$t_{½}$ minutes	10.9	13.7	24.7	30
Protein bound (% liver)	91	57	50	44
Protein bound (% blood)	81	62	78	85
Liver : blood binding ratio	1.12	0.92	0.65	0.51
Molecular weight of anion	535	463	505	436

hippuric acid and 'activated' sulphate. These reactions are catalysed by enzymes, especially the hepatic microsomal enzymes. The induction of microsomal enzymes by various agents inducing phenobarbitone, has been described (Conney, 1967).

Species differences in metabolism of compounds affect the amount of a compound excreted in bile. The domestic cat appears to have a deficient glucuronyl transferase system (Dutton and Greig, 1957) which may affect the following reaction:



UDPGA : Uridine diphosphate glucuronic acid

UDP : Uridine diphosphate

Hence glucuronide formation in the cat is relatively lower than other species (Robinson and Williams, 1958).

In the passage of a compound from blood to bile, it has to go through the permeable endothelium of blood sinusoids and the hepatic cell membrane. Two separate active transport processes may be involved in the uptake and in the excretion of the compound into bile. The endoplasmic reticulum, an intracellular membrane, seems to influence the channeling of the compound in the liver cell and affect the amount secreted into the bile (Levine et al., 1970). Phenobarbitone induces a marked proliferation of the endoplasmic reticulum (Remmer and Merker,

1965), yet, it appears that the newly formed membranes do not participate in the transport of organic anions from the liver to bile (Klassen, 1971).

Chapter II

Models for biliary excretion

Numerous models have been proposed for biliary excretion process (Winkler, 1965). The philosophy of pharmacokinetic modelling has been described in a number of reviews (Garret et al., 1963; Wagner, 1971; O'Reilly, et al., 1972). The basic purpose of such models is to provide a generalized mathematical picture of biological events intended to codify existing knowledge and to have predictive utility. The previous discussion of biliary excretion indicates that the process is varied and complex. To produce a satisfactory model some simplifying assumptions are essential. The basic observation which must be considered for inclusion in a biliary model are:

1. The uptake of material from the blood into the liver.
2. The passage of material from liver to bile.
3. Protein and tissue binding components within the liver and perhaps the blood (Priestly and O'Reilly, 1966).
4. The possible reflux of material from the liver to blood and from bile to liver (Richards, 1965).
5. The nature of the transfer process. Since concentrative transfer from liver to bile occurs (Brauer, 1959), it is probable that some form of saturable or active transfer occurs in one or more of the above processes.

If active transfer processes occur in any of the transport steps then the model proposed must take into account non-linear

or dose-dependent behaviour (O'Reilly, 1972). Such models are mathematically complex and frequently difficult to analyse even with high speed digital computing techniques. However at low dose levels complex active transport systems will behave in a first-order linear manner which greatly simplifies the mathematical description of the process. In this work pharmacokinetic models of the first-order linear type are developed and the data are tested for dose-independent behaviour by the principle of superposition (Dost, 1968). The models are intended to apply to low-dose behaviour (see chapter 3).

Most models so far developed have been based on blood level studies of drugs and dyes known to pass into bile. Much work has been carried out with sulphobromophthalein (BSP) (Winkler, 1965; Richards, 1965; Priestly, 1967; Quarfordt et al., 1971) and other dyes. In general the blood level curve of BSP may be described by a biexponential blood level curve and is usually interpreted in terms of distribution of dye (or drug) between a readily diffusible central compartment which includes the blood and a less readily diffusible peripheral compartment (Riggs, 1963). Such behaviour is commonly found with many drugs and metabolites excreted in either bile or urine and is known as the two-compartment model. In the case of BSP, Richards (1965) and other workers (Quarfordt et al., 1971) have interpreted the biexponential blood curve in terms of a reflux of dye from the liver back to the blood. Such a model (I) has been shown to fit the blood level of BSP in man (Quarfordt et al., 1971).

Comparatively little work has been done on the pharmacokinetics of biliary secretion of drugs and metabolites. The rat has a bile duct structure and bile flow characteristics which permit the relatively simple study of biliary pharmacokinetics. O'Reilly et al. (1971) used biliary excretion measurements and a simple pharmacokinetic model to determine the rate constants for demethylation and biliary excretion of methyl orange in the rat.

Earlier work with dyes including amaranth (Priestly and O'Reilly, 1966) and indocyanine green (Priestly, 1967) indicated that the dye material disappeared from the blood rapidly after injection. The kinetic behaviour of these dyes in the blood was not determined. Work to be described here indicates that the dye disappearance may be of a mono-, bi-, or tri-exponential type. When the rate of biliary excretion of dyes, including amaranth, was determined (Priestly and O'Reilly, 1966; Priestly, 1967) it was found to be triexponential. A model of biliary excretion of these dyestuffs must therefore take into account a mono-, bi-, or a tri-exponential disappearance from blood and a triexponential biliary excretion curve. On the basis of these requirements, numerous models may be developed for biliary excretion (O'Reilly, work in progress). In this thesis, three models (I, II, III)(Fig. 11) will be developed and examined in terms of blood and bile level data.

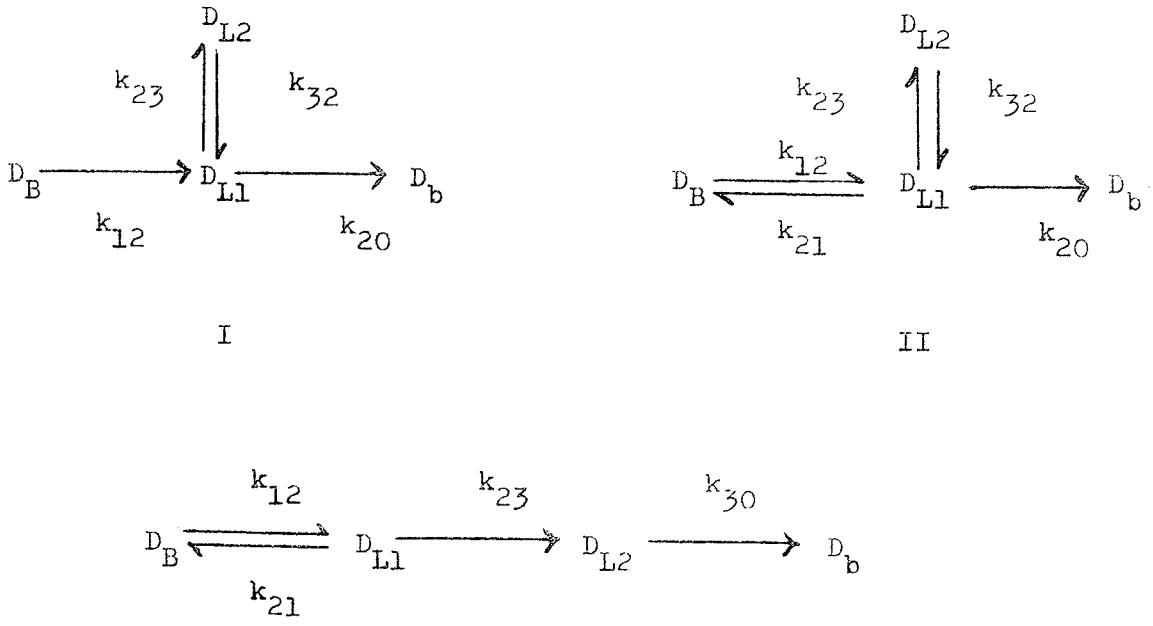


Fig. 11 Proposed pharmacokinetic models for the biliary secretion of dyes in rats. (O'Reilly et al., 1972)

D_B Amount of dye in the blood compartment.

D_{L1} Amount of dye in the unbound compartment of the liver.

D_{L2} Amount of dye in the bound compartment of the liver.

D_b Amount of dye in the bile.

k_{12} First-order rate constant for uptake of dye to the liver.

k_{21} First-order rate constant for reflux from the liver to blood.

k_{20} First-order rate constant for secretion into the bile from L_1.

k_{23} First-order distribution constant from L_1 to L_2.

k_{32} First-order rate constant for reflux from L_2 to L_1.

k_{30} First-order rate constant for secretion into the bile from L_2.

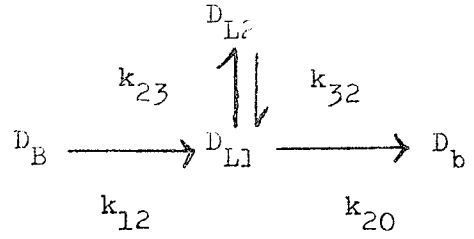


Fig. 12 Pharmacokinetic Model I

Model I

In this model a first-order uptake phase is followed by a distributive process involving tissue binding in the liver (D_{L2}) and first-order passage into the bile.

The differential equations of this model are:

$$\dot{D}_B = -k_{12}D_B \quad (1)$$

$$\dot{D}_{L1} = k_{12}D_B + k_{32}D_{L2} - (k_{20} + k_{23})D_{L1} \quad (2)$$

$$\dot{D}_{L2} = k_{23}D_{L1} - k_{32}D_{L2} \quad (3)$$

$$\dot{D}_b = k_{20}D_{L1} \quad (4)$$

The above equations may be solved by standard methods (Wagner, 1971) to give the following equations for the blood and bile levels of dye.

$$D_B = D_o e^{-k_{12}t} \quad (5)$$

$$D_b = \frac{k_{20}D_o(k_{32} - k_{12})}{(r_1 - k_{12})(r_2 - k_{12})}(1 - e^{-k_{12}t}) + \frac{k_{12}k_{20}D_o(k_{32} - r_1)}{r_1(k_{12} - r_1)(r_2 - r_1)}(1 - e^{-r_1 t}) + \frac{k_{12}k_{20}D_o(k_{32} - r_2)}{r_2(k_{12} - r_2)(r_1 - r_2)}(1 - e^{-r_2 t}) \quad (6)$$

where

$$r_1 = 0.5(k_{20} + k_{23} + k_{32} + \sqrt{(k_{20} + k_{23} + k_{32})^2 - 4k_{20}k_{32}}) \quad (7)$$

$$r_2 = 0.5(k_{20} + k_{23} + k_{32} - \sqrt{(k_{20} + k_{23} + k_{32})^2 - 4k_{20}k_{32}}) \quad (8)$$

This model is analogous to the two-compartment model with absorption (Loo, 1968) and all the parameters of the model may be determined from the biliary excretion plot. The parameters may be estimated as follows: a plot of log rate of biliary excretion versus the mid point of the collection intervals (Wagner, 1971) is constructed. The line (Fig. 18) rises to a maximum and then falls as a biexponential curve. By curve peeling techniques (Riggs, 1963; Wagner, 1971) the line may be fitted to a triexponential curve (equation 9)

$$D_b = -Ae^{-at} + Be^{-bt} + Ce^{-ct} \quad (9)$$

where one of a, b, and c is k_{12} and the other two are r_1 and r_2. If blood level data are available a plot of log C_p (blood level) versus t (time) will have a slope k_{12} (equation 5) and hence will tell us which of a, b, or c is k_{12} and thus completely determine the equation. If k_{12} = a, r_1 = b and r_2 = c, the above

rate equation for this model becomes

$$\dot{D}_b = \frac{k_{20}k_{12}D_o(k_{32} - k_{12})}{(r_1 - k_{12})(r_2 - k_{12})} e^{-k_{12}t} + \frac{k_{20}k_{12}D_o(k_{32} - r_1)}{(k_{12} - r_1)(r_2 - r_1)} e^{-r_1 t} + \frac{k_{20}k_{12}D_o(k_{32} - r_2)}{(k_{12} - r_2)(r_1 - r_2)} e^{-r_2 t} \quad (10)$$

From the coefficients of equation 10 and the following relationships from equations 7 and 8 the parameters of the model may be estimated. If equations 7 and 8 are added, equation 11 results, if multiplied we get equation 12.

$$r_1 + r_2 = k_{20} + k_{23} + k_{32} \quad (11)$$

$$r_1 \cdot r_2 = k_{20}k_{32} \quad (12)$$

If blood level data are not available(as in chapter 3, section 2), then various assumptions must be tried until a satisfactory solution is obtained. In equation 9 if k_{12} is not known then one index (e.g. 'a') could be equated with k_{12} and the values of the parameters calculated on this basis. If $k_{12} = a$, then $r_1 = b$ and $r_2 = c$, since from equations 7 and 8, $r_1 \gg r_2$. The first assumption is that $k_{12} > r_1 > r_2$; the parameters may also be derived on the assumption that $r_1 > k_{12} > r_2$ and $r_1 > r_2 > k_{12}$. Thus three sets of parameter values are theoretically possible from bile rate data if blood level information is not available.

Some sets are excluded if negative values of rate constant are obtained. The reasonable certainty of a unique identification of the parameters of a given set of data comes from computer simulation of data by analog or digital computer and from error evaluation by non-linear square fitting with the program NONLIN (Metzler, 1969). To fit this model on the digital computer a Fortran subroutine (DFUNC) was written for NONLIN, incorporating the algebraic equations of normalized blood and bile measurements (equations 5 and 10). This was the procedure used with amaranth (O'Reilly et al., 1972). With lissamine, dechlorolissamine and geranine, the subroutine involved fitting cumulative equation (equation 6). In each case the graphical estimates were used to start the computer program.

Analog fitting was carried out on the TR 20 Analog Computer (Fig. 13); trial and error estimation of parameter values may be made by this method and used as digital computer input. The analog computer does not give any estimates of error and the fitting of other than simple models to data is difficult (O'Reilly, 1972).

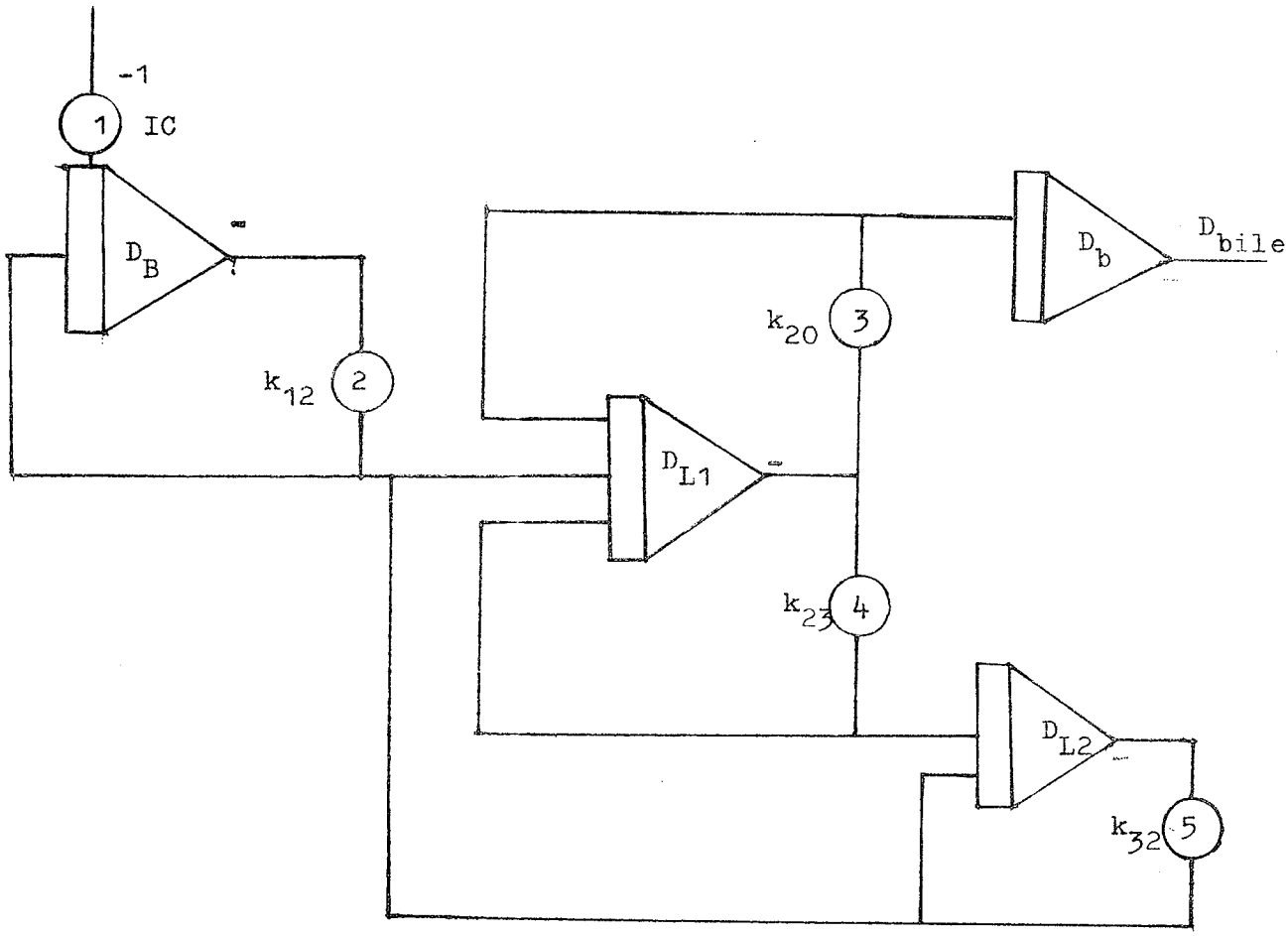
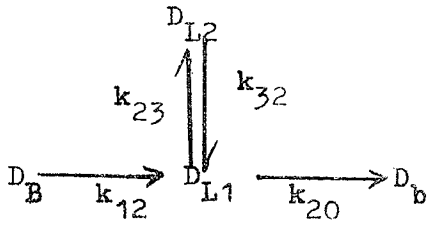


Fig. 13 Analog Circuit for Biliary Excretion Model I



Model I

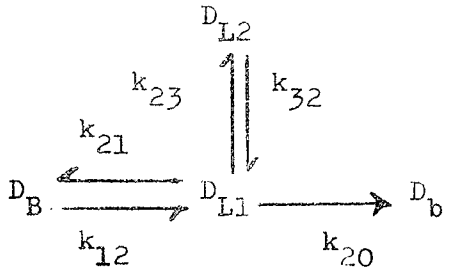


Fig. 14 Pharmacokinetic Model II

Model II

Here the reflux of dye from liver to blood is introduced. Otherwise this model is identical with I. Models similar to this one have been described in the literature for biliary excretion of dyes (Priestly, 1967) and orphenadrine (Hespe and Kafoe, 1970). No solution of this model has been published although similar models have been derived for other purposes (Wagner, 1971; Kruger-Theimer, 1966). The solution given here (O'Reilly, unpublished data) was obtained by Laplace transform techniques. The differential equations of the model are:

$$-\dot{D}_B = -k_{21}D_{L1} + k_{12}D_B \tag{13}$$

$$\dot{D}_{L1} = k_{12}D_B + k_{32}D_{L2} - (k_{21} + k_{20} + k_{23})D_{L1} \tag{14}$$

$$\dot{D}_{L2} = k_{23}D_{L1} - k_{32}D_{L2} \tag{15}$$

$$\dot{D}_b = k_{20}D_{L1} \tag{16}$$

Or solution of these yield:

$$D_B = Ae^{-at} + Be^{-bt} + Ce^{-ct} \tag{17}$$

where $A = \dfrac{D_o(a^2 - \Delta a - \Delta o)}{(b - a)(c - a)}$

$B = \dfrac{D_o(b^2 - \Delta b - \Delta o)}{(a - b)(c - b)}$

$C = \dfrac{D_o(c^2 - \Delta c - \Delta o)}{(a - c)(b - c)}$

and a, b, and c are the complex roots of a cubic equation arising from the Laplace solution of the system of equation. The values of a, b, and c in terms of rate constants are exceedingly complex but the following relationships may be used to estimate values of the parameters (O'Reilly, 1972).

$$\Delta = k_{20} + k_{21} + k_{23} + k_{32} \tag{18}$$

$$\Delta_o = k_{20}k_{32} + k_{21}k_{32} \tag{19}$$

$$abc = k_{12}k_{20}k_{32} \tag{20}$$

$$a + b + c = k_{32} + k_{12} + k_{20} + k_{21} + k_{23} \tag{21}$$

$$ab + ac + bc = k_{20}k_{32} + k_{20}k_{12} + k_{32}k_{12} + k_{21}k_{32} + k_{12}k_{23} \tag{22}$$

For the cumulative excretion of dye into bile (equation 23), the solution is:

$$D_b = \frac{k_{20}k_{12}D_o(k_{32} - a)}{a(b - a)(c - a)}\left(1 - e^{-at}\right) + \frac{k_{20}k_{12}D_o(k_{32} - b)}{b(a - b)(c - b)}\left(1 - e^{-bt}\right) + \frac{k_{20}k_{12}D_o(k_{32} - c)}{c(a - c)(b - c)}\left(1 - e^{-ct}\right) \tag{23}$$

Thus this model predicts that a triexponential disappearance from blood will be followed by a triexponential appearance in the bile. If blood data are available a plot of log C_p (blood level) versus t (time) should fit a triexponential equation (17) and from the values of the coefficient and indices the model parameters may be estimated using equations (17 - 22).

Digital computer programs were written for this model as follows:

a) For equation 17 the estimates of a, b, c, λ, and λ_o were used to obtain a good fit of the blood level curve, and also the refined estimates of the parameters which were then used to re-estimate the parameters.

b) The parameters thus obtained were returned to the computer with a subroutine of the differential equations 13 - 16 which were solved in the computer by a Runge-Kutta procedure. This method had to be used here because of the complex values of a, b, and c in terms of the parameters.

The application of these methods to rose bengal is given in chapter 3, section 3. For amaranth (chapter 3, section 1) a simpler approximate procedure was used. k_{12} was estimated from the initial slope of the blood level curve and a, b, and c estimated from the log rate of biliary excretion plot. Then using k_{12}, a, b, and c in equations 20 - 22 and equation 23 the parameters were estimated. These parameters were refined by non-linear least squares using subroutine b above.

Analog simulations with this model used the program shown in figure 15.

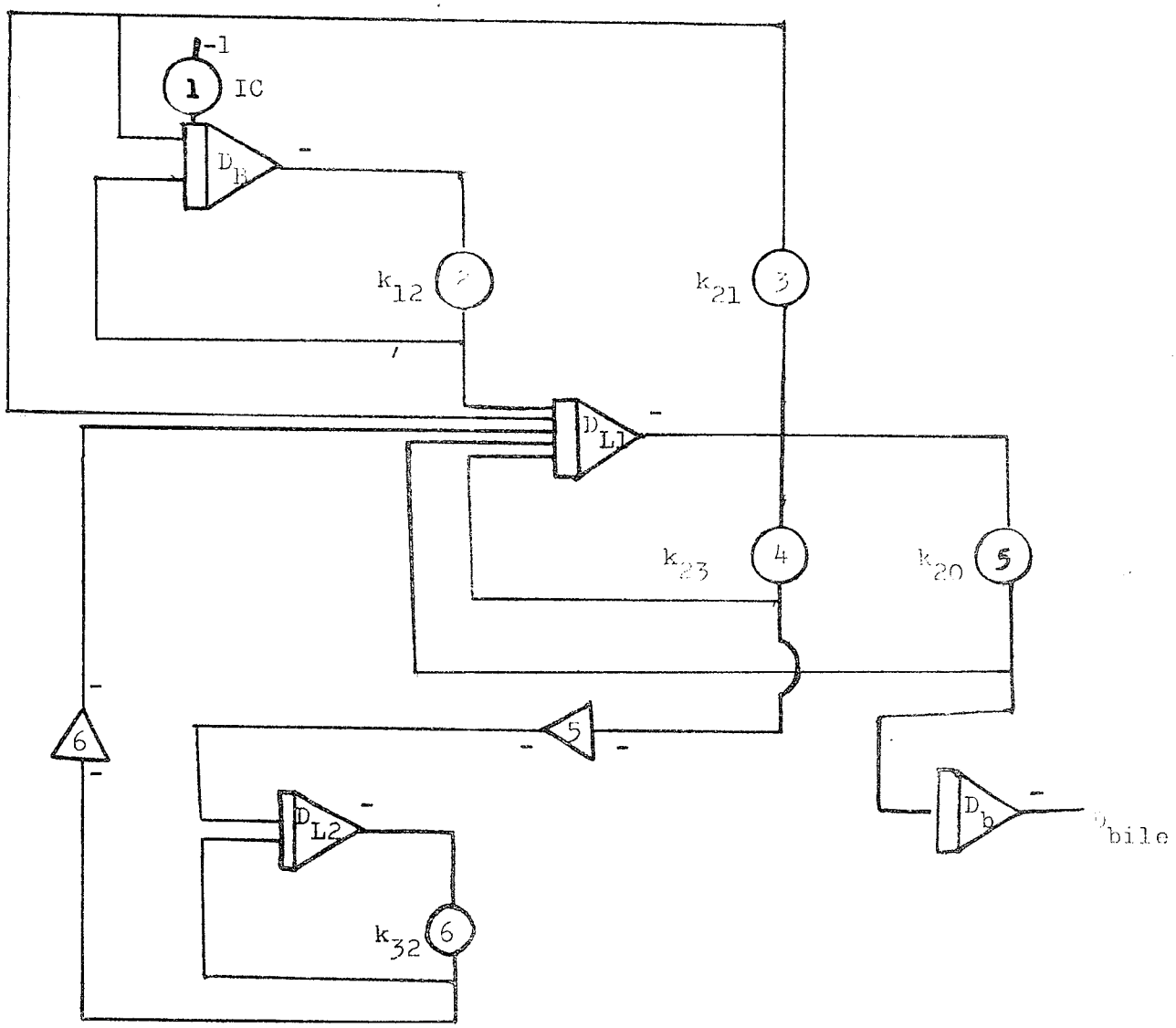
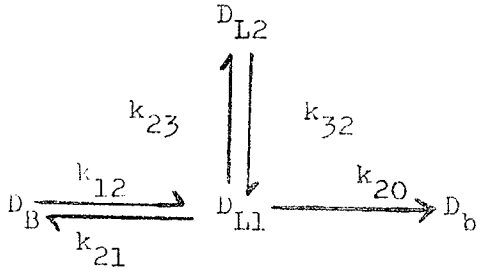


Fig. 15 Analog Circuit For Biliary Excretion Model II



Model II

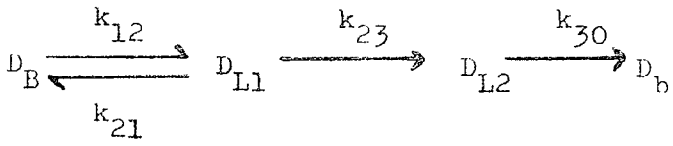


Fig. 16 Pharmacokinetic Model III

Model III

Turco et al. (1966) proposed a model for hepatic uptake and biliary excretion of rose bengal. Their data (blood levels) were fitted to this model by a deconvolution method using a digital simulation program. They did not publish a mathematical analysis of their model and the solution given below was carried out in this laboratory (O'Reilly, to be published). The differential equations of the model are:

$$\dot{D}_B = k_{21}D_{L1} - k_{12}D_B \quad (24)$$

$$\dot{D}_{L1} = k_{12}D_B - (k_{21} + k_{23})D_{L1} \quad (25)$$

$$\dot{D}_{L2} = k_{23}D_{L1} - k_{30}D_{L2} \quad (26)$$

$$\dot{D}_b = k_{30}D_{L2} \quad (27)$$

Solution of these equations gives the following solutions for D_B and D_b

$$D_B = \frac{D_o}{b - a}\left[(k_{21} + k_{23} - a)e^{-at} - (k_{21} + k_{23} - b)\, e^{-bt} \right] \quad (28)$$

$$D_b = \frac{k_{12}k_{23}k_{30}D_o}{a(b - a)(k_{30} - a)}(1 - e^{-at}) + \frac{k_{12}k_{23}k_{30}D_o}{b(a - b)(k_{30} - b)}(1 - e^{-bt}) + \frac{k_{12}k_{23}k_{30}D_o}{k_{30}(a - k_{30})(b - k_{30})}(1 - e^{-k_{30}t}) \quad (29)$$

where

$$a = 0.5\left[k_{21} + k_{23} + k_{12} + \sqrt{(k_{21} + k_{23} + k_{12})^2 - 4k_{12}k_{23}} \right] \quad (30)$$

$$b = 0.5\left[k_{21} + k_{23} + k_{12} - \sqrt{(k_{21} + k_{23} + k_{12})^2 - 4k_{12}k_{23}} \right] \quad (31)$$

and $$a + b = k_{21} + k_{23} + k_{12} \quad (32)$$

$$a.b = k_{12}k_{23} \quad (33)$$

This model predicts a biexponential disappearance of dye from the blood followed by a triexponential appearance in the bile (equations 28 and 29). Blood level data may be fitted to equation 28 and the parameters estimated from the coefficients and indices of the equation with the aid of equations 32 and 33. To estimate k_{30} bile data must be analysed. The fit of these equations to rose bengal pharmacokinetics will be described (chapter 3).

Digital computer subroutines were written for equations 28 and 29 using as starting estimates parameter values obtained graphically as described above. Analog simulations and fitting were carried out using the program outlined in figure 17.

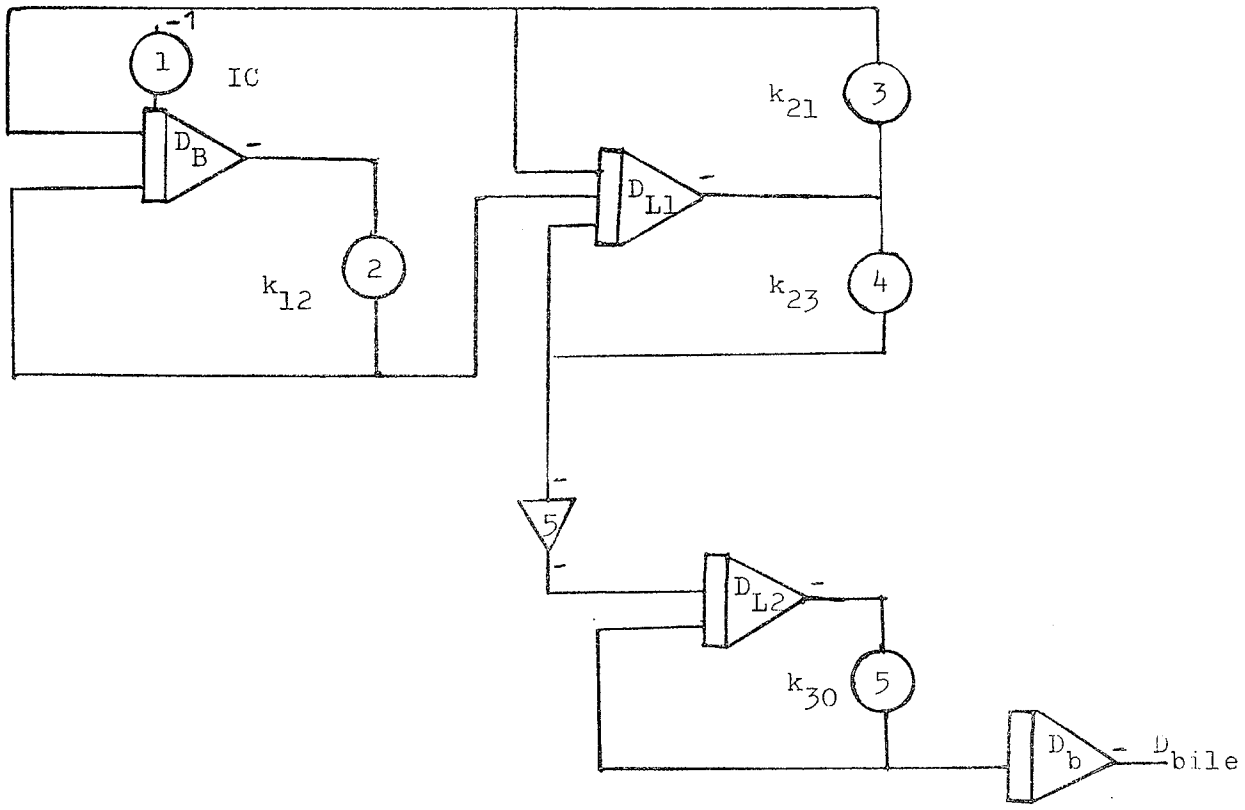


Fig. 17 Analog Circuit For Biliary Excretion Model III

$$D_B \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} D_{L1} \xrightarrow{k_{23}} D_{L2} \xrightarrow{k_{30}} D_b$$

Model III

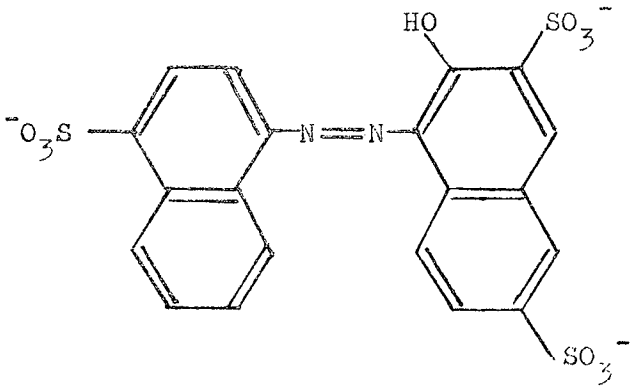
Section 2 Experimental Methods and Materials.

(a) Dyes

The dyes used in all experiments were commercial samples supplied as mentioned below.

1. Amaranth Red (Fisher Scientific Company)

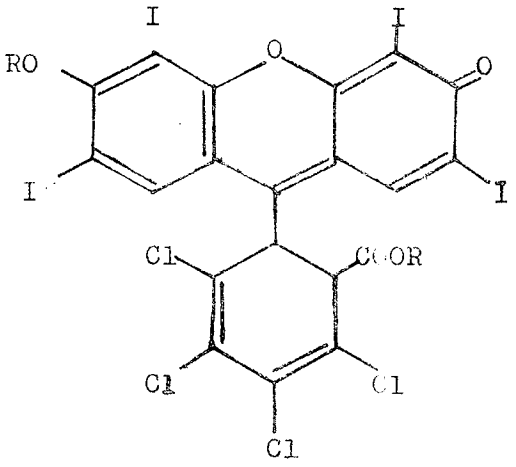
M.W. 535



2. Rose Bengal (British Drug House)

M.W. 974

R = Na



(b) Anesthetic

The anesthetic used was urethane (ethyl carbamate) supplied by Coleman and Bell.

Animals and treatment

Male Sprague-Dawley rats (the colony, Madison) of weight range 275 to 395 grams were used. The rats were maintained at normal body temperature by exposure to a radiator and incisons were surrounded by cotton swabs presoaked in normal saline.

Collection of bile sample

The animals were anesthetized with urethane (125 mg./Kg) injected intraperitoneally. A longitudinal incison was made below the diaphragm and the bile duct was ligated at the duodenal end. A cannula consisting of a 24-gauge hypodermic needle shaft attached to an appropriate length of polyethylene tube was fitted to the bile duct and secured with cotton thread. A blank bile sample was collected before injection of the dye.

Two methods of intravenous injection were employed, either via the femoral vein or the jugular vein. Bile samples at five minutes interval were collected into preweighed tubes for three hours or longer until nearly all the dye was eliminated via the bile.

Collection of blood samples

The method of Van Peten (1970) was adopted. The jugular vein was cannulated with a heparinized polyethylene tube which was secured with thread, 0.5 ml of blank blood sample was withdrawn before adminstration of the dose. The same volume of blood was collected at 2 to 15 minutes intervals for the first half hour after administering the dose. In some experiments the blood and the bile samplings were carried out at the same time.

Analysis

Blood samples: the blood samples were placed in centrifuge tubes containing heparinized normal saline (4.5 mls). In the analysis of amaranth the heparinized normal saline was also acidified with 0.01 M hydrochloric acid. The blood samples were then centrifuged at 600 rpm for 20 minutes in a General Laboratory Centrifuge Model-I. The supernatant was pipetted into the cuvette and the dye content was determined by reading the optical density in a Bausch and Lomb Spectronic 20, at the wavelength of maximal absorption for each dye:

Amaranth: 520 nm

Rose bengal: 580 nm

The standard curve for determination of the dye concentration was prepared by adding known quantities of dye to the blood and reading the respective optical densities.

Bile samples: the bile samples containing amaranth were diluted

with 0.01 M hydrochloric acid while those containing rose bengal were diluted with distilled water to a suitable volume such that the concentration coincided with the range covered by the standard curve of each dye. The dye content was determined by reading the optical density at the wavelength of maximal absorption as quoted in the analysis of blood samples. The standard curve for each dye was prepared by spiking the bile samples with known quantities of each dye and reading the respective optical density. A graph of optical density versus concentration of dye was plotted.

Enzyme induction experiments:

Phenobarbital (75 mg/Kg) was injected intraperitoneally into rats (320 - 360 g) for four days. Twenty-four hours after the last injection, the rats were cannulated and dosed with ten micromoles of amaranth via the femoral vein. Bile samples were collected as described before.

Control rats were treated with normal saline of equal volume as the phenobarbital injection for the same period and then dosed with amaranth as described for the test rats. Analysis of bile samples was as before.

Chapter III

Section 1 Result and discussions on amaranth.

After an intravenous injection, the blood samples and bile samples were obtained simultaneously. The blood level fell rapidly and the dye could not be detected within ten minutes of injection when the blood level had fallen below ten percent of its initial value. The blood level curve followed first-order kinetics with an elimination rate constant k_{12} of 13.52 h^{-} (range 12.94 - 15.69 h^{-}). This value was very similar to one of the exponents in the triexponential biliary excretion rate curve (Fig. 18). A similar rapid disappearance of dye from blood had been observed with indocyanine green in the rat (Paumgartner et al., 1970). The appearance in bile was very rapid after an intravenous administration. Earlier workers (Ryan and Wright, 1961) reported that 53 percent (range 43 - 79 percent) of the dye was excreted in the bile within four hours. With the present series, due to a slightly different technique and a longer period of collection, the recovery of bile was 72 percent (range 54 - 80 percent) for the one to twenty micromoles range. Priestly and O'Reilly (1966) indicated that the log rate of excretion of dye increased initially after injection and then declined in a biexponential fashion. The curve structure was shown to be independent of bile flow rate (Priestly, 1967). In this work a typical rate plot (Fig. 18) was analysed by the method of residuals into a triexponential function. Work with BSP (Priestly, 1967), triphenylmethane,

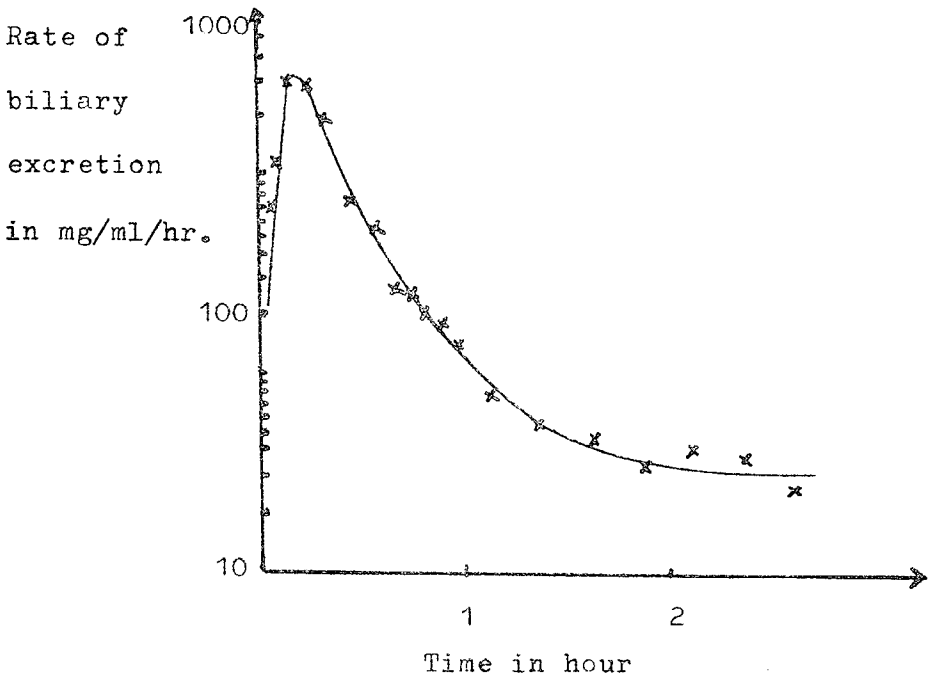


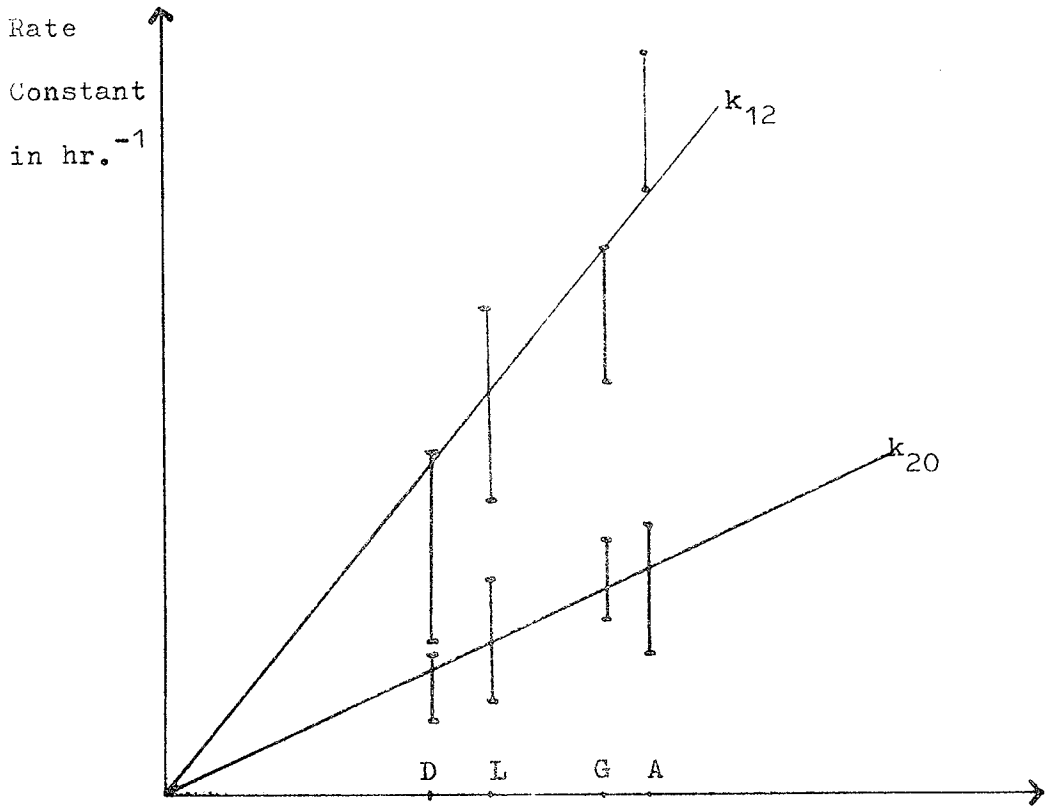
Fig. 18 Log rate of biliary excretion of amaranth in rat 11. The points are plotted versus the mid-point of the collection interval. Graphical analysis of this curve indicates that it may be described by a triexponential function

$(-10.7e^{-13.41t} + 8.5e^{-5.27t} + 0.13e^{-0.66t})$

which was used to estimate the parameters of the model.

xanthene and azo dyes (Iga et al., 1970, 1971a, 1971b) had exhibited similar curves. Iga et al. had interpreted the dye rate excretion curves as composed of two first-order rate components. This does not agree with their data which clearly exhibit triexponential behaviour.

Thus in the case of amaranth there is a first-order disappearance of dye from the blood and a triexponential appearance of dye in the bile. The simplest model which will fulfill these requirements is shown in figure 12, Model I. Amaranth is known to be bound to liver tissue (Priestly and O'Reilly, 1966; figure 19) and the D_{L2} compartment shown in the model is regarded as a tissue binding compartment. The first-order rate constant for uptake into the liver, k_{12}, was obtained as the slope of the log blood level versus time plot, as shown in equation 1. The other exponents in the triexponential function were taken as r_1 and r_2 (equation 2) and used to obtain estimates of the other rate constants of the model. The graphical estimates of rate constants were used as starting estimates in the computer program designed to fit the data to the equation of the model (equations 1 and 2) by an iterative non-linear least square procedure. The computer fit of the model to the data was excellent (Fig. 20). The rate constants for each dose level are shown in table II. Thus it would seem that Model I gives a satisfactory description of the data. However the behaviour of amaranth appears to be different from some other compounds like BSP (Winkler, 1965;



R, protein b nding ratio

Compound	R, protein binding ratio
D--Dechlorolissamine	0.51
L--Lissamine	0.65
G--Geranine	0.92
A--Amaranth	1.12

Fig. 19 Graph of rate constant versus protein binding ratio.

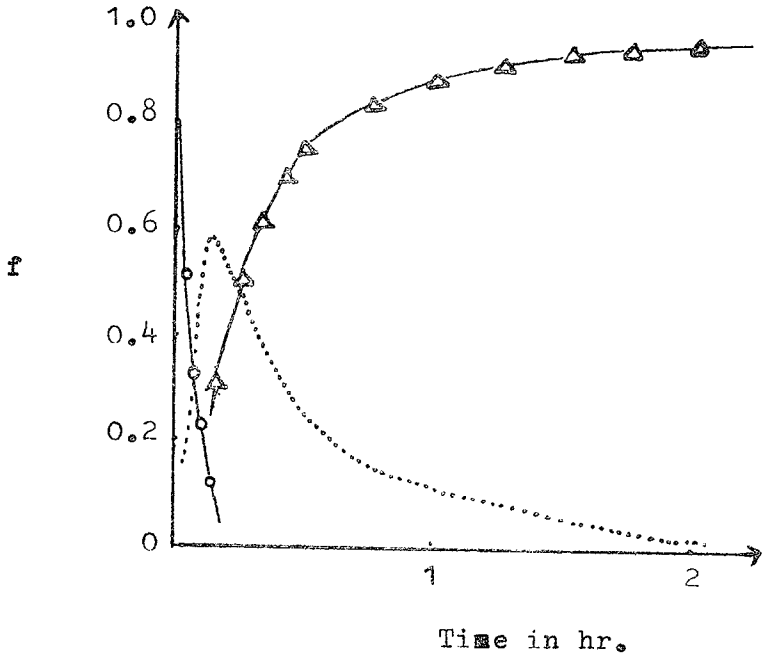


Fig. 20 Cumulative biliary excretion (▲) and blood level (o) curves in rat 6.

Data is calculated as the fraction (f) of the total material in the bile and blood compartment. In the blood, $f = C_b / C^o_b$ where C^o_b is the extrapolated blood level at zero time.

For biliary cumulation, $f = D_b / D^\infty_b$.

The solid lines represent the computer generated lines of best fit of the data for Models I and II.

The broken line is the hepatogram (total liver content of dye).

Richards, 1965), rose bengal (Turco et al., 1966), atropine (Kalser et al., 1965) and benzquinamide (Wiseman et al., 1964) which show biexponential blood curves instead of a mono-exponential disappearance from blood before biliary secretion. Frequently for various reasons, a reflux of material from liver to blood has been suggested to explain the biexponential blood curve (Quarfordt et al., 1971). Two other models (II and III) which predict a triexponential appearance in the bile (equations 6 and 11), are shown in figure 11. Model II predicts a triexponential disappearance from the blood (equation 3) while Model III a biexponential one (equation 10). While this is different from the monoexponential behaviour actually observed with amaranth, simulations of both models on the analog computer (EAI, TR20) indicated that an essentially first-order blood curve such as that obtained with amaranth could arise if $k_{12} \gg k_{21}$ in either model.

Values of the rate constants were estimated for Model II from the triexponential biliary excretion curve, k_{12} was estimated from equations 6 - 9. These estimates were input to the computer with the experimental results and the differential equations of the model, the refined values of the rate constants were obtained. In the three sets of data treated this way the fit to the blood and bile curves was very good (Table III). The rate constants may be compared with those obtained for Model I (Table II). The rate constants k_{12} for liver uptake and k_{20} for biliary excretion are similar in both models but the

Rat	Dose mol	k_{12} h^{-1}	k_{23} h^{-1}	k_{32} h^{-1}	k_{20} h^{-1}
1	1	13.45 ± 0.04	0.73 ± 0.42	2.93 ± 1.88	3.92 ± 0.45
2	5	13.34 ± 0.51	0.49 ± 0.22	3.56 ± 1.74	4.19 ± 0.23
3	5	13.13 ± 0.51	0.69 ± 0.22	3.69 ± 1.37	3.51 ± 0.19
4	10	13.41 ± 0.58	0.73 ± 0.24	3.29 ± 1.27	3.23 ± 0.19
5	10	13.46 ± 0.41	1.17 ± 0.13	1.89 ± 0.27	5.30 ± 0.20
6	10	13.54 ± 0.29	1.09 ± 0.09	1.66 ± 0.18	4.54 ± 0.12
7	10	13.21 ± 0.63	0.95 ± 0.23	2.05 ± 0.63	4.14 ± 0.27
8	10	13.24 ± 0.66	1.14 ± 0.27	2.31 ± 0.70	4.62 ± 0.30
9	10	13.34 ± 0.63	0.99 ± 0.25	2.29 ± 0.69	5.42 ± 0.33
10	10	15.69 ± 0.66	0.91 ± 0.15	2.07 ± 0.43	4.59 ± 0.25
11	10	12.94 ± 0.90	1.15 ± 0.34	3.45 ± 0.68	4.12 ± 1.65
12	20	13.50 ± 0.49	1.26 ± 0.12	1.31 ± 0.16	4.63 ± 0.25
13	20	13.47 ± 0.38	1.41 ± 0.13	1.30 ± 0.15	5.66 ± 0.20

Table II

Model I rate constants for biliary excretion of amaranth at varying dose levels.

The figures are shown ± S.D.* of the parameter.

S.D.* is the standard deviation.

Rat	k_{12} h^{-1}	k_{21} h^{-1}	k_{23} h^{-1}	k_{32} h^{-1}	k_{20} h^{-1}
6	13.74	0.28	0.98	1.52	4.49
	±0.32	±0.16	±0.29	±0.39	±0.20
10	15.94	0.27	0.58	1.31	4.43
	±0.75	±0.32	±0.34	±0.84	±0.31
11	12.77	0.22	0.32	2.51	4.22
	±1.35	±0.20	±0.14	±0.27	±0.57

Table III

Model II rate constants for biliary excretion of amaranth at ten micromoles dose level.

The figures are shown ± S.D. of the parameter.

rate constants for distribution, k_{23} and k_{32}, differ considerably. The rate constant for reflux into the blood, k_{21}, was small.

Thus it appears that Models I and II give a good fit to the data. In a study of the biliary excretion of rose bengal, Turco et al. (1966) measured the hepatogram or overall liver content of radioactive dye versus time curve. To evaluate if this method could be used to distinguish between Models I and II for amaranth, the data and the rate constants were used in an analog computer to generate the amounts of dye in the blood, bile and total liver compartments during the time course of the experiments. All these compartments were identical for both models. Hence the data will not permit any distinction between Models I and II.

Attempts to fit the data to Model III by using similar techniques applied to Model I but with equations 11 - 15 were unsuccessful using either the analog computer or the digital computer methods. It was thus concluded that Model III would not describe the biliary excretion of amaranth.

It must be concluded that as determined by the analytical methods described, the pharmacokinetics of amaranth are best represented by Model I. However, a small contribution of reflux from liver to blood cannot be excluded.

Studies on the biliary excretion of amaranth at different dose levels

Anionic compounds excreted via the biliary route probably

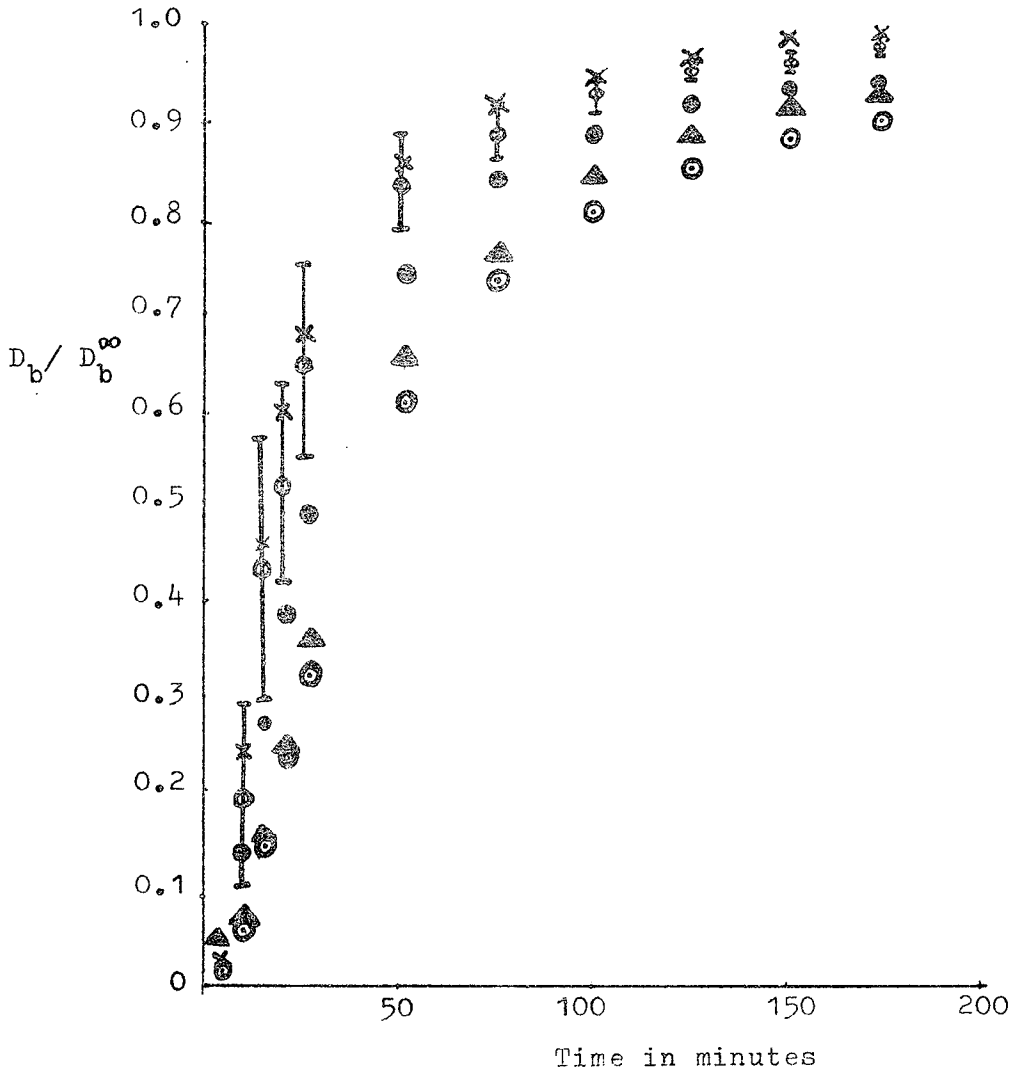


Fig. 21 Superposition of cumulative excretion curves for amaranth at dose levels of 10 mols. (⊕) ± S.D.* , 20 (x), 30 (●), 40 (▲), and 50 (◎) mols. Each point was normalised as a fraction of the total dye excreted in the bile, D_b/D_b^∞

* S.D. Standard deviation

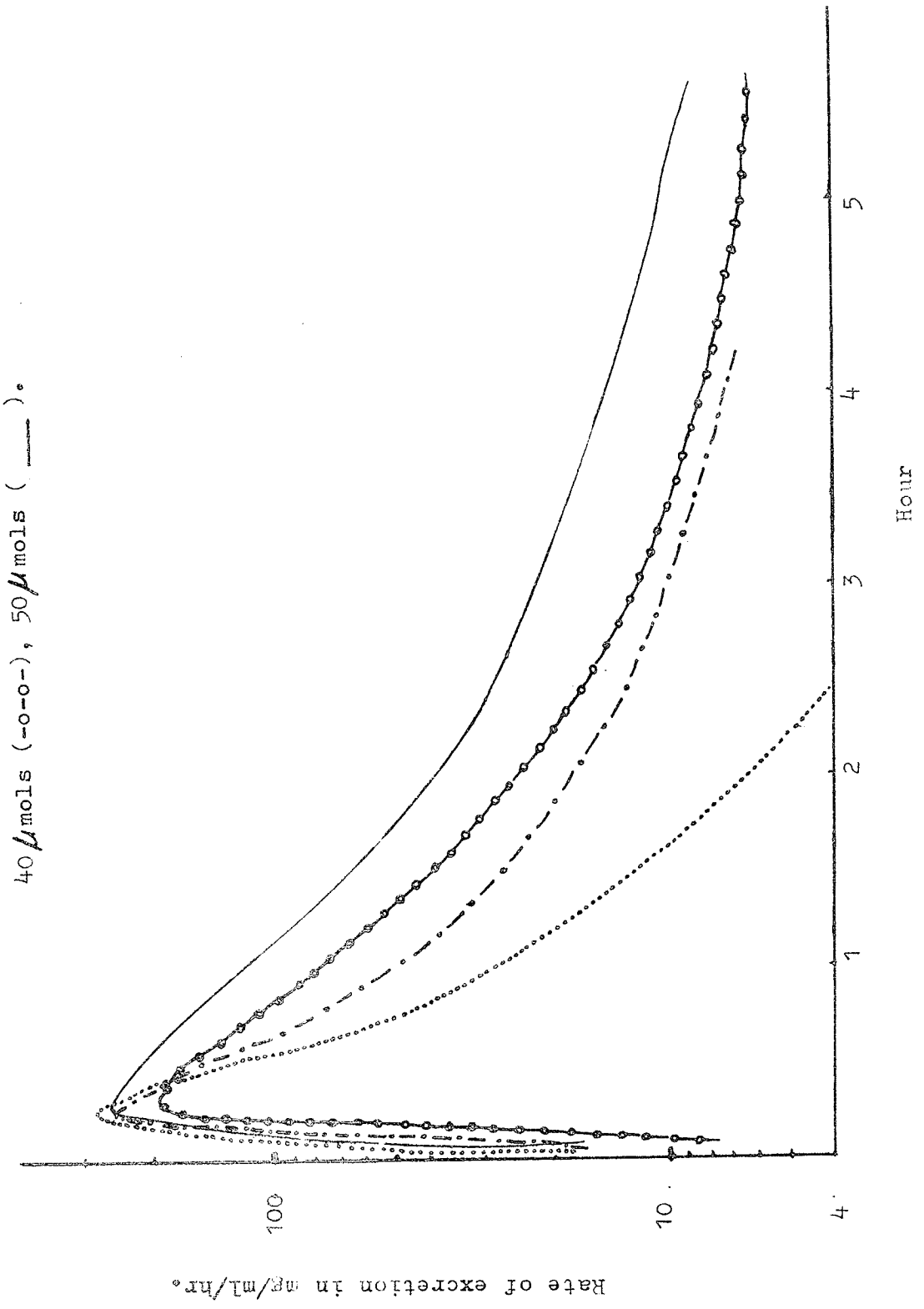


Fig. 22 Graph showing the biliary excretion of amaranth versus time at the dose levels of 20 μmols (•••), 30 μmols (-•-), 40 μmols (-o-o-), 50 μmols (——).

follow a pathway involving one or more transport processes (Sperber, 1959; Millburn, 1970). Hence these compounds should show transport saturation effects which will be reflected in the dose-dependent biliary transport kinetics. To test this possibility the principles of superposition of Dost (1968) was applied. The data for each dose level was normalized as a function of total dye excreted in the bile. The cumulative excretion plots for doses from ten to twenty micromoles superimpose (Fig. 21) indicating that the dye pharmacokinetics are independent of dose. This is not the case for doses above twenty micromoles (Fig. 22). Higher doses exhibit dose-dependent kinetics and probably require a model of a non-linear type.

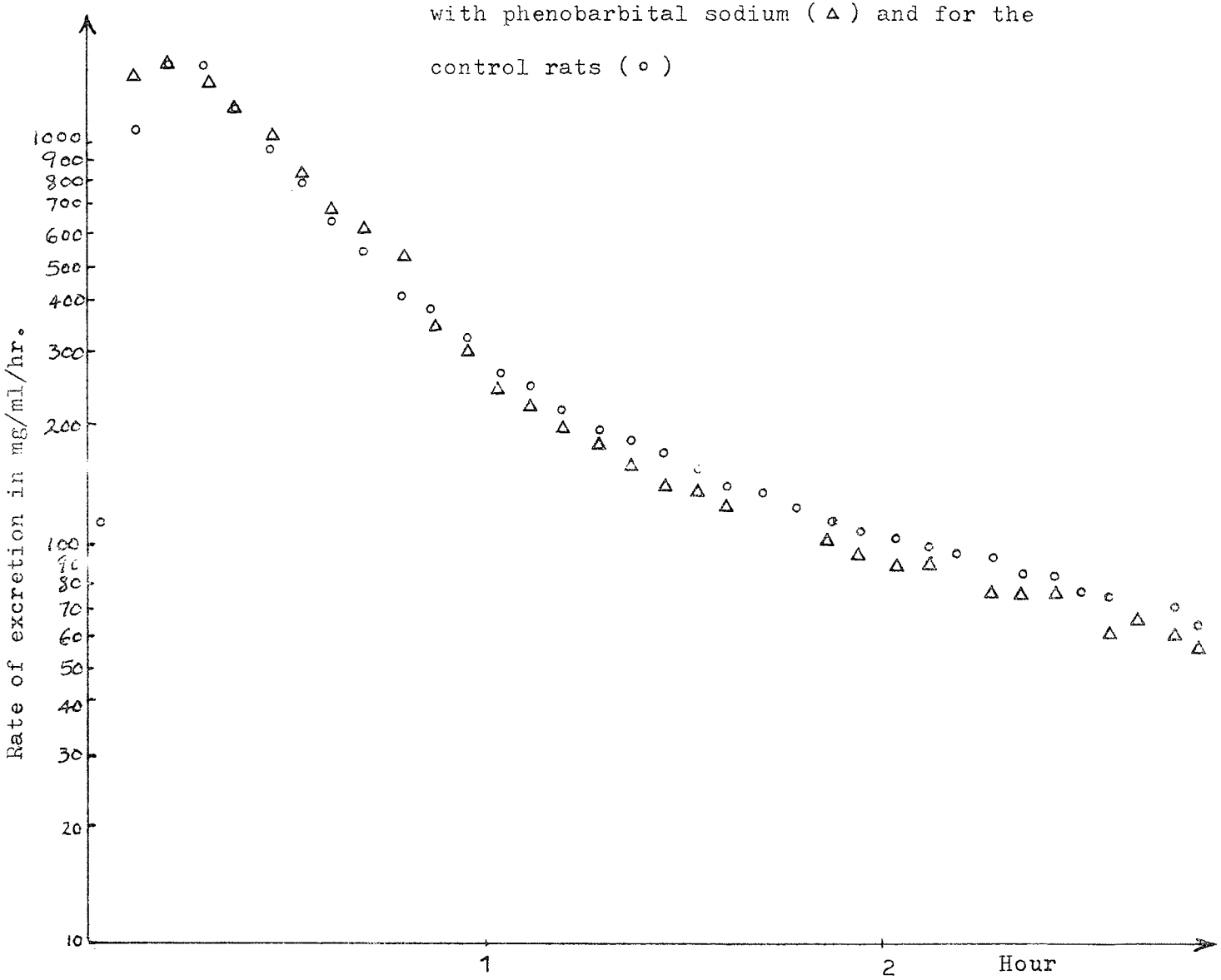
Effect of phenobarbital on biliary excretion of amaranth

A variety of polycyclic hydrocarbons, drugs and hormones have shown enhancing effect on the activities of microsomal and soluble enzymes. Phenobarbital enhances the microsomal activity and thus increases the rate of biotransformation of a large number of drugs. Sodium phenobarbital has been shown to increase hepatic bilirubin conjugating capacity in mice and rabbits and stimulate sulfadimethoxine glucuronide formation in rats (Crigler and Gold, 1969). Pharmacokinetic studies with salicylamide showed that the deficient glucuronide conjugating capacity in jaundiced infants was restored to normal with phenobarbital treatment (Yaffe et al., 1966).

This increased biotransformation rate reduces the biological half life of many drugs. However, phenobarbital also enhances biliary flow (Roberts and Plaa, 1967). This increase in biliary flow appears to play an important role in the accelerated plasma disappearance of BSP (Klassen and Plaa, 1968), indocyanine green (Hart and Adamson, 1968), and thyroxine (Goldstein and Taurog, 1968). The purpose of the present investigation is to observe the effect of phenobarbital treatment on the biliary excretion of amaranth.

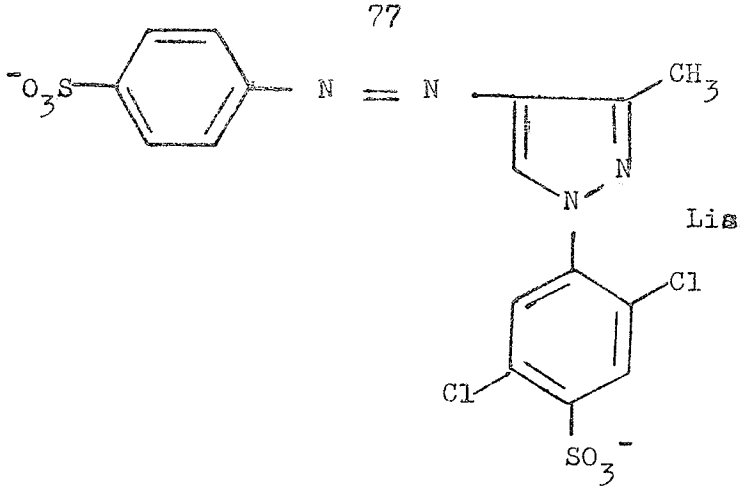
It was observed that rats treated with phenobarbital for a period of four days showed enhanced biliary flow as compared to the control rats treated with normal saline instead. However the biliary rate of excretion expressed as microgram per ml of bile per hour in both the test rats and the control rats were essentially the same (Fig. 23). The rate constants for the flow curves fitted to Model I are essentially the same and it may be concluded that the dye is excreted in both treated and untreated animals by a similar mechanism and independent of bile flow. This agrees with the fact that amaranth was not metabolized by the hepatic microsomal enzymes in rats and the increased biliary output of the dye is a direct effect of the enhanced bile flow. The clinical importance of the enhanced biliary flow after phenobarbital treatment is still not well-known. If phenobarbital has a similar effect in man as in rats, the biological half lives and drug action will be reduced for many compounds eliminated in bile.

Figure 23 Graph showing the rate of biliary excretion of amaranth versus time for rate pre-treated with phenobarbital sodium (△) and for the control rats (○)



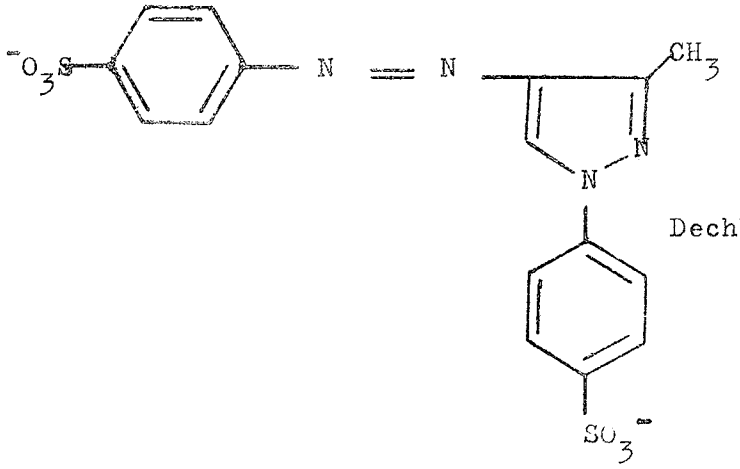
Section 2 Biliary excretion of geranine, lissamine, and dechlorolissamine.

Data on the azo dyes geranine, lissamine, and dechlorolissamine had been obtained earlier (Priestly and O'Reilly, 1966). The data was fitted here to Model I (Fig. 12) as previously described (chapter II--p.44). Initial values of the rate constants were estimated from the triexponential biliary rate of excretion curve and the cumulative curve. The data was processed in the digital computer using the NONLIN Program (Metzler, 1969). Values of the rate constants obtained for these dyes at different dose levels are presented in table IV. Since the blood level data for these dyes were not available, an initial graphical estimation of k_{12} could not be obtained directly. These parameters could be fitted more accurately to the data if blood level determination were available. However the fit obtained was good and the parameters for the various dyes may be usefully compared with that of amaranth (Table IV). The rate constants for hepatic uptake of dye are generally large compared to the other rate constants (Table IV). This would support the view that uptake into the liver is a rapid process. Among the four dyes the hepatic uptake rate is highest for amaranth (Table IV).



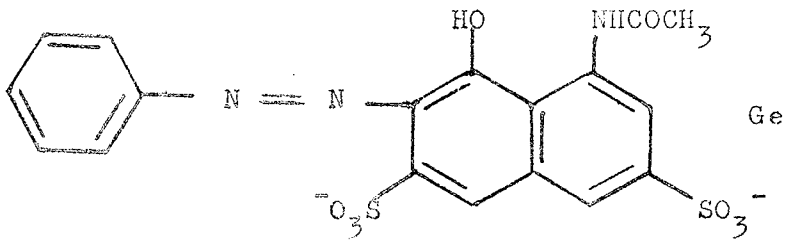
Lissamine Fast Yellow

M.W. 505



Dechlorolissamine

M.W. 436



Geranine

M.W. 463

Fig. 24 Structures of the azo dyes.

Table IV Averaged parameters for various azo dyes for Model I biliary excretion.

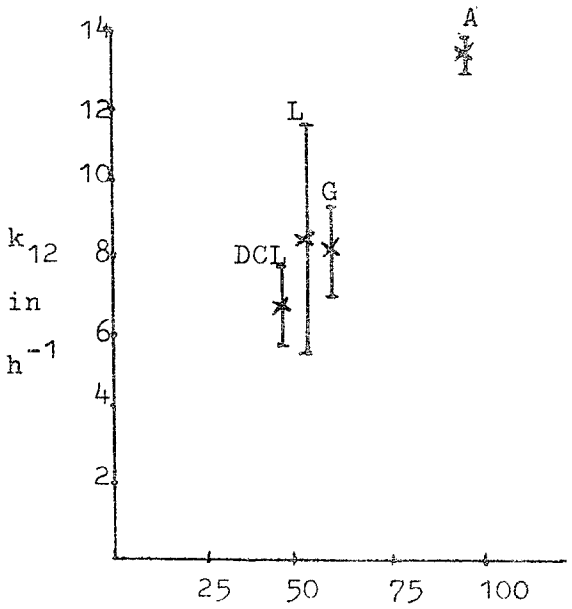
Dye	k_{12} h^{-1}	k_{23} h^{-1}	k_{32} h^{-1}	k_{20} h^{-1}
Geranine 10*	8.19 ± 1.21**	1.07 ± 0.88	2.66 ± 1.94	3.75 ± 0.81
Lissamine 10	8.51 ± 3.24	0.73 ± 0.51	2.71 ± 1.41	3.03 ± 1.53
Dechlorolissamine 7	6.72 ± 1.05	0.51 ± 0.25	2.83 ± 1.08	1.67 ± 0.31
Amaranth 13	13.45 ± 0.40	0.92 ± 0.25	2.56 ± 1.05	4.36 ± 0.33

* number of rats used to obtain the data

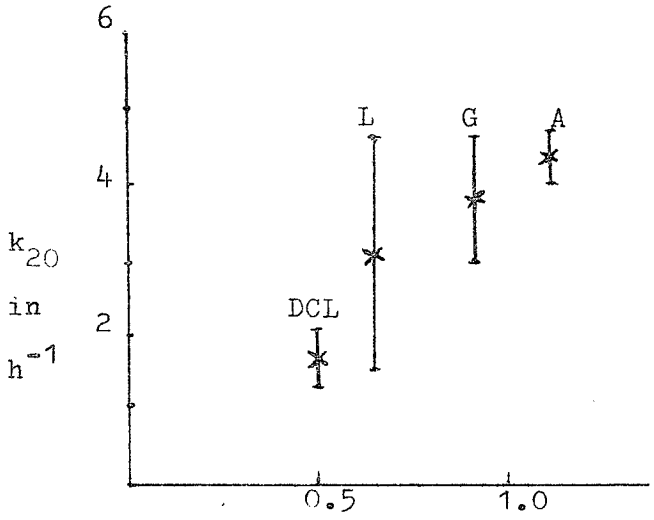
** the standard deviation of the mean of the parameters

The rate parameters were correlated with the protein binding of the dyes in the liver and the liver : blood binding ratio as obtained by Priestly and O'Reilly (1966). Plots to show these correlations are given in figure 27. The linear correlation of k_{12} versus protein binding in liver was excellent (0.995). This strongly supports the earlier view (Priestly and O'Reilly, 1966) that liver protein binding plays an important part in the hepatic uptake of dye. The correlation of protein binding with other model parameters is much less marked.

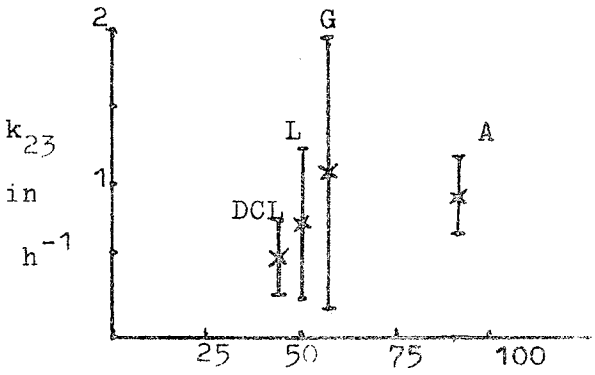
Fig. 27



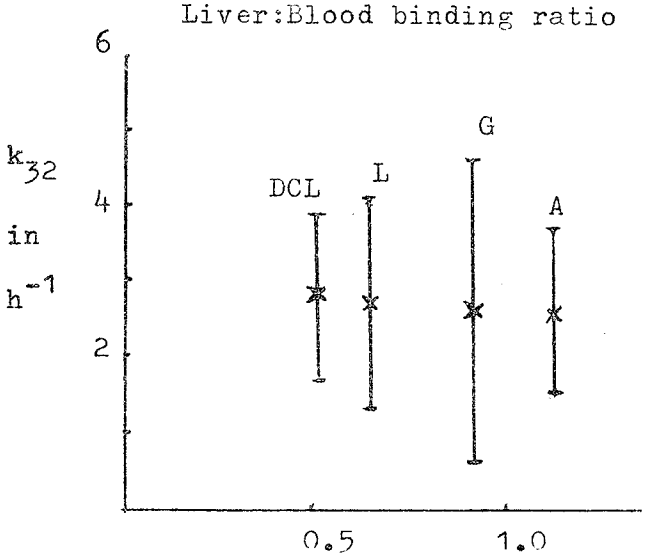
*Percentage protein bound in liver



** Liver:Blood binding ratio



Percentage protein bound in liver



Liver:blood binding ratio

A - Amaranth G - Geronine L - Lissamine DCL - Dechloro-Lissamine

Fig. 27 Diagrams showing the correlation of rate parameters with protein binding of dyes in the liver and the liver:blood binding ratio.

* See Table I for numerical values

** See Table I for numerical values

Section 3 Biliary excretion of rose bengal.

After an intravenous injection of a ten micromoles dose into the rat, both the blood and the bile samples were collected. The dye level in blood fell rapidly to less than 5% of its initial value and dye appeared in the bile within five minutes after adminstration. The collection of both blood and bile samples can severely deplete the animal and necessitates rather infrequent blood sampling. On the other hand mathematical analysis may be better accomplished with a large number of observations. When the blood level data for the test rats were plotted on the same graph, it was observed that they followed the same curve. By normalizing all the data as a fraction of Cp°, combined graph for the blood values of rose bengal was constructed. The composite values were then used in all the computer programs to describe the rose bengal in blood.

Cumulative excretion curves were constructed of the biliary excretion data and an asymptote value was estimated, expressed as D_b^{∞}. The biliary cumulative amount of dye obtained at any given time was then converted to a fraction of the asymptote as D_b/D_b^{∞} and expressed as a percentage.

Earlier workers (Turco et al., 1966) studied the blood kinetics of rose bengal. They proposed a model which described a biexponential disappearance of dye from blood but without any mathematical solution. In this work the blood curve

obtained showed a biexponential disappearance but the possibility of a triexponential disappearance could not be excluded. Hence Model I which requires a biexponential blood curve and Model II which requires a triexponential blood curve were tested in the analog computer and in the digital computer using the NONLIN Program (Metzler, 1969). The analog and digital fit of the blood curve to Model II were good (Fig. 25) but no satisfactory fit could be obtained for Model I and for Model III of Turco et al. (1966). The rate of appearance of dye in bile was found to be triexponential. The parameters of Model II were evaluated by graphical analysis of the data as described earlier (p. 52). The parameter estimates were used to start the computer program, using the differential equations of the model (equations 13 - 16). This method is slow and expensive and was used here with the blood data to obtain estimates of k_{12} and k_{21}. Values of other parameters (k_{32} and k_{23}) were obtained by this method with a rather high standard deviation. To obtain refined values of these parameters, a programme was used in which k_{12} and k_{21}, a, b, and c in equations 17 and 23 were held constant, and k_{20}, k_{32} and k_{23} allowed to vary. By this procedure values for all the rate constants of the model were obtained (Fig. 26) and the calculated curve gave an excellent fit of the data.

Model II was found to represent the biliary excretion of rose bengal better than Model I. This may be explained by the fact that a reflux of the dye from the liver to the blood

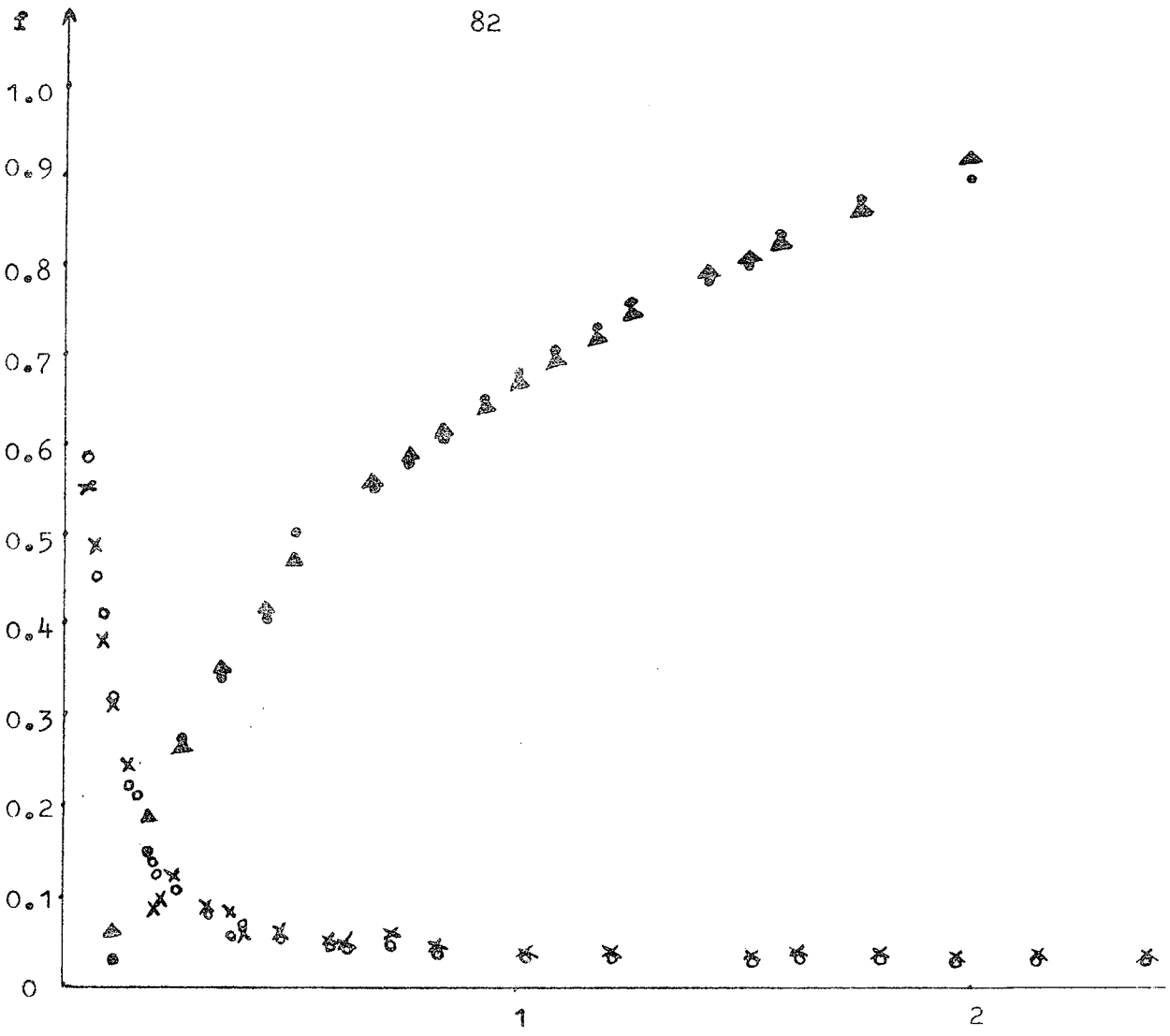


Figure 25 Graph showing the calculated and the observed blood level curves and the cumulative biliary excretion curves for rose bengal as obtained from rat 2.

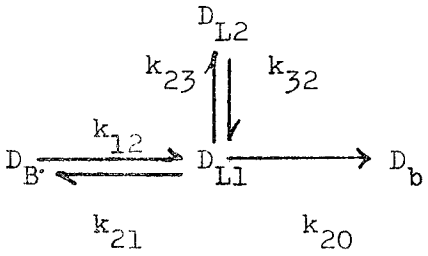
- ● Observed cumulative biliary excretion curve.
- ▲ Calculated cumulative biliary excretion curve.
- ✕ Observed blood level curve.
- ○ Calculated blood level curve.

$f = C_p / C_p^o$ for blood level curves.

$f = D_b / D_b^\infty$ for biliary excretion curves.

Fig. 26 Model II and the values of the rate constants describing the model as obtained from the data for rose bengal.

Model II



Values of the rate constants:

k_{12} 14.47 ± 0.52

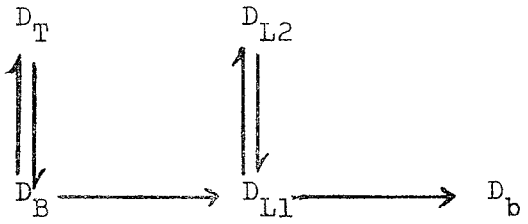
k_{21} 1.67 ± 0.28

k_{12} and k_{21} were held constant for the evaluation of k_{20}, k_{32}, and k_{23}, the estimation of which was illustrated in the following:

Rat	k_{20}	k_{32}	k_{23}
1	2.93 ± 0.06	0.56 ± 0.03	1.12 ± 0.25
2	2.22 ± 0.05	0.77 ± 0.04	1.54 ± 0.18
3	1.97 ± 0.07	0.69 ± 0.04	1.94 ± 0.37

All the rate constants are expressed as h^{-1}

occured which also accounts for the triexponential disappearance of dye from blood. An alternative explanation may be the distribution of the dye from blood to the extrahepatic peripheral compartment as illustrated in the following diagram:



D_T: Extrahepatic tissue compartment.

The hypothesis of 'delayed compartments' proposed by Dedrick and Bischoff (1971) on the metabolism of methotrexate in rats may be applicable to explain the biliary excretion of rose bengal.

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