The Synthesis and Evaluation of Novel Cholegraphic Media

A thesis submitted for the degree of

Doctor of Philosophy

of the

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by

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July, 1976.

Why did I decide to add to the infinite
Series one more symbol? Why, to the vain
Skein which unwinds in eternity
Did I add another cause, effect, and woe?

"The Golem"
Jorge Luis Borges

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INTRODUCTION

The development of modern cholegraphic media has taken place over the last half century. The progress made has been notable. The agents presently available allow the physician to visualize the gall bladder and the biliary tree and to consequently diagnose many pathological conditions. However, the use of cholegraphic media, especially those administered intravenously, is not without hazard. Apart from relatively mild side effects such as nausea and diarrhoea, circulatory collapse and acute renal failure have been noted as a consequence of the use of these agents. The widespread use of these compounds as diagnostic agents makes even infrequent fatalities a matter of concern.

Therefore, the discovery of molecules that exhibit fewer side effects is essential. New agents would preferably be administered via the oral route which is more convenient and less hazardous than the intravenous route. In developing oral cholegraphic agents, it is necessary not only to develop compounds that will be successfully excreted in the bile but also to develop compounds that will be absorbed rapidly and completely from the gastrointestinal tract.

The selection of a direction in the synthesis of new compounds requires the researching of what has been a success and what has been a failure in the past. It also requires that the researcher respond to feedback from the work in progress.

Wallingford (1953) described the discovery of 3-acetamido-2,4,6-triiodobenzoic acid (Urokon), the first triiodinated urographic medium. This discovery followed more than 20 years after the introduction of the first urographic medium. Wallingford described his discovery as being a result of "serendipity" and stated: "In the case of Urokon, the chances of discovering a new and useful compound might have seemed remote, in view of the toxicity of benzoic acid and the extensive exploration we and others had made among closely related compounds. I doubt if the investigation leading to Urokon would have been undertaken if the chances of success had been carefully weighed. An incipient idea is a delicate thing, likely to shrivel in the blast of budgetary control, market analyses, or even literature surveys."

It is hoped that the present study has made a contribution, albeit small, to the knowledge required for the development of safer iodinated cholegraphic media. In the present study, several new iodinated compounds were synthesized. The potential usefulness of these novel compounds was tested by examining the extent of their biliary excretion following intraduodenal administration to rats. Bile samples were analyzed using an x-ray preferential absorption apparatus developed for the present study. The biliary excretion of some of these compounds was also examined following intravenous administration. A preliminary screen of the metabolism of these compounds was also carried out. Finally, the metabolism and excretion of methylglucamine, a cation frequently used in x-ray diagnostic agents, was studied in the rat.

PART I: THE SYNTHESIS AND EVALUATION OF NOVEL CHOLEGRAPHIC AGENTS

LITERATURE SURVEY

Several extensive reviews covering most aspects of iodinated radiocontrast agents can be found in the literature. The reviews of Chenoy (1965), Shockman (1967), Gupta (1970), Herms and Taenzer (1975) and Laval-Jeantet (1975), a conference report of the New York Academy of Sciences (Whitelock, 1959), a book by Knoefel (1961) and a two volume report in the International Encyclopedia of Pharmacology and Therapeutics (Knoefel, 1971) together provide an extensive account of most aspects of radiocontrast agents including an historical overview. The present review is restricted to a brief account of the development of cholegraphic agents with particular attention paid to the orally active media. A summary of the toxicity of these agents is presented as well as a summary of how structural features affect biliary excretion and gastrointestinal absorption. Finally, a brief account of the biotransformation of the orally administered agents is given.

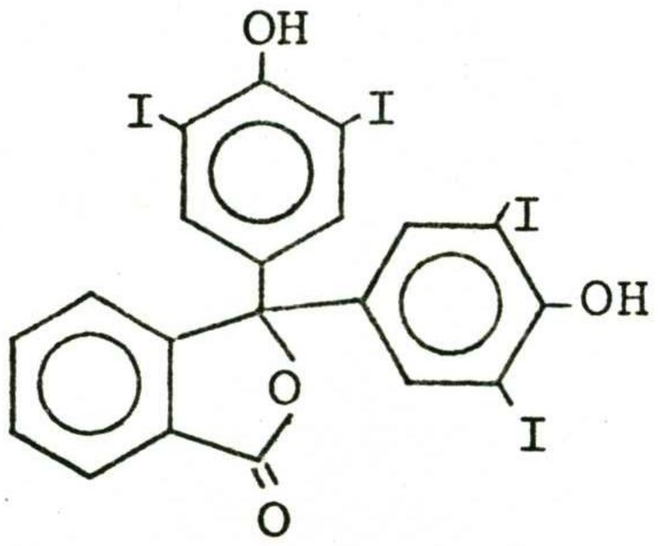
Iodinated radiopaque agents owe their activity to the ability of iodine to absorb x-rays to a greater extent than low atomic number elements. Thus, if an organ is filled with an iodinated contrast agent and is then bombarded by x-rays, the contents of the organ will absorb more strongly than the surrounding tissue and will predominate in the radiogram. This allows the visualization of an organ and permits the diagnosis of many abnormalities.

Cholangiographic agents are those that are excreted by the liver in concentrations high enough so that the bile ducts may be visualized. Cholecystographic agents depend not only on biliary excretion but also on the ability of the gall bladder to concentrate such media to the extent that the gall bladder may be visualized. Such media are often successfully concentrated in the gall bladder but are excreted at concentrations in the bile too low to provide suitable visualization of the ducts and only the gall bladder can be visualized. Ideally, a single agent should do both. The term cholegraphic agent shall be used to indicate media that fill either or both of the two functions described.

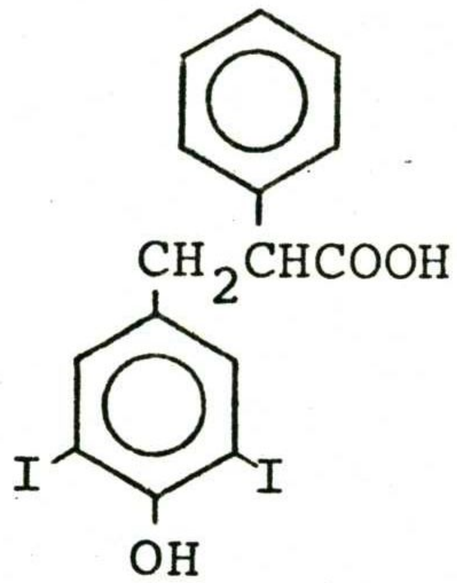
(i) Development of Cholegraphic Agents

An historical overview of cholecystography was presented by Cole (1961). Graham and Cole in 1923 obtained the first cholecystogram using calcium tetrabromophenolphthalein in a fasting dog. Abel and Rowntree (1909) had observed that phenoltetrachlorophthalein was excreted in the bile and it had recently been established that the gall bladder concentrates bile up to 10 times (Rous and McMaster, 1921). Graham concluded from these observations that the corresponding bromo and iodo phenolphthaleins might be useful as cholecystographic agents. Following these studies, sodium tetraiodophenolphthalein (Figure 1, p. 5) and phenoltetraiodophenolphthalein came into use for oral and limited intravenous cholecystography. There was wide use of these agents until iodoalphionic acid was introduced

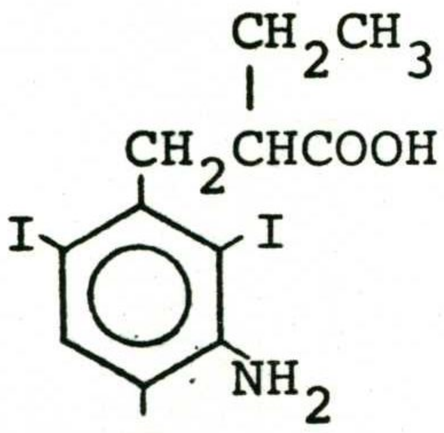
FIGURE 1.* Oral Cholegraphic Media



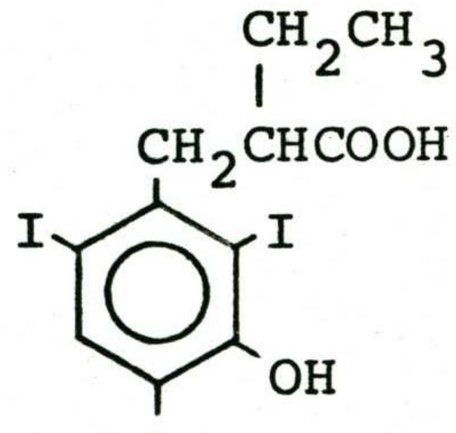
Tetraiodophenolphthalein



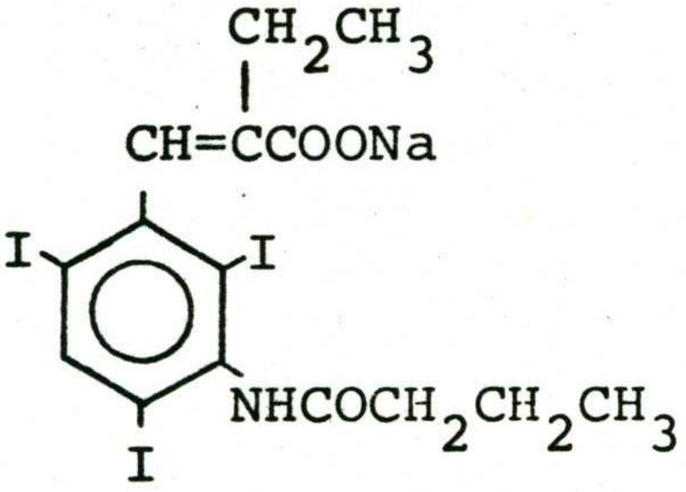
Iodoalphionic Acid



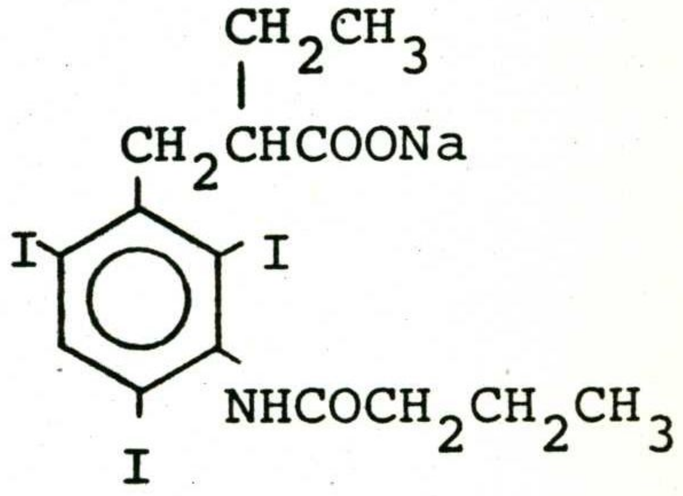
Iopanoic Acid



Iophenoxic Acid



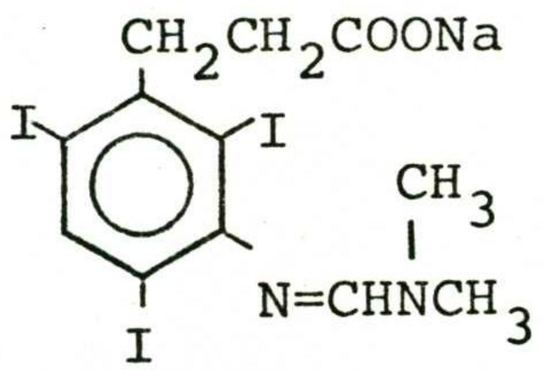
Bunamiodyl Sodium



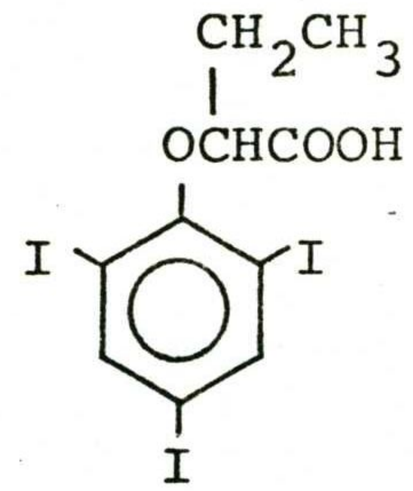
Sodium Tyropanoate

* An unbound copy of Figure 1 can be found in the pocket on the inside back cover.

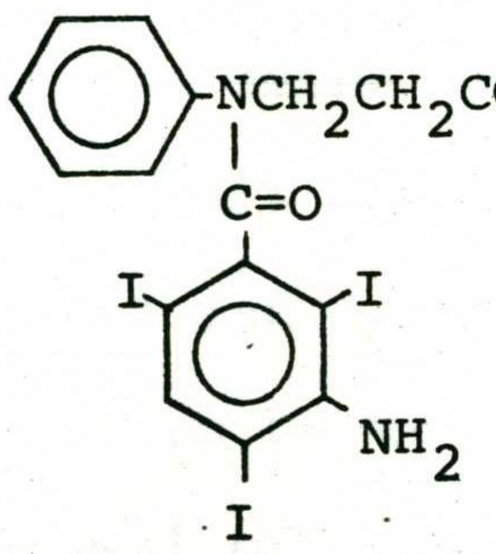
FIGURE 1. (continued)



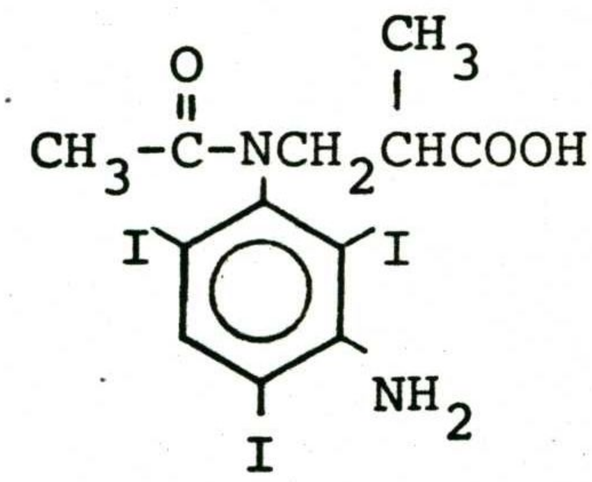
Sodium Iopodate



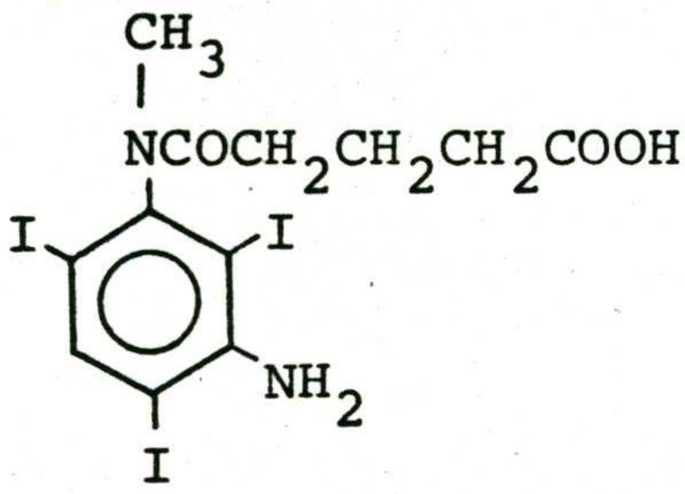
Phenobutiodil



Iobenzamic Acid



Iocetamic Acid



Iomeglamic Acid

in 1940 (Dohrn and Diedrich, 1940). This was a diiodinated-phenylalkanoic acid which possessed the basic chemical structure on which modern oral cholecystographic agents are based. It gave better visualization and fewer side reactions than the phenolphthaleins.

In 1949, Lewis and Archer reported the synthesis of iopanoic acid, a triiodophenylakanoic acid. As might be expected with a third iodine atom in the molecule, Hoppe and Archer (1953) found that it provided greater opacity of the gall bladder of cats than iodoalphionic acid. This was confirmed in human studies. Papa et al (1953) reported the synthesis of iophenoxic acid, a compound analogous to iopanoic acid but with a phenolic hydroxyl instead of the amino group in the 3 position of the aromatic ring. This small change led to very strong protein binding of this compound and a long biological half-life. Astwood (1957) estimated it to be at least 2½ years. This eventually led to the withdrawal of this compound from use.

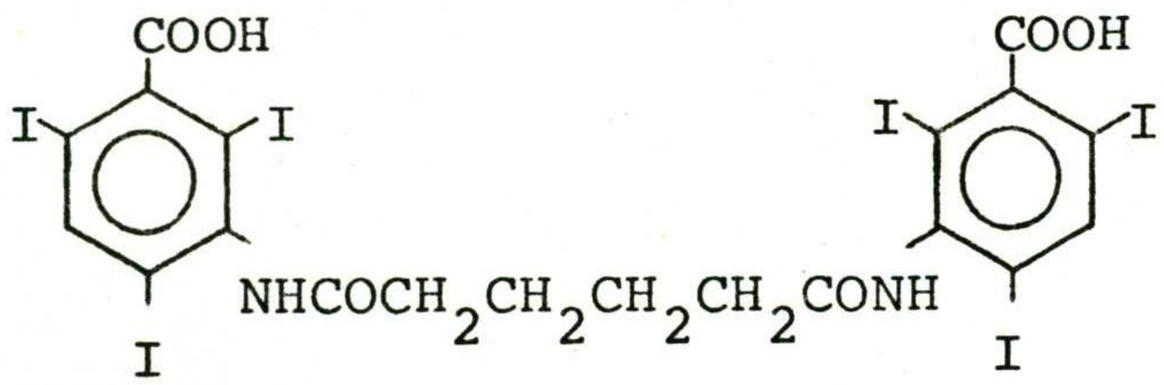
Next, water soluble oral cholecystographic media were introduced. These agents were sodium salts of phenylalkanoic acids and afforded more uniform absorption from the gut than the free acids. Bunamiodyl sodium, a triiodophenylcinnamate with a butyrylamido side chain was popular for a time (Meszaros and Rich, 1960; Teplick et al, 1958). It was implicated in renal toxicity and it was withdrawn from use in some countries. Sodium tyropanoate, the butyramide derivative of iopanoic acid, was introduced recently (Hoppe et al, 1970) and sodium iopodate was introduced by Harwart et al (1959). The ducts are better

visualized with the sodium salts than with iopanoic acid probably due to more rapid absorption from the gastrointestinal tract (p. 19).

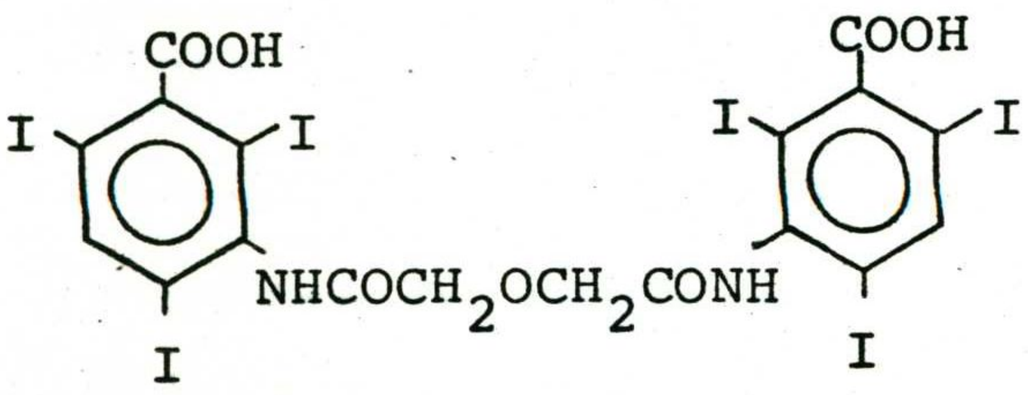
Other oral agents that have been or are in clinical use include phenobutiodil (Redel et al, 1954), iobenzamic acid (Obendorf, 1961), iocetamic acid (Korver, 1968; Janbroers et al, 1969) and iomeglamic acid (Cassebaum et al, 1972; Pfeifer et al, 1972a; Bekker et al, 1972).

Apart from a small amount of intravenous cholegraphy with phenoltetraiodophthalein, the vascular route of administration was not exploited until the discovery of iodipamide (Langecker et al, 1953). This compound is hexaiodinated and its salts are very water soluble. It can be viewed as two 3-amino-2,4,6-triiodinatedbenzoic acids joined at their amino groups by the dibasic adipic acid (Figure 2, p. 9). It is administered as its sodium or methylglucamine(1-deoxy-1-(methylamino)-D-glucitol) salts. This agent is particularly useful in cholangiography. Because this agent is excreted in relatively high concentrations in the bile, the gall bladder may be visualized following the administration of iodipamide without the gall bladder mucosa concentrating the bile further as it normally does. Oral agents, on the other hand, are often excreted at low concentrations and require further concentration of the bile by the gall bladder in order to provide visualization. Therefore, visualization following the administration of oral agents indicates that the concentrating function of the gall bladder is operating normally. However, gall bladder visualization following the administration of intravenous

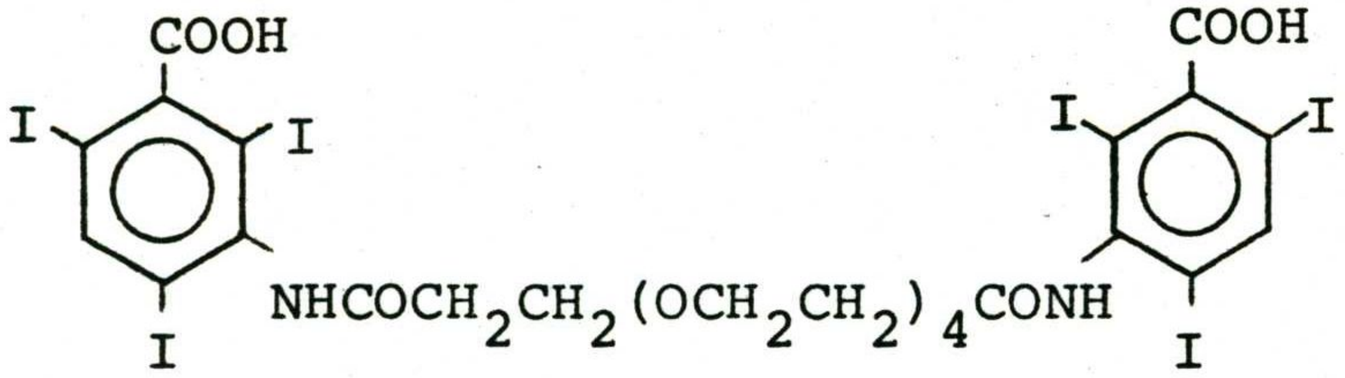
FIGURE 2.* Intravenous Cholegraphic Media



Iodipamide



Ioglycamide



Iodoxamic Acid

* An unbound copy of Figure 2 can be found in the pocket on the inside back cover.

agents does not preclude a diseased gall bladder (Saxton and Strickland, 1972). The administration of oral agents is the method of choice when investigating gall bladder dysfunction. A small but appreciable number of fatalities are experienced with iodipamide usually as a result of circulatory collapse, so the need for a safer agent existed.

Ioglycamide, in which the adipic acid moiety of iodipamide is replaced by diglycolic acid, has been subsequently introduced (Langecker et al, 1964). This agent exhibits lower toxicity and persists in the ducts longer than iodipamide.

Iodoxamic acid, another hexaiodinated bis benzoic acid that forms water soluble salts was synthesized by Felder et al (1973a). Rosati et al (1973) claimed that pharmacological trials proved it to be less toxic and more bilitropic than either iodipamide or ioglycamide. This new agent is capable of producing visualization in jaundiced patients with bilirubin levels as high as 6.5mg percent (Robins et al, 1976). Iodipamide was found to compete with bilirubin for excretion by the liver cells into the bile (Billing et al, 1963) and is useful in jaundiced patients with bilirubin levels of up to 4.0mg percent (Saxton and Strickland, 1972). Therefore, iodoxamic acid represents an improvement in cholegraphy of jaundiced patients.

(ii) Toxicity of Cholegraphic Agents

Both intravenous and oral cholegraphic agents produce nausea and vomitting and oral agents are often responsible for diarrhoea and epigastric discomfort. The most serious side effects of these agents are often attributed to circulatory collapse and

anaphylactic shock. These reactions can be fatal.

Almen (1971) suggested several explanations for these fatal reactions to clinical doses of these agents. One suggestion was that some members of the population will respond to a small dose in much the same way that all members would if given enough of the radiopaque agent. This sensitivity reflected their position in the normal dose-response distribution and such reactions are to be expected in a small number of any given population. This is termed quantitative idiosyncrasy.

Acute allergy has also been cited as the cause for these fatalaties. However, Almen (1971) points out that there are as many arguments against this theory as there are for it. It has been found that patients with a history of allergy exhibited mild toxic reactions to iodipamide 15 times more frequently than patients without a history of allergy (McClenahan et al, 1962). Brasch et al (1976) have demonstrated that certain radiocontrast agents can act as haptens and induce specific antibody production in rabbits. While it was stressed that the experimental conditions did not equate with the clinical situation, this finding does add weight to the argument that allergy may play a role in the toxicity of these agents. However, Lasser et al (1976) reported that a dog exhibited an idiosyncratic response to injections of sodium iothalamate, an intravenous urographic agent. The overt manifestations were vomitting, hypotension and hyperflexia. Equiosmolal solutions of the methylglucamine salt failed to elicit an adverse reaction. Neither circulating nor tissue-fixed antibodies were demonstrated and this was claimed to substantiate the argument that antibody/antigen reactions of either an immediate

or delayed type play little if any role in adverse reactions to injections of contrast media. It has been suggested that histamine release *per se*, as opposed to an antibody/antigen reaction, may account for the toxicity of these agents (Mann, 1961). Mann claimed that allergic patients have a higher content of histamine than normal in their "sensitive" organ (e.g. bronchi of asthmatics) and that this might explain their increased susceptibility to side reactions. Lasser *et al* (1971) have reported that there is evidence for a direct histamine releasing property of contrast media *in vivo*. The methylglucamine cation was implicated since little change in histamine levels of canine liver or lung was noted after the injection of the sodium salts of several media, including iodipamide.

Iodipamide has been implicated in 22 deaths following intravenous cholegraphy (Frommhold and Braband, 1960) involving an estimated 6.2 million injections. Some of the deaths were attributed to cardiovascular collapse and some to anaphylactic shock. These toxic reactions have also been noted to a lesser extent with ioglycamide (Herms and Taenzer, 1975). Animal trials have indicated that iodoxamic acid exhibits fewer signs of cardiovascular effects than either iodipamide or ioglycamide (Tirone and Rosati, 1973). Erythrocyte sedimentation has also been implicated in these serious reactions noted with intravenous cholegraphic agents. These reactions can be ameliorated by administering the injection at a slower rate (Saltzman and Sundstrom, 1960).

Iodipamide has also been found to lower renal blood flow in cats and this action may be involved in its apparent nephrotoxicity (Lindgren et al, 1966).

The breakdown of the blood brain barrier has also been suggested as the cause for fatal reactions in man (Hoppe, 1959). However, in a recent review (Herms and Taenzer, 1975), it was claimed that recent clinical investigations have not confirmed this view.

A positive correlation has been observed between the degree of binding to serum albumin of contrast agents and their toxicity (Lasser, 1971). Strong protein binding has also been found to exist in compounds with no substituent at the 5 position of the 2,4,6-triiodinated aromatic ring. Generally, cholegraphic agents have the 5 position vacant while urographic agents are substituted in this position. Lasser concluded that albumin binding per se had little relevance in systemic toxicity but that it was a predictor of enzyme inhibition and suggested that enzyme inhibition was the important factor in toxicity. The enzymes found to be inhibited were beta glucuronidase, lysozyme, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase and acetylcholinesterase. Lasser also suggested that the inhibition of the latter two enzymes could play a part in the sludging of red blood cells and other alterations of the blood noted after the intravenous administration of radiocontrast agents. Radiopaque media have also been found to inhibit adenosine triphosphatase and carbonic anhydrase (Lang and Lasser, 1975). It was suggested that toxic reactions such as vasomotor collapse associated with both oral and intravenous cholegraphic media could well be related to the same mechanism of cholinesterase activity (Lasser, 1966). Both iodipamide and iopanoic acid are relatively potent acetylcholinesterase inhibitors (Lasser and Lang, 1966). Iodipamide is the weaker of the two.

Fatalities have also been reported with oral cholegraphic agents. Four fatalaties, alleged to be due to coronary insufficiency and myocardial infarction, have been attributed to iopanoic acid and iodoalphionic acid (Littman and Marcus, 1958). Seven fatalaties from an estimated 22 million patients have been reported for iopanoic acid (Shehadi, 1966). Renal failure accounted for four, myocardial infarction for one, liver necrosis for one and overdosage for one. One fatality in 600,000 cases due to anaphylactic shock was reported for iopodate (Shehadi, 1966).

In a study comparing iopanoic acid and iopodate, it was reported that both agents exhibited comparable visualization but fewer cramps and less diarrhoea was noted with iopodate (Fischer and White, 1967). In another study (Benisek and Gunn, 1962), bunamiodyl, iopanoic acid and tyropanoate were compared. It was found that tyropanoate gave the best gall bladder visualization and the fewest side effects. Both iopodate and tyropanoate are administered as sodium salts and are quickly absorbed from the intestine. This may account for the relative freedom from reactions like cramps and diarrhoea found after the oral administration of these agents.

Oral cholegraphic agents, especially bunamiodyl, have been implicated in nephrotoxicity. For example, bunamiodyl produced six cases of renal complication including one fatal case of acute renal failure in 593 patients (Gunn, 1961). Fink et al (1964) observed that these complications tended to be in patients with damaged hepatobiliary functions. This observation appeared to be confirmed by the finding that iopodate, tyropanoate, iopanoic acid and especially bunamiodyl led to impaired renal function in

patients suffering from Laennec's cirrhosis (Teplick et al, 1965). The use of bunamiodyl has decreased because of these indications of renal damage. The high water solubility and rapid intestinal absorption of bunamiodyl may well account for its relatively high toxicity when it is administered at dose levels comparable to other agents.

It has been reported that the hepatic excretion of many radiocontrast agents is by a saturable mechanism (Herms and Taenzer, 1975; Laval-Jeantet, 1975; Nelson et al, 1975). If blood levels are reached such that the ability of the liver to excrete a compound is maximal, any excess will tend to be excreted via the kidneys with the attendant threat of renal damage. Hence there is an optimal dose and dose frequency beyond which biliary excretion will not increase and renal damage will become more likely. Therefore, agents that are more readily absorbed such as bunamiodyl should be given in lower oral doses relative to the more poorly absorbed compounds. This would reduce the risk of toxicity without reducing the relative effectiveness. It has also been found that with tyropanoate, the administration of fractions of a dose over a period of time (fractionated dosing) rather than giving the whole dose at once can lead to lower serum peak levels without reducing radiologic efficacy (Fischer and Burgener, 1974). Therefore, the risk of toxicity can be reduced without reducing the degree of visualization. Cholecystographic agents have been found to possess uricosuric activity (Mudge, 1971). Hence, an attempt to keep serum peak levels of these compounds as low as possible seems prudent because this activity could account for part of the nephrotoxicity

associated with contrast media.

Unlike most drugs, radiocontrast agents are usually given only once or twice to patients and are usually administered in large doses (about 3g), often by the intravenous route. Therefore, the acute toxicity of these compounds is of primary importance. Benness et al (1975) point out that oral agents are easier to administer and exhibit fewer adverse reactions than intravenous agents. In a study comparing iodipamide and iopodate (Kohler and Holsti, 1962), it was found that iodipamide gave better visualization of the biliary ducts but exhibited more changes in blood pressure. A balance is required between the degree of visualization and the risk of side effects. While oral agents exhibit fewer side effects in clinical use, they are more toxic overall than intravenous cholegraphic media. The possibility of obtaining better visualization by using larger doses or by improving the absorption of oral media would depend on the discovery of compounds that are less toxic than those presently in use. In a recent review on the design of contrast media (Herms and Taenzer, 1975), it was concluded that the ultimate goal in cholecystography is to discover orally active substances which would replace the more cumbersome and somewhat dangerous intravenous procedures.

(iii) Chemical Structure and Gastrointestinal Absorption

In a review on the absorption and distribution of anionic benzene derivatives (Saunders, 1974), evidence was reported that indicated a common mechanism of absorption for the whole

gastrointestinal tract. The extent of absorption was claimed to be a function of pKa, which determined the extent of ionization of such drugs at the epithelial boundary. It was hypothesized that all acids with a pKa greater than 3 were unionized at this boundary. Hence, the degree of lipid solubility of the unionized form of a drug determines the rate of absorption of the drug across a membrane that is lipoidal in nature. It was also found that absorption was directly proportional to the concentration of the drug. The presence of similar ionic types did not alter the absorption of each other. Absorption was therefore seen to be passive and non-specific. Because plasma pH is higher than that at the epithelial boundary of the gastrointestinal tract, drugs that were not ionized at the epithelial boundary of the gastrointestinal tract were ionized in plasma. As a result, a gradient was established for diffusion of the unionized species from a position of high concentration to one of low concentration. Dissolution rates of drugs in the gastrointestinal tract also had a large influence on absorption. In a survey on the factors affecting the absorption of acetylsalicylic acid from the gastrointestinal tract, it was found that tablet disintegration time, particle size, aggregation of the acid with tablet ingredients, solution rate, gastric pH, rate of gastric emptying and the presence of interfering substances in the stomach were all important. The rate of solution was found to be the rate limiting step for acetylsalicylic acid (Truitt and Morgan, 1964). Hence biopharmaceutical factors as well as chemical factors play an important role in gastrointestinal absorption.

Lasser (1966), Felder et al (1973b), Taketa et al (1972) and Goldberger et al (1974) support the concept described above. They all suggested that radiocontrast agents crossed the lipoidal barrier of the intestinal mucosa by passive diffusion in the nonionized form. In studies in the dog (Nelson et al, 1973) and the rat (Reinke and Berk, 1971), lymphatic transport was not found to be significant in the intestinal absorption of iopanoic acid. It was found that at pH9.5, sodium iopanoate was rapidly absorbed from all parts of the canine intestine. It was stressed that despite the highly ionized state of iopanoic acid in the alkaline solution used, no conclusion could be drawn as to whether the absorption mechanism involved the ionic or non-ionic species.

In a recent study, the pKa values of various contrast media in a methylcellosolve-water system were determined and the pKa values in water were estimated (Felder et al, 1973b). Urographic agents and intravenous cholegraphic agents tested all had pKa values of less than 2.8 while the oral cholegraphic agents tested all had pKa values of greater than 4.38. This data fitted the theory mentioned above that compounds with a pKa greater than 3 should be unionized and be absorbed while those with a pKa of less than 3 would be ionized at the lipoidal epithelial boundary of the gastrointestinal tract and not be absorbed. Intravenous cholegraphic agents and urographic agents are not appreciably absorbed from the healthy gastrointestinal tract (Wallingford et al, 1952; Laval-Jeantet, 1975). Therefore, oral cholecystographic agents in use are of the general triiodophenylalkanoic acid type. It has been suggested (Archer, 1959) that the alkyl or alkylene side chain joining the carboxylic acid group

to the aromatic ring should be between 4-8 carbons for optimal oral absorption and gall bladder visualization. Compounds with longer chains are not absorbed while those with shorter chains are excreted in the urine.

Oral cholegraphic agents that are administered as sodium salts, such as sodium iopodate, sodium tyropanoate and sodium bunamiodyl generally were found to exhibit a low incidence of gastrointestinal cramps and diarrhoea and gave less frequent bowel residues than did free iopanoic acid (White and Fischer, 1962; Benisek and Gunn, 1962; Meszaros and Rich, 1960; Teplick et al, 1958). The sodium salt of tyropanoic acid was preferred to the free acid because it showed more consistent visualization of the gall bladder (Hoppe et al, 1970). These points all indicate a superior absorption of sodium salts compared to the free acids. It has been found that the use of the sodium salt of iopanoic acid rather than the free acid led to increased absorption in man, the rat and the dog (Saltzman, 1959; Gunnarson, 1959; Taketa et al, 1972; Nelson et al, 1973). With oral administration of the sodium salt, it is likely that the free acid would be formed in the acidic environment of the stomach. It has been found (Goldberger et al, 1974) that recently precipitated iopanoic acid was much more soluble, dissovled more quickly and was more rapidly absorbed than dried iopanoic acid. The recently precipitated product was assumed to be analogous to iopanoic acid which is precipitated in the stomach after the oral administration of the sodium salt while the dried iopanoic acid was assumed to be analogous to the commercially available free acid. When iopanoic acid that is commercially available is administered orally, the

gall bladder cannot be visualized in 15% of cases. A third of these, however, can be visualized after a second study. It was found that the dried iopanoic acid dissolved much more rapidly in the presence of bile salts. Under such conditions, the intestinal absorption of dried iopanoic acid became equal to that of the recently precipitated form. Therefore, it was postulated that the problem of frequent non-visualization of the gall bladder was often related to variability of the intestinal absorption of iopanoic acid due to the lack of bile salts in the duodenum of many fasting patients. This problem could possibly be circumvented by administering iopanoic acid as its sodium salt, by administering a substance such as corn oil along with iopanoic acid in order to stimulate biliary flow or by administering iopanoic acid in fractionated doses. It was stressed that increasing the absorption of the compound could lead to increased toxicity. If improved gall bladder filling occurs only at the expense of toxicity it is not a viable proposition. However, better absorption would decrease the frequency of bowel residues. Because the presence of these residues can interfere with gall bladder visualization, the use of smaller amounts of a better absorbed medium would be beneficial even if the degree of gall bladder filling was not enhanced.

The nature of the substituent at the 3 position of the triiodinated aromatic ring can have an influence on the gastrointestinal absorption of a radiopaque molecule. This effect can be variable from one series of compounds to another. In most commonly used oral cholegraphic agents, the group present in position 3 is a free amino group. These compounds invariably have

an alkyl or aryl substituent on the alkanoic acid side chain. Indeed, sodium iopodate which does not have a branched alkanoic acid side chain has a dimethylaminomethyleneamino side chain in position 3, not a free amino group. It may be that a certain hydrophilic/lipophilic balance is required for gastrointestinal absorption and that the lipophilic portion can be located in the 3 position or as a branch substituent on the alkanoic acid side chain.

Hebky and Jelinek (1965) and Harwart et al (1959), have found that the dimethylaminomethyleneamino side chain in position 3 enhances intestinal absorption. Hebky et al (1970) synthesized a series of 2,4,6-triiodobenzylamino derivatives. It was found that the introduction of a hydroxyalkyl group at position 3 had a favourable effect on the water solubility of the compounds and on their gastrointestinal absorption. It has been found that N-alkylated 3-aminoacyl-2,4,6-triiodophenylalkanoic acids were well excreted after oral administration (Felder et al, 1970). Compounds with various heterocyclic substituents in the 3 position have been found to be successful in cholecystography after oral administration (Fumagalli et al, 1975).

Both bunamiodyl and tyropanoate have a butyramido side chain in the 3 position. Hoppe et al (1970) reported the synthesis and testing of various N-acyl derivatives of iopanoic acid including tyropanoate. The acetamido derivative was found to be the least toxic but the quality of gall bladder visualization was relatively poor following the oral administration of this derivative. The visualization was inferior to that obtained with both iopanoic acid and tyropanoate. This was attributed to a

relatively high level of renal excretion for the acetamido compound. It was reported that all the acyl derivatives containing from 2 to 6 carbon atoms in this side chain exhibited good to excellent gall bladder visualization in cats after oral administration. This suggested that all these derivatives were reasonably well absorbed from the gastrointestinal tract. Therefore, it appears that substitution of the amino group in the 3 position can favour gastrointestinal absorption.

In a series of 2-ethyl-2,4,6-triiodophenylpropionic acids, a vacancy in the 3 position resulted in a compound that exhibited poorer absorption and/or excretion compared to compounds with an amino or hydroxyl group in this position (Papa et al, 1953). However, phenobutiodil has no substituent in this position and has been found to be valuable as an oral cholegraphic agent (Redel et al, 1954). Therefore substitution in this position is not essential for gastrointestinal absorption.

Overall, the oral cholegraphic agents presently in use that exhibit the best absorption from the gastrointestinal tract are the sodium salts of 2,4,6-triiodophenylalkanoic acids that contain an aliphatic side chain at position 3 of the iodinated aromatic ring or an aliphatic side chain as a branching of the alkanoic acid side chain.

(iv) Biliary Excretion of Cholegraphic Media

The biliary excretion of many oral and intravenous cholegraphic media appears to be a saturable phenomenon. A hepatic transport maximum for iodipamide and ioglycamide in the dog has been reported (Rosati and Schiantarelli, 1972). It has been

claimed that the transport maximum for iodoxamic acid was higher than that for iodipamide or ioglycamide (Rosati et al, 1973). Nelson et al (1975) stated that iopanoate was excreted by a hepatic mechanism that was capacity limited and furthermore, pointed out that the administration of sodium salts of some oral cholecystographic agents in the clinical situation could lead to blood levels that exceed the saturation threshold for biliary excretion. Herzog et al (1976) confirmed that iopanoate excretion in the bile of the dog was capacity limited. It has also been reported that there is a hepatic transport maximum for calcium iopodate in the dog (Benness et al, 1975). It has been suggested that a transport maximum also exists for sodium tyropanoate (Fischer and Burgener, 1974). As discussed previously, it would appear that once certain peak blood levels are reached, there would be no further improvement in visualization and the risk of nephrotoxicity would increase due to increased excretion by the kidneys. Whether the overall transport maximum reflects a rate limiting step for accumulation, metabolism or excretion of these compounds or some combination of these factors is still open to question.

It has been found that the extent of biliary excretion of iopanoic acid depends on the presence of bile salts. Bile salts do increase biliary flow and it has been suggested that there is a linear relationship between the amount of iopanoic acid excreted per unit time and the bile flow (Dunn and Berk, 1972). However, in a more recent study on the role of bile salts in the hepatic excretion of iopanoic acid in the dog (Berk et al, 1974), it was concluded that it was not an increase in bile flow per se that stimulated iopanoic acid excretion but it was the influence of the bile salts themselves without regard for the micellar

capacity of the bile salt. It was proposed that an allosteric interaction between bile salts and a carrier for iopanoic acid in the canalicular membrane may have produced an altered enzyme system which would have facilitated the transport of iopanoic acid across the membrane.

Bile salts become depleted in animals with a biliary cannula because of the interruption of the enterohepatic circulation. In the dog, a taurocholate infusion, meant to replace lost bile salts, was found to lead to an increase in iopanoate excretion over a wide range of plasma concentrations of iopanoate (Cooke and Mudge, 1971). Cooke et al (1973) determined that taurocholate replacement increased the biliary excretion of iopanoate in the rat, only in the initial period following the administration of iopanoate at a dose of 50mg/Kg while at a dose of 5mg/Kg no effect was detected. Therefore, it seems that species and dose size are factors that alter the effect of bile salt replacement on iopanoate excretion.

It appears that in studies that provide bile salt replacement the transport maximum for iopanoate was about three times that in which bile salts were depleted (Nelson and Staubus, 1976). While the mechanism of action is unclear it does appear that the depletion of bile salts decreases iopanoate excretion.

Cooke et al (1973) found that bile salt replacement did not increase the biliary excretion of iophenoxate.

Benness et al (1975) noted that calcium iopodate had a lower choleretic effect than iodipamide in the dog. It was concluded that since iopodate was found to form a conjugate, the lack of choloretic effect was due to the inclusion of its glucuronide in bile salt micelles and the concomitant reduction

in its osmotic activity. Iodipamide, on the other hand, did not form a conjugate, was not included in bile salt micelles and as a result of exerting greater osmotic activity, iodipamide had a greater choloretic effect. This is, therefore, another possible mechanism by which bile salts affect the excretion of some radiocontrast agents.

The following is a summary of the structural features necessary for biliary excretion. While much of the information was mentioned earlier in relation to intestinal absorption, it is restated briefly to emphasize its importance in the biliary excretion process. It has been claimed that an assymetrical structure with a hydrophilic and a lipophilic chain is required for biliary excretion of contrast media (Herms and Taenzer, 1975). As mentioned previously, most oral cholegraphic media are 2,4,6-triiodophenylalkanoic acids substituted in position 3 of the aromatic ring. The free carboxylic acid is important in the biotransformation of these compounds and is generally involved in the formation of ester glucuronides. The optimal length of the side chain that joins the carboxylic acid group to the triiodinated aromatic ring was determined to be between 4-8 carbon atoms. The presence of shorter chains favoured renal excretion (Archer, 1959). Usually the aliphatic carboxylic acid side chain is branched. This probably provides a degree of lipophilic character to the molecule. Substitution in the 3 position of the triiodinated aromatic ring can also provide this lipophilic character.

As previously mentioned, all cholegraphic agents presently in use have a vacancy in the 5 position of the aromatic ring.

Substitution in this position predisposes the molecule to renal excretion (Felder et al, 1973a; Hoey et al, 1966; Lasser, 1966). Compounds with a vacancy in the 5 position also show a greater degree of binding to serum albumin than those compounds with a completely substituted aromatic ring. The importance of this binding as regards toxicity has been discussed previously. It also has been implicated in the biliary excretion mechanism. Contrast media that are poorly bound to albumin are excreted via the kidneys and are excreted almost exclusively by the glomeruli (Lasser, 1966). Strongly bound media would be less available for glomerular filtration and would be more prone to biliary excretion. It has also been suggested that the binding of biliary cholegraphic agents might reflect their ability to bind to proteins in the hepatic parenchymal cells and that this might affect their transfer from blood to tissue protein to bile (Lasser, 1966). The hepatic Y and Z binding proteins have been implicated in hepatic excretory mechanisms (Levi et al, 1969). Furthermore, phenobarbital pretreatment has been found to increase the hepatic content of the Y protein fraction in the rat (Reyes et al, 1971). It has been reported that phenobarbital pretreatment in the rat led to increased plasma decay and biliary excretion of iopanoate (Cooke et al, 1973). Herzog et al (1976) also found that phenobarbital pretreatment increased the biliary excretion of iopanoate. It has also been reported that iopanoate is capable of binding to the X and Y proteins (Sokoloff et al, 1973). Therefore, phenobarbital might have influenced the biliary excretion of iopanoate through its ability to increase the hepatic content of the Y protein. This supports the concept of Lasser (1966)

that strong albumin binding reflects a degree of tissue binding that in turn plays a role in the biliary excretion of cholegraphic media.

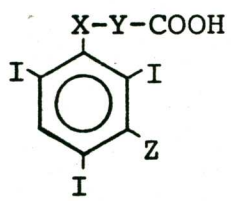
The role played by substituents in the 3 position varies from one series of compounds to another, as noted in the discussion on intestinal absorption. Phenobutiodil has a vacancy in the 3 position and was found useful in oral cholecystography (Redel et al, 1954) while the deaminated derivative of iopanoic acid was described as being disappointing (Papa et al, 1953).

The presence of an acetamido group in the 3 position has been associated with increased renal excretion and relatively poor biliary excretion (Wallingford et al, 1952; Hoppe et al, 1970). In both studies, an increase in the length of the aminoacyl side chain was associated with improved biliary excretion but also led to greater toxicity. The evaluation of a series of compounds related to tyropanoate led to the conclusion that the butyryl side chain was optimal in length. It has also been reported that N-alkylation of the 3-acylamino derivatives of some compounds enhanced their biliary excretion (Felder et al, 1970). The same workers also found that the presence of various heterocyclic groups in the 3 position yielded compounds that exhibited good biliary excretion (Fumagalli et al, 1975). The dimethylaminomethyleneamino side chain in position 3 has also been reported to enhance biliary excretion (Hebky and Jelinek, 1965; Harwart et al, 1959).

In describing the pharmacokinetics of iopanoic acid metabolism, Dunn and Berk (1972) summarized the process of its

absorption and excretion: "It is absorbed from the intestine, presumably by passive nonionic diffusion since it is fat soluble, and then transported in the blood bound to albumin. Part of it is temporarily stored in fat, blood, muscle and most body organs including the liver. Iopanoate is removed from the plasma by the liver, conjugated in the hepatocyte with glucuronic acid and excreted into the bile as the water soluble glucuronide. The mechanisms involved in the transfer from the blood into the hepatocyte and from the hepatocyte into the bile have not been elucidated. The high concentrations obtained in bile implicate an active transport process or processes capable of operating against a strong concentration gradient." This process probably describes the handling of most oral cholegraphic agents.

Hoey et al (1971) presented a summary of basic requirements for successful oral cholegraphic agents. The following generalized structure for an oral cholegraphic agent was presented.



The 2,4,6-triiodo substitution provides iodine in a stable chemical form and gives the molecule its ability to absorb x-rays. A lipophilic/hydrophilic chain (Y-COOH) is linked to the aromatic moiety through "X" and this chain enables the molecule to be absorbed from the intestine and enter the portal circulation. The carboxylic acid group is important in biotransformation. The introduction of iodine into the molecule is facilitated by the presence of "Z" (generally NH_2 or OH) and further substitution at

this position can influence the absorption and excretion of the agent. The lack of substitution at position 5 leads to strong albumin binding and may account for some of the toxicity of these agents. The binding to protein may influence the biliary excretion of these compounds.

About 12-16 hours is required for these agents to accumulate sufficiently in the gall bladder to provide a cholecystogram. Visualization of the biliary ducts with these agents appears to be inferior than that obtained with the intravenous agents. On the evidence so far available, it appears that the hepatic transport of these agents is saturable and that above the saturation threshold, renal excretion takes place.

Overall, a desirable agent would allow visualization of the biliary ducts and the gall bladder following rapid and complete absorption from the gut and excretion into the bile. Then the compound would be safely eliminated via the intestinal and urinary routes.

Smith (1973) proposed that three main factors influence the biliary excretion of foreign compounds in general. It was claimed that if the molecular weight of the excreted form of a compound is 300-400 and upwards, the compound will tend to be excreted in the bile. Secondly, the presence of a polar group was cited as being important for biliary excretion. The polar compounds may be molecules that contain polar groups and are excreted in the bile unchanged or compounds which acquire a polar group by metabolic transformation before undergoing elimination in the bile. Finally, Smith observed that within certain groups of compounds, changes or rearrangements of functional groups in a

molecule could alter the extent of biliary excretion out of proportion to any effect that the change may have had on molecular weight or polarity. Smith called this rather undefined factor, structural features.

In considering this hypothesis of Smith with reference to iodinated radiopaque compounds, it would be of use to compare and contrast urographic agents with both oral and intravenous cholegraphic media.

A typical urographic contrast agent is the sodium salt of diatrizoic acid which is 3,5-diacetamido-2,4,6-triiodobenzoic acid. Urographic agents generally are 2,4,6-triiodinated benzoic acids substituted at the 3 and 5 positions with short relatively hydrophilic groups.

The molecular weights of urographic media are only about half that of the intravenous cholegraphic agents. Also, while urographic agents have approximately the same molecular weight as the oral cholegraphic agents, the urographic agents cannot increase their molecular weight by glucuronide conjugation as can the oral cholegraphic agents. However, the molecular weight of urographic agents is relatively high and exceeds the general threshold level suggested by Smith as necessary for biliary excretion.

The urographic agents and the intravenous cholegraphic agents are acids with similar pKa values. They are stronger acids than the oral cholegraphic agents (Felder et al, 1973b). The presence of long aliphatic groups in the cholegraphic media contrasts with the short chain substituents normally found in urographic media. These longer aliphatic chains probably have the effect of increasing the lipophilic nature of cholegraphic media

and thereby increasing the extent of their biliary excretion.

Finally, in considering what structural features of these compounds seems most important in determining the extent of biliary excretion, one notices the lack of substitution in the 5 position of the aromatic ring of cholegraphic media contrasted with urographic agents which are virtually all substituted in this position. The importance of this feature with regards to protein binding, toxicity and biliary excretion has been discussed previously.

The inter-relationship of the 3 factors enumerated by Smith appears to be complex. The molecular weight of iodinated radiopaque compounds does not in itself appear to be a deciding factor in determining whether or not such a compound will be excreted in the bile. The last two factors appear to be much more important in determining the route of excretion of iodinated radiopaque compounds. While the pKa values of urographic agents are similar to those of intravenous cholegraphic agents, it would appear that urographic agents lack balancing non-polar groups. Smith claimed that a balance of polar and non-polar groups is required for successful biliary excretion. Finally, the presence or absence of substitution at the 5 position of the aromatic ring represents an important structural feature that determines the route of excretion of these compounds.

(v) Biotransformation of Cholegraphic Media

The major metabolic transformation noted with oral cholegraphic media is glucuronic acid conjugation with the carboxylic acid moiety of these agents.

The synthesis of the glucuronide proceeds by way of transfer of the glucuronic acid from uridine diphosphate glucuronic acid (UDPGA) to an acceptor molecule. The reaction is catalyzed by the enzyme glucuronyl transferase. UDPGA is formed from uridine diphosphate glucose by oxidation by UDP glucose dehydrogenase (Smith, 1973).

McChesney (1971) presented a review on the biotransformation of cholegraphic media. Most oral cholegraphic media have been found to form glucuronide conjugates to some extent. The biotransformation of iopanoic acid in the cat, the dog and man was studied by McChesney and Hoppe (1954). A metabolite that appeared to be an ester glucuronide of iopanoic acid was isolated from dog bile. This metabolite cochromatographed with metabolites found in man and the cat. The nature of the metabolite in the cat was not confirmed until McChesney (1964) demonstrated glucuronide formation in the cat from iopanoate, bunamiodyl and tyropanoate. McChesney and Banks (1965) carried out experiments that indicated that approximately 90% of organic iodine in the urine of man following the administration of these three media was in the form of their respective glucuronides. 37% of the administered dose of iopanoic acid was excreted in the urine in 108 hours, 50% of tyropanoate and 44% of bunamiodyl. Further studies indicated that the N-acetyl as well as the N-butyryl (tyropanoate) derivatives of iopanoic acid were excreted as glucuronide conjugates in the bile of dogs (McChesney and Hoppe, 1963). In a study on the absorption and excretion of the isolated glucuronide of iopanoic acid in the cat (McChesney and Hoppe, 1956) it was concluded that iopanoic acid required conversion to the

glucuronide in order to be excreted in the bile and that the conjugate was poorly reabsorbed from the intestinal tract, an important factor in preventing extensive enterohepatic circulation.

In a study on the biliary and urinary excretion of iopanoic acid in the dog, it was concluded that the glucuronide was formed in the liver and was then partitioned between bile and blood. The latter portion was destined for urinary excretion. It was claimed that the sole excretory product in the urine and bile was the glucuronide conjugate (Cooke and Mudge, 1975). That the glucuronide was the sole excretory product in the bile has been questioned recently (Nelson and Staubus, 1976) following a study that indicated that 95% of the total iodine content of the bile of dogs given iopanoic acid migrated on thin layer chromatography with the glucuronide, 2.5% as free iopanoic acid and 2.5% migrated more slowly than the glucuronide. However, by far the greatest amount of iopanoic acid was excreted as its glucuronide. Nelson and Staubus (1976) also noted that taurocholate replacement increased the transport maximum of iopanoic acid in canine bile by a factor of 3 over that found in studies where bile salts were depleted. The results following taurocholate replacement were also stated to approximate the transport maximum determined in dogs with an intact enterohepatic circulation (Herzog et al, 1976). It was concluded that any experimental conclusions regarding iopanoate metabolism that had been obtained in bile salt depleted dogs were therefore open to doubt.

Lasser (1966) described the role of conjugation in the process of gall bladder visualization. The failure of the gall bladder to concentrate a cholecystographic medium is usually considered to be indicative of an unhealthy organ system. It was found that iopanoic acid, presumably as its glucuronide conjugate, was readily reabsorbed through an inflammed gall bladder mucosa. In this case, failure to visualize the gall bladder did indicate a diseased organ. However, it was also found that some patients with a non-visualizing or poorly visualizing gall bladder had levels of endogenous beta glucuronidase that was sufficient to mimic the hydrolysis of opaque bile noted with commercial enzyme systems. It was concluded that the reabsorption of iopanoic acid from the gall bladder of such persons was probably due to prior deconjugation of iopanoic acid by their endogenous beta glucuronidase and not due to a diseased gall bladder mucosa. This also explained why some patients who did not visualize with iopanoic acid, did so with iodipamide. Iodipamide does not form a conjugate and is itself poorly absorbed across a healthy gall bladder mucosa. Therefore, it would not be reabsorbed from the gall bladder of persons with high levels of endogenous beta glucuronidase. Thus, the conjugation of oral cholecystographic agents to glucuronic acid is an important factor in their ability to visualize the normal gall bladder.

The biotransformation of iodoalphionic acid has been studied by Slingerland (1957) and Langecker and Ertel (1957). It was found to be excreted to a considerable extent in the unchanged form and as two compounds which were probably glucuronides. The presence of a minor unidentified metabolite

in rat urine was reported by Slingerland. The glucuronides appeared to be excreted by the kidneys.

The presence of a glucuronic acid conjugate of iocetamic acid in human bile has been reported (Janbroers et al, 1969).

McChesney (1971) reported some inconclusive results on the metabolism of iobenzamic acid and postulated that the metabolic products might have been the result of glucuronide formation, hydroxylation of the unsubstituted phenyl ring or hydrolysis of the amide linkage.

Harwart et al (1959) studied the metabolism of iopodate and found that little unchanged iopodate was excreted by man. Some of the corresponding 3-amino compound was detected in the urine. The major metabolic products were not identified but glucuronic acid conjugates were ruled out. In direct contrast, Benness et al (1975) claimed that preliminary studies have indicated that iopodate is excreted in the dog conjugated, probably to glucuronic acid. Furthermore, they suggested that the conjugate may have been excreted in the bile by inclusion in bile salt micelles. Thus, conjugation may serve the function of creating a molecule suitable for biliary excretion by inclusion in these micelles.

Wade et al (1971) reported that 3 different glucuronides were the major metabolic products following the administration of iophenoxic acid to dogs. The conjugates identified were an ester glucuronide formed with the carboxylic acid group, an ether glucuronide formed with the aromatic hydroxyl group and the diglucuronide. There was a trace of free acid detected plus 4 unidentified metabolites expected to have been products of side

chain oxidation. It was suggested that the glucuronides formed were still lipid soluble to a degree that allowed intestinal reabsorption. The resulting enterohepatic recirculation might account to some degree for the remarkable persistence of this compound in the body. It has been found that a 2 fold increase in the plasma level of iophenoxic acid from 100 to 200μg/ml was accompanied by a 100 fold increase in the rate of conjugation and biliary excretion (Mudge et al, 1971). It was suggested that this phenomenon permitted high biliary excretion at high plasma levels and accounted for the persistence of the compound in the body at low plasma levels.

The metabolism of iomeglamic acid has been studied (Pfiefer et al, 1972b). After oral administration to man, it was reported that the methyl ester, the N-acetate and the glucuronide derivates of iomeglamic acid were present in the urine as well as an undefined deiodinated compound and its methyl ester. Unchanged iomeglamic acid and its glucuronide and N-acetyl derivatives were found in the bile.

Iodipamide (Langecker et al, 1953), ioglycamide (Langecker et al, 1964; Corman et al, 1967) and iodoxamic acid (Pitre and Felder, 1974) were all reported to be excreted in the bile largely unchanged. The presence of small amounts of metabolites has been reported. These probably resulted from the rupture of the aliphatic chain.

The possibility of deiodination of these compounds must also be considered. It has been reported that 2,3,5-triiodobenzoic acid was deiodinated to 2,5-diiodobenzoic acid in the calf (Gutenmann et al, 1967). It has also been demonstrated that

iodoalphionic acid administration to rats and guinea pigs led to the excretion of inorganic iodine in the urine (Slingerland, 1957). Subsequent work in man indicated that the degree of deiodination was much less but still evident.

Deiodination of iomeglamic acid has also been reported (Pfiefer et al, 1972b).

Indications of small amounts of deiodination have also been reported for iopodate (Harwart et al, 1959) and iodipamide and ioglycamide (Langecker et al, 1964). The ability of iopanoate to hinder thyroid uptake of I^{131} implied that it may have been deiodinated to some extent (Clark and Shipley, 1957).

In his review, McChesney (1971) concluded that all iodinated radiopaque compounds should be considered as potentially capable of undergoing deiodination in vivo. Deiodination has been most convincingly demonstrated with 2,3,5-triiodobenzoic acid. McChesney suggested that the deiodination is quite possibly non-enzymatic. Finally, to accept the presence of free iodine as a sign of deiodination, one must be sure the preparation is free of inorganic iodine in the first place. It has been found that contrast media exposed to sunlight released a significant amount of inorganic iodine and traces of inorganic iodine were released as a result of exposure to ordinary room light. No release of iodine was noted after storage in the dark (Lang et al, 1974).

Overall, the major metabolic transformation of these agents involved conjugation with glucuronic acid. The relatively small amounts of other metabolic transformations was probably due to the presence of a highly substituted aromatic ring and, in many cases,

the presence of a substituent alpha to the carboxylic acid group which aided in the prevention of side chain oxidation.

EXPERIMENTAL METHODS

(i) Infrared Spectroscopy

Infrared spectra were obtained with a Unicam S.P.200G spectrophotometer. Compounds were prepared as potassium chloride discs prior to measurement unless otherwise indicated.

(ii) Chromatography

Thin layer chromatography (TLC) was carried out on Silica Gel GF254 according to Stahl (0.25mm layers) and thick layer chromatography was carried out on the same material (1mm layers) (E. Merck AG, Darmstadt, Germany).

The solvents used had the following composition by volume:

Solvent A - Toluene:glacial acetic acid, 95:5

Solvent B - Butanol:glacial acetic acid:water, 2:2:1

Three further solvents were used in the chemical syntheses in order to follow the progress of reactions and to test the purity of intermediates and final products. These were acetone:petroleum ether-2:1, methanol, and dimethylformamide.

Compounds were visualized by fluoresence extinction under short wavelength ultraviolet light. The compounds appeared as dark spots against a green background.

(iii) General

Melting points were determined using a Köfler block and are uncorrected.

Elemental analyses were carried out by the Australian Microanalytical Service, C.S.I.R.O., Chemistry Department, University of Melbourne.

Solvents and commercial chemicals were purified by distillation or recrystallization if necessary.

All temperatures are recorded in degrees Celsius.

Water used was distilled from an all-glass distillation apparatus.

(iv) Enzyme Hydrolyses

Beta glucuronidase was obtained from the Sigma Chemical Co., St. Louis, Missouri, U.S.A. Bile samples (100µl) were mixed with 0.1M acetate buffer, pH4.6 (100µl) and beta glucuronidase (25µl) and incubated for 14 hours at 37° in a shaking incubator (Gallenkamp, England).

(v) Animal Studies

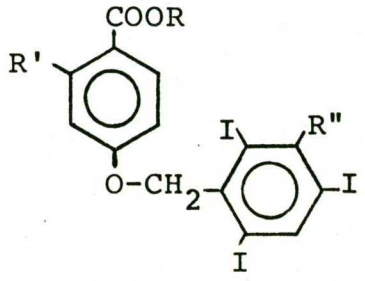
Male white rats of the Wistar strain weighing 220-330g were used throughout.

Rats were anesthetized with an aqueous solution of urethane (25%w/v)(1.25g/Kg) administered intraperitoneally. The common bile duct was exposed through a midline incision and was cannulated using a 23G stainless steel needle shaft attached to 10cm of single lumen polyethylene tubing (I.D.-0.58mm). A 1 hour equilibrium period was allowed to elapse between the time of animal preparation and the administration of the compounds. The bile collected in this period became the blank sample.

Compounds 1-26 (Table 1) were first powdered in a mortar and pestle. Fresh suspensions of each compound

TABLE 1.* CHEMICAL STRUCTURES

A) 4-TRIIODOBENZYLOXYBENZOIC ACID DERIVATIVES

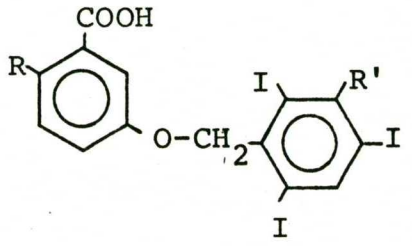


	R	R'	R"
1) Methyl 4-(3-amino-2,4,6-triiodobenzyloxy)benzoate	CH_3	H	NH_2
2) 4-(3-Amino-2,4,6-triiodobenzyloxy)benzoic acid	H	H	NH_2
3) 4-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid	H	H	$NHCOCH_3$
4) 4-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	H	$N{:}CHN(CH_3)_2$
5) Methyl 2-hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)-benzoate	CH_3	OH	NH_2
6) 2-Hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)benzoic acid	H	OH	NH_2
7) 2-Hydroxy-4-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid	H	OH	$NHCOCH_3$
8) Methyl 2-hydroxy-4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoate	CH_3	OH	$N{:}CHN(CH_3)_2$
9) 2-Hydroxy-4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	OH	$N{:}CHN(CH_3)_2$

* An unbound copy of Table 1 can be found in the pocket on the inside back cover.

TABLE 1. CHEMICAL STRUCTURES (continued)

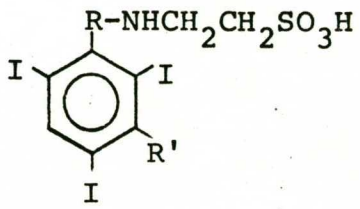
B) 3-TRIIODOBENZYLOXYBENZOIC ACIDS



		R	R'
10)	3-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid	H	$NHCOCH_3$
11)	3-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	$N{:}CHN(CH_3)_2$
12)	2-Hydroxy-5-(3-amino-2,4,6-triiodobenzyloxy)-benzoic acid	OH	NH_2
13)	2-Hydroxy-5-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid	OH	$NHCOCH_3$
14)	2-Hydroxy-5-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	OH	$N{:}CHN(CH_3)_2$

TABLE 1. CHEMICAL STRUCTURES (continued)

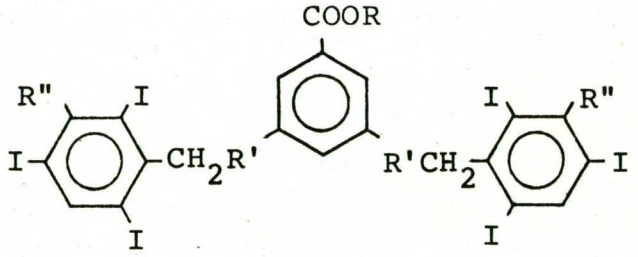
C) TAURINE DERIVATIVES



	R	R'
15) N-(3-(3-Amino-2,4,6-triiodophenyl)propionoyl)taurine	CH_2CH_2CO	NH_2
16) N-(3-Amino-2,4,6-triiodobenzyl)taurine	CH_2	NH_2
17) N-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyl)-taurine	CH_2	$N{:}CHN(CH_3)_2$

TABLE 1. CHEMICAL STRUCTURES (continued)

D) 3,5-BIS(TRIIODOBENZYL)BENZOIC ACID ESTERS



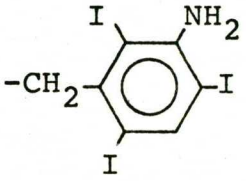
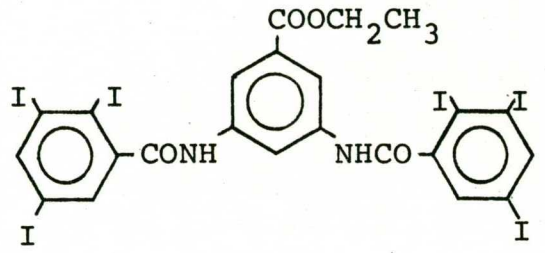
	R	R'	R"
18) Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzylamino)-benzoate	CH_2CH_3	NH	NH_2
19) Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzylamino)-benzoate	CH_2CH_3	NH	$NHCOCH_3$
20) Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)-benzoate	CH_2CH_3	O	NH_2
21) Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzyloxy)-benzoate	CH_2CH_3	O	$NHCOCH_3$
22) 3-Amino-2,4,6-triiodobenzyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)benzoate		O	NH_2

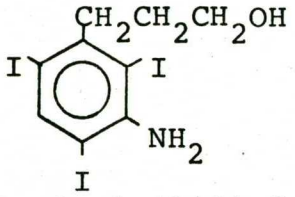
TABLE 1. CHEMICAL STRUCTURES (continued)

E) MISCELLANEOUS COMPOUNDS

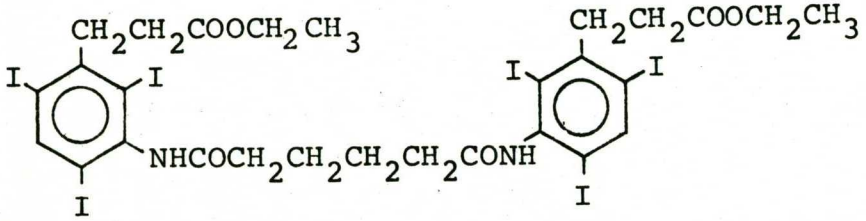
23) Ethyl 3,5-bis(2,3,5-triiodobenzamido)benzoate



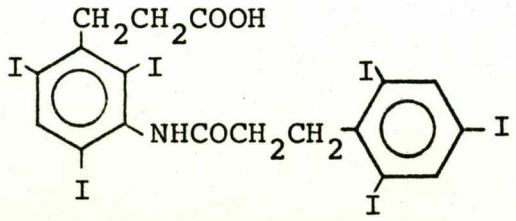
24) 3-(3-Amino-2,4,6-triiodophenyl)propan-1-ol



25) N,N'-bis(3-(2-carbethoxyethyl)-2,4,6-triiodophenyl)-adipamide



26) 3-(3-(3-(2,4,6-triiodophenyl)propionamido)-2,4,6-triiodophenyl)propionic acid



(35.0mg/ml) were made using 2% compound tragacanth powder (Australian Pharmaceutical Formulary). The suspensions were then injected into the duodenum (100mg/Kg). Samples of bile were collected hourly in tared containers for six hours after dosing. The peritoneum was perfused hourly with 0.9% saline to prevent dehydration. Hourly samples were weighed and corresponding samples from 2 or 3 rats were bulked so as to provide a sample large enough for analysis (>0.68ml). Compounds that showed evidence of excretion after administration to one group of rats were then administered to a second group of 2 or 3 rats. Samples were analyzed as described below without any further treatment.

For intravenous administration, compounds 4-12, 14, 15 and 20 were prepared as solutions (3.0mg/ml) in aqueous dimethylsulphoxide (DMSO). Compound 15 was administered in 10% DMSO, compounds 5 and 8 in 85% DMSO, compound 20 in 95% DMSO and the remaining compounds in 70% DMSO. These solutions were injected at a dose level of 20mg/Kg into the left femoral vein of rats over a period of 5-8 minutes except for the 95% DMSO solution that required 15 minutes to inject, so as to avoid fatalities. Zero time was taken from the start of the injection. The injections were made with the aid of an Arnold Micro-Applicator (Burkhard Manufacturing Co. Ltd., Rickmansworth, Herts, England) which allowed the controlled injection of the solutions in increments of 10μl. These compounds were administered to 2 groups of rats each. Samples were handled as described for intraduodenal administration.

For each compound excreted, the hourly bile sample with the highest iodine concentration was hydrolyzed with beta glucuronidase and compared on TLC(Solvent B) with a blank bile sample treated similarly.

A suspension of iopanoic acid and a solution of sodium iopodate were also administered intraduodenally to rats (35mg/ml; 100mg/Kg).

(vi) Isolation of Compound 6 from Rat Bile

Compound 6, (2-hydroxy-4-(3-amino-2,4,6-triiodo-benzyloxy)benzoic acid), was administered to 2 male Wistar rats (320g) intraduodenally at a dose of 100mg/Kg as described previously. Bile was collected for 4 hours following administration and bulked giving a total volume of 9.4ml. This was hydrolyzed with beta glucuronidase as previously described. The hydrolyzed sample was reduced in volume on a rotary evaporator at room temperature. This was then applied to 5 thick layer chromatographic plates 20cm wide and developed in Solvent B. The streak cochromatographing with the parent compound was scraped off and extracted from the silica with dimethylformamide. After precipitation with water, the solid was redissolved in a small volume of dimethylformamide and run once more on the same thick layer chromatography system. A further extraction, precipitation and drying yielded 1.3mg of an amorphous powder. m213-5°. An infrared spectrum was obtained.

(vii) Iodine Analysis

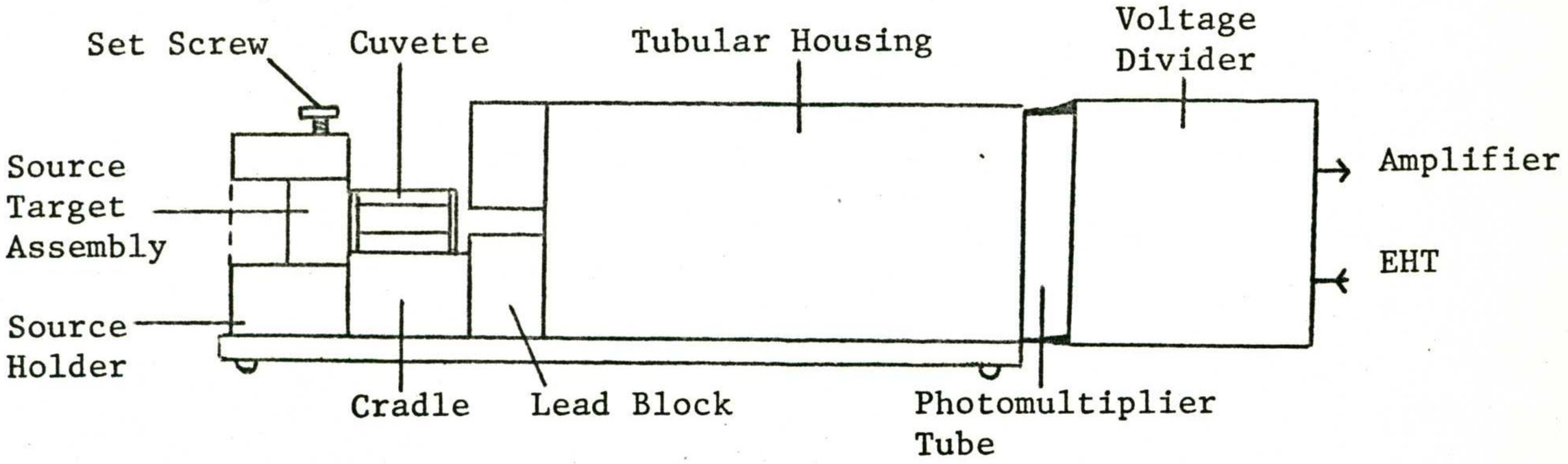
The iodine content of the bile samples was determined using an x-ray absorption technique. A cylindrical source target

assembly (the Radiochemical Centre, Amersham, England, Code No. AMCV970) combining an annular 100 millicurie americium-241 source and a disc shaped cerium oxide target was used to provide an x-ray beam with predominant energies of 34.72 and 34.28 keV. The assembly was aligned with a cylindrical perspex cuvette 2cm in length and with an internal diameter of 0.6cm. After exiting from the cuvette, the beam was collimated by passing it through a 0.5cm hole in a 16mm thick lead block. The detector was a scintillation counter with a sodium iodide (thallium) crystal 1 inch in diameter and 1mm thick associated with a photomultiplier tube 1.5 inches in diameter (Bicron Model IXM040/1.5B X-Ray Detector; Bicron Corporation, U.S.A.). The pulses were then passed through a voltage divider (Bicron Model P-12) and amplified with a gain of 1x1000 using an EKCO Pulse Amplifier Type N640 A (EKCO Electronics Ltd., Essex, England). A Head Unit Power Supply (Australian Atomic Energy Commission) supplied +300 volts to the amplifier. An EHT unit supplied +1100 volts to the photomultiplier tube (Isotope Developments Ltd., Berks, England). The amplified signal was fed into an automatic timer-scaler (Australian Atomic Energy Commission, Type 2B) with a preset time of 10 seconds, a preset count of 100,000 and a deadtime of 2.5 microseconds. The apparatus is illustrated in Figure 3.

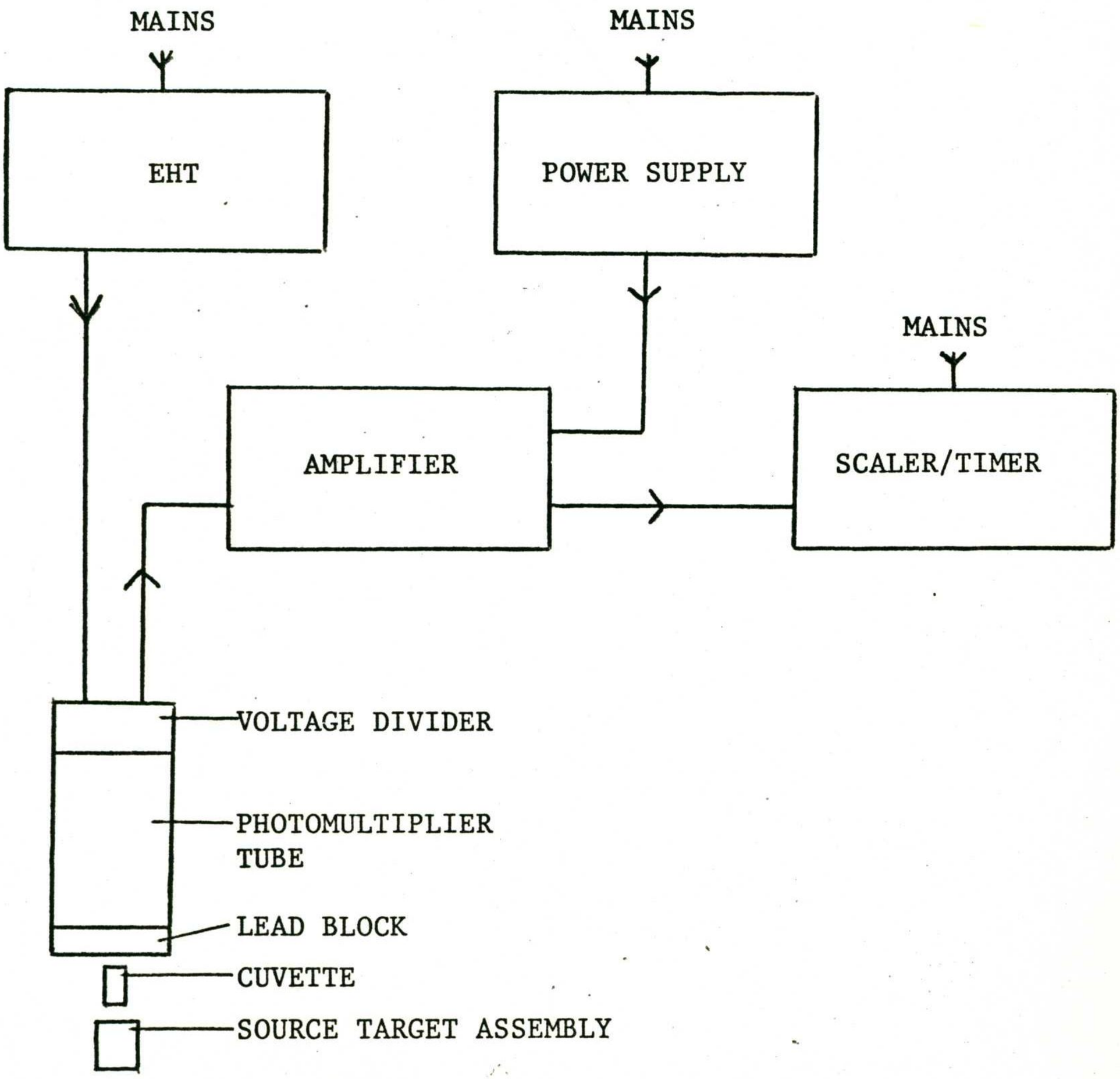
A standard curve was prepared for the apparatus. Solutions of potassium iodide (analytical grade) over a range of 0.2-10mg/ml of iodine were prepared from a stock solution of 6.5402g of potassium iodide in bile (25ml). The solutions were counted for 4 successive 10 second periods each. A blank bile sample was also counted. The common logarithms

FIGURE 3. X-Ray Analysis Assembly

A) SOURCE-DETECTOR ASSEMBLY (½ scale)(cross section)



B) BLOCK DIAGRAM OF X-RAY ANALYSIS ASSEMBLY



of the ratios of the sample counts over the blank bile count were plotted against the iodine content (mg/ml). Blank bile containing known amounts of organic iodine were also tested.

Samples of bile from rats fed experimental compounds were also counted for 4 consecutive 10 second periods and were compared to the blank bile sample obtained from the same group of rats. A value for the concentration of iodine in the bile (mg/ml) was obtained from the standard curve. This value multiplied by the weight of the sample (mg) gave the total quantity of iodine present (mg).

To test the error inherent in the instrumentation, 3 solutions of sodium iopodate in bile (equivalent to 0.46, 3.24 and 6.64mg/ml iodine) were analyzed eight separate times. The means and standard errors for each concentration were calculated.

In order to obtain a measure of the long term stability of the instrumentation and of the variation from one sample of bile to the next, the mean and standard deviation were calculated for blank bile samples from 56 separate groups of rats collected and counted over a period of 4 months.

(viii) Chemical Syntheses

The progress of all synthetic reactions and the purity of intermediates and final products were ascertained with the aid of thin layer chromatography. The structures of the compounds prepared are illustrated in Table 1.

Triiodobenzyloxybenzoic Acids

3-Aminobenzyl alcohol

3-Aminobenzyl alcohol was prepared from 3-aminobenzoic

acid according to Cerny and Malek (1970) by reduction with sodium bis (2-methoxyethoxy) aluminium hydride. Yield 65% m 95-6° (reported 94.5-95°).

3-Amino-2,4,6-triiodobenzyl alcohol

3-Amino-2,4,6-triiodobenzyl alcohol was prepared from 3-aminobenzyl alcohol according to Hebky and Karasek (1964) by treatment with iodine monochloride in hydrochloric acid. Yield 82% m 153-3.5° (reported 150°).

3-Amino-2,4,6-triiodobenzyl chloride

3-Amino-2,4,6-triiodobenzyl chloride was prepared from the alcohol according to Hebky and Karasek (1964) by treatment with thionyl chloride. Yield 68% m 178-80° (reported 168-71°).

3-Acetamido-2,4,6-triiodobenzyl chloride

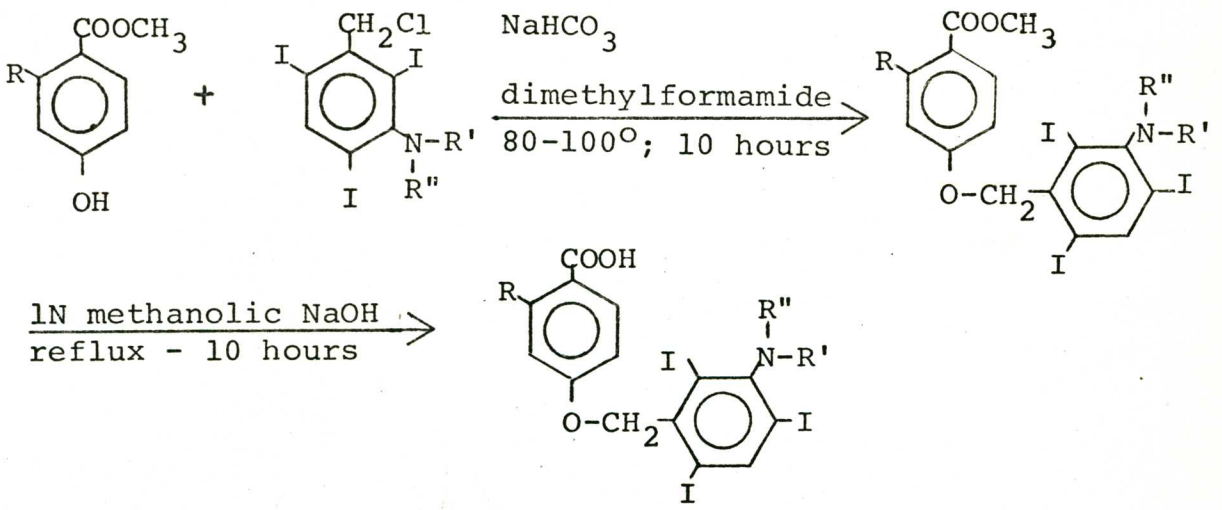
3-Acetamido-2,4,6-triiodobenzyl chloride was prepared from the corresponding amino derivative according to Hebky and Karasek (1964) by treatment with acetic acid and acetic anhydride. Yield 64% m 251-3° (reported 257-8°).

3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyl chloride

3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyl chloride was prepared from the corresponding amino derivative according to Hebky *et al* (1970) by treatment with phosphorus oxychloride in dimethylformamide. Yield 76% m 132-3° (reported 132-4°).

The above 3 benzyl chlorides were reacted with the methyl esters of 4-hydroxybenzoic acid, 3-hydroxybenzoic acid, 2,4-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid as detailed below. The general reaction sequence for the synthesis

of compounds 1-14 is outlined below.



A) 4-Triiodobenzyloxybenzoic Acid Derivatives

Compound 1: Methyl 4-(3-amino-2,4,6-triiodobenzyloxy)benzoate

Methyl 4-hydroxybenzoate (0.0033mole), 3-amino-2,4,6-triiodobenzyl chloride (0.0030mole) and sodium bicarbonate (0.0033mole) in dry dimethylformamide (50ml) were heated at 80-100° for 10 hours with constant stirring. Water (200ml) was added to the cooled reaction mixture. The precipitate was centrifuged and then washed with water and dried. It recrystallized from chloroform-petroleum ether. Yield 79% m 167-8°. $C_{15}H_{12}I_3NO_4$ requires C28.3%, H1.9%, I60.0%, N2.2%; found C28.4%, H1.9%, I59.9%, N2.1%.

I.R. $\nu_{max}(cm^{-1})$: 760, 850, 1005, 1100, 1110, 1161, 1241, 1282, 1315, 1430, 1510, 1605, 1712, 2920, 3318, 3400.

Compound 2: 4-(3-Amino-2,4,6-triiodobenzyloxy)benzoic acid

The ester (compound 1)(0.001mole) was suspended in 50ml of 1N methanolic sodium hydroxide and was refluxed with

stirring for 10 hours. The free acid was precipitated by the addition of water. The suspension was neutralized with dilute hydrochloric acid. The precipitate was collected by centrifugation and washed with water. It recrystallized from glacial acetic acid. Yield 65% m 263-4.5°. $C_{14}H_{10}I_3NO_3$ requires C27.1%, H1.6%, I61.4%, N2.3%; found C27.4%, H1.6%, I61.3%, N2.1%.

I.R. $\nu_{max}(cm^{-1})$: 768, 849, 990, 1003, 1160, 1248, 1280-1300, 1315, 1413, 1510, 1605, 1685, 1690, 3330, 3425.

Compound 3: 4-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid

This compound was prepared as described for compounds 1 and 2 but the pure ester was not isolated. The acid recrystallized from glacial acetic acid. Yield from the benzyl chloride 43% m 275-6°. $C_{16}H_{12}I_3NO_4$ requires C29.0%, H1.8%, I57.5%, N2.1%; found C29.2%, H1.8%, I57.7%, N2.2%.

I.R. $\nu_{max}(cm^{-1})$: 768, 840, 1000, 1165, 1240, 1422, 1510, 1605, 1660, 1690, 2920, 3200, 3380.

Compound 4: (3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy) benzoic acid

This compound was prepared as described for compound 3. It recrystallized from aqueous methanol. Yield from the benzyl chloride 51% m 211-6°. $C_{17}H_{35}I_3N_2O_3$ requires C30.2%, H2.2%, I56.4%, N4.1%; found C30.2%, H2.3%, I56.5%, N3.9%.

I.R. $\nu_{max}(cm^{-1})$: 768, 846, 1003, 1105, 1166, 1240, 1348, 1380, 1425, 1510, 1605, 1645, 1696, 2920, 3400.

Compound 5: Methyl 2-hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)benzoate

This compound was prepared in a similar manner to compound 1. It recrystallized from chloroform. Yield from the benzyl chloride 87% m 196-7°. $C_{15}H_{12}I_3NO_4$ requires C27.6%, H1.8%, I58.5%, N2.2%; found C27.4%, H1.8%, I58.8%, N2.2%.

I.R. $\nu_{max}(cm^{-1})$: 690, 760, 852, 1008, 1090, 1140, 1170, 1218, 1250, 1265, 1285, 1295, 1345, 1430, 1440, 1460, 1500, 1580, 1595, 1620, 1675, 2920, 3340, 3430.

Compound 6: 2-Hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)-benzoic acid

Compound 5 was hydrolyzed as previously described for compound 3. The free acid recrystallized from glacial acetic acid. Yield from the benzyl chloride 72% m 213-4.5°. $C_{14}H_{10}I_3NO_4$ requires C26.4%, H1.6%, I59.8%, N2.2%; found C26.4%, H1.6%, I59.8%, N2.2%.

I.R. $\nu_{max}(cm^{-1})$: 770, 825, 1008, 1089, 1141, 1180, 1245, 1380, 1425, 1455, 1500, 1598, 1620, 1645, 3330, 3420.

Compound 7: 2-Hydroxy-4-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid

This compound was prepared as described for compound 3. The free acid recrystallized from glacial acetic acid. Yield from the benzyl chloride 44% m 238-41°. $C_{16}H_{12}I_3NO_3$ requires C28.3%, H1.8%, I56.1%, N2.1%; found C28.6%, H1.9%, I56.0%, N2.1%.

I.R. $\nu_{max}(cm^{-1})$: 758, 1009, 1151, 1180, 1245, 1265, 1345, 1368, 1425, 1450-60, 1500, 1545, 1582, 1621, 1648, 1651, 1660, 3200, 3350.

Compound 8: Methyl 2-hydroxy-4-(3-dimethylaminomethylene-amino-2,4,6-triiodobenzyloxy)benzoate

This compound was prepared in a manner analogous to that for compound 1. It recrystallized from methanol. Yield 49% m 169°. $C_{18}H_{17}I_3N_2O_4$ requires C30.6%, H2.4%, I54.0%, N4.0%; found C30.5%, H2.4%, I53.9%, N3.8%.

I.R. $\nu_{max}(cm^{-1})$: 690, 772, 1008, 1100, 1145, 1182, 1220, 1255, 1350, 1380, 1440, 1504, 1585, 1625, 1636, 1645, 1671, 2920, 3400.

Compound 9: 2-Hydroxy-4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid

Compound 8 was hydrolyzed as described previously for the preparation of compound 2 except that a reaction time of 26 hours was used. The acid recrystallized from aqueous methanol. Yield 68% from the ester m 143-5°. $C_{17}H_{15}I_3N_2O_4$ requires C29.5%, H2.2%, I55.1%, N4.0%; found C29.5%, H2.1%, I54.7%, N4.2%.

I.R. $\nu_{max}(cm^{-1})$: 688, 775, 828, 1005, 1101, 1150, 1180, 1240, 1376, 1439, 1500, 1585, 1631, 1641, 1705, 2910, 3380.

B) 3-Triiodobenzyloxybenzoic Acids

Compound 10: 3-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid

This compound was synthesized as described for compound 3 using methyl 3-hydroxybenzoate. It recrystallized from methanol. Yield from the benzyl chloride 73% m 234-6°.

$C_{16}H_{12}I_3NO_4$ requires C29.0%, H1.8%, I57.5%, N2.1%; found C28.8%, H1.8%, I57.2%, N1.9%.

I.R. $\nu_{max}(cm^{-1})$: 758, 1021, 1039, 1110, 1252, 1290, 1350, 1385, 1428, 1455, 1500, 1550, 1595, 1685, 1700, 3230, 3380.

Compound 11: 3-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid

This compound was prepared as described for compound 3. The ester separated out as a gummy residue and was hydrolyzed without purification. The free acid recrystallized from methanol. Yield from the benzyl chloride 30% m 216-8°. $C_{17}H_{15}I_3N_2O_3$ requires C30.2%, H2.2%,I56.4%, N4.1%; found C30.0%, H2.3%, I55.8%, N4.1%.

I.R. $\nu_{max}(cm^{-1})$: 680, 755, 805, 1038, 1105, 1215, 1245, 1290, 1331, 1382, 1405, 1455, 1588, 1648, 1700, 2920, 3400.

Compound 12: 2-Hydroxy-5-(3-amino-2,4,6-triiodobenzyloxy)-benzoic acid

This compound was prepared as described for compound 3. The formation of methyl 2-hydroxy-5-(3-amino-2,4,6-triiodobenzyloxy)benzoate was incomplete. The ester was purified by recrystallization from acetone prior to hydrolysis to the free acid. The acid recrystallized from glacial acetic acid. Yield from the benzyl chloride 12% m 226-9°. $C_{14}H_{10}I_3NO_4$ requires C26.4%, H1.6%, I59.8%, N2.2%; found C26.7%, H1.6%, I59.7%, N2.5%.

I.R. $\nu_{max}(cm^{-1})$: 715, 786, 810, 825, 870, 1015, 1079, 1215, 1390, 1430, 1469, 1488, 1602, 1678, 2920, 3350, 3445.

Compound 13: 2-Hydroxy-5-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid

The methyl ester of compound 13 was prepared as described for compound 1. The formation of the ester was incomplete. The unreacted starting materials were removed from the precipitated reaction mixture by extraction of the precipitate with boiling methanol (300ml). The resultant crude ester was hydrolyzed as described for compound 2. The free acid recrystallized from methanol. Yield from the benzyl chloride 12% m 252-5°. $C_{16}H_{12}I_3NO_5$ requires C28.3%, H1.8%, I56.1%, N2.1%; found C28.5%, H2.1%, I55.8%, N2.4%.

I.R. $\nu_{max}(cm^{-1})$: 705, 730, 793, 820, 870, 1020, 1035, 1080, 1222, 1250, 1275, 1348, 1379, 1429, 1445, 1471, 1491, 1545, 1622, 1671, 1882, 2910, 3220, 3380.

Compound 14: 2-Hydroxy-5-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid

The methyl ester was prepared as described for compound 13. The ester was hydrolyzed as described for compound 2. The free acid recrystallized from methanol. Yield from the benzyl chloride 14% m 224-5°. $C_{17}H_{15}I_3N_2O_4$ requires C29.5%, H2.2%, I55.1%, N4.0%; found C29.7%, H2.4%, I55.1%, N3.8%.

I.R. $\nu_{max}(cm^{-1})$: 725, 775, 817, 860, 1036, 1088, 1112, 1137, 1225, 1250, 1288, 1330, 1360, 1385, 1460, 1495, 1590, 1650, 1715, 2920, 3390.

C) Taurine Derivatives

3-(3-Amino-2,4,6-triiodophenyl)propionic acid

3-(3-Amino-2,4,6-triiodophenyl)propionic acid was prepared from 3-nitrocinnamic acid according to Scaglia (1958) by reduction with Raney nickel followed by iodination using iodine monochloride in hydrochloric acid. Yield 85% m 223-5° (reported 224-5°).

Compound 15: N-(3-(3-Amino-2,4,6-triiodophenyl)propionoyl)-taurine

This was prepared by a method analogous to that described by Goldberg et al (1946) for benzoyl taurines.

A solution of 3-(3-amino-2,4,6-triiodophenyl)propionic acid (0.0055mole) in thionyl chloride (20ml) was refluxed for 3 hours. Toluene (40ml) was added to the solution and the excess thionyl chloride was distilled over in 25-30ml of the toluene. The remaining solution in toluene was evaporated to dryness and the acid chloride was dissolved in acetone (25ml). The acetone solution was then added dropwise, at 0-5° to a stirred solution of taurine (0.0056mole), sodium hydroxide (0.0056mole) and sodium bicarbonate (0.01mole) in water (10ml). Stirring was continued for 2 hours after the addition was complete. The reaction mixture was then evaporated to dryness and the dried residue was ground in a mortar and pestle. The ground residue was then extracted with boiling acetone and filtered yielding the free sulphonic acid which was recrystallized from aqueous acetone. Yield 15.6% m 280-1°. $C_{11}H_{13}I_3N_2O_4S$ requires C20.3%,

H2.0%, I58.6%, N4.3%; found C20.0%, H1.9%, I58.5%, N4.1%.
I.R. $\nu_{max}(cm^{-1})$: 875, 1055, 1211, 1376, 1430, 1455, 1557, 1605, 1640, 1650, 2920, 3340, 3420.

Compound 16: N-(3-Amino-2,4,6-triiodobenzyl)taurine

3-Amino-2,4,6-triiodobenzyl chloride (0.0019mole), taurine (0.0038mole) and sodium bicarbonate (0.0038mole) in dry dimethylformamide (20ml) were heated at 100° for 10 hours with constant stirring. The sulphonic acid was precipitated when the reaction mixture was filtered into acetone (150ml). The precipitate formed was filtered and dissolved in water with the aid of 1-2 drops of 10% sodium hydroxide. The solution was treated with charcoal and filtered. The solution of the sodium salt was then warmed on a steam bath and made just acid by the addition of a few drops of concentrated hydrochloric acid. The free acid crystallized out upon cooling. Yield 9% m 246-50°.
$C_9H_{11}I_3N_2O_3S$ requires C17.8%, H1.8%, I62.7%, N4.6%; found C17.9%, H1.7%, I62.7%, N4.7%.
I.R. $\nu_{max}(cm^{-1})$: 732, 1039, 1150, 1179, 1222, 1270, 1299, 1395, 1435, 1452, 1605, 2920-3000, 3330, 3420.

Compound 17: N-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyl)taurine

This compound was prepared in a manner analogous to compound 16 using the corresponding benzyl chloride. The free acid precipitated from the acetone suspension and was filtered and recrystallized from aqueous acetone. Yield 32% m 201-5°.
$C_{12}H_{16}I_3N_3O_3S$ requires C21.7%, H2.4%, I57.5%, N6.3%; found

C22.0%, H2.1%, I57.5%, N6.7%.

I.R. $\nu_{max}(cm^{-1})$: 732, 786, 870, 1046, 1109, 1170-1210, 1265, 1317, 1327, 1375, 1395, 1405, 1446, 1510, 1535, 1640, 1650, 2920, 3400.

D) 3,5-Bis(Triiodobenzyl)benzoic Acid Esters

Ethyl 3,5-dinitrobenzoate

3,5-Dinitrobenzoyl chloride was treated with ethyl alcohol to yield ethyl 3,5-dinitrobenzoate. It recrystallized from ethanol. Yield 89% m 94-5° (reported 91°(Brill, 1921).

Ethyl 3,5-diaminobenzoate

Ethyl 3,5-dinitrobenzoate (10g) in ethanol (200ml) was hydrogenated using a Parr Hydrogenation Apparatus (Parr Instrument Co., Moline, Illinois, U.S.A.) and 10% palladium on activated charcoal (700mg)(E. Merck A.G., Darmstadt, Germany). The diamino compound recrystallized from ethanol-petroleum ether. Yield 68% m 77-8° (reported 84° (Brill, 1921).

Ethyl 3,5-dihydroxybenzoate

3,5-Dihydroxybenzoic acid was esterified by Vogel's "Azeotropic Mixture Method" (Vogel, 1956). The toluene-ethanol solution was evaporated to dryness to yield the pure ester. Yield 76% m 125-8° (reported 128.5° (Suter and Weston, 1939).

Compound 18: Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzylamino)-benzoate

Ethyl 3,5-diaminobenzoate (0.001mole), 3-amino-2,4,6-triiodobenzyl chloride (0.002mole) and sodium bicarbonate

(0.004mole) in dry dimethylformamide (30ml) were heated at 80-100° for 12 hours with constant stirring. Water (200ml) was added and the precipitate was collected, washed with water and dried. The crude ester was washed with acetone, dissolved in dimethylformamide, treated with activated charcoal, precipitated by the addition of water and dried. Yield 11% m 127-30°. $C_{23}H_{20}I_6N_4O_2$ requires C24.1%, H1.7%, I66.5%, N4.9%; found C24.3%, H2.0%, I64.5%, N4.8%.

I.R. $\nu_{max}(cm^{-1})$: 761, 795, 850, 1025, 1095, 1210, 1238, 1305, 1375, 1428, 1518, 1600, 1713, 2860, 2930, 3360, 3440.

Compound 19: Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzylamino)benzoate

This compound was prepared in a manner analogous to compound 18 from 3-acetamido-2,4,6-triiodobenzyl chloride. Yield 9% m 256-8° . $C_{27}H_{24}I_6N_4O_4$ requires C26.3%, H2.0%, I62.0%, N4.6%; found C26.5%, H2.0%, I58.3%, N4.7%.

I.R. $\nu_{max}(cm^{-1})$: 760, 863, 1027, 1095, 1205, 1233, 1309, 1335, 1369, 1410, 1450, 1465, 1508, 1605, 1678, 2830, 2900, 3360.

Compound 20: Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)-benzoate

This was prepared in a manner analogous to compound 18 from ethyl 3,5-dihydroxybenzoate. The precipitate was recrystallized from aqueous dimethylsulphoxide. Yield 39% m 240-1° . $C_{23}H_{18}I_6N_2O_4$ requires C24.0%, H1.6%, I66.4%, N2.4%;

found C24.1%, H1.9%, I64.0%, N2.5%.

I.R. $\nu_{max}(cm^{-1})$: 720, 762, 853, 870, 970, 1025, 1049, 1100, 1149, 1230, 1298, 1315, 1345, 1368, 1390, 1430, 1445, 1598, 1720, 2910, 2950, 3315, 3415.

Compound 21: Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzyloxy)-benzoate

This compound was prepared as described for compound 18 from ethyl 3,5-dihydroxybenzoate and 3-acetamido-2,4,6-triiodobenzyl chloride. Yield 9.4% m 314-8°. $C_{27}H_{22}I_6N_2O_6$ requires C26.3%, H1.8%, I61.9%, N2.3%; found C26.6%, H1.8%, I57.6%, N2.3%.

I.R. $\nu_{max}(cm^{-1})$: 710, 762, 855, 1039, 1101, 1153, 1200, 1232, 1298, 1315, 1342, 1368, 1420, 1442, 1500, 1540, 1598, 1670, 1715, 2900, 2950, 3380.

Compound 22: 3-Amino-2,4,6-triiodobenzyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)benzoate

3,5-Dihydroxybenzoic acid (0.0005mole), 3-amino-2,4,6-triiodobenzyl chloride (0.001mole) and sodium bicarbonate (0.002mole) in dry dimethylformamide (10ml) were heated at 80-100° with constant stirring for 12 hours. The crude ester was precipitated by the addition of water (200ml). The precipitate was washed with water and hot dimethylformamide and dried. Yield 23% m 294-7°. $C_{28}H_{18}I_9N_3O_4$ requires C21.0%, H1.1%, I71.3%, N2.6%; found C21.0%, H1.2%, I70.9%, N2.6%.

I.R. $\nu_{max}(cm^{-1})$: 718, 759, 860, 1022, 1036, 1092, 1150, 1220, 1297, 1358, 1388, 1428, 1600, 1725, 2900, 3335, 3430.

E) Miscellaneous Compounds

Compound 23: Ethyl 3,5-bis(2,3,5-triiodobenzamido)benzoate

A solution of 2,3,5-triiodobenzoic acid (0.002mole) in thionyl chloride (5ml) was refluxed for 1 hour. Chlorobenzene (20ml) was then added to the solution and the excess thionyl chloride was distilled over in 10ml of chlorobenzene. The amide formation in compounds 23, 25 and 26 was performed in a manner analogous to that described by Priewe et al (1954). The solution of the acid chloride in chlorobenzene was added dropwise over 15 minutes to a stirred refluxing solution of ethyl 3,5-diaminobenzoate (0.001mole) in chlorobenzene (20ml). The ester precipitated out and was washed with chlorobenzene and extracted with boiling ether. Yield 28% m 195°. $C_{14}H_9I_3N_2O_3$ requires C24.1%, H1.2%, I66.6%, N2.4%; found C24.2%, H1.3%, I66.7%, N2.4%.

I.R. ν_{max}(cm^{-1}): 709, 765, 862, 1020, 1110, 1185, 1248, 1280, 1370, 1395, 1430, 1451, 1565, 1615, 1678, 1710 (shoulder), 2930, 2990, 3080, 3380.

Compound 24: 3-(3-Amino-2,4,6-triiodophenyl)propan-1-ol

3-(3-Aminophenyl)propionic acid was reduced using a method of Cerny et al (1969). To a refluxing solution of 3-(3-aminophenyl)propionic acid (0.03mole) in dry benzene (100ml), a solution of sodium bis(2-methoxyethoxy) aluminium hydride (0.09mole) (Redal-Aldrich Chemical Co. Inc., Milwaukee, Wisconsin, U.S.A.) in dry benzene (104ml) was added dropwise over 30 minutes in a nitrogen atmosphere. The solution was refluxed for 4 hours

after the addition was complete. The excess Redal was destroyed using 5-10ml of 17% sulphuric acid. The suspension formed was then filtered and the filtrand was washed with ethanol. The ethanol fraction was then combined with the original filtrate and evaporated to dryness on a rotary evaporator. The residue obtained was dissolved in ether. The ether solution was washed with 10% aqueous sodium carbonate solution and water and then evaporated to dryness to yield the crude 3-(3-aminophenyl)-propan-1-ol which was further purified through its hydrochloride salt. The residue was first dissolved in dry ether and dry hydrogen chloride gas was passed through the solution. The hydrochloride salt was extracted into water and the free amine was precipitated by the addition of ammonia. The precipitate was redissolved in ether, the ether dried with magnesium sulphate and filtered. The solution was evaporated to dryness to yield 3-(3-aminophenyl)propan-1-ol as a crude oil (1.8g).

The oil was dissolved in concentrated hydrochloric acid (100ml) and this solution was diluted with water (800ml). This solution was then slowly heated to 60^{o} with stirring. Iodine monochloride (8.2g) in concentrated hydrochloric acid (10ml) was added all at once. 3-(3-amino-2,4,6-triiodophenyl)-propan-1-ol began to precipitate out. The reaction mixture was stirred for a further 2 hours allowing the temperature to rise to 75^{o}. The reaction mixture was allowed to cool and the iodinated alcohol was filtered off. It recrystallized from chloroform. Yield from 3-(3-aminophenyl)propionic acid 5% m 153-4^{o}. $C_9H_{10}I_3NO$ requires C20.4%, H1.9%, I72.0%, N2.6%; found C20.6%, H1.9%, I71.8%, N2.5%.

I.R. $\nu_{max}(cm^{-1})$: 721, 879, 973, 1020, 1060, 1385, 1435, 1468, 1610, 2860, 2930, 3340, 3400.

Ethyl 3-(3-amino-2,4,6-triiodophenyl)propionate

The ester was prepared from the corresponding acid by Vogel's "Azeotropic Mixture Method" (Vogel, 1956). It recrystallized from acetone. Yield 94% m 125-6°. $C_{11}H_{12}I_3NO_2$ requires C23.1%, H2.1%, I66.7%, N2.5%; found C23.3%, H2.2%, I67.5%, N2.4%.

I.R. $\nu_{max}(cm^{-1})$: 868, 1152, 1200, 1288, 1305, 1360, 1379, 1431, 1459, 1521, 1608, 1660, 1710, 1735, 2850, 2920, 3310, 3390.

3-(2,4,6-Triiodophenyl)propionic acid

3-(3-Amino-2,4,6-triiodophenyl)propionic acid was deaminated via its diazonium salt by treatment with cuprous oxide (Papa et al, 1953). The final product recrystallized from acetone. Yield 57% m 103-4°. $C_9H_7I_3O_2$ requires C20.5%, H1.3%, I72.1%; found C20.8%, H1.6%, I71.8%.

I.R. $\nu_{max}(cm^{-1})$: 740, 778, 865, 1055, 1193, 1295-1305, 1735 (Nujol mull).

Compound 25: N,N'-bis(3-(2-carbethoxyethyl)-2,4,6-triiodophenyl)-adipamide

To a solution of ethyl 3-(3-amino-2,4,6-triiodophenyl)-propionate (0.0025mole) in refluxing chlorobenzene (20ml), a solution of adipoyl chloride (0.00125mole) in chlorobenzene (10ml) was added dropwise over 0.5 hour. The reaction mixture was refluxed for a further 4 hours. A white solid (0.75g)

precipitated. The precipitate was washed with chlorobenzene and extracted with boiling ether to yield an amorphous powder. Yield 20% m 286-9°. $C_{28}H_{30}I_6N_2O_6$ requires C26.8%, H2.4%, I60.9%, N2.2%; found C27.1%, H2.4%, I56.9%, N1.9%. I.R. $\nu_{max}(cm^{-1})$: 860, 1040, 1180-1200, 1298, 1345, 1362, 1419, 1450, 1498, 1659, 1730, 2910, 2960, 3210.

Compound 26: 3-(3-(3-(2,4,6-triiodophenyl)propionamido)-2,4,6-triiodophenyl)propionic acid

A solution of 3-(2,4,6-triiodophenyl)propionic acid (0.0115mole) in thionyl chloride (10ml) was refluxed for 1 hour. Chlorobenzene (25ml) was added to the solution and the excess thionyl chloride was distilled over in 10ml chlorobenzene. The solution of the acid chloride was added dropwise over 20 minutes to a refluxing solution of ethyl 3-(3-amino-2,4,6-triiodophenyl)-propionate (0.0115mole) in chlorobenzene (25ml). The solution was refluxed for a further 3 hours. The crude ester precipitated from the reaction mixture (1.55g) and was filtered and washed with chlorobenzene and acetone. It was then suspended in 35% potassium hydroxide in 25% aqueous methanol (30ml; Claisen's Alkali) and heated on a water bath until solution was effected. Immediately, the solution was acidified with concentrated hydrochloric acid. The precipitated free acid was purified by 4 successive precipitations with water from solutions in dimethylformamide preceded by treatment with activated charcoal. Yield from 3-(2,4,6-triiodophenyl)propionic acid 4% m 324.5-5.5°. $C_{18}H_{13}I_6NO_3$ requires C20.5%, H1.2%, I72.4%, N1.3%; found C20.8%,

H1.4%, I68.7%, N1.4%.

I.R. $\nu_{max}(cm^{-1})$: 860, 1190, 1414, 1449, 1500, 1515, 1548, 1668, 1712, 2920, 3225, 3405.

RESULTS

(i) X-Ray Absorption Analysis

The concentration of iodine required for a clear radiogram has been estimated to be at least 1% (Sperber and Sperber, 1971). This agrees with the conclusion of Edholm and Jacobsen (1959) who claimed that a concentration of 10-12mg iodine per ml of bile gave fairly good visibility of the bile ducts whereas ducts containing bile with 5-7mg iodine per ml could hardly be detected.

While cholecystographic media are concentrated in the gall bladder yielding concentrations higher than those found in the biliary ducts, it was decided to determine the level of excretion of the experimental compounds in rats, which do not possess a gall bladder. In order to have a reference as to what level of excretion could be expected in rats from agents presently in clinical use, iopanoic acid and sodium iopodate were administered to these animals. These compounds gave peak concentrations in the bile of 5 and 6.5mg/ml of iodine respectively. It was decided that an assay that covered concentrations of compounds up to the equivalent of 10mg/ml of iodine would be suitable.

Microchemical techniques have been used to determine iodine concentrations directly (e.g. Zak and Boyle, 1952). The drawback of these methods is the relatively long time required to work up a sample. Ultraviolet spectrophotometric techniques have also been described (e.g. Medzihradsky *et al*, 1975) for the determination of radiocontrast agents in biological fluids. In the case of radiocontrast agents in bile, considerable interference

is noted from bile itself and a time consuming work up of the sample would be required. Also, spectrophotometric techniques depend on a common wavelength of absorption for each compound and its metabolites if these techniques are to be convenient. Different intensities of absorption must also be taken into consideration. Several methods for the analysis of iodipamide were presented in an analytical profile of that compound (Lerner, 1974). Methods mentioned included flame photometry, polarography, elemental analysis, spectrophotometric analysis, x-ray flourescence and x-ray absorption.

Investigation of the literature indicated that x-ray preferential absorption could provide a rapid, relatively inexpensive method for analysis that would be suitable for analyzing large numbers of different iodine containing compounds in biological fluids (e.g. Edholm and Jacobsen, 1959).

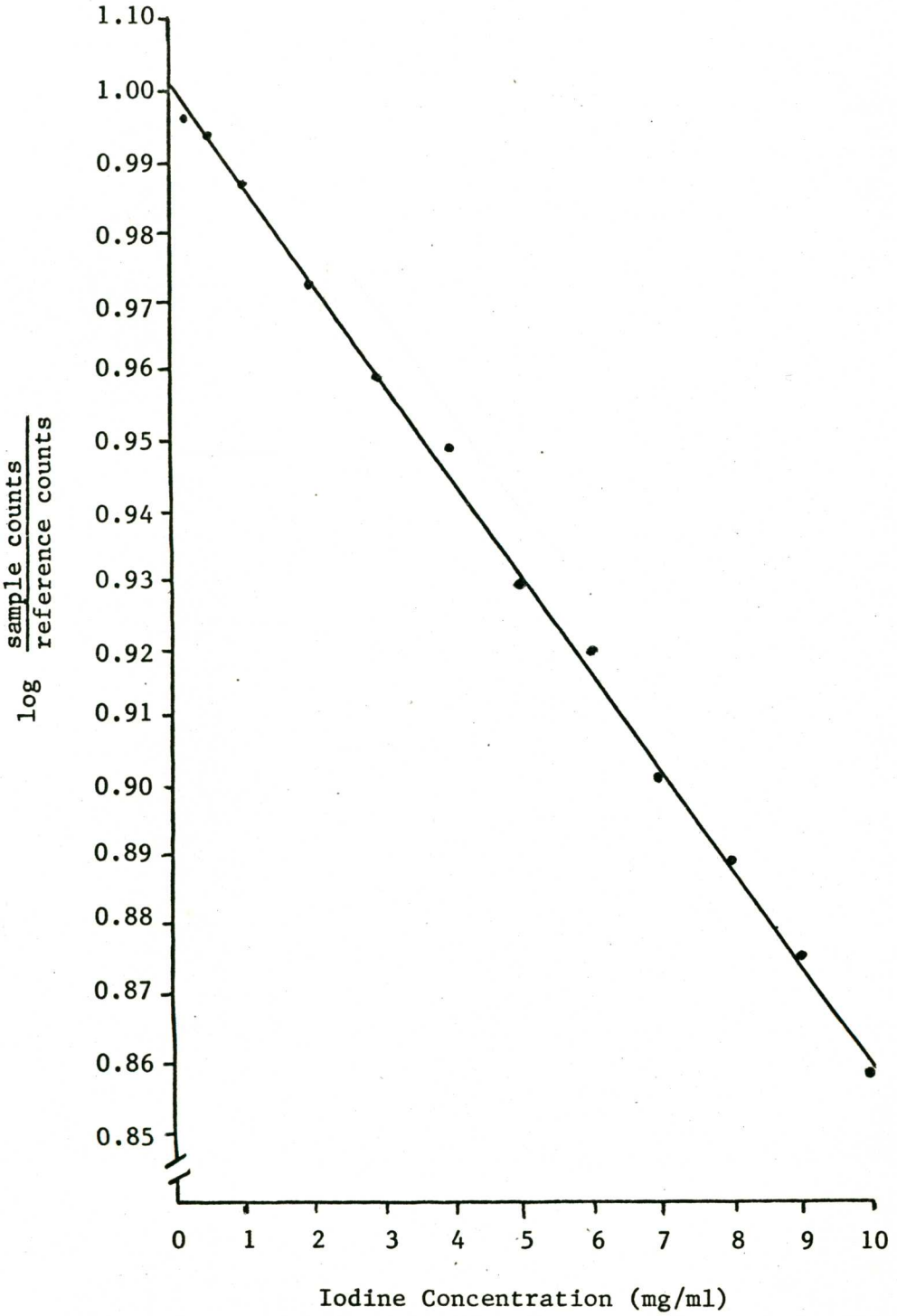
Therefore, the x-ray absorption apparatus described earlier (p. 47) was developed for the present study. The standard curve for the apparatus was determined as described earlier. The data for the standard curve is summarized in Table 2. A linear least squares regression line was fitted to the data points. The data points and the calculated regression line are illustrated in Figure 4. The equation of the regression line was calculated to be: $y = 1.0015 - 0.0141x$. A good fit was indicated by a coefficient of determination of 0.9992. A linear relationship was assumed over the range of 0.2-10mg/ml of iodine.

10 bile samples (100μl) were weighed. The mean was 100.2mg with a standard deviation of 0.4. Therefore, it was assumed that the specific gravity of bile was close enough to

TABLE 2. Data for the standard curve of the x-ray preferential absorption analysis

Concentration of KI in bile as mg/ml of iodine	log $\frac{\text{sample counts}}{\text{reference counts}}$
0.20	0.9972
0.50	0.9947
1.00	0.9877
2.00	0.9731
3.00	0.9594
4.00	0.9472
5.00	0.9298
6.00	0.9179
7.00	0.9018
8.00	0.8898
9.00	0.8753
10.00	0.8587

FIGURE 4. Standard curve for x-ray preferential absorption analysis relating iodine concentration to the log of the ratio of the sample counts and the blank bile counts



unity that weights of bile samples (mg) obtained from rats given test compounds could be converted directly into volume measurements (μl).

In order to illustrate that organically bound iodine absorbed x-rays to the same extent as the inorganic iodine on which the standard curve was based, 3 samples containing known concentrations of sodium iopodate in bile were determined on the x-ray absorption apparatus. Eight replicate determinations of samples containing 0.46, 3.24 and 6.64mg/ml of iodine as sodium iopodate in bile gave results of 0.55 ± 0.05, 3.15 ± 0.03 and 6.73 ± 0.04 respectively (mean $\pm$ standard error). The standard errors were constant over a wide concentration range. Therefore, there was a higher absolute error at low concentrations and a lower absolute error at high concentrations. The error was considered acceptable in the context of a procedure intended to provide a screen of potentially useful cholegraphic agents.

It was also of interest to determine whether or not blank bile samples from different rats exhibited different degrees of absorption of the x-rays. The analysis of 56 separate bulked bile samples collected and analyzed over a 4 month period gave a mean of 116,216 counts over 40 seconds with a standard deviation of 532 counts. This result not only illustrated that there was little variation in the values obtained with bile from different groups of rats but also indicated that the instrumentation was stable over the 4 month period.

(ii) Biliary Excretion Studies

A) Intraduodenal Studies

Compounds 1-26 were administered intraduodenally (ID). It was considered that those compounds that upon analysis gave a ratio of sample count to blank count of less than 0.9950 in at least one of the hourly samples were excreted in the bile. A ratio of 0.9950 was equivalent to 0.26mg/ml of iodine. Therefore, compounds that were not excreted at levels greater than 0.26mg/ml of iodine were considered to exhibit no apparent excretion in the bile. Of all 26 compounds, only compounds 4, 6, 9, 10, 11, 12 and 14 exhibited any apparent biliary excretion. None of the other compounds appeared to be excreted following intraduodenal administration. Appendix 1 gives details of the concentration and weight of iodine in each hourly bile sample of both runs performed with each of the excreted compounds. The total weight of iodine excreted in 6 hours and the percentage of the administered dose that was excreted in 6 hours are also recorded for each run. Table 3 summarizes these results giving the average hourly concentrations of iodine excreted for each compound, as well as the average percentage of the administered dose excreted in 6 hours. The results were expressed as concentrations of iodine rather than concentrations of compound because it is the iodine concentration that is clinically significant. Therefore, a ready comparison of the potential usefulness of each compound can be made. The iodine content of the 7 compounds excreted ranged from 55.1% to 59.8%. All compounds were administered at the level of 100mg of compound per kilogram of body weight.

TABLE 3. Biliary excretion of iodine after intraduodenal administration of test compounds (100mg/Kg). Results for each compound represent the average of two runs.

Sample time (hour)	1	2	3	4	5	6	
COMPOUND	CONCENTRATIONS (mg/ml I)						% of dose excreted in 6 hours
4	0.67	1.21	1.36	1.20	1.16	1.04	16.7
6	0.52	0.78	0.79	0.68	0.59	0.55	11.5
9	0.36	0.93	1.20	1.24	1.30	1.15	12.9
10	0.28	0.35	0.34	0.39	0.33	0.34	4.6
11	0.38	0.60	0.65	0.65	0.61	0.52	7.7
12	0.59	1.41	1.33	1.07	1.10	0.86	15.8
14	0.22	0.48	0.60	0.78	0.71	0.65	9.3
Iopanoic Acid	2.76	5.02	4.77	4.17	3.47	2.99	42.8
Sodium Iopodate	4.04	6.51	5.88	4.90	3.98	3.30	58.6

Compounds 6 and 9 were excreted in the bile at levels of 12-13% of the administered dose in 6 hours. 8-9% of the administered dose of compounds 11 and 14 was excreted in the same time. Compound 10 exhibited a low but consistent excretion giving a total of about 5% of the administered dose in 6 hours. Compounds 4 and 12 exhibited the best excretion with levels of 16-17% of the dose excreted in 6 hours. The biliary concentration of all these compounds peaked between 2 and 5 hours after administration. Compounds 4, 9 and 12 peaked at 1.3-1.4mg/ml of iodine and compounds 6, 11 and 14 peaked at 0.7-0.8mg/ml of iodine. Compound 10 peaked at 0.4mg/ml of iodine.

Appendix 1 and Table 3 also show the results obtained following the ID administration of iopanoic acid and sodium iopodate. Iopanoic acid peaked at 5.0 mg/ml of iodine in the second hour and exhibited a total excretion of 43% of the dose in 6 hours. Sodium iopodate peaked at 6.5 mg/ml of iodine in the second hour and exhibited a total excretion of 59% of the dose in 6 hours.

As mentioned previously, these two compounds were administered so as to provide data that was indicative of a clinically useful oral cholegraphic agent. While compounds may reach higher concentrations in the gall bladder than in the bile ducts in a clinical situation, it was assumed that if the experimental compounds did not approach the values obtained following the administration of iopanoic acid and sodium iopodate to the rat, they would not be successful as cholangiographic or cholecystographic agents. None of the experimental compounds approached the levels of excretion exhibited by the two commercial agents.

It was of interest to know whether or not the compounds excreted in the bile following intraduodenal administration had an effect on the bile flow over 6 hours. Therefore, the bile flow over 6 hours of rats to whom the excreted compounds had been administered was compared to that of 10 reference rats. The mean and standard deviation for the reference bile flow was found to be $2.30g \pm 0.5$. The mean and standard deviation of the bile flow of the 4 rats to which compound 6 had been administered was found to be $3.15g \pm 0.4$. Therefore, this compound appeared to have some choloretic activity. However, the mean bile flows of rats to which compounds 4, 9-12 and 14, iopanoic acid and sodium iopodate had been administered were all within the range of $2.30g \pm 0.5$ and it appeared that these compounds had no marked effect on bile flow.

B) Intravenous Studies

Following the administration of compounds 1-26ID, the question arose as to whether or not a lack of intestinal absorption accounted for the non-excretion of compounds 1-3, 5, 7, 8, 13 and 15-26 and the relatively low excretion of compounds 4, 6, 9-12 and 14. Therefore, all the compounds that were excreted following ID administration were administered intravenously (IV). Also, 5 of the remaining compounds were selected to be given IV in order to represent the various structural types that had not exhibited any excretion after ID administration.

Due to the insolubility of these compounds in water, they were administered as solutions in aqueous dimethylsulphoxide (DMSO). While the DMSO noticeably affected the behaviour of the

rats and possibly the excretion of these compounds, the aim of this experiment was simply to illustrate whether or not compounds that were not excreted after ID administration would be excreted after IV administration, and whether or not the extent of excretion of those compounds that were excreted after ID administration would change after IV administration.

Compounds 4-12, 14, 15 and 20 were administered to rats intravenously. All compounds administered were excreted. Appendix 2 gives the details of the hourly concentrations (mg/ml) and weights (mg) of iodine excreted in both runs of each of the 12 compounds administered intravenously. Also the total weight excreted in 6 hours and the percentage of the dose excreted in 6 hours are recorded. These results are summarized in Table 4 which gives the average hourly concentrations of iodine (mg/ml) of both runs for each compound and the average percentage of the administered dose excreted in 6 hours. About 90% of compound 4 was excreted in 6 hours, 85% of compound 11, 77% of compounds 9 and 14, 72-73% of compounds 6 and 8, 65-66% of compounds 5, 7 and 12, 58% of compounds 10 and 15 and 22% of compound 20.

Compound 11 gave the highest peak iodine concentration at about 2mg/ml followed by compounds 4, 5 and 15 at 1.6-1.7mg/ml, compounds 7-10, 12 and 14 at 1.3-1.4mg/ml, compound 6 at 1.1mg/ml and compound 20 at 0.5mg/ml. Compounds peaked 1-2 hours after administration.

(iii) Beta Glucuronidase Hydrolyses

Because glucuronide conjugation is the major metabolic transformation of many oral cholegraphic media, a screen of the

TABLE 4. Biliary excretion of iodine after intravenous administration of test compounds (20mg/Kg). Results for each compound represent the average of two runs.

Sample time (hour)	1	2	3	4	5	6	% of dose excreted in 6 hours
COMPOUND	CONCENTRATIONS (mg/ml I)						
4*	1.60	1.69	1.14	0.87	0.67	0.57	90.4
5	1.66	1.64	1.01	0.82	0.64	0.49	64.8
6*	1.05	1.12	1.04	0.90	0.68	0.60	71.8
7	1.34	1.09	0.79	0.57	0.54	0.42	65.0
8	1.36	1.17	0.56	0.59	0.60	0.57	73.3
9*	0.90	1.28	1.12	1.08	0.92	0.77	76.5
10*	1.36	0.81	0.69	0.56	0.57	0.44	57.7
11*	1.95	1.66	0.98	0.75	0.64	0.48	85.3
12*	1.18	1.26	0.87	0.72	0.56	0.47	65.6
14*	1.39	1.31	0.97	0.62	0.52	0.39	77.2
15	1.60	0.94	0.69	0.65	0.64	0.56	58.4
20	0.49	0.46	0.39	0.41	0.50	0.45	22.1

* - also excreted after intraduodenal administration

biotransformation of the compounds excreted in the present study was carried out with emphasis on glucuronide formation. Bile from rats to which these compounds had been administered was analyzed on TLC before and after hydrolysis with beta glucuronidase.

Table 5 summarizes the results obtained from the thin layer chromatography (TLC) of bile samples containing peak concentrations of iodine after intraduodenal administration of compounds 4, 6, 9-12 and 14. Samples were run in Solvent B before and after hydrolysis with beta glucuronidase. A blank bile sample was also run before and after hydrolysis. Spots corresponding to those evident in the TLC of the blank samples were disregarded. Bile from rats given iopanoic acid intraduodenally was also screened.

The TLC of samples collected from rats given compounds 4, 9, 10, 11 and 14 appeared the same before and after hydrolysis. Compounds 4 and 14 appeared to be excreted unchanged. Compounds 9 and 10 exhibited one spot apart from the parent compound and compound 11 exhibited two spots other than the parent compound. The disappearance after beta glucuronidase hydrolysis of a moderately intense spot at Rf 0.49 in the TLC for compound 6 suggests the presence of a glucuronide metabolite. The disappearance after hydrolysis of a moderately intense spot at Rf 0.42 and the reduction in relative intensity of another spot at Rf 0.70 in the TLC for compound 12 suggests the presence of a glucuronide metabolite plus one other metabolite apart from unchanged compound 12.

The reversal in the relative intensities of the two spots found in the TLC of iopanoic acid before and after hydrolysis

TABLE 5. Thin layer chromatography of bile before and after hydrolysis with β-glucuronidase from rats treated with compounds that were excreted after intraduodenal dosing.

COMPOUND #	Rf	UNHYDROLYSED SAMPLE Rf	Intensity	HYDROLYSED SAMPLE Rf	Intensity
4	0.63	0.63	++	0.63	++
6	0.90	0.91	+	0.90	++
		0.49	++		
9	0.77	0.77	++	0.77	++
		0.71	++	0.71	++
10	0.89	0.90	++	0.89	++
		0.49	++	0.49	++
11	0.74	0.86	++	0.85	++
		0.74	++	0.73	++
		0.60	++	0.59	++
12	0.97	0.98	++	0.96	++
		0.69	++	0.70	+
		0.42	++		
14	0.49	0.47	++	0.47	++
iopanoic acid	0.91	0.91	+	0.91	++
		0.45	++	0.43	+

Run in solvent B

\+ : faint intensity

++ : moderate intensity

with beta glucuronidase, suggests the presence of a glucuronide conjugate for this compound as well.

The TLC of the bile of rats to which test compounds were administered intravenously is summarized in Table 6. Compounds 4, 7, 8, 14 and 15 exhibited only one spot corresponding to the parent compounds both before and after hydrolysis. Hence, these five compounds appeared to be excreted unchanged. Compounds 9 and 10 exhibited two spots and compound 11 exhibited three spots before and after hydrolysis suggesting that some metabolism may have taken place that did not involve glucuronide formation. Compounds 5 and 6 exhibited a faint spot corresponding to the parent compound and another stronger spot before hydrolysis and only one spot corresponding to the parent compound after hydrolysis. This suggested the formation of a glucuronide. Compound 12 (Rf 0.96) exhibited three equally intense spots (Rf 0.98, 0.73 and 0.47) before hydrolysis and one moderately intense spot (Rf 0.97) and one faint spot (Rf 0.70) after hydrolysis. This suggested glucuronide formation as well as the presence of one other metabolite apart from unchanged compound 12. Compound 20 could not be detected in bile by TLC either before or after hydrolysis with beta glucuronidase.

(iv) <u>The Isolation of Compound 6 from Rat Bile</u>

Compound 6 was administered to 2 male rats ID. The collected bile was hydrolyzed with beta glucuronidase. The hydrolyzed bile was purified twice using thick layer chromatography. The band cochromatographing with compound 6 was scraped off the

TABLE 6. Thin layer chromatography of bile before and after hydrolysis with β-glucuronidase from rats treated with compounds that were excreted after intravenous dosing.

COMPOUND #	Rf	UNHYDROLYSED SAMPLE Rf	Intensity	HYDROLYSED SAMPLE Rf	Intensity
4	0.63	0.60	++	0.60	++
5	0.84	0.83	+	0.83	++
		0.31	++		
6	0.87	0.87	+	0.87	++
		0.43	++		
7	0.83	0.83	++	0.83	++
8	0.63	0.62	++	0.63	++
9	0.80	0.83	+	0.82	+
		0.71	++	0.70	++
10	0.89	0.89	++	0.89	++
		0.45	++	0.47	++
11	0.73	0.85	++	0.88	++
		0.72	++	0.72	++
		0.59	++	0.61	++
12	0.96	0.98	++	0.97	++
		0.73	++	0.70	+
		0.47	++		
14	0.51	0.51	++	0.51	++
15	0.14	0.15	++	0.16	++
20	0.92	-	-	-	-

Run in solvent B

\+ : faint intensity

++ : moderate intensity

plates, dissolved in dimethylformamide, precipitated with water, filtered and dried. No metabolites were observed on the thick layer plates. The white powder obtained melted at 213-5^{o} and cochromatographed on TLC with compound 6 in Solvents A and B. The infrared spectrum obtained for the powder was identical to that described for compound 6. It was concluded that the isolated product was compound 6.

DISCUSSION

(i) Chemical Syntheses

The N- and O-alkylations performed in the present study were carried out in dimethylformamide (DMF) in the presence of sodium bicarbonate. The use of DMF in the condensation of potassium phthalimide with organic halides was found to give higher yields and require lower temperatures and shorter reaction times than with the use of other solvents (Sheehan and Bolhofer, 1950). The use of DMF as a solvent in O-alkylations has also proved advantageous compared to other solvents (Chopin and Durual, 1965). It was found that an excess of alkyl chloride was not necessary and that high yields of benzyloxy-2'chalcones were obtained in a short period of time following the treatment of hydroxychalcones with benzyl chloride and anhydrous potassium carbonate in DMF. The opportunity of conserving the iodinated benzyl chlorides, in the present study, led to the use of DMF. Sodium bicarbonate was used in place of potassium carbonate. The simplicity of the synthetic procedure and the reasonable yields obtained led to the adoption of this method for both N- and O-alkylations in the present study.

The synthesis of many of the hexaiodinated compounds was much more troublesome than was at first expected. The original intention was to obtain the free acids of compounds 18-21, 23 and 25. In all cases except for compound 20, attempts to obtain crystalline esters failed and the necessity to purify the amorphous esters contributed to the low yields. Attempts to hydrolyze these

esters to the corresponding acids were unsuccessful. Either the ester resisted hydrolysis completely or if stronger conditions were used the hydrolysis led to the formation of several by-products from which the pure acid could not be isolated. Compound 26 was the only hexaiodinated compound that was obtained as the free acid. Other investigators have indicated difficulty in obtaining certain iodinated compounds in a state of purity (Felder et al, 1973a; Carnmalm et al, 1974).

Difficulties were encountered with microanalytical results obtained for iodine. In the case of large hexaiodinated molecules, the analysts returned replicate results that varied by as much as 4-5%. However, the results for the carbon, hydrogen and nitrogen were consistently acceptable. Therefore, the problem was assumed to be with the microanalytical technique and the compounds concerned (18-21, 25 and 26) were accepted as pure. Inconsistent results were obtained with an analytically pure sample of calcium iopodate. This confirmed the above conclusion. Other investigators have reported microanalytical results for iodine which differed from the theoretical by more than 1% (Priewe and Poljak, 1960; Hebky et al, 1964: Hoey et al, 1966; Felder et al, 1973a). With the smaller and generally more soluble triiodinated molecules, no difficulty was encountered with the microanalyses.

Repeated attempts to synthesize 3-(3-amino-2,4,6-triiodobenzyloxy)benzoic acid were met with failure. This compound is the first member of the series of compounds that include compounds 10-14. The product synthesized (as described for compound 3) exhibited only one spot on TLC and melted at

303.5-5°. The microanalysis of this product indicated that the carbon content was low ($C_{14}H_{10}I_3NO_3$ requires C27.1%, H1.6%, I61.4%, N2.3%; found C25.0%, H1.6%, I60.3%, N2.1%) compared to the theoretical value. The infrared spectrum exhibited no absorption in the region of 1650-1750cm^{-1}, indicating the absence of a carbonyl group. The microanalysis also did not conform to the theoretical value for the corresponding decarboxylated compound.

(ii) X-Ray Absorption Analysis

In testing compounds as potential cholegraphic agents, the degree of excretion of the compounds in the bile of experimental animals serves as the basic criterion of acceptability. In the present study, rats were used because of their availability, their ease of handling and their low cost.

The use of ultraviolet spectrophotometry for the analysis of test compounds excreted in bile was limited to a crude quantitative estimation because bile itself exhibited considerable absorption that interfered with the absorption of the compounds even after suitable dilution of the bile. Also, the contribution made by metabolites might be ignored if their absorption spectra were different from that of the parent compound. Alternatives included direct chemical analysis for iodine, the use of radioactive iodine, ultraviolet spectroscopy preceded by chemical manipulation to separate biliary metabolites from interfering contaminants, polarography, x-ray fluourescence and x-ray absorption.

The latter two techniques were attractive because of their potential specificity for elemental iodine and their insenitivity to the low atomic number elements found in biological fluids.

Despite the high sensitivity and simplicity of the x-ray fluorescence technique of Moss et al (1972), the cost of the necessary multichannel analyzer and solid state detector was prohibitive. Holynska and Jankiewicz (1969) described an x-ray absorption analysis using gamma rays emitted by an americium-241 source to analyze the iodine content of radiocontrast media. However, the use of relatively high energy gamma rays failed to take advantage of the k-absorption edge of iodine and hence, lacked a degree of sensitivity to iodine that could have been attained using a more carefully selected source energy. Methods of iodine determination using its k-absorption edge have been described (Rose and Flick, 1964; Jacobsen, 1953; Jacobsen, 1964; Heedman and Jacobsen, 1964; Edholm and Jacobsen, 1959; Atkins et al, 1972; Kraner et al, 1973; Oldendorf et al, 1974; Roy et al, 1962; MacKay, 1960). These methods use two or three x-ray energies, with one above and one below the k-absorption edge. The system developed in the present study utilized a single x-ray source just above the k-absorption edge of iodine.

Any single element can be quantitatively analyzed by measuring the attenuation of one monochromatic x-ray beam according to the Beer-Lambert law (Edholm and Jacobsen, 1959). Generally, absorption decreases with increasing photon energy but at critical energies that are characteristic for each element, the absorption increases markedly for a small increase in energy. These discontinuities are called absorption edges. When an x-ray beam with an energy just above the binding energy of the K-shell

electrons of iodine interacts with an iodine atom in the sample cuvette, it will be absorbed by that atom and lead to the ejection of one of the K electrons. An L-shell or M-shell electron will then fall into the K-shell vacancy and the atom will emit a photon with an energy characteristic of iodine. These flourescent x-rays are emitted isotopically from the atom and would interfere very little with the x-ray beam being measured. Therefore, each interaction of a photon in the incident beam with an iodine K electron reduces the intensity of the beam. Hence, the degree to which the beam is absorbed by the sample is a measure of the amount of iodine present.

Maximum sensitivity for the element of interest is obtained when the photon energy is selected to be just above an absorption edge of that element. Significant errors can be caused by variation from sample to sample in the content of high atomic number elements such as lead and by elements of atomic number just below that of the element of interest. Variation in the content of light elements such as sodium, oxygen, carbon or nitrogen will produce only small errors.

A biological sample containing iodine as the only high atomic number element will strongly absorb x-rays with an energy just above the iodine K-absorption edge. Variations in the transmitted intensity of such x-rays will, therefore, be a sensitive measure of changes in iodine content.

For iodine, the K-absorption edge or K-binding energy is 33.17keV. In the present study, a primary excitation source (americium-241, 100mCi) was used to irradiate a cerium oxide target. The K x-rays emitted by the cerium target provided an

incident beam of predominantly 34.72 and 34.28keV. This formed the basis for a non-destructive assay for non-radioactive iodine in bile. The concentration of iodine was related to the absorption of the incident beam involving the principles explained above. Using this method both organic and inorganic iodine may be measured quickly and accurately and the apparatus may be assembled at a relatively low cost. The actual analysis took less than 5 minutes per sample.

As with all analyses which determine total iodine, metabolites could not be differentiated in the present study. However, the purpose of this sytem was to screen compounds for use as potential cholegraphic agents and total biliary iodine levels certainly are the basic criterion in determining this usefulness.

Standard errors are relatively large at low iodine concentrations. However, compounds with low biliary excretion are not of any potential use as cholegraphic agents and the accuracy of measurements obtained at these low levels of excretion still allowed conclusions to be drawn regarding further chemical syntheses. Much better accuracy was obtained in the medium to high concentration range of this assay. This is in the range where potentially useful agents would be found.

The small standard deviation found for the 56 blank bile samples collected and counted over 4 months indicated that the variation in the absorption of cerium K x-rays from one bile sample to another was small. Also, the long term stability of the instrumentation was shown to be acceptable.

This system was found to be very suited to the preliminary screening of experimental compounds in rats provided

bile from 2 or 3 animals was bulked so as to provide a large enough sample for analysis. The use of larger animals would circumvent the need for bulking bile samples. The greater production of bile by larger experimental animals would also permit more frequent sampling and allow the use of a cuvette with a longer path length which would lead to greater potential sensitivity.

(iii) Biliary Excretion Studies

The biliary excretion of the compounds synthesized in the present study was examined after administration of the substances by the intraduodenal route (ID). ID administration was selected rather than the oral route because it overcame the problems associated with gastric emptying. Also, the effect of laparotomy on peristalsis would not affect the results (Janbroers et al, 1969). Since the abdomen was already opened for the biliary cannulation, the ID route was also particularly convenient. Compounds were administered as suspensions in compound tragacanth powder to allow the measurement and delivery of an accurate dose. Care was taken that no leakage of the suspensions into the peritoneum occurred.

Twelve compounds were also administered by the intravenous route (IV). In order to inject compounds intravenously as solutions, the compounds were dissolved in various percentages of aqueous dimethylsulphoxide (DMSO). Compound 15, a sulphonic acid, was soluble in 10% DMSO and was more water soluble than the rest of the compounds. The two triiodinated esters required 85% DMSO and the hexaiodinated ester required 95% DMSO. The carboxylic

acid derivatives were administered as solutions in 70% DMSO. When these solutions were administered rapidly, the rats convulsed and died. However, a gradual injection over 6-15 minutes prevented all fatalities except in the case of 95% DMSO. A slow steady injection was accompanied only by a marked tremor.

The dose level of 20mg/Kg was determined by balancing the solubilities of the compounds with the maximum dose of DMSO that the rats could tolerate. The injection of approximately 2ml of solutions of 3mg/ml of the compounds was considered optimal. Dose volumes larger than 2ml would not be tolerated and concentrations greater than 3mg/ml could not be obtained using aqueous DMSO. The large volumes of DMSO administered could have had an effect on the distribution and elimination of the compounds administered intravenously. The aim of this experiment, however, was to illustrate that compounds administered intravenously would be excreted in the bile even though they were not excreted after ID administration. Indeed, all compounds administered were excreted including compounds 5, 7, 8, 15 and 20 even though they showed no excretion after ID administration. These 5 extra compounds were chosen to be administered by the IV route to ascertain whether or not the various structural types not excreted after ID administration would be excreted after IV administration. Thus, two triiodinated esters, a hexaiodinated ester, an acetamido derivative and a taurine derivative were administered IV.

It seems that absorption from the gut is the greatest obstacle in the design of new oral cholegraphic agents and that once good absorption is obtained, biliary excretion is most likely to occur with a wide range of structural types.

In the present study, iopanoic acid was administered intraduodenally to rats in the same manner as the experimental compounds. Sodium iopodate was given intraduodenally as an aqueous solution. They gave peak iodine concentrations in the bile of 5.0 and 6.5mg/ml respectively. None of the compounds synthesized in the present study approached these values.

The concentration of sodium iopodate peaked at 10.6mg/ml of bile in the present study. This is equivalent to about 1% of sodium iopodate in the bile. This compares favourably with the result of 0.8% reported by Harwart et al (1959). Harwart et al report a total excretion in 4 hours of 81-2% of the administered dose in contrast with 42-4% found in the present study. This discrepancy could possibly be due to the fact that they used young female rats of an unreported strain as opposed to the mature Wistar derived males used in the present study. It has been reported, for example, that female rats excrete twice the amount of indocyanine green in the bile in 1.5 hours than do male rats (Hart et al, 1969). It is quite possible that the differences in the animals account for the difference in the total biliary excretion of sodium iopodate in the two studies.

A) Intraduodenal Administration

All 26 compounds were administered ID. Only seven were excreted in the bile. These were compounds 4, 6, 9-12 and 14.

None of the compounds containing six atoms of iodine (compounds 18-21, 23, 25 and 26) were excreted, including compound 26 which possessed a free carboxyl group. It was thought that a hexaiodinated molecule that was capable of being absorbed from

the gastrointestinal tract might produce better visualization by virtue of the greater number of iodine atoms per molecule compared with the triiodinated oral cholegraphic agents presently in use. It was also thought that a hexaiodinated phenylpropionic acid derivative might be better absorbed than the hexaiodinated benzoates such as iodipamide. Compound 26 was the only such hexaiodinated carboxylic acid synthesized. Compounds 18-21, 23 and 25 were all given as ethyl esters because the pure acids could not be obtained. The lack of excretion of the 7 hexaiodinated compounds probably resulted from a failure to be absorbed from the gut. Compound 20 was found to be excreted in the bile at a level of 22% of the administered dose in 6 hours following intravenous administration. This supports the hypothesis that the absence of excretion of these compounds following intraduodenal administration was due to a lack of gastrointestinal absorption. Iodipamide is very slowly absorbed from the gut (Langecker et al, 1953). A series of 40 hexaiodinated compounds containing two benzoic acid moieties were all reported to be poorly absorbed after oral administration (Felder et al, 1973a). The introduction of a phenylpropionic acid moiety in compound 26, represented no improvement over the hexaiodinated benzoates.

Compound 22 contained 9 iodine atoms per molecule. There was no apparent excretion of this compound. It was found to be extremely insoluble, even in dimethylformamide. This extremely low solubility probably excluded it from being successfully absorbed from the gastrointestinal tract.

Compound 24, a triiodinatedphenylpropanol, did not show any apparent signs of biliary excretion after ID administration.

The 3 taurine derivatives (compounds 15, 16 and 17) did not exhibit any apparent biliary excretion after ID administration. It was thought that a triiodinated aromatic ring coupled to taurine might be able to exploit the absorption and excretion mechanisms of taurocholic acid. This did not appear to be the case. Neither the compounds with the 3-amino nor the 3-dimethylaminomethyleneamino side chains were excreted. References to iodinated sulphonic acids in the patent literature indicate they are useful as x-ray diagnostic agents for pyelography. Routes of administration and biliary excretion levels were not reported (Goldberg and Besley, 1946; DeLaMater, 1960).

Compound 23 is a 2,3,5-triiodinated compound as opposed to the 2,4,6-triiodinated compounds generally used. As reported earlier, this compound showed no apparent biliary excretion after ID administration. It was decided to synthesize further compounds having only the 2,4,6 symmetrical configuration as other configurations have been reported to be prone to metabolic deiodination (Gutenmann et al, 1967).

It was thought that if the alkanoic acid moiety commonly found in oral cholegraphic agents was replaced by a benzoic acid moiety, the compounds produced might be absorbed from the gastrointestinal tract and be excreted in the bile. There have been many reports in the literature of compounds of the general 2,4,6-triiodophenylalkanoic acid type. It has been found that such compounds with additional phenyl or benzyl substituents on the alkanoic acid side chain exhibit good bilitropism (Papa et al, 1953; Fumagalli et al, 1975; Suter and Zutter, 1971; Cassebaum et al, 1968; Hebky and Polacek, 1970). Also, α-phenoxy and

α-phenyl-β-(diiodophenyl)propionic acid derivatives have been found to successfully visualize the gall bladder (Papa et al, 1950; Diedrich, 1962).

Felder et al (1969) found a high degree of bilitropism with many compounds in a series of 2,4,6-triiodobenzamidophenyl- and 2,4,6-triiodobenzamidophenoxyalkanoic acids although poor intestinal absorption was reported.

Therefore, it seems the aromatic substitution of iodinated phenylalkanoic acids can favour biliary excretion. It has also been found that 2-(4-hydroxy-3,5-diiodobenzyl)benzoic acid produced visualization of the gall bladder and biliary ducts of the dog after intravenous administration (Jones et al, 1948). Tetraiodobenzamidobenzoic acids have also been reported as being potentially valuable in cholecystography after oral administration (Obendorf and Meindl, 1962; Oesterreichische Stickstoffwerke A.G., 1963).

Therefore, it was decided to synthesize a series of compounds in which the alkanoic acid side chain often used in previous investigations would be substituted by benzoic acid or substituted benzoic acids. These were compounds 1-14. All seven of the compounds found to be excreted after intraduodenal administration were from this series (compounds 4, 6, 9-12 and 14).

Of the 14 compounds synthesized in this series, 3 carboxylic acid derivatives (compounds 2, 6 and 12) had a free amino group in the 3 position of the iodinated aromatic ring. Compounds 6 and 12 which contained a hydroxyl group ortho to the carboxylic acid group were excreted at levels of 12 and 16% of the administered dose in 6 hours. Compound 2, on the other

hand, which did not possess an ortho hydroxyl group, was not excreted. Papa et al (1953) synthesized a series of 2-ethyl-3-(2,4,6-triiodophenyl)propionic acids. After oral administration to dogs, they found that the compound with a hydroxyl group in the 3 position of the aromatic ring was outstanding. The compounds that had an iodine in the 3 position of the aromatic ring or had that position vacant were described as disappointing. They concluded that amino or hydroxyl groups in the 3 position of these compounds markedly altered their absorption and/or excretion and thus had a direct influence on the efficacy of these compounds as cholegraphic agents. Shtacher and Dayagi (1972) prepared a series of 2-amino-2-methyl-4-(2,4,6-triiodophenyl)butyric acid derivatives and gave them orally to dogs and cats. No visualization of the gall bladder was noted with the derivatives that had an amino, acetamido or hydroxy group in the 3 position of the aromatic ring. So, these groups in themselves cannot be considered crucial for all compounds. Indeed, Redel et al (1954) prepared a series of 2,4,6-triiodophenoxyalkanoic acids including phenobutiodil in which the 3 position on the aromatic ring was vacant. Phenobutiodil showed good cholecystographic activity in man after oral administration. It would appear that substitution in the 3 position exerts a variable effect and depends on the overall structure of the molecule.

Of the 4 carboxylic acid derivatives with an acetamido group in the 3 position of the iodinated aromatic ring (compounds 3, 7, 10 and 13), only compound 10 exhibited excretion and this was very marginal. Compounds 3, 7 and 13 showed no apparent biliary excretion after ID administration. In testing

2,4,6-triiodophenylglutaric and phenyliminodiacetic acid derivatives, Shtacher (1968) observed that derivatives containing an acetamido group in position 3 of the iodinated aromatic ring gave no visualization of the gall bladder after oral administration to cats and only moderate visualization following intravenous administration. However, some triiodinated phenylalkanoic acids with acetamido groups in the 3 position of the aromatic ring can be absorbed from the gut. Sodium tyropanoate is the sodium salt of the butyryl derivative of iopanoic acid. In a study on this oral cholecystographic agent, Hoppe et al (1970) reported on the efficacy of other N-acyl derivatives which contained 1-6 carbon atoms in the acyl group. All except the formyl derivative showed good to excellent gall bladder visualization and with the exception of the formyl group, toxicity increased with increasing chain length. The 3-acetamido derivative did not give such efficient gall bladder visualization. This was attributed to loss through urinary excretion rather than a lack of intestinal absorption. The success of the butryl derivative confirmed earlier conclusions (Epstein et al, 1946; Hoppe and Archer, 1953) that the addition of methylene groups to a potential cholecystographic agent may increase its biliary excretion.

The concept that alkylation of side chain increases the bilitropism of a molecule is borne out by the observation that N-alkylation of the acylamino group in the 3 position of the aromatic ring increased the biliary-urinary excretion ratio of various 2,4,6-triiodophenyl- and 2,4,6-triiodophenoxyalkanoic acids (Fumagalli et al, 1975; Felder et al, 1969). Indeed a new oral

agent with an N-ethyl-N'-acetyl side chain has been investigated and recommended for clinical trial (Rosati et al, 1972). In the present study, it was also found that alkylation improved absorption and excretion. The 4 carboxylic acids with a dimethylaminomethyleneamino (DMAMA) side chain in the 3 position of the triiodinated moiety were all excreted. Compound 4 exhibited a total excretion of 17% of the administered dose in 6 hours, compound 9 - 13% and compounds 11 and 14 - 8-9%. This side chain is found in the oral cholegraphic agent, sodium iopodate (Harwart et al, 1959). Hebky and Jelinek (1965) claim this side chain improves gastrointestinal absorption and biliary excretion.

In the present study, the acetamido group in the 3 position of the iodinated aromatic ring did not appear to aid the absorption of the media from the gut. The amino group gave variable results and seemed to depend on the presence of the hydroxyl group ortho to the carboxylic acid. The DMAMA side chain, on the other hand, consistently afforded the absorption and excretion of this group of compounds.

Compounds 1, 5 and 8 were all methyl esters. None of these compounds exhibited any apparent biliary excretion after ID administration. McChesney and Hoppe (1954) suggested that a proper balance between lipophilic and hydrophilic groups is needed to ensure the absorption of these compounds from the gut. Perhaps it was the masking of the hydrophilic carboxyl group that prevented absorption and excretion of these compounds by destroying the required balance. Not even the presence of the successful DMAMA side chain in the 3 position of compound 8 aided the absorption and excretion of that ester.

As mentioned above, compounds 6 and 12 which contained a 3-amino group and a hydroxyl group ortho to the carboxylic acid group were excreted in the bile after ID administration while compound 2 which contained a 3-amino group but no ortho hydroxyl group was not excreted. Apart from the apparent effect of the ortho hydroxyl in this instance, the presence of this group (compounds 5, 7-9, 13 and 14) or its absence (compounds 1, 3, 4, 10 and 11) appeared to have no marked effect.

Compounds in which the benzyl moiety was para to the carboxylic acid group (compounds 1-9) did not show any consistent difference from those in which the benzyl substitution was in the meta position (compounds 10-14). Compounds of both types exhibited good absorption and excretion and no clear cut advantage could be observed for either pattern of substitution.

B) Intravenous Studies

All 12 compounds administered intravenously (IV) were found to be excreted in the bile (compounds 4-12, 14, 15 and 20). The peak biliary concentrations obtained with a dose level of 20mg/Kg IV were generally high compared to those following ID administration at 100mg/Kg. The total excretion in 6 hours was 58-90% of the administered dose for all the compounds administered IV with the sole exception of compound 20, a hexaiodinated benzoic acid ester. This was excreted at low levels totalling 22% of the administered dose in 6 hours.

Compounds 4, 8, 9, 11 and 14 all contained the DMAMA side chain and exhibited the best total excretion in 6 hours of the 12 compounds tested. Other investigators have also found

that this group leads to good biliary excretion (Hebky and Jelinek, 1965; Felder et al, 1969). This group of compounds includes compound 8, a methyl ester. This reinforces the concept that the hydrophilic carboxylic acid group is more important in promoting absorption from the gut than in promoting biliary excretion. This group of 5 compounds also includes both meta and para substituted benzylbenzoates as well as compounds with and without a hydroxyl group ortho to the carboxylate group. This indicates that these variations in the general structure do not exert a marked or consistent effect upon the biliary excretion of these compounds.

The compounds containing 3-amino (compounds 5, 6 and 12) and 3-acetamido (compounds 7 and 10) groups were roughly equivalent in their levels of excretion. This suggested that the acetamido group presents more a barrier to intestinal absorption than to biliary excretion. That the acetamido compounds (7 and 10) were perhaps more rapidly excreted in the urine than those with the DMAMA side chain in the 3 position (compounds 4, 8, 9, 11 and 14), may have accounted for their lower excretion in the bile.

The taurine compound tested, compound 15, was excreted to the extent of 58% of the administered dose in 6 hours. Considering the relatively high water solubility of this compound as shown by its ready solution in 10% DMSO in water and the rapidity with which its concentration in the bile dropped, it would be reasonable to assume that most of the remainder of the compound was excreted in the urine. This is supported by the fact that 2,4,6-triiodinated sulphonic acids reported in the literature were alleged to be useful as potential pyelographic

media (Goldberg and Besly, 1946; DeLaMater, 1960). It would appear that compound 10 was also excreted to a relatively high extent in the urine. Indeed, in a study on 2,4,6-triiodoacylaminobenzoic acids, Wallingford et al (1952) observed that after IV administration, the compounds were excreted in progressively greater amounts in the bile as the acylamino side chain was lengthened. In this series, the acetamido group favoured urinary excretion.

The high initial levels of compounds 4 and 11 in the bile and their rapid overall excretion in the bile indicated a particular affinity for biliary excretion.

Overall, compounds with the DMAMA side chain in the 3 position of the iodinated aromatic ring proved most successful. They were relatively well absorbed from the gut and very well excreted in the bile. The 3-amino group appeared to have a variable effect while the 3-acetamido derivatives were poorly absorbed from the gut and appeared to be only moderately well excreted in the bile, possibly due to enhanced urinary excretion. A free carboxylic acid group was essential for intestinal absorption and the free acids were also best excreted in the bile following IV administration. The methyl esters, however, did exhibit reasonably high biliary excretion after IV administration. The most successful compound was compound 4, 4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid.

The three main factors proposed by Smith (1973) as being important in biliary excretion have been discussed earlier (p. 29). The compounds that were excreted following intravenous administration in the present study all had molecular weights over 600

and therefore fitted the molecular weight requirement cited by Smith.

The presence of a polar group was also cited as being important for biliary excretion. Of the 12 compounds tested, only compound 20, the hexaiodinated carboxylic acid ester, did not have a polar group. That compound 20 exhibited the lowest biliary excretion, supports Smith's hypothesis. Compounds 6, 7, 9, 12 and 14 all had a free carboxylic acid group and a free phenolic group. Compounds 4, 10 and 11 all had a free carboxylic acid group and compound 15 had a free sulphonic acid group. Even the two methyl esters tested, compounds 5 and 8, had a phenolic group ortho to the esterified carboxylic acid moiety. Smith also proposed that a lipophilic-hydrophilic balance is required for successful biliary excretion. The two compounds that exhibited the best biliary excretion (compounds 4 and 11) both possessed a free carboxylic acid group and the lipophilic DMAMA side chain. Compound 20 which lacked a strong hydrophilic moiety was only excreted at a level of 22% of the administered dose in 6 hours. The two compounds that exhibited the lowest biliary excretion, apart from compound 20, were compounds 10 and 15. Compound 10 contained a carboxylic acid moiety and a short relatively hydrophilic acetamido group while compound 15 was a sulphonic acid. Therefore, it appears that a balancing of hydrophilic and lipophilic groups in a molecule produces a compound that is better excreted in the bile compared to compounds which are either strongly hydrophilic or lipophilic.

Finally, Smith recognized that within a group of compounds, certain structural features exert an influence on

biliary excretion that is not related to an effect on molecular weight or polarity. The most prominent such feature of cholegraphic media concerns substitution in the 5 position of the iodinated aromatic ring. As with all cholegraphic media, all the compounds synthesized in the present study were not substituted in the 5 position of the triiodinated aromatic ring. This has been cited frequently as a prerequisite for successful cholecystographic agents. Substitution in this position decreases the ability of compounds to bind to plasma albumin and favours urinary excretion for both oral and IV radiopaque substances (Felder et al, 1973a; Hoey et al, 1966; Gupta et al, 1970; Lasser, 1966; Lasser, 1971). Unsubstituted compounds are found to be more toxic than those compounds substituted in the 5 position.

(iv) Biotransformation

The most frequently cited metabolic transformation exhibited by oral cholegraphic agents is glucuronide conjugation. It is often the only transformation of any quantitative significance.

Compounds 4, 6, 9-12 and 14 exhibited biliary excretion after administration to rats ID and IV. Compounds 5, 7, 8, 15 and 20 exhibited biliary excretion only after administration to rats IV. In each case, the hourly bile sample that showed the highest iodine concentration was submitted to hydrolysis by beta glucuronidase. The hydrolyzed samples were compared with the unhydrolyzed samples using thin layer chromatography.

This experiment was intended to be a qualitative screen of biliary metabolites. The procedure was designed to detect glucuronides and by the absence of any chromatographic behavioural

alteration, indicate the presence of non-glucuronide metabolites.

For compounds administered both ID and IV, the pattern of the results was the same regardless of the route. Compounds 4, 7, 8, 14 and 15 appeared to be excreted unchanged. The excretion of compounds 9 and 10 appeared to be accompanied by one transformation product each and compound 11 by two metabolites. However, none of the transformation products of these 3 compounds showed any susceptibility to hydrolysis by beta glucuronidase.

Compound 5 and 6, on the other hand, appeared to be excreted along with a metabolite which disappeared after the bile sample was hydrolyzed with beta glucuronidase. Compound 12 was excreted along with two apparent metabolites, one of which disappeared after hydrolysis with beta glucuronidase. The non-hydrolyzable metabolite decreased in intensity relative to the parent compound after hydrolysis, probably as a result of the transformation of the apparent glucuronide into the parent compound during the hydrolysis making the spot corresponding to the parent compound relatively more intense. Compounds 5, 6 and 12, therefore, all appeared to undergo conjugation with glucuronic acid. An ester glucuronide from the free carboxylic acid groups of compounds 6 and 12 is a possibility. The TLC of compound 6 is similar to that of compound 5, the corresponding methyl ester. Therefore, it is possible that compound 5 was transformed *in vivo* to the free carboxylic acid and that the glucuronide formed after the administration of compound 5 may have been an ester glucuronide as well. However, the other free carboxylic acids (compounds 4, 7, 9-11 and 14) did not exhibit glucuronide formation. Compound 8, the other ester tested, also did not exhibit glucuronide

formation. This suggested that the glucuronides of compounds 5, 6 and 12 did not form from the carboxylic acid or the phenol. A structural feature that was shared by compounds 5, 6 and 12 that was not shared by compounds 4, 7-11 and 14 was the presence of a free amino group. This suggested that the hydrolyzable metabolites of compounds 5, 6 and 12 were amine glucuronides. Amine glucuronide formation has not been reported for these types of compounds although iophenoxic acid is known to form an ether glucuronide in this position (Wade et al, 1971). It should be noted, however, that the sulphonic acid derivative, compound 15, also had a free amino group but did not exhibit any apparent glucuronide formation.

Compound 20 could not be detected on TLC, probably because of its low concentration in the bile.

Iomeglamic acid, a 3-amino-2,4,6-triiodophenylamino-alkanoic acid was reported to be metabolized to the corresponding N-acetyl, methyl ester and ester glucuronide derivatives (Pfiefer et al, 1972b). Sodium iopodate is reported as being transformed into the corresponding 3-amino compound. Deiodinated metabolites have been reported for iomeglamic acid. These and other metabolic transformations such as the hydroxylation of the unsubstituted phenyl ring appear possible for the compounds used in the present study.

The degree of apparent metabolism had no consistent effect on the absorption and excretion of the compounds in the present study. Compounds showing no apparent metabolism, showing signs of metabolism other than glucuronide formation or exhibiting apparent glucuronide formation, all were absorbed and excreted in

the bile to varying extents after ID administration. This is not to say that metabolism played no role in determining the extent of excretion of these compounds but the role did not appear to be a major one.

The result for iopanoic acid indicates that it was excreted largely as a glucuronide. This finding corresponded with numerous reports in the literature (e.g. McChesney, 1964). It was also noted that the hydrolysis did not go to completion. This problem was also experienced by Wade et al (1971) with the beta glucuronidase hydrolysis of the glucuronides of iophenoxic acid. However, the hydrolysis of the three experimental compounds that exhibited glucuronide formation did apparently go to completion as shown by the disappearance of the glucuronide spot on TLC following hydrolysis with beta glucuronidase.

(v) The Isolation of Compound 6 from Rat Bile

Bile from rats to which compound 6 had been administered ID was hydrolyzed with beta glucuronidase. Upon isolation and purification a substance was obtained which cochromatographed with compound 6 and had an identical infrared spectrum and melting point with compound 6. After beta glucuronidase hydrolysis, unchanged compound 6 appeared to be the sole metabolite present in the bile and for this compound, glucuronide formation appeared to be the only major biotransformation that had taken place.

PART II: METABOLISM AND EXCRETION OF METHYLGLUCAMINE IN THE RAT

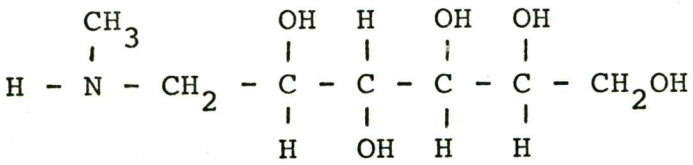
INTRODUCTION

Many of the iodinated organic acids used as radiocontrast agents are administered as their sodium salts. The introduction of salts of methylglucamine (1-deoxy-1-(methylamino)-D-glucitol) led to preparations that were less toxic to the cardiovascular system than were the preparations containing the sodium salts (Fischer and Cornell, 1965). The methylglucamine salts are used primarily in intravascular radiology; however, Gastrografin, a mixture of the sodium and methylglucamine salts of diatrizoate, is used in gastroenterology in place of barium sulphate. When barium sulphate is administered to patients with a gastrointestinal perforation, the barium sulphate can leak into the peritoneum and lead to the formation of granulomas (Cockran et al, 1963). Water soluble iodinated organic contrast media may be used in cases of impending or possible perforation without danger (Rubin et al, 1960). While the use of Gastrografin is apparently safe in the case of perforation, its use has been implicated recently in hypertonic dehydration in the neonate (Poole and Rowe, 1976) and in hemorrhage following its use in the treatment of postoperative stomal dysfunction (Gallitano et al, 1976).

Therefore, the use of Gastrografin is not without hazard and the fate of methylglucamine following oral administration is of interest. Because Gastrografin is used in patients with possible gastrointestinal perforations, it also means that methylglucamine may find its way into the peritoneum. Therefore, the metabolism and excretion of methylglucamine-^{14}C following oral

and intraperitoneal administration was studied.

The chemical structure of 1-deoxy-1-(methylamino)-D-glucitol is as follows.



EXPERIMENTAL METHODS

(i) Scintillation Counting

A Packard Tri-Carb Model 3003 Scintillation Spectrometer, fitted with an automatic external standard (Packard Automatic Standardization Model 3950) was used for the measurement of radioactivity. Counting efficiency was determined from standard curves which had been previously prepared.

PPO, dimethyl POPOP, methanolic hyamine hydroxide solution and Cab-O-Sil thixotropic gel were purchased from the Packard Instrument Co. Inc., La Grange, Illinois, U.S.A.

The scintillation solvent consisted of naphthalene (6%), PPO (0.4%), dimethyl POPOP (0.02%), methanol (10%) and ethylene glycol (2%) dissolved in purified dioxan (Bray's Solution) (Bray, 1960).

(ii) Chromatography

Thin layer chromatography (TLC) was carried out on Silica Gel GF_{254} according to Stahl (0.25mm layers) E. Merck AG, Darmstadt, Germany).

The solvent used had the following composition by volume:- ethylacetate : acetic acid : methanol : water, 15 : 3 : 3 : 2.

(iii) Methylglucamine

1-Deoxy-1-(methylamino)-(D-glucitol-^{14}C(U))(methyl-glucamine-^{14}C) was obtained from the Radiochemical Centre,

Amersham, England. Specific activity was 8.3μci/mg and it was at least 98% radiochemically pure by paper and thin layer chromatography (manufacturer's specifications).

(iv) Animal Studies

150μg of methylglucamine-^{14}C in water was administered to rats (male, Wistar derived, 250-330g) either intraperitoneally or orally. The animals were individually kept in sealed all-glass metabolism cages (Metabowls, Jencons Laboratory Furnishings, Hemel, Hampstead, England) through which a stream of air was passed. The effluent gases were passed through 3 traps containing 20% aqueous sodium hydroxide (80ml). Urine and faeces were collected for 24 hours. Food and water was available ad libitum. One group of rats was given neomycin sulphate orally (500mg/rat) 24 hours prior to oral dosing and was then given water containing 1% neomycin sulphate throughout the experiment. The collection of urine and faeces extended to 72 hours for these animals so as to collect a high percentage of the administered radioactivity.

Urine was diluted to 250ml with water and 0.5ml aliquots counted in Bray's solution (10ml). Faeces were dried under vacuum over phosphorus pentoxide and 20mg aliquots were shaken with methanolic hyamine hydroxide solution (0.4ml,1M) at 60^{o} for 12 hours. The coloured solution was bleached with a few drops of 30% hydrogen peroxide solution and mixed with Bray's solution (15ml) for counting.

The alkaline traps were made up to 500ml with water. Aliquots (0.5ml) were mixed with Bray's solution (10ml) and Cab-O-Sil (300-400mg), shaken and counted after storage in the

dark for several hours to allow luminescence to subside.

Urine samples from the experimental animals were analyzed on TLC. The dried chromatograms were divided into 1cm segments. These were scraped into scintillation counting vials, Bray's solution (10ml) added and counted.

RESULTS

The excretion of methylglucamine-^{14}C is summarized in Table 1. Intraperitoneal dosing led to rapid and complete excretion. About 93% of the dose was found in the urine, with small amounts in expired carbon dioxide and faeces after 24 hours. Oral dosing showed a different pattern with about 75% of the dose excreted in 24 hours. Urine contained the least activity with about 15% of the dose. Expired carbon dioxide accounted for 20% and 40% was found in the faeces.

Rats pretreated with neomycin sulphate were maintained in the metabolism cages for 72 hours. A relatively small amount of faeces was collected from these animals after 24 hours and inter-animal variation was large. This was likely a result of gastro-intestinal upset following neomycin pretreatment. After 72 hours, however, the faecal collection was larger and more uniform and a total of 69% of the dose was found in the faeces. The urine contained 19% and expired carbon dioxide accounted for only 3% of the dose.

The TLC of urine from experimental animals was identical after intraperitoneal dosing, oral dosing and oral dosing preceded by neomycin treatment. The TLC of methylglumaine-^{14}C is illustrated in Figure 1. The TLC of urine from the experimental animals given methylglucamine-^{14}C is illustrated in Figure 2. The chromatogram of methylglucamine-^{14}C corresponded well with the chromatograms of the urine suggesting that unchanged methylglucamine-^{14}C is a metabolite. However, metabolites cochromatographing with methylglucamine-^{14}C have not been excluded.

TABLE 1. Excretion of Methylglucamine-^{14}C in rats.

	Rat	% Administered radioactivity excreted			
		In urine	In faeces	As CO_2	Total
1) IP Dosing	1	93.2	4.8	1.0	99.0
	2	93.9	1.6	1.7	97.1
	3	91.3	4.5	2.4	98.1
	4	96.8	1.3	2.0	100.1
	5	88.6	5.0	1.8	95.4
	Average	92.8 ± 3.1*	3.4 ± 1.8	1.8 ± 0.5	97.9 ± 1.8
2) Oral Dosing	1	24.6	42.9	5.6	73.1
	2	13.6	41.1	24.8	79.5
	3	19.7	35.7	20.4	75.8
	4	9.1	38.1	20.6	67.8
	5	15.6	39.7	29.1	84.4
	Average	16.5 ± 5.9	39.5 ± 2.8	20.1 ± 8.9	76.1 ± 6.3
3) Oral Dosing	1	21.5	66.8	3.2	91.6
	2	18.1	68.4	3.5	90.0
	3	15.1	67.1	3.8	86.0
	4	17.6	77.6	3.8	99.0
	5	22.9	63.6	2.6	89.1
	Average	19.1 ± 2.8	68.7 ± 4.7	3.4 ± 0.4	91.1 ± 4.3

1 & 2 - Collected over 24 hours

3 - Collected over 72 hours
- Pretreated with neomycin sulphate

* - $\pm$ Standard Deviation

FIGURE 1. TLC of Methylglucamine-^{14}C

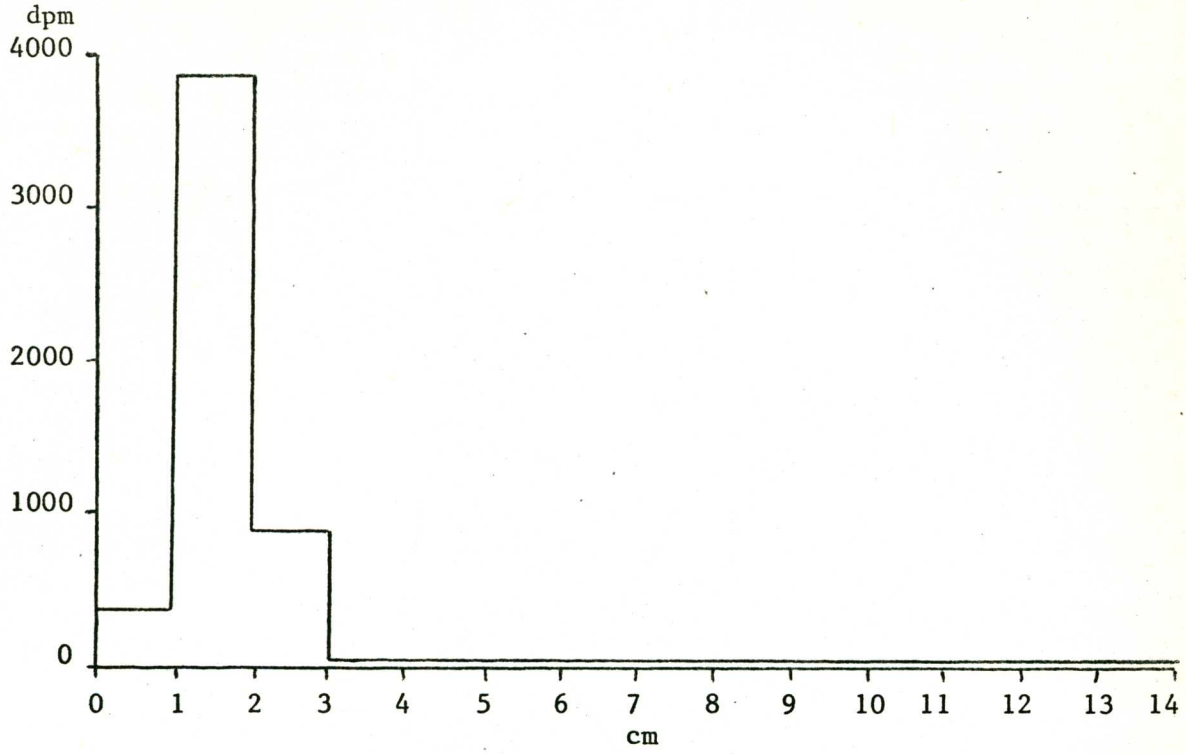
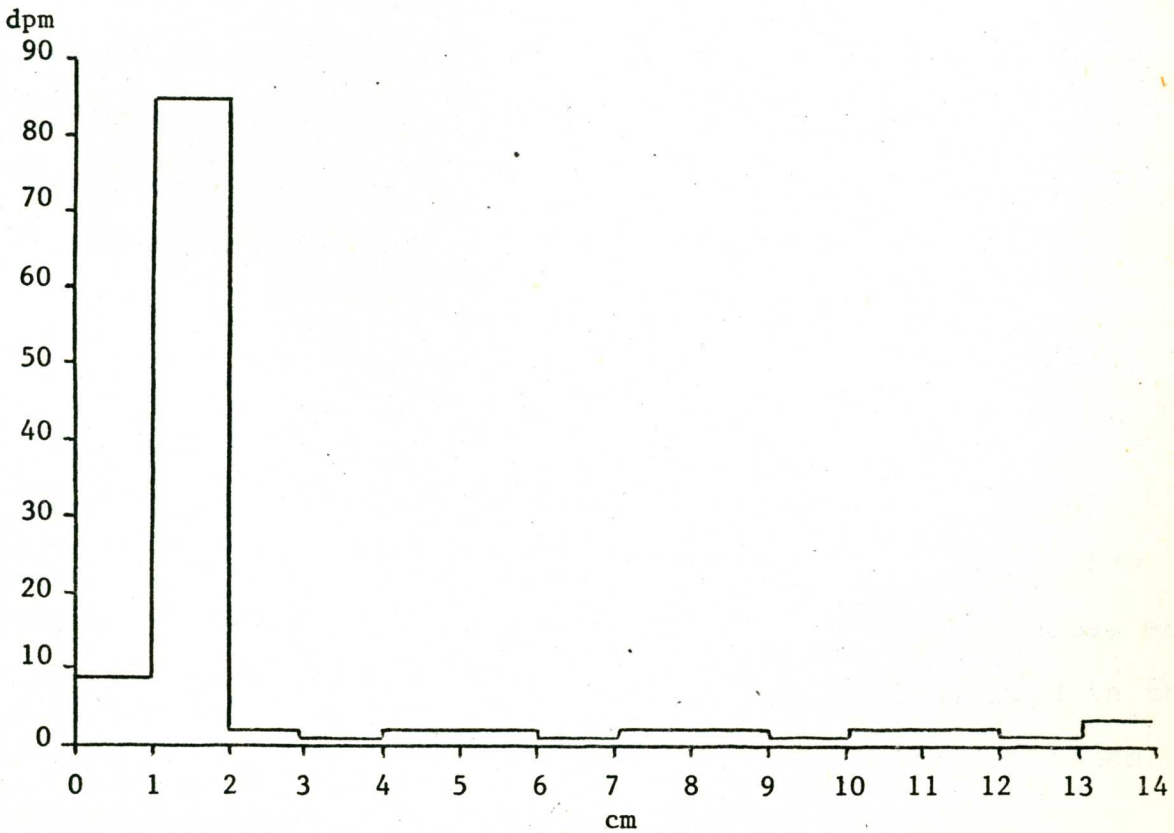


FIGURE 2. TLC of Urine from Rats fed Methylglucamine-^{14}C



DISCUSSION

The route of administration appeared to have a marked effect on the pattern of excretion and metabolism of methyl-glucamine-^{14}C in the rat. Given intraperitoneally, virtually the entire dose was excreted in 24 hours. The small amount excreted as carbon dioxide is evidence that there is some metabolism taking place and the low faecal excretion is indicative of little or no biliary excretion.

Oral administration, on the other hand, led to 76% of the dose being excreted in 24 hours. Half of the excreted radioactivity was eliminated in the faeces and a quarter each in the urine and expired air. The high faecal excretion after oral administration in conjunction with the low faecal excretion levels after intraperitoneal administration show that methylglucamine is very poorly absorbed from the gastrointestinal tract. The relatively high level of excretion of radioactivity as carbon dioxide was indicative of oxidation of methylglucamine in the gastrointestinal tract, possibly by the gut microflora.

The results from rats pretreated with neomycin sulphate confirmed that the gut microflora are active in the metabolism of methylglucamine following oral administration. In the case of these animals, the gut microflora of which had been substantially depleted, 91% of the dose was excreted in 72 hours. Of the excreted radioactivity, most was eliminated in the faeces, about 19% in the urine and only a small amount was eliminated in the expired air. The slow overall excretion exhibited by these

animals was probably due to gastrointestinal upset because of the neomycin treatment and the resultant irregularity of defaecation. The small amount of radioactive carbon dioxide detected relative to the amount of radioactivity excreted in the urine and faeces, supports the idea that the gut microflora are involved in the oxidation of methylglucamine to carbon dioxide.

The detection of radioactive carbon dioxide after oral administration implicates the gut flora in the oxidation of methylglucamine or its metabolites either directly to carbon dioxide or to a product that the rat is capable of metabolizing completely to carbon dioxide. Because the N-methyl group was not labelled, the radioactive carbon dioxide must have resulted from oxidation of the glucitol residue and not resulted from N-demethylation.

Ryan et al (1972) studied the metabolism and excretion of another modified sugar, hydroxyethylated starch. It was concluded that after oral administration, this compound was metabolized largely by the gut flora. As in the present study, no mechanism of microbiological degradation of the sugar could be suggested.

Because methylglucamine is used as a cation for contrast agents in gastroenterology when gastrointestinal perforation is possible, the results from the oral and intraperitoneal administration of this compound are of interest. Most use of methylglucamine salts of radiocontrast agents is in intravascular radiography. It is likely that methylglucamine given by an intravascular route would be handled much the same as after intraperitoneal administration. It would be excreted rapidly and almost solely in the urine with little or no biliary excretion.

APPENDIX 1. Hourly and total excretion of iodine in the bile after intraduodenal administration of the test compounds (100mg/Kg)

Sample time (hr)		1		2		3		4		5		6		TOTAL	
COMPOUND	RUN	CONC*	WT+	CONC	WT	CONC	WT	CONC	WT	CONC	WT	CONC	WT	WT (mg)	% of dose excreted in 6 hours
4	1	0.65	0.86	1.24	1.46	1.27	1.49	1.16	1.29	1.07	1.25	1.00	1.13	7.48	17.9
	2	0.69	0.91	1.17	1.70	1.44	1.43	1.23	1.22	1.24	1.24	1.08	1.08	7.58	15.4
6	1	0.44	0.50	0.70	0.89	0.73	0.72	0.63	0.71	0.51	0.59	0.47	0.53	3.94	10.5
	2	0.59	0.61	0.85	1.02	0.84	0.84	0.73	0.79	0.67	0.67	0.62	0.66	4.59	12.4
9	1	0.39	0.51	0.94	1.19	1.13	1.34	1.18	1.23	1.24	1.27	1.20	1.22	6.76	13.3
	2	0.33	0.39	0.92	1.01	1.26	1.27	1.30	1.20	1.35	1.18	1.09	1.08	6.13	12.4
10	1	0.30	0.32	0.32	0.29	0.33	0.30	0.35	0.30	0.37	0.35	0.35	0.32	1.88	4.3
	2	0.26	0.21	0.38	0.27	0.35	0.29	0.43	0.30	0.28	0.20	0.33	0.25	1.52	4.8
11	1	0.41	0.49	0.60	0.66	0.62	0.56	0.62	0.55	0.56	0.50	0.48	0.41	3.17	8.3
	2	0.34	0.33	0.60	0.50	0.67	0.60	0.68	0.58	0.65	0.58	0.55	0.51	3.10	7.0
12	1	0.59	0.64	1.45	1.32	1.43	1.34	1.17	1.11	1.16	1.12	0.92	0.85	6.38	14.8
	2	0.59	0.61	1.36	1.07	1.23	0.92	0.97	0.80	1.04	0.87	0.79	0.71	4.98	16.7

APPENDIX 1. (continued)

Sample time (hr)		1		2		3		4		5		6		TOTAL WT (mg)	TOTAL % of dose excreted in 6 hours
COMPOUND	RUN	CONC*	WT+	CONC	WT	CONC	WT	CONC	WT	CONC	WT	CONC	WT		
14	1	0.28	0.40	0.48	0.61	0.58	0.72	0.74	0.97	0.66	0.81	0.53	0.69	4.20	9.0
	2	0.16	0.22	0.48	0.57	0.62	0.62	0.81	0.93	0.75	0.66	0.77	0.72	3.72	9.5
Iopanoic Acid	1	2.72	3.53	4.82	5.33	4.50	4.69	4.11	4.18	3.60	3.64	3.28	3.16	24.53	42.1
	2	2.79	2.72	5.21	4.85	5.04	4.26	4.23	4.47	3.33	3.46	2.69	2.90	22.66	43.5
Sodium Iopodate	1	4.18	4.85	6.20	5.98	5.64	5.87	4.60	5.00	3.89	4.52	3.25	3.76	29.98	58.2
	2	3.89	4.56	6.82	6.37	6.12	6.17	5.19	5.33	4.06	3.83	3.34	3.57	29.83	58.9

* CONC - mg/ml

+ WT -mg

APPENDIX 2. Hourly and total excretion of iodine in the bile after intravenous administration of the test compounds (20mg/Kg)

Sample time (hr)		1		2		3		4		5		6		TOTAL	
COMPOUND	RUN	CONC*	WT+	CONC	WT	CONC	WT	CONC	WT	CONC	WT	CONC	WT	WT (mg)	% of dose excreted in 6 hours
4	1	1.64	1.66	1.43	1.46	0.97	0.92	0.72	0.63	0.65	0.52	0.57	0.45	5.64	90.1
	2	1.55	2.15	1.95	2.14	1.30	1.37	1.02	1.03	0.69	0.70	0.57	0.58	7.97	90.6
5	1	1.54	1.45	1.71	1.72	0.98	0.84	0.86	0.65	0.67	0.48	0.59	0.42	5.56	63.2
	2	1.77	2.01	1.56	1.78	1.04	1.08	0.78	0.75	0.60	0.53	0.38	0.35	6.50	66.3
6	1	1.06	1.16	1.12	1.08	0.88	0.74	0.77	0.58	0.57	0.41	0.46	0.33	4.30	71.3
	2	1.03	1.51	1.11	1.19	1.20	1.39	1.04	1.04	0.79	0.70	0.73	0.62	6.45	72.3
7	1	1.30	1.29	0.96	0.93	0.71	0.64	0.48	0.42	0.46	0.33	0.33	0.25	3.86	62.6
	2	1.37	1.22	1.21	1.02	0.86	0.65	0.65	0.46	0.61	0.44	0.51	0.37	4.16	67.4
8	1	1.18	1.01	1.08	1.23	0.60	0.55	0.62	0.51	0.62	0.48	0.62	0.49	4.27	71.6
	2	1.53	1.09	1.26	1.17	0.52	0.49	0.55	0.46	0.57	0.43	0.52	0.36	4.00	74.9
9	1	0.87	1.07	1.30	1.52	1.16	1.28	1.03	1.14	0.82	0.92	0.69	0.77	6.70	73.7
	2	0.92	1.12	1.25	1.48	1.08	1.09	1.13	1.06	1.01	0.93	0.84	0.76	6.44	79.2

APPENDIX 2. (continued)

Sample time (hr)		1		2		3		4		5		6		TOTAL WT (mg)	TOTAL % of dose excreted in 6 hours
COMPOUND	RUN	CONC*	WT+	CONC	WT	CONC	WT	CONC	WT	CONC	WT	CONC	WT		
10	1	1.23	1.78	0.85	0.85	0.67	0.56	0.60	0.47	0.63	0.48	0.48	0.37	4.51	55.8
	2	1.48	2.34	0.76	0.83	0.70	0.76	0.51	0.54	0.50	0.52	0.39	0.39	5.38	59.6
11	1	1.85	1.64	1.54	1.37	0.96	0.72	0.68	0.48	0.55	0.39	0.44	0.31	4.91	82.7
	2	2.04	1.86	1.78	1.56	1.00	0.86	0.82	0.65	0.72	0.54	0.52	0.38	5.85	87.8
12	1	1.13	1.68	1.22	1.41	0.75	0.79	0.71	0.62	0.60	0.60	0.41	0.35	5.45	62.9
	2	1.23	1.28	1.29	1.21	0.99	0.85	0.72	0.58	0.52	0.40	0.52	0.38	4.70	68.2
14	1	1.35	1.04	1.41	1.11	1.11	0.79	0.67	0.46	0.55	0.36	0.40	0.24	4.00	76.6
	2	1.42	2.08	1.20	1.58	0.83	0.95	0.56	0.60	0.48	0.47	0.37	0.33	6.01	77.8
15	1	1.52	2.10	0.84	1.09	0.70	0.79	0.63	0.69	0.65	0.72	0.62	0.70	6.09	55.6
	2	1.67	2.29	1.03	1.31	0.68	0.79	0.66	0.75	0.63	0.74	0.50	0.58	6.46	61.1
20	1	0.53	0.63	0.50	0.60	0.40	0.40	0.40	0.33	0.53	0.37	0.45	0.31	2.64	20.7
	2	0.45	0.86	0.42	0.78	0.38	0.57	0.42	0.55	0.46	0.53	0.45	0.50	3.79	23.5

* CONC - mg/ml
+ WT - mg

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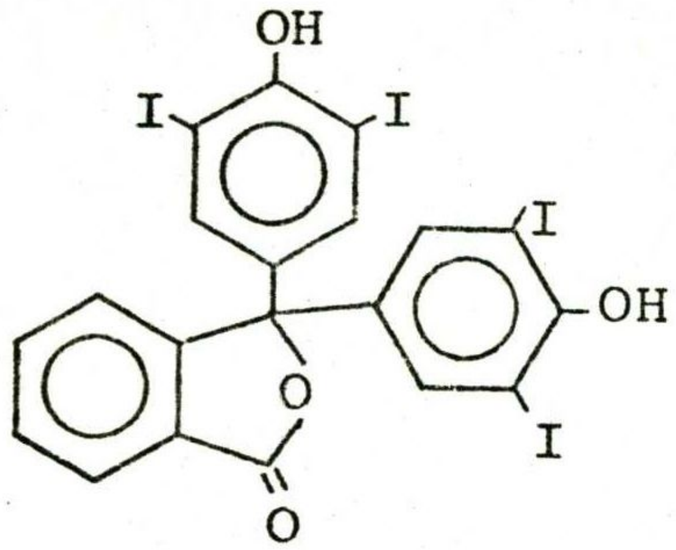
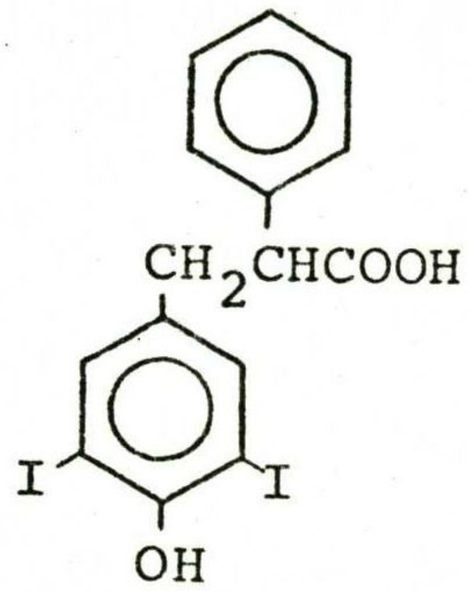
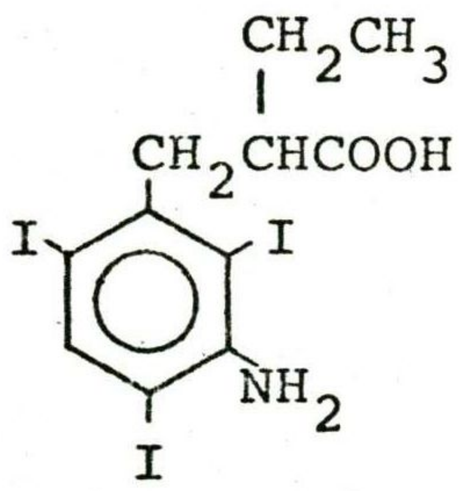
FIGURE 1. Oral Cholegraphic Media



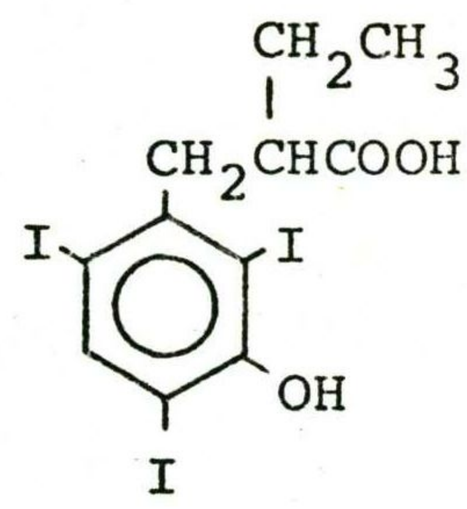
Tetraiodophenolphthalein



Iodoalphionic Acid



Iopanoic Acid



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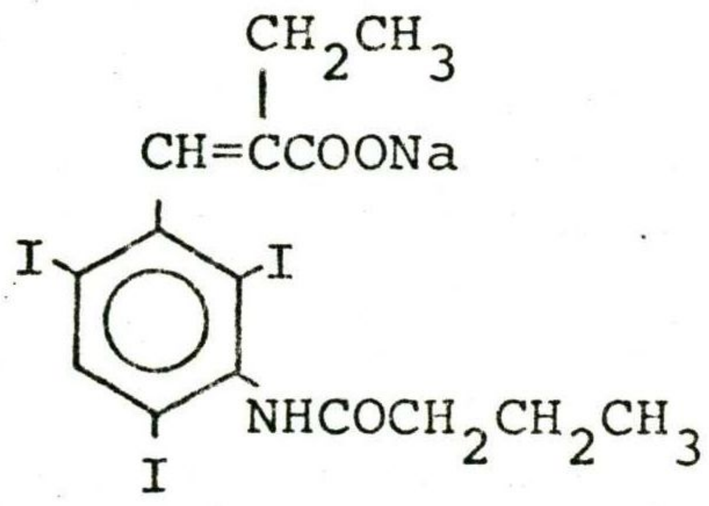
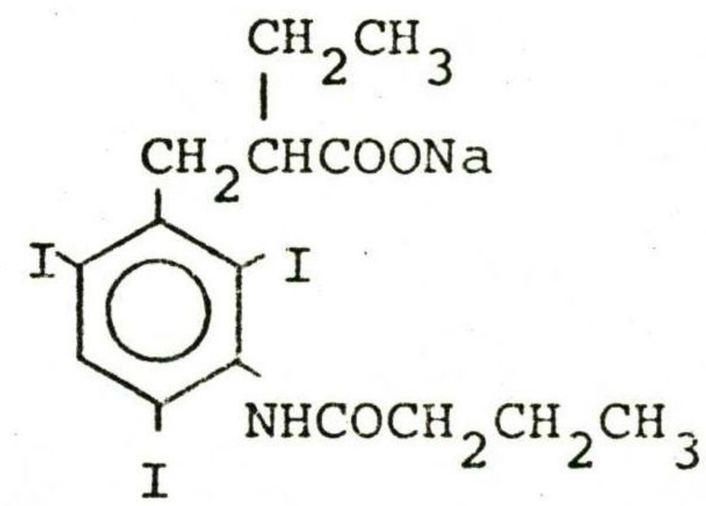
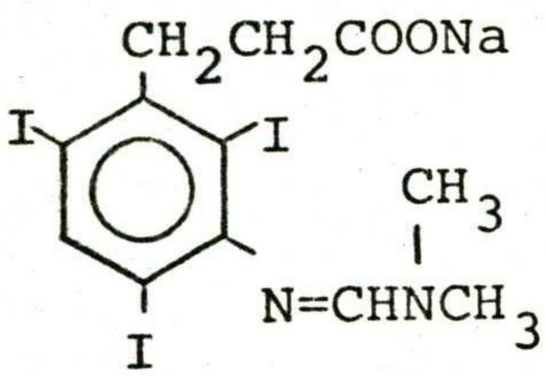


Bunamiodyl Sodium

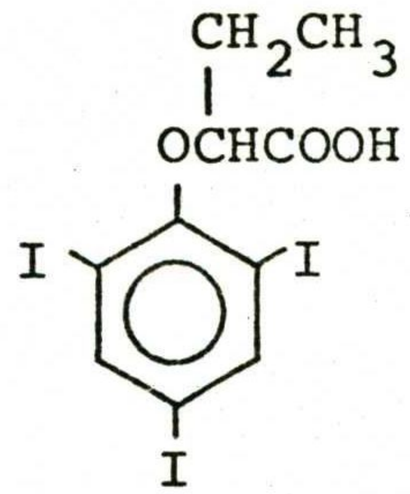


Sodium Tyropanoate

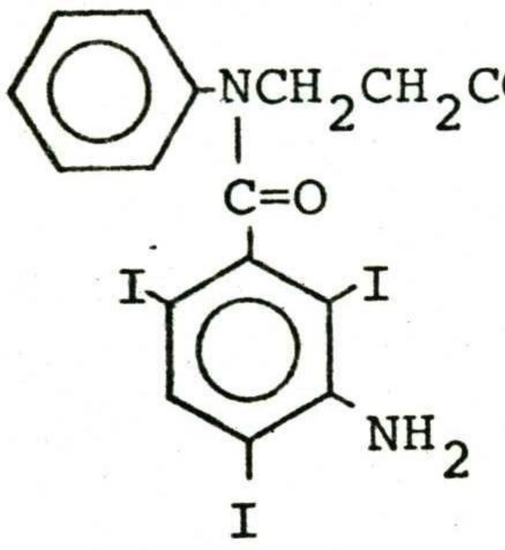
FIGURE 1. (continued)



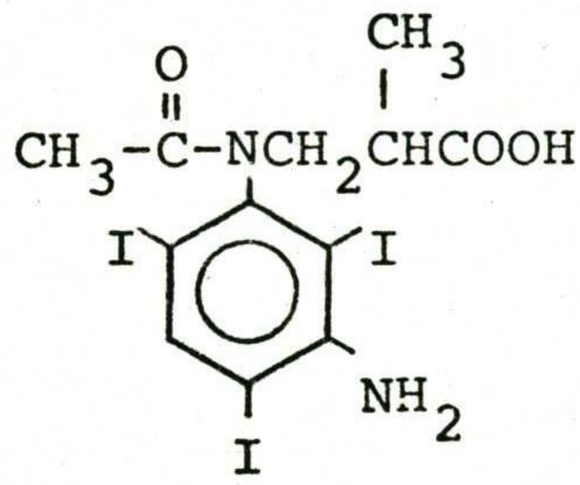
Sodium Iopodate



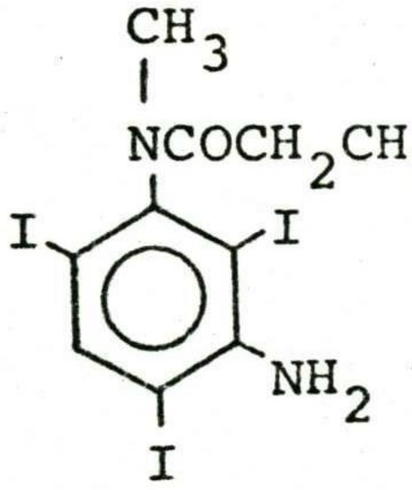
Phenobutiodil



Iobenzamic Acid

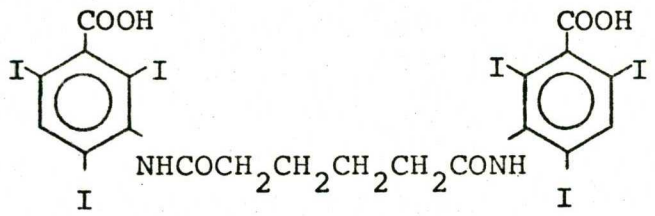


Iocetamic Acid

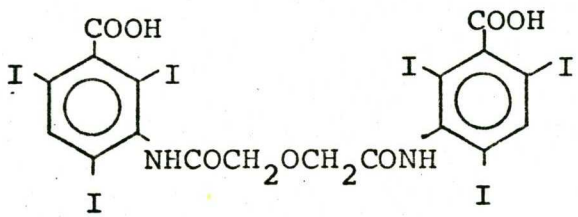


Iomeglamic Acid

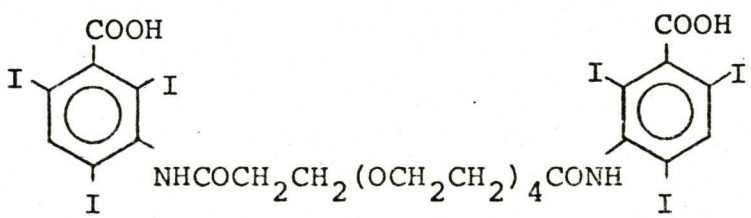
FIGURE 2. Intravenous Cholegraphic Media



Iodipamide



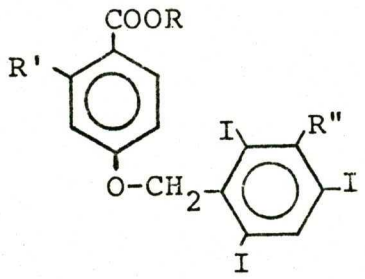
Ioglycamide



Iodoxamic Acid

TABLE 1. CHEMICAL STRUCTURES

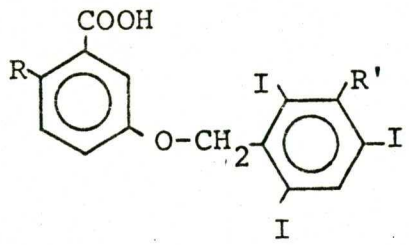
A) 4-TRIIODOBENZYLOXYBENZOIC ACID DERIVATIVES



	R	R'	R"
1) Methyl 4-(3-amino-2,4,6-triiodobenzyloxy)benzoate	CH_3	H	NH_2
2) 4-(3-Amino-2,4,6-triiodobenzyloxy)benzoic acid	H	H	NH_2
3) 4-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid	H	H	$NHCOCH_3$
4) 4-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	H	$N:CHN(CH_3)_2$
5) Methyl 2-hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)-benzoate	CH_3	OH	NH_2
6) 2-Hydroxy-4-(3-amino-2,4,6-triiodobenzyloxy)benzoic acid	H	OH	NH_2
7) 2-Hydroxy-4-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid	H	OH	$NHCOCH_3$
8) Methyl 2-hydroxy-4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoate	CH_3	OH	$N:CHN(CH_3)_2$
9) 2-Hydroxy-4-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	OH	$N:CHN(CH_3)_2$

TABLE 1. CHEMICAL STRUCTURES (continued)

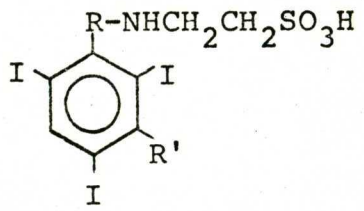
B) 3-TRIIODOBENZYLOXYBENZOIC ACIDS



	R	R'
10) 3-(3-Acetamido-2,4,6-triiodobenzyloxy)benzoic acid	H	$NHCOCH_3$
11) 3-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	H	$N{:}CHN(CH_3)_2$
12) 2-Hydroxy-5-(3-amino-2,4,6-triiodobenzyloxy)-benzoic acid	OH	NH_2
13) 2-Hydroxy-5-(3-acetamido-2,4,6-triiodobenzyloxy)-benzoic acid	OH	$NHCOCH_3$
14) 2-Hydroxy-5-(3-dimethylaminomethyleneamino-2,4,6-triiodobenzyloxy)benzoic acid	OH	$N{:}CHN(CH_3)_2$

TABLE 1. CHEMICAL STRUCTURES (continued)

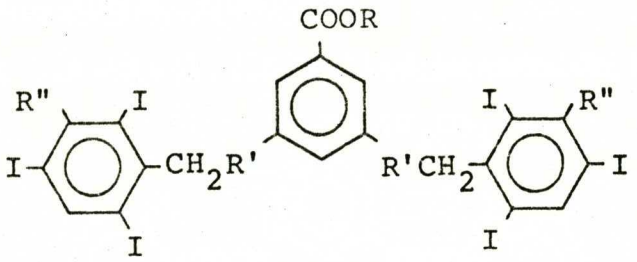
C) TAURINE DERIVATIVES



	R	R'
15) N-(3-(3-Amino-2,4,6-triiodophenyl)propionoyl)taurine	CH_2CH_2CO	NH_2
16) N-(3-Amino-2,4,6-triiodobenzyl)taurine	CH_2	NH_2
17) N-(3-Dimethylaminomethyleneamino-2,4,6-triiodobenzyl)-taurine	CH_2	$N:CHN(CH_3)_2$

TABLE 1. CHEMICAL STRUCTURES (continued)

D) 3,5-BIS(TRIIODOBENZYL)BENZOIC ACID ESTERS



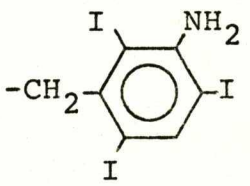
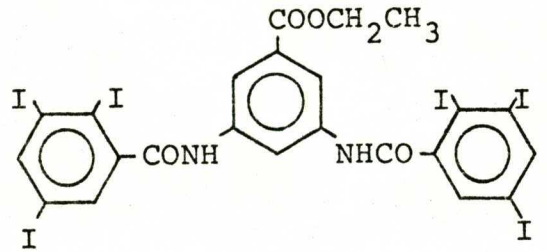
	R	R'	R"
18) Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzylamino)-benzoate	CH_2CH_3	NH	NH_2
19) Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzylamino)-benzoate	CH_2CH_3	NH	$NHCOCH_3$
20) Ethyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)-benzoate	CH_2CH_3	O	NH_2
21) Ethyl 3,5-bis(3-acetamido-2,4,6-triiodobenzyloxy)-benzoate	CH_2CH_3	O	$NHCOCH_3$
22) 3-Amino-2,4,6-triiodobenzyl 3,5-bis(3-amino-2,4,6-triiodobenzyloxy)benzoate		O	NH_2

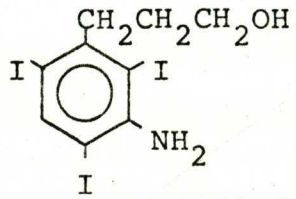
TABLE 1. CHEMICAL STRUCTURES (continued)

E) MISCELLANEOUS COMPOUNDS

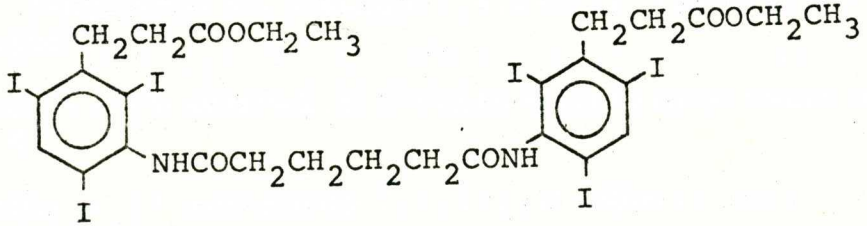
23) Ethyl 3,5-bis(2,3,5-triiodobenzamido)benzoate



24) 3-(3-Amino-2,4,6-triiodophenyl)propan-1-ol



25) N,N'-bis(3-(2-carbethoxyethyl)-2,4,6-triiodophenyl)-adipamide



26) 3-(3-(3-(2,4,6-triiodophenyl)propionamido)-2,4,6-triiodophenyl)propionic acid