INTRAHEPATIC CHOLESTASIS: A REVIEW OF BIOCHEMICAL-PATHOLOGICAL MECHANISMS

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ABSTRACT

Intrahepatic cholestasis involves impaired excretion of bile via the hepatobiliary system as a consequence of one or more lesions within the liver. In humans, intrahepatic cholestasis most often results as a side-effect of drug therapy and the clinical manifestation of this condition, jaundice, has been estimated to account for hospitalization in 2 to 5% of the cases for the general population and approaches as much as 20% in the elderly. With the aging of the population and the common occurrence of poly-drug therapy in geriatric patients, it is to be expected that jaundice due to drug-induced intrahepatic cholestasis will become even more prevalent, and accordingly the need to understand the basic mechanisms of this disease condition will become more urgent. The list of culprit agents implicated in the induction of intrahepatic cholestasis in humans is continually expanding. These include various steroid hormones, bile acids, drugs and other chemicals. Experimentally, a wide spectrum of agents has been shown to precipitate intrahepatic cholestasis.

Over the years, a number of hypotheses on the biochemical and pathological mechanisms of intrahepatic cholestasis has emerged, including the following: impaired sinusoidal membrane function; interference with the distribution and binding of cytoplasmic endogenous carrier proteins; interference with mitochondrial energy supply; defects in the canalicular membrane including altered Na^+/K^+-ATP-ase activity; impairment of microfilament and microtubule functions; interference with bile secretion involving bile acid dependent and independent fractions, and altered bile acid metabolism due to "hypoactive hypertrophic smooth endoplasmic reticulum". In partial agreement with the latter hypothesis, our studies indicated that impairment of the endoplasmic reticulum might represent one of the early stages in the development of intrahepatic cholestasis. Various experimental conditions that induce intrahepatic cholestasis to different degrees resulted in an interference of the synthesis of microsomal phospholipids and altered microsomal function. The conditions included the administration of various hepatotoxic compounds or steroids, pregnancy, delayed development of the endoplasmic reticulum in neonates, and dietary methyl donor or choline deficiency.

This review reports the biochemical-pathological mechanisms postulated to be involved in the genesis of intrahepatic cholestasis with specific reference to experimental models of drug-induced intrahepatic cholestasis. The important practical implications of cholestasis are also briefly surveyed.

I. INTRODUCTION

One of the most important functions of the liver is to produce bile and secrete it into the duodenum. The bile contains bile acids, phospholipids, cholesterol, bile pigments, various salts, water and very low amounts of proteins (Fig. 1). Many lipid soluble intermediates and end-products of metabolism including various foreign substances are eliminated in the bile. Failure of the normal bile flow leads to stagnation resulting in the retention of biliary substances. This can originate from various causes including: (a) primary hepatocellular damage consisting of parenchymal lesions associated with a defect in the oxidation of bile acids; (b) alterations of several subcellular organelles around the bile canaliculus followed by in-

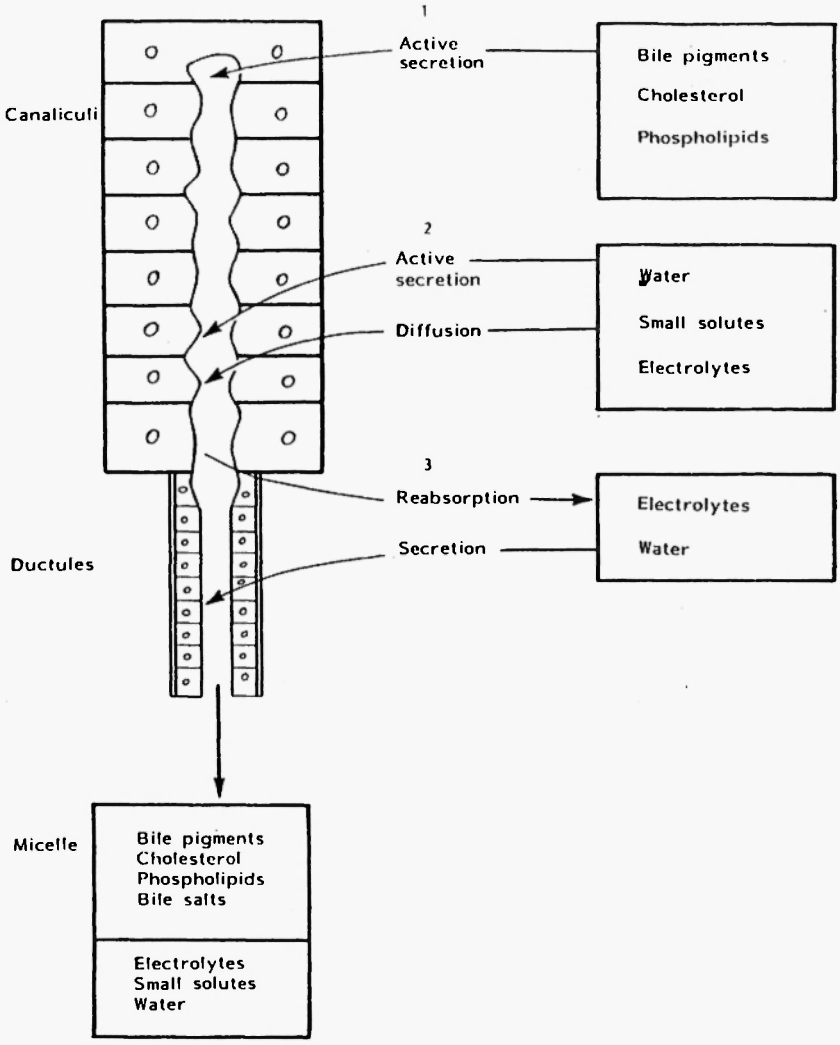

Fig. 1: Mechanism of bile formation. Bile is formed in the canaliculi in response to the secretion of bile salts and other solutes and modified in the bile ducts either by reabsorption or secretion of water and electrolytes. 1: Active secretion is dependent on bile salts. 2: Bile salt independent active secretion, probably related to Na^+/K^+-ATP-ase activity. 3: Secretin dependent secretion. Adapted from Wheeler HO: Secretion of bile. In: Diseases of the Liver, Fourth ed. Schiff L (ed), Lipincott, Philadelphia-Toronto, 1975, p. 103 /645/.

creased intracellular pressure and impaired transport processes of the bile; (c) changes in subcellular structures coinciding with the formation of bile thrombi, resulting in a mechanical obstruction and bile content regurgitation into the sinusoids; (d) overproduction of bilirubin from heme which exhausts the hepatic systems available for transport and metabolism, leading to impaired metabolism and secretion of bilirubin and other bile constituents; (e) interaction between endogenous substances (mainly steroid hormones) and exogenous foreign compounds (e.g., drugs, food additives, environmental pollutants) which are processed and eliminated by the same hepatic system, resulting in an accumulation of various metabolites, and (f) obstructive or destructive phenomena occurring in the intralobular structures and bile ducts due to inflammation or cirrhosis. Whatever the cause of failure of the bile outflow, obstruction in passing of bile from hepatocytes is associated either with abnormal retention of bile within the liver cell or with the accumulation of abnormal amounts of biliary substances in the blood /1-3/. These anomalies represent the symptoms of extrahepatic or intrahepatic cholestasis.

Intrahepatic cholestasis represents a toxic side effect of a variety of drugs and chemicals, several commonly used compounds such as estrogens, erythromycin, phenothiazines and some oral antidiabetics /4/. This disorder should be distinguished from two other forms of hepatobiliary injury: extrahepatic cholestasis due to mechanical obstruction of the bile ducts or massive production of bilirubin from the excessive breakdown of erythrocytes and general cell injury with an enhanced release of cytosolic enzymes. In most cases, the changes associated with intrahepatic cholestasis are rather discrete compared to the gross alterations evident following extrahepatic cholestasis or other abnormal hepatobiliary conditions (Fig. 2). It is relatively simple to identify intrahepatic cholestasis by exclusion of other pathological states. Differentiation can be made between intrahepatic and extrahepatic cholestasis by discriminant analysis /980/.

Extrahepatic cholestasis involves a mechanical obstruction such as common duct stones, sclerosing cholangitis or cancer of the pancreas. However, in the case of intrahepatic cholestasis there is no apparent anatomical obstruction. Intrahepatic cholestasis is caused by an impairment of normal biochemical processes within the liver cell. This may be obstructive or functional in nature, since the cause of inadequate bile flow may result from an obstruction of the in-

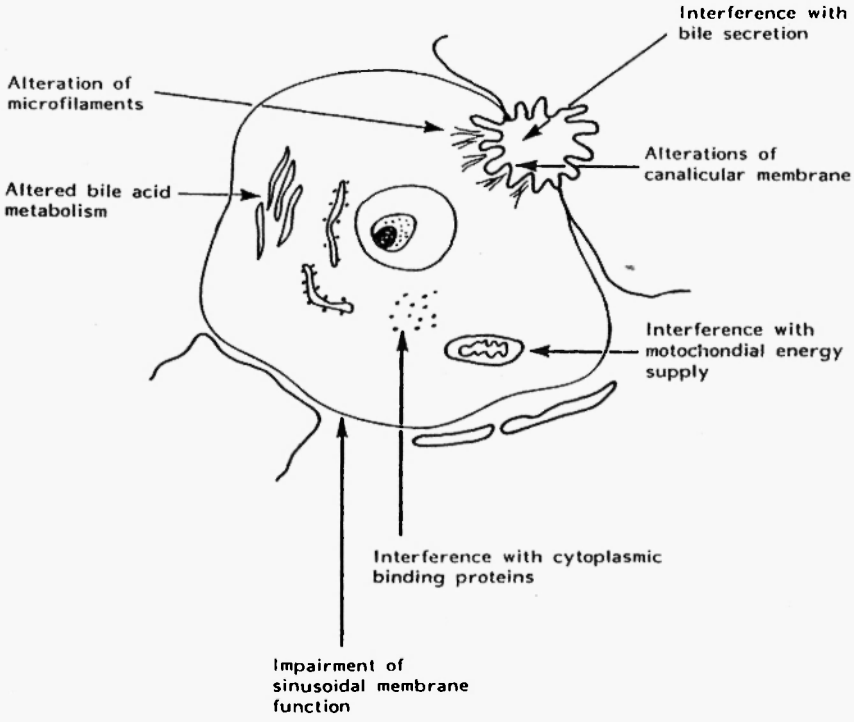

Fig. 2: Hypothetic sites of lesions as the initiating event in intrahepatic cholestasis.

trahepatic biliary passages, such as cholestasis during cirrhosis, or during inflammatory processes from viral hepatitis /5, 6/. In other cases the primary lesion is a secretory disturbance of the liver cell, such as cholestatic actions of various foreign compounds.

Bile secretion can be divided into two different but interdependent processes: (a) concentration and transport of solutes from the sinusoids to the biliary space and (b) continuous fluid secretion. It is known that specific carrier proteins are responsible for solute transport /7-13/; however, the mechanism of fluid secretion has not yet been well defined. It is known that sodium ions play an essential role in both processes. Bile acids and other anions are actively secreted into the bile together with their cations resulting in an iso-osmotic bile fluid. Disturbances anywhere in these mechanisms can initiate intrahepatic cholestasis. Either the capacity of the hepatocytes to

concentrate cholephilic solutes or the capacity to secrete bile fluid can be impaired in cholestasis /14, 15/.

It is important to study intrahepatic cholestasis since liver damage occurs frequently as a side effect of drug therapy representing a considerable health care problem. In a recent survey of patients hospitalized due to jaundice, drug-induced hepatotoxicity accounted for 2-5% of the cases /16/. Administration of drugs to pregnant women or during lactation may cause adverse cholestatic reactions in the newborn and infants. Moreover, in elderly patients with jaundice, the incidence of liver disease attributed to drugs is close to 20% /17/. Severe hepatic dysfunction from massive liver necrosis is attributable to adverse drug reactions in about 25% of cases /18/. This highly significant incidence of hepatotoxic reactions has existed for decades and occurs for several reasons: Firstly, the liver is particularly susceptible to injury as a consequence of its extensive blood supply. Orally ingested and absorbed drugs are delivered to the liver directly by the portal vasculature, so it is exposed to drugs to a greater extent than any other organ. Secondly, the liver cell is more vulnerable to drug-mediated injury than other cells because of its major role in the concentration and metabolism of drugs /19/.

Our understanding of the biochemical-pathological mechanisms underlying drug-induced intrahepatic cholestasis is still far from complete. Several hypotheses have been suggested based on investigations in experimental animals and reports from clinical observations. This review is based mainly on experimental studies and is complemented with clinical studies, wherever possible. Although no experimental model has been developed which duplicates all features of intrahepatic cholestasis in man, it must be emphasized that the cholestatic lesions produced by various substances in experimental animals often show similar morphological, biochemical and clinical characteristics to those seen in humans.

There are many extensive reviews on intrahepatic cholestasis, especially reference books containing chapters on the mechanism of bile formation /20-26/, impaired excretory functions /27-29/, morphological characteristics /30-34/ and the possible mechanisms involved in its development /35-39/. Experimental studies as well as hypotheses on mechanisms leading to cholestasis have also been reviewed /40/. These reviews were published between 1965 and 1976, and with new developments in this field during the past decade, we considered this an opportune time to provide an updated review on recent

contributions to the understanding of cholestatic mechanisms. In addition to a survey of the pertinent literature, we also present the results of our own recent investigations on drug-induced intrahepatic cholestasis.

II. VARIOUS FEATURES OF INTRAHEPATIC CHOLESTASIS

2.1 Clinical changes

The primary lesion in cholestasis is manifest in the liver. The patient develops jaundice slowly and during later stages skin color may become greenish due to the retention of pigments other than bilirubin, such as biliverdin (Fig. 3). Pruritus results from irritation of cutaneous sensory nerves by retained bile salts. Skin xanthomas

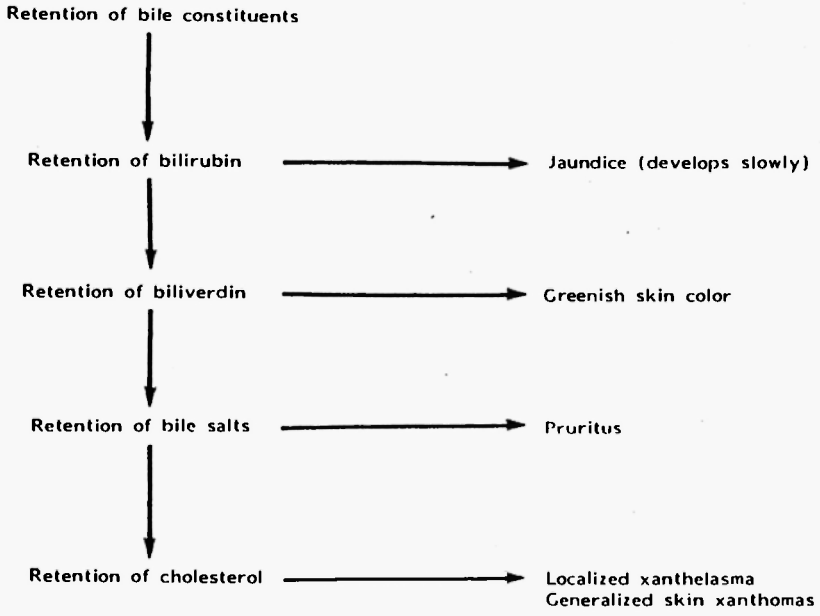

Fig. 3: Clinical features of acute intrahepatic cholestasis.

develop if total serum cholesterol levels exceed 11.6 mmol/l for three months. The liver is often enlarged, with a firm, smooth, non-tender edge. The feces are usually pale, due to diminished bilirubin in the intestines. Inadequate bile salts in the small intestine to achieve micellar solution of lipids results in steatorrhea and malabsorption of calcium and fat-soluble vitamins /41/. Malabsorption of vitamin D and calcium can result in osteomalacia. The bone is weakened, osteoblasts multiply, and this accounts for a rise in serum alkaline phosphatase. Osteomalacia may also be accompanied by osteoporosis, a defect in bone matrix, which may be due to protein deficiency of chronic steatorrhea and hepatocellular disease, or to some retained toxic factor acting on the bone. Bone changes develop slowly but their occurrence is unlikely, unless the cholestasis is deep and of longer than two years' duration. If the cholestasis is sufficiently prolonged, malabsorption of vitamin A can lead to night blindness. Malabsorption of vitamin K and Factor VII can result in prolonged prothrombin time and excessive bleeding with trauma.

2.2 Morphological changes

Cholestasis is the most common form of hepatic disease in which several organelles are altered around the bile canaliculus due to increased intracellular pressure and impaired bile transport processes (Fig. 4). Changes in the endoplasmic reticulum and mitochondria may be associated with the detergent action of bile acids. However, changes in the bile duct system are probably secondary to the parenchymal lesions. In many cases, cholestasis includes primary hepatocellular damage resulting in a defect in bile acid oxidation and qualitative changes in bile composition. Changes in intracellular structures coincide with the formation of bile thrombi, which bring about a mechanical obstruction or induce a defect in the excretion of conjugated bilirubin with regurgitation into the sinusoids. Cell membranes and extralobular ductules develop an increased permeability to water and electrolytes, and a lesion of the sodium pump leads to changes in the concentration of canalicular bile. Obstructive or destructive phenomena occurring in the intralobular ductules and bile ducts are also involved in the pathogenesis of certain types of intrahepatic cholestasis, particularly in primary biliary cirrhosis /31, 42, 43/.

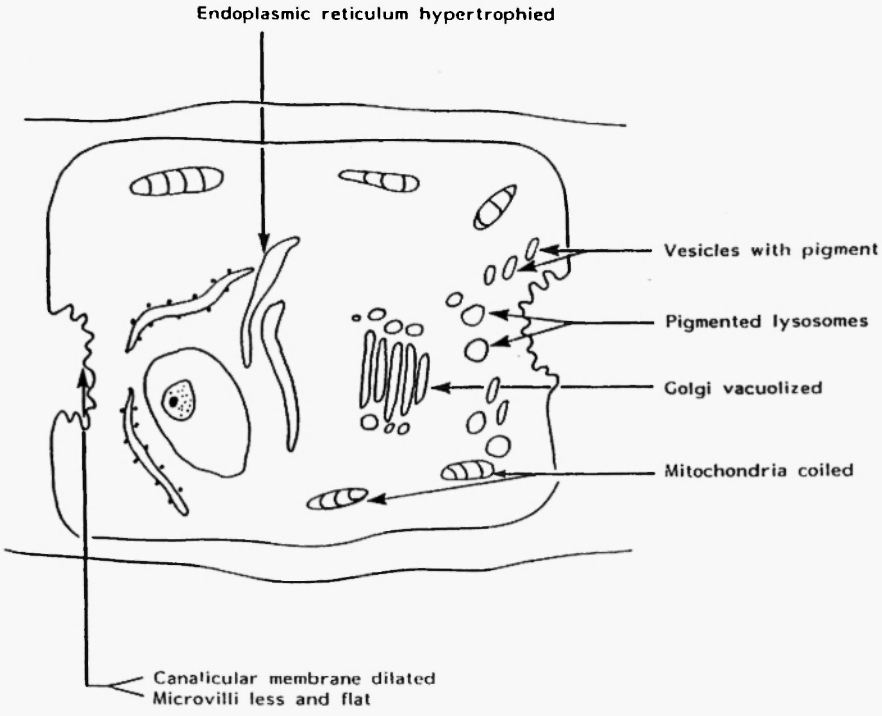

Fig. 4: Morphological features of intrahepatic cholestasis.

Several agents cause cholestatic injury that spare the parenchyma and mainly cause a reduction of bile flow /44-49/. Histologic manifestations may consist only of bilirubin casts in canalicular spaces and little injury to bile ducts or parenchyma. Cholestatic lesions in some cases, such as those induced by chlorpromazine, may be associated with minor parenchymal changes /47-49/.

Some toxicants, such as α-naphthylisothiocyanate (ANTT), selectively destroy the bile ducts, followed by cholangio-proliferative lesions /44/. It seems that there are two types of jaundice caused by drugs /50/. One group of agents produces mainly canalicular jaundice, while for others the lesion extends to bile canaliculi and parenchymal cells (Table 1). However, many agents, such as aflatoxin B_1 /50/ and ethionine /51/, cause cytotoxic hepatic injury and also produce ductal lesions to some degree. As a consequence, acute intra-

TABLE 1
Common causes of intrahepatic cholestasis

Disease	Causes
Familial	
Classic cholestasis	Benign recurrent cholestasis Familial intrahepatic cholestasis
Selective cholestasis	Dubin-Johnson syndrome Rotor syndrome
Developmental	Neonatal
Intrinsic liver disease	Cirrhosis Primary biliary cirrhosis Viral hepatitis Inflammatory bowel disease
Hepatocanalicular	Chemicals Estrogens and oral anabolic steroids Drugs Hepatotoxins
Infiltrative liver disease	Hemochromatosis Steatosis Amyloidosis Leukemia Granulomas Gaucher's disease
Focal liver disease	Abscess Carcinomas Metastases
Canalicular Hepatocanalicular	Chemicals Estrogens and oral anabolic steroids Drugs Hepatotoxins
Secondary causes	
Direct involvement	Alcoholic hepatitis Cholangiocarcinoma Intrahepatic biliary atresia Pericholangitis Primary biliary cirrhosis Viral hepatitis
Indirect involvement	Erythropoietic protoporphyria Sickle cell anemia crisis Hodgkins' disease Hypophysectomy Endotoxins Total parenteral nutrition Sepsis Postoperative open heart surgery

Modified from references /242, 901-920/.

hepatic cholestasis can lead to chronic disease which resembles primary biliary cirrhosis.

Generally, structural changes due to intrahepatic cholestasis include pigmented lysosomes and secondary vesicles, coiled mitochondria, vacuolized Golgi apparatus and changes in the endoplasmic reticulum (Fig. 4). Bile canaliculi are dilated and there is swelling, blunting, distortion and sparsity of microvilli. Bile plugs, containing mainly conjugated bilirubin, are evident in the bile canaliculi /52/ and bile pigment is present in Kupffer cells and hepatocytes. Lysosomes are more numerous and the endoplasmic reticulum is hypertrophied. Abnormal mitochondria consisting of intermitochondrial structures with myelin-like figures, and elongation and irregular coiling of the cristae may also occur. In severe forms of intrahepatic cholestasis, additional features are feathery degeneration, bile infarcts and accumulation of foamy cells.

Abnormalities in bile formation associated with intrahepatic cholestasis caused by various clinical conditions and chemical agents are summarized in Table 2.

2.3 Biochemical changes

a) Clinical level

During intrahepatic cholestasis, serum levels of all bile constituents are increased due to their impaired excretion (Table 3). Serum bilirubin is raised, containing mainly the conjugated fraction. There is an increase in serum alkaline phosphatase, 5'-nucleotidase and γ-glutamyl transpeptidase to more than twice the normal level; cholesterol and total bile acids also rise, and there is usually an increase in low density lipoproteins, but a decrease in high density lipoproteins /53/. The increase in lipoproteins is largely due to an abnormal protein called lipoprotein X, which is probably catabolized normally in the liver. It accumulates during intrahepatic cholestasis because of hepatic dysfunction /54/. Serum albumin level is usually normal until the stage of terminal liver cell failure, when it falls; however, serum globulin is usually increased. Urinary urobilinogen is diminished, since less bilirubin reaches the duodenum; the elevated conjugated bilirubin in the serum leads to a spill-over and elimination in the urine. After prolonged chronic jaundice, development of ascites, edema, and a lowered serum albumin level indicate liver cell failure.

TABLE 2
Abnormalities in bile formation associated with intrahepatic cholestasis

Site and abnormality	Condition or Chemicals	Reference
Sinusoidal uptake		
Decreased exogenous carriers	Not known	
Decreased driving forces	Hypothyroidism	402
	Chlorpromazine	230,539
	Endotoxins	954,955
	Estrogens	195,406,416
	Porphyrins	956
Decreased bile salt delivery	Bile duct drainage	239,356
	Ileal resection	
Competitive inhibition	Various drugs	198,428,768,957
Altered cellular permeability	Bilirubin-manganese	151
	Taurollthocholate	147
Membrane fluidity	Chlorpromazine	229,540
	Ethinyl estradiol	135,143
	Taurolithocholate	149,225,471
Cellular translocation		
Microfilaments	Cytochalasin B	153,165
	Norethandrolone	958
	Phalloidin	145,146,763
Microtubules	Colchicine	146
Calcium deprivation		139,397
Transcellular regurgitation	Bile duct ligation	137,144,239,347
	Calcium deprivation	390,397
	Ethinyl estradiol	135,143,411
	α-Naphthylisothiocyanate	367,664-666
	Phalloidin	136,145,146
	Taurolithocholate	138
Excretion		
Decreased endogenous carriers	Cycloheximide	484

Modified from Reichen J, Simon FR: Cholestasis, In The Liver: Biology and Pathobiology, Second Edition, Arias JM, Jacoby WB, Popper H, Schachter D, Shafritz DA (Eds.). Raven Press, New York pp 1105-1124, 1988 /133/, and references given in the Table.

TABLE 3
Biochemical features of intrahepatic cholestasis

Serum
↑Bilirubin (Conjugated) ↑Bile acids ↑Cholesterol ↑Liver enzymes - Alkaline phosphatase - γ-Glutamyl transpeptidase - 5'-Nucleotidase ↑Low density lipoproteins, Lipoprotein X ↓High density lipoproteins ↑Globulin Albumin - Normal until terminal liver cell failure when↓
Urine
+Conjugated bilirubin -Urobilinogen

↑, increased; ↓, decreased; +, present; -, absent

b) Cellular level

There are many biochemical changes at cellular level. Bile secretion from the liver cell can be divided into two fractions: one is bile acid-dependent and the other bile acid-independent (Fig. 1). Both fractions are altered by sex steroid hormones or exogenous bile acids. The bile acid-independent fraction is modulated by the sodium pump catalyzed by the sodium/potassium-activated adenosine triphosphatase enzyme (Na^+/K^+-ATP-ase). This fraction contains mainly electrolytes and the greater part of the excretion product is reabsorbed in the bile duct and gall-bladder. The bile acid-dependent fraction contains bile acids and heterogeneous micelles which are composed of polyionic complexes formed from bile salts, cholesterol and phospholipid. Hyperbilirubinemia is produced when the elimination of this fraction is disturbed /55-59/. Hydrophilic bile acids promote the formation of these aggregates which also carry other anions including bilirubin. In normal circumstances the micelle and bile flow follow a unidirectional route from the sinusoids towards the bile canaliculi. Normally only trace amounts of monohydroxy cholic acids are pres-

ent in the bile; in pathologic conditions there may be a considerable increase.

Variation in the concentration or type of bile acid synthesized disturbs micelle formation resulting in impaired accumulation and production of liquid crystals. The abnormal mixture of cholesterol, lecithin and bile salts form these liquid crystals and sometimes amorphous materials are also present. These structures precipitate in intracanalicular spaces and produce thrombi leading to fragmentation of the microvilli and pericanalicular cell membranes, with altered cytoplasmic constituents including the endoplasmic reticulum and mitochondria /59-61/. Disturbances are probably associated with alterations of the metabolic capacity of these cellular organelles. Differences in antipyrine metabolic clearance rate provided evidence that the drug metabolizing function of the hepatic endoplasmic reticulum is impaired in intrahepatic cholestasis /62/. This decrease could be caused by a reduced functional parenchymal mass associated with some degree of hepatic necrosis.

Microsomal enzymes are responsible for the transformation of cholesterol to bile acids and mitochondrial enzymes for the side chain cleavage of this molecule, providing more sites for further hydroxylation /63-66/. In intrahepatic cholestasis due to faulty hydroxylation, the process of bile acid production is incomplete. Following this defect, excess amounts of mono- and dihydroxy bile acids are formed at the expense of cholic acid. The excretion of lithocholic acid may damage the entire excretory apparatus from the microvilli to the ductules. The accumulating monohydroxy bile acids such as lithocholic acid are poor micelle formers and therefore these components may be involved in the development of cholestasis. In these circumstances the activities of hepatic hydroxylating enzymes and cytochrome P-450 are reduced /67/. The conversion of dihydroxy acids to monohydroxy derivatives by intestinal bacteria can also be considered as a secondary factor. The prevalence of chenodeoxycholic acid in the intestines enhances the formation of lithocholic acid which is then reabsorbed via the enterohepatic circulation in abnormally high amounts.

In both intrahepatic and extrahepatic cholestasis many enzymes leak out from the liver cell and appear in the serum. Besides a suggestion of general membrane damage or dilatation of pores in the membrane and adequate enzyme impairment, the mechanism of elevation of serum enzyme activity is not clear. Animal experiments

have shown that 1 to 2 days following intrahepatic cholestasis brought about by a single dose of ANIT, a five-fold and sixty-fold increase occurs in γ-glutamyl transpeptidase activity and of the corresponding mRNA. From day 2 to day 14 γ-glutamyl transpeptidase activity in the serum and bile progressively returned to levels predominant in the liver cells /68/. It seems, therefore, that in intrahepatic cholestasis the increase in serum γ-glutamyl transpeptidase activity is of biliary cell origin and is not released from hepatocytes.

2.4 Changes due to aging

Drug-induced hepatotoxicity causes a high degree of hospitalization in elderly patients. This is mainly due to the aging process. The liver like most other organs is morphologically and functionally altered in aged organisms /69, 70/. The exact nature of these changes has not been established largely due to major sex, strain and species differences occurring in the liver structure and activity during aging. In some strains of rats cytochrome P-450 dependent enzyme activity shows a decline, in others there is no change with advancing age /69/. Age-related changes manifest in the morphology and function of the human liver include: (a) decreased weight, (b) decreased blood flow, (c) reduced responsiveness to certain hormones, (d) increased proliferation of bile ducts, (e) increased fibrosis, (f) altered drug metabolism and (g) slow regeneration.

In aging animals, the alterations in liver function are relatively minor; still, many age related changes occur at cellular and subcellular levels. These include: (a) decreased proteolysis, (b) decreased transcription and translation, (c) increased synthesis of lipofuscin and other abnormal proteins, (d) increased cell size and protein content, (e) increased chromosomal abnormalities, (f) increased number of abnormal nuclei and (g) increased number and size of lysosomes. Similar changes characterize senescent human fibroblasts and other cell types /71/. It seems that the mechanism of aging has common characteristics operating at the cellular level and aging causes a widespread decline of many physiological and biochemical processes. In the liver the aging process is somewhat delayed as compared to other organs due to its ability to regenerate.

Probably the most important aspect of cellular aging may be caused by altered protein breakdown processes. Due to the decreased lysosomal pathways of protein degradation abnormal

proteins accumulate in the senescent cells /72-74/. These abnormal proteins affect cytosolic pathways of protein catabolism by activation or inhibition also leading to the degradation or alteration of normal cellular proteins with short lives. Such proteins may represent a small percentage of cellular constituents but these short-lived proteins may have an essential role as precursors or constituents in regulating and coordinating metabolic pathways.

The alteration of hepatic protein composition in aged cells is mainly related to cytosolic pathways. Whether protein degradation is increased or decreased may depend on what the rate limiting steps are. Certain forms of abnormal proteins may represent poor substrates so that they can accumulate in the hepatocytes during aging. If such proteins are bound to other components of the catabolic pathways, these can delay the degradation process, and can become highly competitive inhibitors of cell function /40, 44, 45/.

Parallel with the age dependent accumulation of cellular proteins and age-related increase of lysosomes /75/ this rise in number and size may represent an attempt by the cell to compensate diminished lysosomal function or inhibition. Lysosomal inhibitors can lead to the build-up of abnormal materials in the cell such as lipofuscin /76, 77/. The accumulation of lipofuscin in aging may reflect a reduced ability of the lysosomal scavenging enzymes to degrade certain types of macromolecules within the cell. Similar compensatory processes occur in young cells, where the size and number of lysosomes are increased in response to enhanced lysosomal proteolysis /78/.

Due to the development of altered cytosolic and lysosomal functions in the senescent cells a variety of abnormal proteins and complex macromolecules are formed. Resulting from the reduced rate of proteolysis the longer life span of proteins may lead to the accumulation of various non-enzymatic components which can interfere with other cell functions /79, 80/. The consequence of these changes is that the function of the hepatic endoplasmic reticulum is also impaired and the reduced drug metabolizing activity may be responsible for the increased frequency of drug side-effects and drug-induced intrahepatic cholestasis in elderly people /69, 81/.

The effect of aging on hepatic function can be influenced by life-long caloric restriction in rats /82/. Bile flow rate is decreased with age in control animals, associated with a reduction of the bile acid dependent fraction at early life and both bile acid dependent and independent fractions at late life. In rats fed a restricted diet, bile

flow was higher than in controls, containing enhanced amounts of bile acids, cholesterol and phospholipids. The increased bile flow was due to elevated bile acid dependent and independent fractions. It seems that dietary restriction is very effective in reducing the age related decline of hepatic function.

2.5 Effects in the newborn

There are conditions when cholestasis occurs more frequently, such as in the newborn and in pregnancy /83-87/. Most detoxication reactions are not present in early postnatal liver /88-90/. Particularly important is the low activity of glucuronidation /91, 92/. Glucuronidase activity has not been found in human placenta and therefore the maternal liver UDP-glucuronyltransferase takes care of the elimination of many endogenous and exogenous metabolites. Bilirubin conjugation appears after birth. Drugs compete with bilirubin for the conjugation site and for transport and secretion. Thus the low conjugating capacity of the newborn is probably the cause for direct poisoning by drugs and for the development of cholestasis and jaundice at that age. Abnormal steroids from the mother's milk via breast feeding can also lead to adverse liver reactions in the neonate /39, 93-98/.

The application of total parenteral nutrition in neonates causes hepatic dysfunction in newborns as indicated by increases in serum alkaline phosphatase, γ-glutamyl transpeptidase, bilirubin and total bile acid levels /99/. Administration of metronidazole in adequate doses prevented the elevation of transaminase brought about by total parenteral nutrition. This indicates the possible involvement of intestinal anaerobic bacterial flora in the pathogenesis of liver dysfunction associated with the diet.

2.6 Effects during pregnancy

The drug metabolizing capability of the maternal liver in late pregnancy is reduced, and at the same time there are increased demands that drain some of the energy needed in detoxication reactions. This results in a more frequent occurrence of drug-induced side effects and cholestasis during pregnancy /100-106/. The adverse cholestatic response during pregnancy may be due to the significant increase in steroid hormones affecting bilirubin and drug metabolism

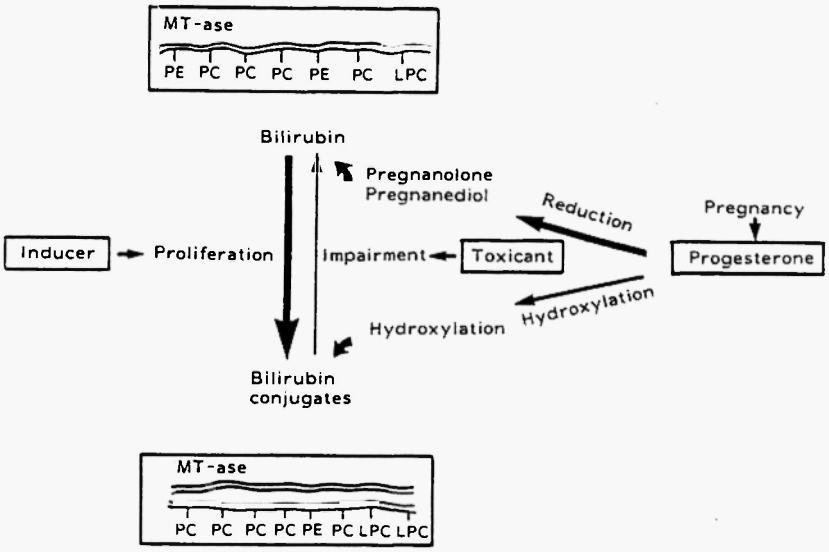

Fig. 5: Scheme for the mechanism of action of pregnancy on the hepatic endoplasmic reticulum. During pregnancy the metabolism of progesterone is shifted to the reductive pathway. Increased levels of pregnenolone and pregnanediol inhibit drug metabolism and may lead to intrahepatic cholestasis. The proliferation of the membranes is also inhibited associated with impaired protein synthesis and reduced phospholipid methylation.

(Fig. 5) /107-112/. Anabolic steroids and oral contraceptives also cause cholestasis /113-115/.

Liver dysfunction leading to intrahepatic cholestasis during pregnancy was first described a long time ago /39/. It is characterized by pruritus (pruritus gravidarum) and jaundice /116/. In this condition jaundice may appear in severe forms and liver function tests show increased serum levels of conjugated bilirubin, alkaline phosphatase and 5'-nucleotidase activities /13, 108, 117/; bile acids, particularly cholic acid, are also significantly raised /118-121/. Morphological examinations reveal canalicular bile plugs and some evidence of cellular necrosis. Intrahepatic cholestasis of pregnancy is infrequently associated with the production of fatty liver /981/. Moreover, intrahepatic cholestasis developed during pregnancy may represent a perinatal risk /1000/. It may be that increased availability of opiate agonist ligands at opiate receptor sites in the brain is the major factor contributing to the pruritus of cholestasis /122/.

Intrahepatic cholestasis is considered a benign disease, but often clinical signs of a mild nutritional impairment can be detected. Fat malabsorption has been demonstrated in patients with obstructive jaundice /13/, in some diseases characterized by intrahepatic cholestasis /118-120/ and in patients with anicteric chronic liver disease /8, 9/. Increased fecal fat excretion could be detected as early as 3 weeks after the clinical onset of intrahepatic cholestasis of pregnancy, that remained stable during the pregnancy and returned to normal between 3 to 9 weeks after delivery /123/. The steatorrhea is correlated with the severity of the cholestasis as estimated by measurements of serum bilirubin, bile acids and aspartate aminotransferase activity.

Intrahepatic cholestasis has also been found in a few women taking oral contraceptive pills soon after their application /13/. The correlation between cholestasis of pregnancy and cholestasis attributable to oral contraceptives was soon recognized. It has been shown that 50% of women with intrahepatic cholestasis caused by oral contraceptives also had intrahepatic cholestasis in pregnancy /124, 125/, suggesting that the estrogenic component is the active agent /13/. Early investigations reported that the administration of ethinyl estradiol or high doses of estradiol or estriol causes hepatic impairment /126/. This accords with the suggestion that pregnancy related intrahepatic cholestasis is connected with the elevated hormone levels /127/.

Oral administration of S-adenosylmethionine reversed intrahepatic cholestasis in pregnant women /128/. Serum alkaline phosphatase activity and total and conjugated bilirubin were significantly reduced by the treatment. Subjective symptoms such as pruritus and fatigue were also improved.

Various causes of intrahepatic cholestasis in neonates and adults are listed by order of frequency in Table 4.

III. MECHANISMS OF CHOLESTASIS

The term cholestasis was originally coined to indicate bilirubin stagnation in hepatocytes and biliary passages as observed by light microscopy /129/. It was considered that defects of bile secretion were connected with mechanical obstruction and with abnormal processes occurring in the liver cell. Electron microscopic studies have shown that in cholestasis the number of biliary canaliculi are reduced. They are dilated, containing electron-dense amorphous

F-actin /145/. The action of polymerization mainly occurs around the canaliculi and the tight junction /144, 146/. In phalloidin cholestasis the permeability of the biliary tree to inert solutes is increased /136, 146/. A motility disorder of the canaliculi may also be associated with the action of phalloidin on the liver /157, 166/. Chlorpromazine- /71, 167/ and norethindrone-induced /168/ cholestasis and the American Indian childhood cirrhosis /169/ are followed by changes similar to the action of phalloidin.

d) Microtubules

Studies with colchicine, an inhibitor of tubulin polymerization, show no effects at low levels on basal bile flow but bile salt excretion is reduced. Although colchicine alone is ineffective on bile flow, when given together with phalloidin it acts in a synergistic fashion, and decreases bile flow /123/. In high concentrations cholchicine inhibits bile acid uptake /153/. These investigations suggest that microtubules are important structures in the non-specific secretion of various proteins /170, 171/, and in specific receptor-mediated pathways such as in the transfer of lysosomal enzymes /172/, and in the translocation of immunoglobulin A /173/.

Further investigations revealed that colchicine or vincristine and vinblastine inhibit bile acid stimulated bile flow and bile acid secretion in the rat and potentiated the inhibitory effect of phalloidin /146, 962/. Colchicine also significantly reduced taurocholate excretion in control animals and inhibited biliary secretion of taurocholate in rats previously depleted of bile salts. It seems that the transport and excretion of bile salts is dependent on the microtubular system when the transcellular flow and biliary excretion of bile salts increases; it has little effect in normal basal conditions.

e) Endoplasmic reticulum

It has been suggested that hypoactivity of the smooth endoplasmic reticulum is connected with the mechanism of cholestasis /30/. As a consequence of this hypoactivity bile acid hydroxylation is reduced. Cholestasis also affects the activity of several enzymes bound to the endoplasmic reticulum /174-177/. Most changes are associated with reduced protein synthesis /174, 178/; it may be, therefore, that these are consequences rather than the cause of cholestasis. This view is

supported by the report that induction of hypoactive hypertrophic smooth endoplasmic reticulum by phenobarbital and cobalt chloride does not alter bile flow /179/. Modification of drug metabolizing enzymes may lead to different pathways connected with toxic or cholestatic compounds. Such effects may occur in estrogen-induced cholestasis, where toxic glucuronides are formed /180/, or in chlorpromazine-induced cholestasis, where the inhibition of the drug metabolizing enzyme system leads to the accumulation of chlorpromazine thus accelerating its toxicity /181/.

f) Lysosomes

These subcellular cell organelles participate in some forms of hepatocellular changes, but there is no evidence of direct specific involvement of lysosomes in the production of cholestatic lesions. It has even been reported that ethinyl estradiol-induced cholestasis exerts no effect on the export of lysosomal proteins /30, 174/.

g) Cytosol

Among cytosolic constituents, glutathione transferases play a role in cholestasis. Some of these enzymes bind bile acids and reduce the toxicity of compounds such as lithocholate /182, 183/. The canaliculi contain a carrier protein for glutathione /184, 185/ and the exported glutathione is hydrolyzed in the bile to the component amino acids and thus provides osmotically active substances /163/. It seems that normal glutathione homeostasis is necessary for bile acid secretion /186/ and perhaps the impaired redox state of the cells is connected with the impaired bile acid excretion.

In various models of cholestasis the concentration of glutathione transferase in the cytosol is reduced including cholestasis caused by ethinyl estradiol /187/. The mechanism of enzyme reduction is not known but could be associated with a decreased anion transport /188/.

h) Bile salt transport proteins

Secretion of bile salts is the major process in bile production, and the determination of their amount provides a semiquantitative measurement of the degree of cholestasis /189/. Secretion of bile salts is dependent on the delivery from the intestine, hepatic uptake, excre-

tion and translocation. Intestinal absorption and portal circulation account for 90% of biliary acids. The hepatic uptake of bile acids, especially taurocholate, is a carrier-mediated, sodium-dependent process /190, 191/. Several receptors have been identified for the specific uptake process /192-194/. In contrast, biliary secretion of bile salts is independent of sodium ions /192, 195/; it requires different transport proteins and binding sites. In cholestasis, taurocholate transport and the number of binding sites are reduced. This has been found in hepatocytes isolated from the liver of ethinyl estradiol-treated rats /198/ and in cholestasis induced by taurolithocholate /196/, androgenic steroids /197/, chlorpromazine /198/ and bile duct ligation /137/.

In the transport of bile salts, albumin and high-density lipoproteins (HDL) play a major role /199-201/. There is a close relationship between the apolipoproteins of HDL and bile salts. The fraction of individual bile salts associated with HDL is dependent on their individual properties. The more hydrophilic derivatives have greater affinity to HDL; in contrast, the more hydrophobic bile salts are bound to albumin /202-204/. Modifications in the composition and concentration of individual bile salts in the blood and in the proportion of bile salt binding serum proteins may result in an altered distribution of bile salts between albumin and HDL. These alterations could have profound consequences on clearance processes in the liver and kidney.

In normal subjects albumin carries about 80% of total serum bile acids and about 20% is bound to HDL, although the concentration of the latter carrier protein is low compared to that of albumin /202/. The distribution may be altered under pathological circumstances when concentrations of albumin or lipoproteins in the serum are abnormal. The distribution pattern of ^{3}H-taurocholate in the sera of non-icteric patients is not different from healthy controls /205/. The portion of taurocholate in the albumin fraction was between 75 and 80%, and in the HDL fraction between 20 and 25%. The relative amounts of taurocholate in these fractions were dependent on the concentrations of albumin and HDL. In the sera of deeply jaundiced patients the distribution pattern of ^{3}H-taurocholate between the carrier proteins was different from that in healthy controls and non-icteric patients. It was markedly shifted from albumin toward HDL. The taurocholate protein in the HDL fraction was higher than could be expected from the serum concentrations of HDL apoprotein and

albumin. The altered distribution roughly corresponded to the degree of cholestasis. The ^{3}H-taurocholate distribution pattern in the HDL fraction showed two distinct peaks. No association was found between ^{3}H-taurocholate and lipoprotein X indicating that this protein does not play a significant role in bile salt transport in cholestasis.

The elevation of relative taurocholate distribution in the HDL fraction is probably related to some factors which interfere predominantly with bile salt binding to albumin without affecting the degree of association of bile salts with HDL. These factors, which are known to be elevated generally in liver diseases, are some amino acids /206/, fatty acids /207/, bile acids and bilirubin /208-211/. In the taurocholate shift from albumin to HDL only bilirubin was active. Both unconjugated and conjugated bilirubin exhibited an effect and they are at least in part responsible for the enhanced proportion of ^{3}H-taurocholate in the HDL fraction. In the sera of cholestatic patients conjugated and unconjugated bilirubin and another bilirubin species were found to be bound tightly to albumin /208-211/. It may be that bilirubin, when firmly attached to albumin, also influences bile salt binding and consequently bile salt distribution between serum components.

In vitro studies have also shown that bilirubin caused a shift of taurocholate binding from albumin to HDL. Other organic anions, such as methionine, phenylalanine, tyrosine, proline and oleic acid, elicited no influence on the distribution of ^{3}H-taurocholate between serum carrier proteins /205/. It is known that drugs can interfere with bilirubin binding to albumin /212/; it may be important to consider this effect in the mediation of bile salt uptake in the liver /213, 214/ and in the action of bile salts on enterohepatic circulation.

3.3 Changes in membrane composition

a) Enzymes

Using reliable methods, bile canalicular (apical or luminal) membranes can be separated from sinusoidal plasma (basolateral) membrane fractions /215-218/. Canalicular fractions can be identified using γ-glutamyltransferase /219/ and leucine amino peptidase marker enzymes /220/. Measurement of the activity of Na^{+}/K^{+}-ATP-ase is generally applied in connection with glucagon-stimulated

adenylate cyclase for the specific identification of the sinusoidal surface /221/. Although the activity of these enzymes characterizes different membrane domains in the hepatocyte, there are still only small differences in the polypeptide pattern /218/.

Changes in various marker enzymes have been investigated in cholestasis using different animal models as well as in clinical cases. Earlier, it was considered that Na^+/K^+-ATP-ase regulates the active transport of Na^+ and K^+ ions across the plasma membrane. This enzyme also regulates the bile-salt independent bile flow. Since Na^+/K^+-ATP-ase activity is dependent on optimal membrane fluidity for the required conformational changes /222/, it was suggested that this enzyme is functionally inactive on the canalicular membrane because of its viscous lipid environment. Fast changes in canalicular membrane fluidity or redistribution of ATP-ase enzyme units from inactive canalicular sites to sinusoidal surfaces are probably connected with the regulation of membrane function /223, 224/. Recently, however, the weaknesses of this hypothesis have also been commented on /158, 159/.

The activity of Na^+/K^+-ATP-ase controls many functions including cytoskeletal changes, extra- and intracellular electrolyte distribution, internal pH, intracellular free calcium and distribution of secondary active transport components which in turn may control bile acid-independent flow and bile acid transport. Na^+/K^+-ATP-ase is inhibited by monohydroxy bile salts /225, 226/, ethinyl estradiol /227, 228/, chlorpromazine /229, 230/, and obstruction of the bile duct /231/.

The role of Mg^{++}-ATP-ase in cholestasis is not well understood. This enzyme is bound to the plasma membrane and is localized predominantly on the canalicular surface of the hepatocyte /232/. It is also present in the ductular epithelium, but its activity is much higher in plasma membranes /233/. Most bile acids inhibit Mg^{++}-ATP-ase *in vitro* /223, 234/. Bile ligation enhances enzyme activity /134/; the effects of ethinyl estradiol /134, 218, 235/ and chlorpromazine /181, 230/ on this enzyme are controversial. Production of cholestasis by cytochalasin B, an inhibitor of microfilaments, leads to a loss of Mg^{++}-ATP-ase activity indicating an association between microfilaments and the Mg^{++}-ATP-ase enzyme /165/.

In clinical medicine one of the most important marker enzymes is alkaline phosphatase. Using bile duct ligation as a cholestasis model, alkaline phosphatase activity is increased in the serum as well as in the liver /134, 136, 236/. This enzyme is found in hepatocellular and

ductular epithelial cells, hepatocellular cells being more stimulated than ductular cells after bile ligation /237/. The electrophoretic patterns of the two alkaline phosphatases are different /238/.

Alkaline phosphatase is also present in the cytosol fraction of the hepatocyte, but only the membrane bound fraction is elevated in cholestasis /134/. It is probable that during cholestasis in man the alkaline phosphatase is derived from the basolateral membranes since the activity spreads from the primary canalicular location to the whole membrane /236, 239/. Using precursor incorporation methods it has been found that following bile duct ligation the increased alkaline phosphatase is derived from increased synthesis /136/, although the RNA content of hepatocytes remains unchanged, indicating that the rise of alkaline phosphatase activity is translationally regulated /134/. In cultured hepatocytes as well as *in vivo*, bile salts stimulate specific alkaline phosphatase synthesis; increased bile salt concentration may represent the role of a stimulatory messenger in cholestasis /240-242/. Alkaline phosphatase activity is enhanced in both extra- and intrahepatic cholestasis /134/.

b) Lipids

The composition and structural properties of lipids in hepatocyte plasma membranes are different from those present in intracellular organelles. The major lipid components are phospholipid and free cholesterol. Membrane-bound lipid also shows marked variations. The canalicular membrane is relatively rich in free cholesterol, phosphatidylserine and sphingomyelin /198, 243-245/ indicating a less fluid membrane. Bile canalicular fractions show greater viscosity than the sinusoidal membranes /245/. There are, however, limitations in the interpretation of the significance of the alterations in lipid composition of plasma membranes, and their role in structure and function as related to the injury occurring during the manifestation of cholestasis. Moreover, the physiological significance of the marked membrane lipid differences has not yet been established. The high cholesterol and sphingomyelin concentration in the epithelial membranes is probably associated with the rapid turnover of membrane components. Acute but not chronic administration of cholic acid depletes total plasma membrane phospholipids without modifying specific phospholipid species /246/.

Changes in membrane-bound free cholesterol are known to influence enzyme function, water permeability, lipid fluidity and transport processes in most membranes /247-249/. In hepatocyte membrane fractions, taurolithocholic and lithocholic acids increase free cholesterol content two- and six-fold, respectively /225/. Ethinyl estradiol elevates membrane-bound cholesteryl ester content /227/; however, lower doses of this steroid exert no action on cholesteryl ester accumulation, although bile flow, membrane fluidity and Na^+/K^+-ATPase activity are affected /250/.

Membrane lipids play an essential role in the formation of the lipid bilayer in the membrane which determines the physical state of this structure /251/. This bilayer also regulates cross membrane movements associated with the function of the ion pumps and transporters /245/. In the physical state, the major determinant of membrane fluidity is the lipid composition of the membrane and specifically the cholesterol:phospholipid ratio is an essential factor. Other influencing factors include phosphatidylcholine:sphingomyelin ratio, phospholipid head groups, and the degree of saturation and length of fatty acid side chains /252/.

Dietary factors can modify membrane fluidity and through fatty acid changes they may partially reverse ethinyl estradiol-induced cholestasis /253/. In several cholestasis models, plasma membrane fluidity has been measured. Following chlorpromazine /229/ or ethinyl estradiol administration /173/, or thyroidectomy /233/, the ensuing cholestasis is associated with a decreased membrane fluidity. It is likely that alteration of membrane lipid composition and consequent fluidity changes are involved in many phases of cholestasis.

3.4 Cholestatic factor in liver tissue

From lymphocytes sensitized by stimulation with specific antigens a new lymphokine has been isolated, acting as a cholestatic factor /254-257/. This factor can produce a marked reduction in bile flow and bile acid excretion. The cholestatic factor can be produced *in vitro* from peripheral blood lymphocytes of patients with drug-induced allergic hepatitis by activating them with a drug in the presence of a soluble liver fraction containing liver-specific lipoprotein. It can also be isolated from the lymphocytes of patients with viral hepatitis showing definite characteristics of intrahepatic cholestasis /258/. Potent cholestatic factor activity could be detected in the supernatants

of tuberculin-sensitized guinea-pig lymph node cell cultures after stimulation with a purified protein derivative /259/.

Injections of the cholestatic factor containing lymphocyte culture fluid into rats through the mesenteric veins induced typical histological changes such as dilated bile canaliculi with a decrease in microvilli, which are similar to morphological changes observed in biopsy specimens from patients with intrahepatic cholestasis. The cholestatic factor was found in the supernatant of sensitized lymphocyte cultures of patients with cholestatic and viral hepatitis /260/. In a further study, the presence of this cholestatic factor was confirmed in the liver tissue of patients with acute intrahepatic cholestasis including drug-induced allergic hepatitis, alcoholic hepatitis, lupoid hepatitis and cholestasis due to hepatitis A type, hepatitis B type and hepatitis non-A, non-B type infections /261/. In contrast, the cholestatic factor was undetectable in the liver tissue of patients without intrahepatic cholestasis. Moreover, although severe jaundice was found in patients with extrahepatic obstructive jaundice, cholestatic factor was not detectable in the liver tissue of these patients. It is likely that this novel lymphokine cholestatic factor plays an important role in the development of intrahepatic cholestasis associated with various liver diseases.

IV. STRUCTURAL ABNORMALITIES OF INTRAHEPATIC CHOLESTASIS

Cholestasis is a frequent side-effect of drug therapy /4/. Administration of oral contraceptives /262/, C-17 alkylated anabolic steroids (such as methyltestosterone /263/), phenothiazines, especially chlorpromazine /6/, methyldopa /264/, niacin /54/, griseofulvin /265/, Atromid-S /266/, azathioprine /267/, erythromycin /268, 269/, sulfanilamide /270/, isoniazid /271/, coumarin /272, 273/ and warfarin /274-276/, to mention but a few, has been reported to precipitate liver lesions as side effects, mainly manifested as intrahepatic cholestasis in a number of patients /277/.

Two major experimental approaches have been used in investigating the pathogenesis of drug-induced intrahepatic cholestasis: (a) morphological or biochemical studies using intact animals after treatment with cholestatic drugs; (b) biochemical studies using the isolated perfused liver, hepatocyte culture, or subcellular material to quantify the effects of cholestatic drugs on specific cellular functions

that are manifested in the whole cell, in the endoplasmic reticulum or in other subcellular organelles, and in canalicular membranes.

Cholestatic lesions induced in animals with drugs and other chemical agents are structurally and functionally similar to those seen in clinical conditions in man. Animal studies have contributed to the emergence of several theories on the mechanism of intrahepatic cholestasis. Based on these studies, the following major hypotheses have been formulated.

4.1 Impairment of sinusoidal membrane function

Integrity of the sinusoidal cell membrane is essential for the mediation of hepatocellular uptake of substances from the blood. Inhibition of carrier-mediated uptake of bile acids into isolated liver cells /278/, and into isolated perfused liver /279/ by cholestatic steroid hormones provided evidence for the involvement of impaired sinusoidal membrane function in the pathogenesis. A decreased rate of bile acid uptake was postulated to cause cholestasis, and decreased intracellular levels may trigger off bile acid synthesis /280/.

4.2 Impairment of the endoplasmic reticulum

The hepatic endoplasmic reticulum is one of the major sites where drugs and other foreign compounds are metabolized. Disturbances in their function may lead to reduced capability of these membranes to process these substances. Impairments brought about by toxicants may also modify the structure and function of the endoplasmic reticulum (Fig. 6).

Since steroid hormones also inhibit microsomal hydroxylations /281/, the biosynthesis of mono- and dihydroxy bile acids (lithocholic and chenodeoxycholic acid) would be dominant, rather than trihydroxy bile acid (cholic acid). Mono- and dihydroxy bile acids exert lesser micellar action resulting in bile stagnation and intrahepatic cholestasis /148, 282/.

4.3 Interference with intracellular binding and distribution

The liver contains cytoplasmic carrier proteins /188, 262/. Organic anions, such as bilirubin and bile acids, are strongly bound to these proteins. If the binding capacity of these proteins is restricted,

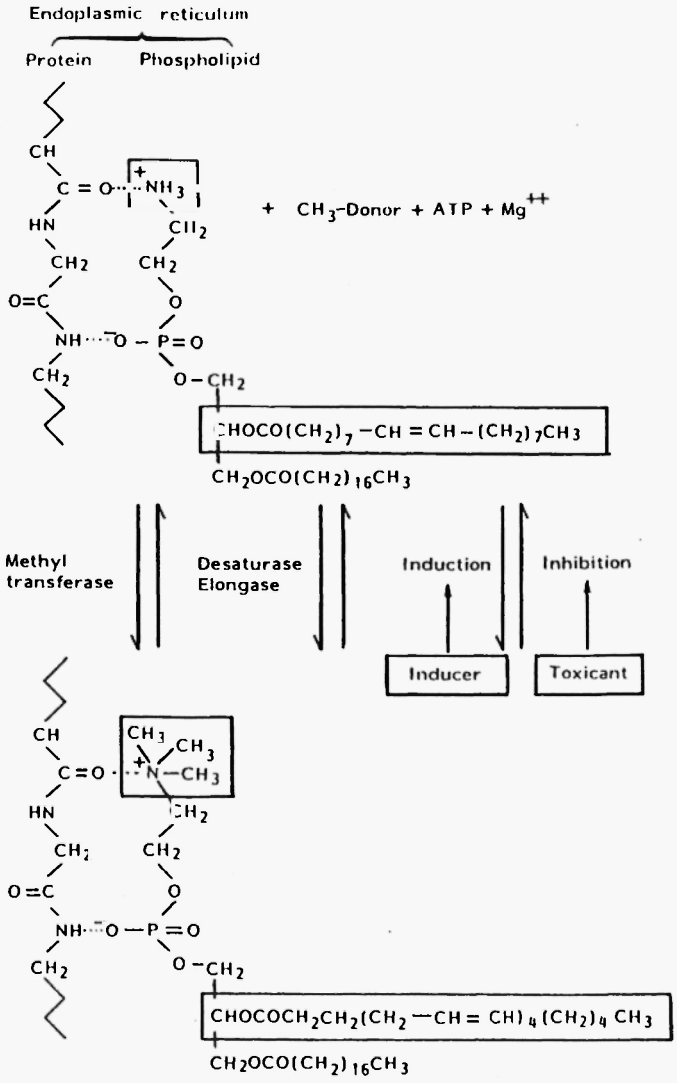

Fig. 6: Schematic presentation of molecular changes associated with the hepatic endoplasmic reticulum brought about by inducers of drug metabolism or hepatotoxicants. Inducers shift membrane bound methyltransferase, fatty acid desaturase and elongase activities producing membranes rich in methylated phospholipid (lecithin) and polyunsaturated fatty acids, thus changing membrane fluidity. On the other hand, toxicants cause a reduction of methyl transferase, fatty acid desaturase and elongase activities, resulting in membranes deficient of methylated phospholipids and reduced fluidity.

increased intracellular concentrations of these bile acids, especially mono- and dihydroxy bile acids, could exert adverse effects on microsomal and mitochondrial enzymes, resulting in exacerbation of the elevated bile acids. Ethinyl estradiol, a known cholestatic drug, and bile duct ligation have both been shown to decrease the amount of carrier proteins /188/. On the other hand, phenobarbital has an opposite effect by increasing the amount of binding proteins /55, 283/. This further suggested a close correlation between the availability of hepatic proteins and cholestasis.

4.4 Altered bile acid metabolism

In intrahepatic cholestasis the smooth endoplasmic reticulum is increased in quantity but decreased in activity /283/. This led to the concept that due to a "hypoactive hypertrophic smooth endoplasmic reticulum", reduced hydroxylation of bile acids is the initiating event in cholestasis /30/. This hypothesis suggests that in particular an impairment of 7α- and 12α-ring hydroxylation of cholesterol to form di- and trihydroxy bile acids, would lead to excessive amounts of monohydroxy bile salts within the hepatocyte and consequent bile stagnation due to their low micellar activity (Fig. 7). It has also been suggested that impairment of conjugating rates of bile acids may lead to cholestasis /284/ since only conjugated bile acids supposedly enter the bile.

4.5 Interference with mitochondrial energy supply

Most of the cellular ATP, which serves as the metabolic energy supply, is synthesized in the mitochondria. In cholestasis, steroid hormones and bile acids inhibit electron transfer in the mitochondrial respiratory chain *in vitro* /281-284/. This causes a reduced ATP synthesis and consequently intrahepatic cholestasis, since the uptake, metabolism and excretion of bile acids as well as bile flow are energy-dependent /285-289/.

4.6 Alterations of the canalicular membrane

Intrahepatic cholestasis is accompanied by bile canaliculi dilatation and loss of microvilli, but there are also alterations in canalicular membrane composition and enzyme activities. Several reports have

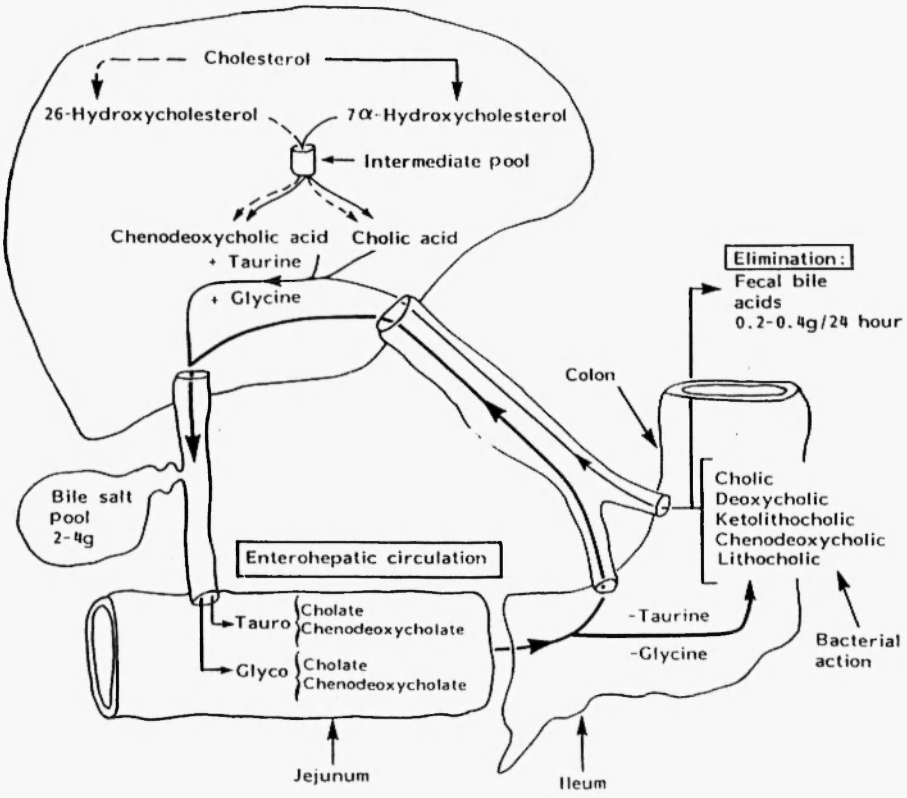

Fig. 7: Bile acid metabolism. Adapted from Javitt NB: Bile salts and hepatobiliary disease. In: Diseases of the Liver, Fourth ed. Schiff L. (ed), Lipincott, Philadelphia-Toronto, 1975 p. 111 /961/.

indicated that ethinyl estradiol decreases canalicular membrane Na^+/K^+-ATP-ase activity, together with an increase in alkaline phosphatase and sialic acid contents /43/. Decreased Na^+/K^+-ATP-ase may result from diminished membrane enzyme content or a change in the enzyme activity /290, 291/, leading to reduced bile flow and consequently cholestasis. Altered membrane composition has been

suggested as the cause of impaired membrane function and drugs such as steroid hormones elicit this effect through their capacity to interact with the membrane and alter the microenvironment of carrier proteins /292/.

4.7 Alterations in microtubules and microfilaments

Microtubules and microfilaments, situated at both the canalicular and sinusoidal membranes, are important for the stability of cell structure and are involved in various transport processes /293/. Interference with microtubule formation by colchicine resulted in a decreased secretion of protein and phospholipid into the blood, but had no apparent effect on biliary secretion or bile flow. This may indicate that biliary secretion does not occur via vesicles /294/. In contrast, cholestasis with characteristic dilatation of bile canaliculi and loss of microvilli is produced by the administration of cytochalasin B which reacts with microfilaments /295/.

4.8 Interference with bile secretion

Decreased bile flow is not necessarily due to a decreased secretory rate, but could also result from increased permeability of the canalicular membrane or tight junctions, which may result in stasis due to reflux of secreted material /296/.

Formation of precipitates by lithocholate, or other monohydroxy bile acid derivatives, which are poorly solubilized by biliary micelles, has been suggested as the cause of bile stasis due to obstruction of the bile canaliculi /148/. Such precipitates of lithocholate have been observed under the electron microscope in experimental conditions /297/. In the case of chlorpromazine, precipitate formation has been proposed via an immune response /298/.

4.9 Interference with the synthesis of membrane-bound phospholipid

Systematic studies from our laboratory indicated that in conditions that favor the development of mild cholestatic changes the synthesis of phosphatidylcholine from phosphatidylethanolamine is particularly affected. These conditions include: (a) pregnancy associated with high serum estrogen and progesterone levels; (b) treat-

ment with certain progesterone derivatives; (c) pre- and neonatal hepatic development of hepatocyte structural elements; (d) impaired function of the liver and subcellular organelles especially endoplasmic reticulum due to the action of some drugs. Drug treatment /19, 299-308/, pregnancy /84, 309-313/, various reductive progesterone derivatives /314, 315/, neonatal hepatic development /316/, dietary methyl donor /302, 306, 309/ and choline deficiency /301, 317, 318/ particularly affected membrane-bound phospholipid synthesis /295, 319-322/.

The impairment of the hepatocyte function brought about by the treatment of rats with low levels of carbon tetrachloride, coumarin, reduced progesterone derivatives (5α- or 5β-pregnane-3β-20-one, 5α- or 5β-pregnane-3β-20β-diol), pregnancy and delayed development of drug metabolizing enzymes in the newborn is associated with a reduction of membrane bound phospholipids /309, 316, 325-332/. This is probably due to a decreased phospholipid synthesis as measured by total liver phospholipid content, *de novo* incorporation of the methyl group from [^{14}C-Me]-L-methionine into phosphatidylethanolamine and S-adenosyl-L-methionine:microsomal phosphatidylethanolamine methyl transferase activity /306/. The reduction of phospholipid in hepatocytes and endoplasmic reticulum is mainly manifest in phosphatidylcholine, phosphatidylethanolamine and lysophosphatidylcholine fractions. Phospholipid fatty acid content is somewhat diminished in saturated acids, palmitic and stearic acids, and in several unsaturated components including arachidonic, oleic and linoleic acids. All these changes are reversible: both hepatic and microsomal phospholipids and fatty acids return to the control levels 2-4 weeks after cessation of drug treatment, or reach adult levels 5-6 weeks after birth, or return to non-pregnant levels 2-3 weeks following delivery.

Some progesterone derivatives induce phospholipid synthesis, increase hepatic and microsomal phosphatidylcholine content and enhance the production of unsaturated and polyunsaturated fatty acids bound in phospholipids /328, 333, 334/. These steroids include 16α-hydroxyprogesterone and pregnenolone-16α-carbonitrile /310/. These compounds show a mild induction effect on the hepatic endoplasmic reticulum similar to barbiturates, polycyclic aromatic hydrocarbons or organohalogen pesticides /323/. Among other enhanced enzyme activities the inductive action increases UDP-glucuronic acid:bilirubin glucuronate transferase activity which actually leads to

reduced hepatocyte bilirubin content and faster elimination through bile canaliculi /324, 325/.

The view that S-adenosyl-L-methionine reverses abnormal membrane fluidity is controversial /335, 336, 339, 340/; the effect of S-adenosylmethionine on estrogen induced cholestasis can be explained by the increased methylation enhancing the drug metabolizing activity of the endoplasmic reticulum membranes. Thus, the estrogens can be converted to non-toxic substances (Fig. 6) /337-339, 341/. The metabolism of other agents is improved by S-adenosylmethionine administration /342, 343/. Another view is that S-adenosyl-L-methionine as a methyl donor participates in the increase of the membrane phosphatidylcholine content which alters membrane fluidity /291, 339, 340/. This effect is related to an antagonizing action of S-adenosylmethionine on bile cholesterol supersaturation brought about by ethinyl estradiol /344/. Studies from our laboratory revealed that methylated drugs enhance the proliferation of the endoplasmic reticulum in the rat liver parallel with increased activity of drug metabolizing enzymes. These drugs also increase the phospholipid content of microsomes /301, 306/. This prompted the hypothesis that methylation of phospholipids may be intrinsically associated with the drug metabolizing function of microsomes. Methyl group containing substances included 4-methylcoumarin, butylated hydroxytoluene-O-methyl ether, methamphetamine and nicotine /301, 319/. Sex differences in drug metabolism between male and female rats are also related to the phospholipid composition of hepatic microsomes /299, 300/.

In the absence of natural methyl donors, such as methionine or choline, methylated drugs served as precursors for the *de novo* formation of phosphatidylcholine (lecithin) /304, 306, 319/. Variations of dietary protein and phospholipid precursors exerted changes in the activities of both methyl transferase and drug metabolizing enzyme systems in a similar fashion /301, 302/. The latter group of enzymes includes UDP-glucuronic acid transferase involved in the metabolic elimination of bilirubin from the liver cell. Dietary manipulations that altered the source and amount of methyl donors, also affected the induction of drug metabolism by drugs, paralleled by changes in the synthesis of microsome-bound phospholipids, particularly the formation of phosphatidylchloline through the action of methyl transferase. The administration of the parent compounds which contain no methyl groups led to a reduction of proliferation, de-

creased drug metabolizing enzyme activity, and decreased phospholipid synthesis. Prolonged administration of these compounds may cause an impairment of hepatocyte function similar to the effect of hepatotoxic chemicals. These observations strongly support our view that the methyl transferase enzyme provides a major controlling point in the metabolism of foreign compounds. Especially in the synthesis of methylated phospholipids, phosphatidylcholine may play a key role in this regulatory function. By modifying microsomal phospholipid synthesis in hepatocytes, the action of various foreign compounds including drugs may reflect subclinical changes involved in the primary stages of cholestasis.

V. EXPERIMENTAL MODELS OF CHOLESTASIS

Various animal models have been used that simulate cholestasis in man for the elucidation of different steps in the pathogenesis of this disease. There are certain limitations in the interpretation of these experimental models, such as the doses used to cause cholestasis are generally much greater than those producing cholestasis in clinical conditions and dose-response relationships have not been explored adequately. Moreover, the different experimental conditions rarely point to a specific selective site of action. It is also difficult to establish, among the observed abnormal changes, which are the primary or secondary events associated with bile stagnation in the hepatocytes leading to reduced secretion. Usually in a special model only one of several potential changes due to the cholestatic action can be studied. It may be that cholestasis connected with a reduced bile, water and solute excretion represents the terminal manifestation of complex abnormalities resulting from the disturbed intracellular metabolism.

Some of the most important and well established experimental models are discussed in this review. Several of these studies have been summarized in earlier reviews and books /2, 4, 33, 37, 345, 346/.

5.1 Bile duct ligation

The experimental procedures represent a close approximation for studying extrahepatic mechanical obstruction in man. Changes

brought about by bile duct ligation in gap and tight junctions have been well characterized /347-350/.

These investigations show distortion of the tight junctional strands and a loosening of the network indicating an enhanced permeability of the biliary tree /349, 350/. In physiological circumstances the increased permeability is reversible and returns to normal after the relief of the obstruction /137/. Morphological changes of the canaliculi resemble the processes of perinatal maturation associated with increased formation of new canaliculi which rapidly disappear when conditions are normalized /239/.

Bile duct ligation is a widely studied model, clearly demonstrating that changes in cholestasis are manifest in many parts of the hepatocytes and afflicts not only the excretory structures. Bile duct ligation causes a loss of the cell polarization connected with an alteration of many enzymes; a particularly characteristic change is the elevated synthesis of alkaline phosphatase /236, 351-353/. This increase is due to an induction process related to a heat-stable low-molecular weight compound present in the bile. Bile acids can only partly stimulate this action /236, 354/. Bile duct ligation also causes changes in the hormonal responses of the hepatocytes /303, 304/. This procedure affects the function of the endoplasmic reticulum which may be connected with the raised level of bile acids /354/. In the rat, prolonged ligation is connected with a biliary type of cirrhosis /355/. Using a model of selective biliary obstruction, changes brought about by increased biliary pressure due to the obstruction, and the systemic effects of retained substances can be separated /356, 357/.

Another animal experiment related to the choledochocaval fistula in the rat provided a better model for the clearer separation of the effects due to biliary stasis and those caused by obstruction /358/. It has been shown that the retention of substances normally occurring in the bile represents a minor factor in the cellular damage. The morphological changes are minimal in the choledochocaval fistula model as compared with rats with obstructed livers. High biliary pressure causes an increase in the resistance of the biliary outflow. In this model the excretion of the bile-salt-independent fraction of the bile, the net secretion of canalicular water /359, 360/, and the maximum secretory rate of bile salts are all greatly reduced /361/.

In a recent study using dogs, elevated serum bile acid concentration following experimentally induced hyperbilirubinemia was reported /362/. Obstructive jaundice was produced by surgical ligation

of the common bile duct and hepatocellular jaundice was created by dimethylnitrosamine administration. Serum bile acid concentration was increased in both conditions; it increased slowly after dimethylnitrosamine, but rose rapidly following bile duct ligation. The serum bile acid concentration is correlated with cholestasis and bile duct proliferation observed in liver biopsy specimens, but did not show any relation with inflammation or necrosis. There was also a positive correlation with serum alkaline phosphatase and alanine transaminase activities, and bilirubin and cholesterol concentrations. The controversies concerning hepatocyte bile secretion have also been pointed out in some publications /2, 363/.

5.2 Isolated liver perfusion

The methodology of isolated liver perfusion is relatively simple /364-367/; still, this model has not been frequently applied for studying the pathomechanism of intrahepatic cholestasis. In a recent study using isolated rat livers three basic mechanisms could be differentiated /368-370/: (a) In the livers of rats treated with α-naphthylisothiocyanate (ANIT), the biliary-sinusoidal barrier showed increased permeability associated with morphological alterations of the tight junctional complexes as revealed by electron microscopy. Consequences of these changes included reflux of bile constituents such as taurocholate, dyes such as sulfobromophthalein and increased access of paracellular markers such as sucrose and inulin to the biliary space. (b) In the development of cholestasis induced by estrogens, the primary event seemed to be associated with an inhibition of the basic process of fluid secretion. Rats were treated with ethinyl estradiol and in the isolated liver bile flow was diminished. However, the permeability of the biliary tract to sucrose and inulin remained unaffected. The maximum concentration of taurocholate in the bile was raised, indicating that its secretion was sustained. The administration of estradiol valerate to rats resulted in the same effect in isolated perfused livers. However, after 3 weeks of treatment, taurocholate concentration in the bile was decreased and sucrose clearance was raised. These results indicate that in the development of cholestasis estrogens exert a primary action to inhibit bile flow and as a consequence they have the potential to increase tight junctional permeability /136, 371/. (c) Secretory inhibition could be induced by a reduction of the concentration of Ca^{2+} in the perfusate. Using

ANIT-pretreated livers which have a very low capacity to secrete dyes, the high rate of sulfobromophthalein excretion into the perfusate indicates that due to Ca^{2+} deprivation the efflux of cholephilic solutes is stimulated across the sinusoidal membranes /370, 372/.

These results show that intrahepatic cholestasis may be related to the action of the cholestatic agent on a number of different sites and interference with the normal function of the hepatocytes, particularly with the complex process of bile secretion. Cholestasis induced by ANIT appears to be mediated by a relatively simple mechanism, a primary lesion on the tight junction. Still, we do not know the primary attack leading to this lesion. The inhibition of bile flow by estrogens seems to be more complex; perhaps the plasma membrane-bound Na^+/K^+-ATP-ase is the mediator of this phenomenon /227/. The cholestatic changes following Ca^{2+} reduction may be associated with the diverse cellular regulatory functions of calcium, especially the general dependence of secretory processes on calcium concentration /366, 373-375/.

5.3 Calcium depletion

Calcium plays an important role in the regulation of metabolism, secretion, cytoskeletal structural integrity, motility, cell division and many cellular activities /376-379/. The modulation of such diverse functions is regulated by calcium homeostasis connected with active transport and sequestration into subcellular compartments /379, 380/. The endoplasmic reticulum is a key site for calcium binding and storage /381, 382/. Part of this bound calcium is released rapidly during the process of signal transduction after the binding of hormones and growth factors to cell surface receptors /383/. Calcium also serves as a cofactor in the action of catabolic enzymes such as phospholipases, proteases and endonucleases. Its release from storage has been suggested to be involved in some type of cell death /384, 385/. The discharge of calcium into the cytosol has been considered as an early event in hepatotoxicity brought about by cystamine /386/ or menadione /387/.

Cholestasis induced by perfusion of isolated rat livers with hypocalcemic media produces several defects in bile secretion including an increased biliary permeability /366, 388-390/, probably caused by an impairment of the tight junctions sealing the canaliculi. Recent studies, however, report controversial correlations between perme-

ability and the structure in hepatocellular tight junctions /391-393/. Using quantitative stereological analysis it was found that removal of calcium from the medium did not modify the morphological organization of the tight junctions /394/. It decreased the volume fraction of hepatocytes and caused a marked efflux of potassium, hence it disturbed the osmotic equilibrium in the cell, probably by an impairment of the ion transport system involved in the control of the hepatocytes' volume.

In another study using isolated perfused rat liver, the cholestatic effects of lithocholic acid or taurolithocholic acid was tested on calcium homeostasis /395/. No changes were found in the intracellular distribution of calcium and especially there was no depletion or release of calcium from the endoplasmic reticulum. The presence of extracellular calcium affected taurocholate transport in isolated perfused livers. Removal of calcium leads to cholestasis /364, 397/ associated with diminished biliary taurocholate excretion and bile flow and increased paracellular permeability. The uptake of taurocholate by the hepatocytes and its release are independent of extracellular calcium. Recent studies have shown that free bile acids and bile acids bound to micelles and phospholipids can bind calcium /398, 399/. The hydroxyl groups of bile acids are involved in this binding, therefore various bile acids may bind calcium with different affinity. The affinity of binding calcium by cholestatic bile acids is an order of magnitude greater than that of non-cholestatic bile acids /400/. Thus it can be considered that some cholestatic agents, such as bile acids and certain drugs, may bind enough calcium to decrease canalicular calcium concentration below a critical level which disturbs permeability. It is likely that changes in extra- and intracellular calcium concentrations are involved in the pathogenesis of cholestasis, but other factors may alter cellular events at early stages of cholestasis before paracellular permeability is affected /396/. It has been reported that impairment of calcium absorption during childhood was associated with chronic cholestasis /983/.

5.4 Hypophysectomy and hypothyroidism

Thyroid hormones probably exert a role in the regulation of hepatic excretion. In experimental studies it has been found that hypophysectomy is connected with a reduced bile flow and modified

sulfobromophthalein and bilirubin retention. These effects could be reversed by the addition of thyroid hormones /401/. In hypothyroid rats the bile-salt-independent fraction of the bile flow is decreased in association with reduced membrane-bound Na^+/K^+-ATP-ase activity /402/. Thyroid hormones increase bile-salt-independent bile flow and Na^+/K^+-ATP-ase activity /402/ and decrease membrane fluidity /403/. The effect of thyroid hormones on the function of hepatic plasma membranes may be mediated through the regulation of synthesis of specific proteins or phospholipids.

In some hyperthyroid patients signs of intrahepatic cholestasis are described /404/ associated with markedly increased serum alkaline phosphatase, γ-glutamyltransferase and leucine aminopeptidase activities, and overt jaundice. Methimazole therapy further enhanced the hepatic lesion. Therapy with propranolol and methimazole together was connected with transient deterioration of the liver function. After euthyroidism was achieved and propranolol administration was discontinued, liver function became normalized despite continuation of methimazole therapy. Propranolol seems to exert further deterioration of liver function in patients with cholestasis due to hyperthyroidism.

5.5 Immunologic model

Immunologic techniques have also been used to produced experimental intrahepatic cholestasis. Heat-killed *Propionibacterium acnes* was injected intravenously to tuberculin-sensitized guinea-pigs and 7 days later purified protein derivative was also administered intravenously. This treatment resulted in a significant reduction of bile flow and bile acid excretion, increased serum levels of alkaline phosphatase and leucine aminopeptidase activities, and bile acid and cholesterol contents. Morphologic examinations have shown a decrease of microvilli and dilatation of bile canaliculi indicating that intrahepatic cholestasis was brought about by the treatment /405/. In this model, intrahepatic cholestasis was induced when tuberculin-sensitized lymphocytes infiltrated the liver and the cholestatic factor was produced by the administration of purified antigen proteins.

5.6 Hormones

a) Estrogens

In studying intrahepatic cholestasis, the administration of estrogen to experimental animals and to humans represents an excellent model /107, 406-409/. The clinical observation was made that as a side effect of oral contraceptive steroids containing synthetic estrogens, a cholestatic syndrome developed in some women; this led to the initiation of animal studies with estrogens /410-413/. Following high doses of megestrol acetate for metastatic breast carcinoma, intrahepatic cholestasis developed /414/. Similarly, cholestasis was induced by estrogen therapy after liver transplantation /415/. In late pregnancy when steroid hormone synthesis is significantly increased, hepatocyte changes are apparent as reflected by decreased maximum excretory transport of bromsulfophthalein (BSP). Estrogens reduce bile flow of both bile acid dependent and independent fractions /411, 412, 416/. These effects are associated with increased permeability of the biliary tree /410, 417/, increased viscosity of hepatocyte membranes /403, 418/, or decreased activity of the Na^+/K^+-ATP-ase /227, 291, 419/. The excretion of bilirubin, bile salts, and other organic anions and the elimination of BSP are also decreased following treatment /126, 291, 420-423/.

Electron microscopic studies demonstrated only minor abnormalities in the arrangement of the strands of the tight junctions /368, 369, 371, 417/, and serum markers of cholestasis are not elevated by ethinyl estradiol administration /407, 424/. Ethinyl estradiol produces subclinical changes with minimal lesions. This action indicates that biochemical changes in hepatocytes affect the primary involvement in the pathogenesis of reduced bile secretion following treatment with estrogens. The primary abnormality in estrogen-induced cholestasis is at the sinusoidal membrane, probably connected with abnormal lipids and proteins affecting normal membrane fluidity.

There is some evidence indicating that the D-ring glucuronide conjugate of estradiol is cholestatic in rats /423/. Several other steroids conjugated with glucuronic acid on the D-ring produce a dose related cholestasis which is reversible after the removal of the steroid. A-ring conjugates do not induce cholestasis. It has been suggested that the glucuronide formation is required for the cholestatic effects of various steroids /416/.

In experimental studies using rats, the administration of estradiol 17β-valerate caused a reduction of basal and taurocholate-stimulated bile flow /424/. In contrast, the concentration of taurocholate in the bile was elevated. Clearance studies indicated that the altered permeability of the biliary tree is not the primary event, but it occurs subsequent to the cholestasis brought about by estrogens.

There are two current theories explaining the pathogenesis of cholestasis induced by various estrogens. According to the first view, estrogens do not affect bile secretion from the hepatocytes, but cause an increase of the permeability of the biliary canaliculi which allows the back diffusion of water, electrolytes and other substances into the cell /409, 410, 425/. Electron microscopic studies provide evidence for the feasibility of this theory /142, 368, 369, 432/, but cholestasis induced experimentally with estrogen in rats shows some biochemical effects, including defective transport of taurocholate, which are somewhat contradictory to this view /406, 411, 426-428, 433/.

According to the second theory the reduced transport of electrolytes and organic anions is due to a lesion of the transport function secondary to the impaired plasma membrane fluidity of the hepatocytes /373, 227, 291, 373, 419/. The decreased fluidity is specially manifest at the sinusoidal surface. Further studies indicate that membrane fluidity changes are connected with changes in the composition of phospholipid fatty acids /424, 429, 434/.

A recent publication questions that the fluidity of liver plasma membranes is the rate-limiting determinant of bile flow in estrogen-induced cholestasis /431/. In this study the effects of ethinyl estradiol and spironolactone on bile flow and membrane fluidity were compared. Spironolactone does not have a phenolic D-ring necessary for the estrogens to cause cholestasis. Bile flow was reduced by ethinyl estradiol and increased by spironolactone; however, both compounds reduced fluidity and membrane Na^+/K^+-ATP-ase activity, and increased cholesterol ester and sphingomyelin content of the membrane. These findings indicate that although both compounds elicit similar changes in the lipid composition and fluidity of the plasma membrane, they have a diverse action on bile flow leading to cholestasis, and thus the association between these parameters may be secondary.

The role of estrogen receptors in mediating the changes occurring in estrogen-induced cholestasis has been questioned /409/. Hepatic estrogen receptors have been well characterized /435, 436/ and there

is evidence that these take part in a variety of estrogen effects, such as the elevated synthesis of sex-steroid binding globulin /437/, thyroxine-binding globulin /438/, ceruloplasmin /407/ and renin substrate /439/. Estrogen receptors may mediate an increased synthesis of alanine carriers and hepatic plasma membrane low density lipoprotein receptors and a parallel decrease of bile acid and organic anion carriers which may be related to the early stages in the development of intrahepatic cholestasis. Identification of proteins in the membranes at the receptor site may also be responsible for the transport of organic anions such as bile acids, and interference or competitive binding to these sites may provide a new insight into the mechanism of estrogen-induced cholestasis /193, 440-443/.

We have found that the hepatic endoplasmic reticulum contains bound progesterone receptors /444, 447/, which may have some role in the regulation of drug metabolizing activity of these membranes. Impairment of the endoplasmic reticulum by steroid hormones or drugs and other chemicals may be mediated via the progesterone receptors. Recent experiments have reported that ^{3}H-estradiol-17β(β-D-glucuronide) specifically attached to a high affinity binding site in canalicular membranes. The cholestatic D-ring glucuronides and taurocholate also bind to this site, but in contrast the non-cholestatic A-ring glucuronides do not /963/. The nature of this binding site has not yet been established but it may be the bile acid carrier site in the canaliculi where taurocholate regulates bile flow. It seems, furthermore, that the increased paracellular permeability induced by estradiol-17β(β-D-glucuronide) is causally associated with cholestasis and during chronic estrogen administration sufficient amounts of estrogen glucuronides are produced causing estrogen cholestasis /964, 965/.

b) Other steroid hormones

Some estrogen metabolites, such as estradiol-17-glucuronide, are potent inhibitors of bile flow /411, 416/. The effect of this glucuronide is immediate in a dose-dependent fashion. The administration of other D-ring glucuronide conjugates of estrogen derivatives to rats caused an immediate dose-dependent and reversible cholestasis established by BSP retention /409, 416, 417/. These steroids include estrone, estradiol, estriol, epi-estriol, estradiol-17-acetate, estradiol-3-acetate, estradiol-3-methyl ether, 11β-hydroxyestrone, 17-dioxy-

estrone, 16-ketoestrone, 2,17α-dimethylestradiol, mestranol, ethinyl estradiol 16- and 17-conjugates, equilenine and diethylstilbestrol /235, 422, 449/. A-ring conjugates (3-glucuronides) elicited choleresis /416/. The mechanism of induction of cholestasis by these compounds is not known, but it is consistent with the view that some specific receptor sites are involved.

In studying the structure-activity relationship it was established that the incidence of BSP retention was highest with norethindrone and normethandrone, moderate with methandriol, methyltestosterone and fluoxymesterone and low with ethisterone /448/. The presence of a keto group in position 3 represented a greater potential for BSP retention than the 3-hydroxyl group in the same position; the C17-alkyl group could modify this response. The effect of these steroids on BSP retention is unrelated to progestational, anabolic or androgenic properties of the steroid.

In experimental animals taurocholate administration provides protection against estradiol-17-glucuronide-induced cholestasis /412/. The interaction between estradiol-17-glucuronide and taurocholate is not purely competitive. Taurocholate stimulates the biliary excretion of estradiol-17-glucuronide and excess estradiol-17-glucuronide enhances taurocholate secretion, indicating an interaction between these compounds at their respective carriers. The detergent Triton WR-1339 reverses ethinyl estradiol-induced bile secretory failure /235/. This observation has been contradicted by another report /413/. In humans, estradiol, estriol and oral contraceptives induce a reversible retention of BSP and a rise of plasma alkaline phosphatase activity /406, 411, 450/. Some progesterone metabolites produced by mothers were also shown to cause pathological jaundice in the newborn /93, 451-456/.

Several anabolic steroids are involved in cholestasis in man. Methyl testosterone and norethandrolone impair the transport of BSP /457/. This report, however, was later questioned /458/. Norethandrolone may affect microfilaments in the cell /459/. Norbolethone also impairs the clearance of BSP and in higher concentrations decreases bile flow in isolated perfused rat livers /460/. Estradiol, progesterone and norethandrolone inhibit taurocholate uptake into hepatocytes /427/. Some androgens also impair the uptake of bile salts in isolated hepatocytes /427/. It was suggested that probably various steroid metabolites are responsible for the development of cholestasis. In another study it was shown that

17β-alkylation represents a specific requirement for BSP retention in rabbits treated with steroids /461/. The degree of BSP retention was varied and unrelated to the androgenic or myotropic actions of these steroids. The tested steroids included methyltestosterone, methandrostenolone, methylandrostenediol, ethylestrenol, norbolethone, norethandrolone, oxymetholone, oxandrolone, oxymesterone, bolasterone, fluoxymesterone and stanosol. The administration of methyltestosterone brings about fibrosing cholangiolitis /992/.

Although the precise mechanism by which various steroid hormones induce cholestasis is not known, the animal models provide an important insight into the factors that regulate the generation of bile flow and transport of bile acids and organic anions. Application of these methods has been useful in the identification of proteins in the canalicular and basolateral membranes which are probably responsible for the transport of organic anions /462-466/.

5.7 Bile acids

Taurolithocholate is described as the first bile acid to cause morphological changes in the liver connected with cholestasis /467/. Acute treatment with lithocholate and its glucuronide brings about cholestasis in experimental animals /999/. Chronic administration of lithocholic acid also induces cirrhosis and bile acid retention in a number of species /468/. A variety of other monohydroxy bile salts can elicit immediate cholestasis and secondary biliary cirrhosis /469/, such as 3-hydroxy-5-cholenoic acid /470/, allo-monohydroxy bile salts /471/ and any bile acid given in sufficiently high doses /449, 472, 473/. Chronic administration of an uncharged bile acid derivative induces cirrhosis in the mouse /474/. Although this compound enters the hepatocyte, it is not secreted along the normal biliary route. Various monohydroxy bile acids may be pathogenic to man in some form, leading to cholestasis /475, 476/. These compounds are metabolized by conjugation and excreted in sulfate or glucuronidate forms. Conjugation, however, does not protect from the cholestatic action of these compounds. Some of the conjugates are even more cholestatic than the parent compounds /477-479/. Monohydroxy bile acids and their derivatives or analogues can cause hepatocellular damage and abrupt cessation of bile flow. These substances occur normally in the body in very small amounts. It is still questionable whether these are

formed from endogenous bile acids by metabolic impairment, or as a result of bacterial degradation and absorption from the intestine by enterohepatic recycling. The effect of some substances other than bile salts, such as alcohol, various drugs and chemicals, or viruses causing hepatic lesion, may be mediated through a promoting action on the production of abnormal amounts of monohydroxy bile acids.

a) Lithocholic and taurolithocholic acid

Lithocholate induces morphological changes in the liver, including loss of microvilli, abnormal lamellar transformations, ultrastructural changes of the canalicular plasma membrane and canalicular dilatations /149, 225, 467/. These effects are connected with a reversible dose-dependent cholestasis /467/. Administration of taurolithocholate shows similar effects /149/.

Lithocholic acid produces a six-fold increase of cholesterol content in the canalicular membrane; the taurine conjugate causes only a two-fold elevation /225/. Several membrane enzymes, including Na^+/K^+-ATP-ase /226, 480/, show profound changes corresponding to loss of subcellular particles /149, 470/. Lithocholate causes an increased canalicular permeability to inert solutes /138/, connected with a defect of the canalicular membrane /481/. Parallel administration of taurocholate blocks the induction of cholestasis brought about by lithocholate or taurolithocholate /138, 467/ by forming a complex molecule /481/. Pregnenolone-16α-carbonitrile reverses the toxic effect of lithocholic acid in the rat /482/. Other steroid and non-steroid hormones are only partially effective. Pregnenolone-16α-carbonitrile is a potent inducer of microsomal drug metabolism. The role of enzyme induction in the protective action is not clear since other microsomal enzyme inducers are partially protective such as phenobarbital or ineffective such as diphenylhydantoin /485-487/.

The enhanced cholesterol level in canalicular membranes is due to increased *de novo* cholesterol biosynthesis /483/ which can be prevented by cycloheximide /484/. The cholestatic effect of monohydroxy bile acids is connected with abnormalities of calcium metabolism together with changes in the integrity of the tight junction /391-393/. Bile acids may be precipitated and thus mechanical obstruction leads to cholestasis /488/.

There is some evidence that simultaneous infusion of primary bile acids with lithocholate provides protection against the development

of intrahepatic cholestasis in experimental animals. In rats following a combined infusion of cholic acid and lithocholic acid, more than 95% of the lithocholic acid was excreted in free form /489/. The increased secretion of lithocholate was associated with a five- to seven-fold rise in biliary cholesterol secretion and a significant increase in phospholipid secretion. It seems that cholic acid protects against lithocholic acid cholestasis by the formation of a lithocholic-cholic acid complex that prevents its toxic effect on the bile canalicular membrane.

b) Chenodeoxycholic acid

Chenodeoxycholic acid has been employed in the therapy of patients with cholelithiasis. In a clinical study, evidence has been provided that intrahepatic cholestasis developed in 64% of patients treated with chenodeoxycholic acid for 9 to 24 months indicating that this bile acid exerts clinically significant hepatotoxicity /490/. In several cases, the development of intrahepatic cholestasis is considered as a common subclinical abnormality in patients with cholelithiasis. The severity of the condition, however, increases during chenodeoxycholic acid therapy.

c) Monohydroxy bile acid conjugates

Observations with bile acid sulfate derivatives indicate that conjugation leads to protection against the hepatic action of bile salts /491-493/. This observation, however, has been recently modified. Taurolithocholate sulfate does not affect bile flow, but lithocholate sulfate and taurine conjugates cause a 20% transient decrease in bile flow. Neither of these compounds exerts any morphologic effects /483, 494/. Glycolithocholate sulfate, on the other hand, diminishes bile flow in a dose dependent fashion and to a greater extent than lithocholate sulfate. A similar effect was found recently with bile salt glucuronide derivatives /367/. The hepatic effect of these conjugates is connected with the appearance of membrane-bound vacuoles which is different from the action of the monohydroxy bile acid parent compounds /494/. It seems, therefore, that the mechanism by which these conjugate compounds produce cholestasis differs from that connected with lithocholate or taurolithocholate-induced cholestasis.

d) Allo bile salts and 5β-hydroxy-5-cholenoic acid

5β-Hydroxy-5-cholenoic acid has been detected in different types of cholestasis and in hepatic waste products. The effect of 5β-hydroxy-5-cholanic acid on the liver is similar to that of lithocholate /470/. This effect is blocked by the coadministration of cholate and chenodeoxycholate /467/. The action of 3β-hydroxy-5α-cholanic acid and its sulfate ester was tested on rats /495/. It significantly reduced bile flow and the secretion of bile salts, cholesterol and phospholipids in a dose-dependent manner. The canaliculi showed dilatation and partial or total loss of microvilli. It seems that allo-monohydroxy bile acids have a four-times greater cholestatic potential than 5β-analogues and that sulfation does not protect against the cholestatic action.

5β-Hydroxy-5α-cholanic acid and its sulfate ester produce a dose-dependent cholestasis, connected with a pronounced decrease of bile salt and lipid secretion /477/. Allo compounds are about 4-fold more potent than their β-analogues. Their effect is connected with dilatation, loss of microvilli, and the presence of intracanalicular precipitates /471/.

VI. XENOBIOTIC-INDUCED CHOLESTASIS

Intrahepatic cholestasis is a toxic side effect of a variety of drugs and chemicals (Table 5). Certain classes of pharmacologic agents have been associated with this adverse cellular response; however, chemical similarities between all these substances have not yet been established. Moreover, the pathogenesis and clinical features of drug-induced cholestasis show differences /986/. The effects of xenobiotics causing intrahepatic cholestasis must be distinguished from other forms of hepatobiliary lesions, namely extrahepatic cholestasis due to mechanical obstruction of the bile ducts and general cytotoxic cellular lesions connected with a massive release of cytosolic enzymes. In many instances the biotransformation of xenobiotics leads to biologically active metabolites, often to highly reactive substances producing acute hepatotoxicity. In contrast, in most cases of intrahepatic cholestasis the changes brought about by foreign chemicals are rather discrete. Only a few drugs with cholestatic potential have been shown to produce reactive metabolites *in vitro*. These

TABLE 5
Various chemicals and drugs causing cholestasis

Inorganic compounds
- Manganese, cobalt

Organic compounds
- Greater than 2% incidence
  - Erythromycin estolate, propionate, lauryl sulfate
  - Norethandrolone
  - Triacetyloleandomycine
- About 1% incidence
  - Chlorpromazine
  - Methyltestosterone and related anabolic steroids
  - Contraceptive steroids
  - Iprindole
  - Acetohexamide, carbutamide, diclorpropamide
- Rare incidence
  - Drugs
    - Steroids: estrogens, estrogen-glucuronides, spironolactone
    - Alcohol
    - Neuroleptics: promazine, prochlorperazine, thioridazine, trifluoperazine, chlorprothixene
    - Anxiolytics: chlordiazepoxide, diazepam, fluorozepam, triazolam
    - Tricyclic antidepressants: amytriptiline, imipramine
    - Tranquilizer-sedatives: ectylurea, mepazine
    - Analgesic: maltrexone
    - Anticonvulsants: carbamazepine, diphenylhydantoin, valproic acid
    - Diuretic: chlorothiazide, ethacrynic acid, perhexiline maleate, tienilic acid
    - Antibacterials, antibiotics: nitrofurantoin, novobiocin, oxacillin, penicillin, rifampicin, sulfadiazine, sulfanylamide, sulfamethoxazole, flucloxacillin, amoxicillin, triacetyloleandomycin, clavulanic acid, azathioprine, thiomethoprim
    - Antituberculotic: p-aminosalicylic acid
    - Antisyphilitic: arsphenamine
    - Antihistaminic: cinnarizine
    - Antithyroids: carbimazole, methimazole, propylthiouracil, thiouracil
    - Hypoglycemics: acetohexamide, carbutamide, chlorpropamide, metahexamide, tolazamide, tolbutamide, glibenclamide
    - Antihypertensive: methyldopa
    - Anticoagulants: phenindione, warfarin
    - Antirheumatics: gold salts, phenylbutazone
    - Hypolipidemic: Atromid-S
    - Immunosuppressive: azathioprine
    - Antiseptic: iodoform
    - Antiamebic: carbarsone
    - Antihelminthic: thiabendazole
    - Antiinflammatory: indomethacin, buculome
    - Antiarrhythmic: procainamide, N-acetylprocainamide ethobromide, prajmaline
    - Neuromuscular blocking agent: D-tubocurarin
    - ß-Adrenergic blocker: propranolol
    - Autacoid: captopril
    - Laxative: dioctylsulfosuccinate
    - Transport inhibitor: probenecid
    - Immunosuppressant: cyclosporins
    - Antineoplastic agents: busulfan, 1,3-bis (2-chloroethyl)-1-nitroso urea, colchicine, vincristine, vinblastine, etoposide urea
    - Radiopaque contrast agents: iodipamide, iodoxamate, ioglycamide

cont.

TABLE 5 continued

Miscellaneous drugs: thioxanthene, nafenopin

Chemicals

Dyes: eosin, fluorescein, bromosulfthalein, BSP-glutathione, 1-chloro-2,4-dinitrobenzene-5-glutathione, phenol red

Amines: diamino-anthropyrimidine, niacin, 4,4'-diaminodiphenylmethane, p-monomethylamino azobenzene, toluenediamine, 2-ethyl-2-phenyl butyramide

Food additive: sucrose acetate isobutyrate

Miscellaneous: dinitrophenol, α-naphthylisothiocyanate, 1,2-dibromoethane, dihydroxy dibutyl ether, diethyl maleate, monochloroacetate

Natural toxins

Plant products: Concavalin A, phalloidin, cytochalasin B, crotalaria, icterogenin, lantana toxins, lectins, theophyllin, cyclopiazonic acid, griseofulvin, sporidesmin, gempin, patrinoside, 6,7-dimethylesculetin, 22ß-angeloyloxyoleanolic acid, ethionine, flavaspidic acid

Bacterial/fungal products: sporidesmin, E. coli endotoxin, aflatoxin B_1

Animal products: thyroxine, hydrocortisone, cyclic AMP, glucagon, vasopressin

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Adapted from Zimmerman HJ: Hepatotoxicity: The adverse effects of drugs and other chemicals on the liver. Appleton-Century-Crofts, New York 1978 /534/, Plaa GL, Priestly BG: Intrahepatic cholestasis induced by drugs and chemicals. Pharm Rev 28:207-273, 1977 /37/; Goldberg DM: Am J Clin Pathol 71:537, 1979 /960/, and from original publications (references see in the text).

include ethinyl estradiol /496, 497/, norethisterone /498/, norethynodrel /499/ and imipramine /500/. In these cases, however, correlations with hepatic injury have not been demonstrated.

Biotransformation might be a prerequisite in cholestasis and in other forms of hepatic lesions, and this might provide an explanation for some of the unique features of drug-induced intrahepatic cholestasis in man, namely the relatively low incidence, the lack of dose-response relationships, and the failure to reproduce some of these liver injuries in experimental animals. Hepatic drug side effects that manifest in multiple locations and associated with various abnormalities in the hepatocytes may reflect the actions of different metabolites. In this review we describe most drugs, chemicals, bacterial toxins and endogenous components that cause intrahepatic cholesta-

sis. Structural modifications and possible biotransformation reactions are also mentioned in some instances.

6.1 Drugs

In normal circumstances, the metabolism and disposition of drugs are adequate and thus therapeutic doses have no harmful effects on hepatic function. The efficiency and duration of the drug effect depend on the rate at which drugs are activated or inactivated. Under certain conditions, however, adverse reactions occur, related either to hepatic dysfunction or to the toxic nature of the foreign compound /501/. These reactions result in hepatic disorders which may be reversible, like drug-induced cholestasis, or irreversible causing long term damage, like cirrhosis.

Intrahepatic cholestasis occurs as a side effect of drug therapy in humans /502-504, 509/ and in animal models /113, 505, 506/. Subsequent hepatic lesions represent substantial health care problems, especially in elderly patients. Drug-induced cholestasis causes 2-5% of the hospitalizations for jaundice and is increasing because orally ingested and absorbed drugs are delivered to the liver directly by the portal vasculature, thus exposing it to drugs in concentrations higher than other organs. Moreover, the liver cell is vulnerable to drug-mediated injury because of its role in the accumulation and biotransformation of drugs.

Drug-induced cholestasis results in the stagnation of bile formed by hepatocytes and the retention of biliary substances in the blood due to failure of clearance. Several drugs produce this type of cholestasis as an adverse effect and drug hypersensitivity manifests itself also in cholestasis /113, 507-571/.

a) Phenothiazines and tricyclic antidepressants

The incidence of cholestasis associated with the therapeutic use of phenothiazines and tricyclic antidepressants is due to side effects related to many factors. The occurrence of cholestasis varies considerably with the chemical structure of these compounds; still, this is the most important group of drugs causing drug-induced intrahepatic cholestasis. It has not been clearly established whether the liver lesion is due to the direct hepatotoxic action of the drugs, or their metabolites, or whether it is connected with a hypersensitivity reac-

tion. Although the effects of several phenothiazines (in particular chlorpromazine) and more recently thioxanthenes and tricyclic antidepressants have been studied in experimental animals, the mechanism of action of these drugs is still not clear. In some cases the morphological appearance of the hepatic damage shows the features of hepatocellular necrosis or hepatitis; in other cases it resembles biliary obstruction.

There is an important species variation in the hepatobiliary effect of chlorpromazine. After intravenous injection in the dog, chlorpromazine causes a decrease in the bile flow accompanied by diminished bile viscosity; bilirubin concentration is increased in hepatocytes /512/. Another investigation confirmed the reduced bile flow which is associated with an enhanced intrabiliary pressure /513/. It is questionable whether the effect of chlorpromazine is direct or mediated by neurohumoral factors since the neural tissue has a great specific affinity to chlorpromazine /514/.

Intravenous infusion of chlorpromazine to rhesus monkeys produces cholestasis, connected with reduced bile flow /515/. The rate of recovery is dependent on the dose administered. Change in bile flow is associated with biliary lipid secretion and it has been concluded that chlorpromazine causes a reduction in both bile lipid-independent and lipid-dependent flow.

In the rat, chlorpromazine exerts no effect on liver function when given either in acute or chronic doses; no change is observed in liver histology /516/. Chlorpromazine elicits ultrastructural changes consisting of dilatation and vacuolization of the pericanalicular microvilli. However, the microvilli are not shortened as in cholestatic lesions /517/. BSP retention has been reported in rats given chlorpromazine /518/. This phenomenon is related to its biliary clearance since BSP is eliminated from the liver conjugated to glutathione. Chlorpromazine decreases the critical amount of glutathione, but hepatic function is not impaired.

Plasma BSP retention is found in mice treated with chlorpromazine /519/, but the effect is related to a decreased hepatic blood flow produced by this drug and other phenothiazine derivatives. A decrease in blood flow is observed in isolated perfused rat livers following chlorpromazine or thioridazine treatment /520/, and in anesthetized dogs due to an increased hepatic resistance /521/. Acute chlorpromazine administration has no effect on BSP metabolism in rats and mice /522/; the effect of phenothiazine derivatives on plasma

BSP retention is related to a reduction of hepatic blood flow and not to liver dysfunction. Phenothiazine-induced cholestasis is probably due to a hypersensitivity reaction.

Several *in vitro* studies demonstrated chlorpromazine hepatotoxicity. In isolated perfused rat livers, bile secretion and BSP excretion diminished when chlorpromazine /523/, other phenothiazines /524/, or chlordiazepoxide /525/ were present in the medium. In the perfused rat liver chlorpromazine produced cholestasis and changes of portal pressure /985/. Using rat hepatocyte suspensions, tricyclic antidepressants /526/, thioxanthenes /527/ and phenothiazine derivatives /528, 529/ caused a significant dose-related release of intracellular enzymes. These *in vitro* effects are probably related to a direct hepatotoxicity on the cell membrane by these surfactant drugs. The relative potency of phenothiazines correlates well with their critical micelle concentration /530/. Similar correlations are found between cytotoxicity and surfactant action in the effects of erythromycins, bile salts /531/, oxyphenisatin and dioctylsulfosuccinate /523/, indicating that surfactant actions of these substances may represent an important step in the mechanism of intrahepatic cholestasis.

Using isolated hepatocytes, a dose-related release of intracellular enzymes has been reported after exposure to phenothiazines /289, 528, 540/, thioxanthenes /527/ and tricyclic antidepressants /526/. These *in vitro* observations may reflect a direct toxicity of the surfactant properties of these drugs /587-590/, indicating that surfactant interactions could be related to the mechanism of intrahepatic cholestasis produced by these drugs.

Chlorpromazine-mediated jaundice occurs in 1-2% of patients treated with this drug /534/. The side effects associated with morphological changes and increased serum alkaline phosphatase and aminotransferase levels are manifest in about 50% of patients during treatment.

In a recent paper, in experimentally induced cholestatic liver injury in rats by chlorpromazine, the release of mitochondrial aspartate aminotransferase into the serum was studied /528/. It was shown that the relative aminotransferase activity in the intermembrane space of the mitochondria was significantly increased after chlorpromazine administration, suggesting that the mitochondrial aspartate aminotransferase, which is dominantly located in the mitochondrial matrix, transmigrated to the intermembrane space via the inner membrane under the drug effect. The release of this

enzyme into the serum preceded the increase of total aminotransferase activity, indicating that the initial action of chlorpromazine takes place in the mitochondria.

Due to the high frequency of liver impairment, chlorpromazine and its metabolites are considered hepatotoxins. Adverse hepatic reactions included decreased Na^+/K^+-ATP-ase activity, probably connected with the inhibition of bile salt-independent bile flow /198/, changes in plasma membrane fluidity /230, 535/ and active aggregation in the membrane /167/. Chlorpromazine and thioridazine also modify the distribution of blood flow /198, 520/. Comprehensive studies using various derivatives revealed that the adverse hepatic reactions brought about by chlorpromazine are partly due to hydroxylated metabolites, such as 7,8-dihydroxy-chlorpromazine, which are more toxic than the parent compound /230/. In contrast, sulfoxides are less toxic than chlorpromazine /519/.

Chlorprothixene-induced cholestasis is also characterized by a reduction of bile acid-dependent bile flow which depresses the plasma clearance of BSP /536/. Other neuroleptics, *cis*-thiothixene and *cis* and *trans* isomers of clopenthixol and flupenthixol, cause a dose-dependent decrease in the elimination of BSP and indocyanine green and reduction of bile flow /537/. These data do not reveal the mechanism of the depression of bile acid-independent flow.

Hepatotoxicity has been observed in isolated perfused rat livers as assessed by the reduction of bile flow and BSP excretion when chlorpromazine /198, 515, 523, 538/, chlorprothixene /536/, chlordiazepoxide /525/ or other phenothiazines /539/ are added to the perfusate. Chlorpromazine also suppresses bile acid excretion in isolated rat liver preparations /540, 541/. In this condition hepatic perfusion is reduced; however, the inhibition of taurocholate excretion by chlorpromazine is predominantly associated with a generalized action on the plasma membranes of hepatocytes /540, 548/.

Studies using hepatocyte cultures have shown that the primary action of chlorpromazine is on the membrane at the sinusoids /542/ which is in agreement with the increased bile acid uptake /540/ and with the inhibition of membrane-bound Na^+/K^+-ATP-ase activity /230/. In this *in vitro* model, peroxidase reaction is decreased, indicating a diminished endocytosis /543/. The membrane effect of chlorpromazine is probably connected with its cationic detergent properties /544, 545, 547/ and related to an interaction with the non-polar moieties of membrane phospholipids /546, 549, 550/.

b) Erythromycins

There has been a number of clinical reports that the antibiotic lauryl sulfate salt of erythromycin propionate causes a mild and reversible cholestasis /551-555/. Hepatic dysfunction has been clearly established in susceptible individuals /556/. A number of erythromycin salts and esters have been applied in therapy, and it seems that the propionyl ester and the lauryl sulfate salt are the major compounds with cholestatic potential. It is possible that this adverse reaction is connected with the enhanced absorption and greater stability of this erythromycin derivative resulting in a higher serum level than the other erythromycins /557/. Other derivatives show smaller effects and no correlation has been observed between hepatotoxicity and plasma levels /558, 559/.

Comparative studies with various erythromycin derivatives *in vitro* using isolated perfused rat liver /557/, isolated rat hepatocytes /529/, human liver cells /560, 561/ and cultured mouse liver cells /562/ confirm that erythromycin propionate and its lauryl sulfate salt exert the greatest toxicity. The relative hepatotoxicity of these compounds correlates well with their ability to lower surface tension. In the human hepatocyte experiments, erythromycin cetyl sulfate shows the highest anti-surfactant activity which is congruent with its most toxic character /89/.

Although extrapolation from these *in vitro* studies provides a good indication of cholestatic potential in man, no evidence exists for erythromycin-induced cholestasis in experimental animals. When erythromycin propionate or its lauryl salt is administered to animals, the only relevant change is a reduction of bile flow in isolated perfused rat liver /563/. The lack of development of genuine cholestasis in experimental studies may be due to species differences in erythromycin metabolism.

In man, the metabolism of erythromycin derivatives is relatively slow and after oral administration of erythromycin propionate the ester:base ratio in the plasma is consistent at 3.5:1 /564/. However, in the rat the metabolism of erythromycin propionate follows two routes: it is degraded relatively quickly to the base by hydrolysis and partly by N-demethylation /565/. The half-lives of the propionate hydrolysis and the N-demethylated metabolite are 5.5 and 1.5 h, respectively. However, the route of elimination of erythromycin and

its metabolites in rats occurs through biliary excretion; in man the importance of this route is questionable.

c) Miscellaneous drugs

Many Na^+/K^+-ATP-ase inhibitors decrease bile salt-independent bile flow in the rabbit /56/. These drugs include cardiac glycosides and ouabain. The hepatic effects have led to the interpretation that this fraction of the bile flow is connected with the function of the sodium pump. This view has not been widely accepted since cardiac glycosides in cholestatic doses exert their action predominantly by a vascular event /566/. Moreover, in the rat ouabain shows choleretic action, probably due to its osmotic effect /567/.

The antibiotics rifampicin and novobiocin have some cholestatic potential /568, 576, 987/, but they mainly produce retention of BSP and jaundice /569-572/. These effects are related to a selective action on hepatic uptake of organic anions and inhibition of glucuronyl transferase /573, 574/. When rifampicin was combined with isoniazid, the latter itself a hepatotoxin, clinical jaundice occurred at much higher rates associated with cytotoxic and cholestatic lesions /572, 576/. Rifampicin and isoniazid exert synergistic hepatotoxic actions in the rat /577/. Rifampicin also reduces the biliary excretion of warfarin /578/. Some cholecystographic agents can also produce several features of cholestasis.

Nitrofurantoin occasionally brings about hepatic impairment in man /579, 580/, although the changes are more of a hepatocellular necrotic nature, rather than showing intrahepatic cholestatic features /581, 582/. Animal experiments with nitrofurantoin are few; it is excreted in the bile of dogs and its excretion is associated with marked choleresis /568/. The oral hypoglycemic drugs, carbutamide, tolbutamide and chlorpropamide, elicit a very low incidence of hepatic reactions including cholestatic jaundice and elevated serum alkaline phosphatase activity /583/.

Several other xenobiotics produce choleresis in various experimental animals, such as rats, rabbits, guinea-pigs and dogs. These xenobiotics include organic anions, cations and neutral compounds /779-781/, and their choleretic effect partly depends on the species and the basal bile flow rate. Generally some substances are choleretic and the increased bile production is associated with the osmotic activity of the substance in the bile /23/. Substances such as

eosin, fluorescein, bromosulfthalein, phenol red, phlorizin and ioglycamide exert a dose-dependent action on bile flow. Lower doses of bromosulfthalein exert choleresis in dogs /782/, higher doses cause cholestasis in mice /780/ and rats /783/. Various drugs bring about choleresis in experimental animals, such as nafenopin /784/, perhexiline maleate /785/, tienilic acid /786/, bucolome /787-790/, dihydroxydibutyl ether /611/, D-tubocurarine /795/, N_4-acetyl procainamide ethobromide /795/. Other substances that induce choleresis by stimulating bile acid-independent flow include theophylline /791/, thyroxine /402/, hydrocortisone /792, 793/, cyclic AMP /791/, glucagon /791/ and probably vasopressin /794/. Several microsomal enzyme inducers are choleretic, such as diazepam /796/, pregnenolone-16α-carbonitrile /797/, spironolactone, cortisol /797-800/, carbutamide /801/, and phenobarbital /863/. Recently the chemical-induced interference with hepatocellular transport has been suggested to play a role in cholestasis /984/.

In present literature many drug-induced cases of intrahepatic cholestasis have been reported in man. These include phenylbutazine /584/, triazolam /585/, flurazepam /586/, captopril /587, 994/, methimazole /588/, propranolol /589, 590/, trimethoprim /591/, thiabendazole /592/, tolazamide /593/, glibenclamide /594, 989/, chlorpropamide /594/, cinnarizine /992/, sulfamethoxazole /595/, prajmaline /995/, indomethacin /596/, etoposide /990/, procainamide /991/, flucloxacillin /597/, amoxycillin /598-600/, triacetyloleandomycin /588/, busulfan /601/, cyclosporins, especially cyclosporin A /602-606/, and alcohol /607/. It has been suggested that altered biliary secretion of bile acids is the possible reason for the drug-induced cholestasis /394, 424/. Some of these findings have been confirmed by animal experiments. Cyclosporin A causes transient changes in the half time of the elimination of technetium-99m EHIDA and bilirubin elimination in rabbits /601/. In contrast, cyclosporin exerts some beneficial effect in man, by slightly diminishing the cholestasis in patients with primary biliary cirrhosis /603, 604/. In other cases the side effects were more unfavorable than benefits.

Several xenobiotics stimulate bile flow in dogs and rats and induce choleresis or intrahepatic cholestasis. These include ethacrynic acid /608-610/, diethyl maleate /610/, dihydroxydibutyl ether /611/, probenecid /612/, valproic acid /613-616/, naltrexone /617/, iodipamide /618, 862/, ioglycamide /619, 620/, iodoxamate /621-623/, 1-chloro-2,4-dinitrobenzene-5-glutathione /624/, BSP-glutathione /625, 626/,

6,7-dimethylesculetin /627/, gempin and patrinoside /628/. Choleresis brought about by these agents develops immediately after acute doses and in most cases it is associated with the osmotic activity of the test compound or its metabolites. Some other measurements of canalicular secretion are also influenced by several compounds /641-645/. Chemically-induced cholestasis occurs with flavaspidic acid /629/, sporidesmin /630-634/, icterogenin /457, 630, 634-636/, dinitrophenol /630, 637-640/, arsphenamine /639/, toluenediamine /638/, niacin /54/, azathioprine /267, 993/, atromid S /266/, griseofulvin /265/ and warfarin /274/.

The lesions caused by these compounds include impaired transport of bilirubin and other organic anions from sinusoids into the hepatocyte, and reduced bile flow. This interference can be associated with: (a) reduced ATP synthesis /630/; (b) membrane injury /641, 642/; (c) injury of bile ducts /640/; (d) precipitation of insoluble complexes in or around the canaliculus /641, 642/; (e) impairment of bile salt dependent flow by blocking the synthesis or transport of bile acids; (f) interference with micelle formation in the bile; (g) abnormal reabsorption or secretion of water and electrolytes; (h) impairment of the bile acid-independent bile flow by affecting membrane-bound Na^+/K^+-ATP-ase activity /643-645/. It is likely that the various xenobiotics producing cholestasis may cause defects at many sites rather than a single lesion.

Flucloxacillin therapy caused prolonged hepatic cholestasis and debilitating pruritus in patients /597/. Treatments with amoxycillin/clavulanic acid preparations also resulted in the development of cholestasis and cholestatic hepatitis /598/. It seems likely that clavulanic acid is responsible for this adverse effect /599/. Several days of indomethacin therapy led to mild cholestasis with a moderate elevation of alkaline phosphatase /597/. The cholestasis was resolved soon after the medication was stopped. In contrast, hepatic dysfunction brought about captopril therapy /587/ was associated with jaundice, pruritus, anorexia and hepatomegaly as indicated by high alkaline phosphatase, lactate dehydrogenase and alanine aminotransferase activities and total bilirubin in the serum. The recovery was slow but complete after discontinuation of the drug.

Phenylbutazone can cause intrahepatic cholestasis and jaundice, probably as a result of a hypersensitivity reaction /583/. This was found in a patient treated with this drug for one month due to swollen knees. Liver biopsy revealed pericentral cholestasis and infiltration

of lymphocytes and eosinophilic leukocytes, indicative of a drug reaction. Clinical and biochemical parameters returned to normal 2 months after cessation of therapy. A later provocation test, however, resulted in a marked elevation of serum alkaline phosphatase and transaminases. Transaminases became normal within a few days, whereas alkaline phosphatase remained increased for a month.

Glibenclamide therapy of a diabetic patient led to intrahepatic cholestasis and cutaneous bullae /594/. Similar liver dysfunction may occur with chlorpropamide, a comparable sulfonylurea agent. Flurazepam hydrochloride is one of the most commonly used nighttime sedatives in North America and, like other benzodiazepines, it has very few side effects /646/. Still a case has been reported of this drug causing intrahepatic cholestasis /586/. In contrast, however, another benzodiazepine, triazolam, caused severe pruritus and jaundice which subsequently proved fatal /585/. Liver histology revealed intense cholestasis consistent with a cholestatic drug reaction.

Patients treated with triacetyloleandomycin who had also been taking oral contraceptives for 1.5 to 2.5 years developed obstructive jaundice after one week /588/. This jaundice showed biological and histological features of intrahepatic cholestasis due to steroids. Serum alkaline phosphatase activity was high but γ-glutamyl transpeptidase was normal or slightly elevated. Jaundice disappeared after cessation of the pill and triacetyloleandomycin. The mechanism of action of this drug is still unknown; it may facilitate the development of intrahepatic cholestasis in women taking oral contraceptives.

6.2 Chemicals

Many chemicals produce the cholestatic syndrome. Some of these effects are connected with genuine cholestatic actions, others are related to mixed cholestatic-hepatic lesions; some chemicals are true hepatotoxins causing primarily hepatocellular necrosis. Chemically-induced hepatic side effects occur in man due to accidents at the workplace or at home, in connection with suicide attempts or related to environmental contaminations. It is generally accepted that, similarly to drugs, the effects of chemicals on the liver seem to be associated with a hypersensitivity reaction /37/. In several instances it was considered that the adverse liver response to chemicals or drugs, associated with host idiosyncrasy, may be mediated by the hepatic metabolism of the substance leading to toxic metabolites, or

allergic reactions may be playing a part in side effects /45/. In this review we discuss the action of only those chemicals which either produce clinical jaundice in man, or are used in animal experiments for studying the mechanism of cholestatic changes in the liver.

a) α-Naphthylisothiocyanate (ANIT)

Acute administration of this chemical causes a cholestatic response; chronic administration leads to bile duct hyperplasia and biliary cirrhosis. The cholestasis and hyperbilirubinemia are dose-dependent in rat and mouse and, therefore, these models have been used extensively for establishing the mechanism of chemically-induced cholestasis /647-650/. Acute doses of ANIT bring about morphological and biochemical changes /651/. These include mild fatty changes, the immediate appearance of small necrotic foci which are widespread within 24 hours and are combined with desquamation of the epithelium in some of the larger bile ducts. The lumen of these ducts contains amorphous material composed of cellular debris, fatty substances and plugs of mucus. Two weeks after exposure the hepatocytes appear entirely normal. A recent report revealed that morphological changes in the bile duct lining precede changes in hepatocytes /652/. Four hours after a single dose of ANIT, electron microscopy showed dilatation of the bile ducts, loss of microvilli from bile duct epithelial cells and an apparent opening of the tight junctions between some bile duct epithelial cells. After six hours these changes were more pronounced and detachment of the nuclear membrane occurred in some bile duct lining cells parallel with a loss of γ-glutamyl transpeptidase activity and vacuolization of the endoplasmic reticulum. Twenty-four hours following ANIT administration the majority of the bile ducts were destroyed, and by forty-eight hours there was some evidence of regeneration.

Acute administration of ANIT results in an intrahepatic biliary obstruction due to cholangiolitis in rodents /653-658/. The biochemical changes start with a gradual depletion of glycogen from the hepatocytes and a decrease of the canalicular ATP-ase activity, particularly around necrotic areas. Alkaline phosphatase is increased, 5'-nucleotidase activity is reduced in the area of the canaliculi /659, 660/.

Since ANIT causes damage of cell membranes in the rat /661/, the activity of enzymes bound to liver plasma membranes has been

extensively studied /524/. Na^+/K^+-ATP-ase, Mg^{2+}-ATP-ase and 5'-nucleotidase activity are all decreased in isolated membrane preparations and return to normal level 7 days after the administration of ANIT. This suggests that ANIT has a generalized injurious effect on plasma membranes.

In rats /662, 663/ and dogs /649/, ANIT produces a moderate rise in plasma alanine aminotransferase activity. This can be detected in the rat 2 hours after administration of ANIT, much earlier than the signs of cholestasis become apparent. Plasma 5'-nucleotidase is also elevated but the time course of this increase is similar to the changes in bile flow brought about by ANIT.

Electron microscopic studies revealed that the lumina of the bile canaliculi are dilated with some reduction of microvilli, the number of lysosomes is increased and the Golgi network seems to be extended /664, 665/. Some alterations in mitochondria are also observed /666, 667/. It seems that the early effects of ANIT are correlated with a gradual and progressive alteration of the parenchymal cells, and later changes occur in the bile ductular cells due to obstruction. In rodents the bile duct epithelium appears to be the site of action of ANIT /668-670/.

In non-rodent species the effect of ANIT is similar. In sheep and calves, acute administration of ANIT results in an increased serum bilirubin level and a rise of several cytoplasmic enzymes /671/. Histochemical procedures show a marked cellular swelling, vacuolation and loss of periportal cell staining. The activity of succinic tetrazolium reductase, glutamate dehydrogenase and non-specific esterase is reduced in the whole liver lobule. Sinusoidal alkaline phosphatase is enhanced. It seems that various species respond differently to the administration of ANIT /672/. Most of the changes are in the bile duct epithelium in rodents, but in sheep and calves, the hepatocytes are primarily affected by ANIT.

ANIT enhances bilirubin excretion in the rat /673, 674/. This rise occurs before cholestasis sets in /675/. Probably the source of the increased bilirubin is not erythropoietic; however, an increased bilirubin synthesis is involved in ANIT-induced hyperbilirubinemia /676, 677/. Cessation of the blood flow leads to hyperbilirubinemia. ANIT also affects BSP retention at a time before bile stasis occurs, and it also reduces the activity of drug metabolizing enzymes bound to the hepatic endoplasmic reticulum in rats and mice. Hexobarbital and pentobarbital sleeping time and zoxazolamine-paralysis time were

prolonged in rats treated with a cholestatic dose of ANIT /663, 678, 679/. Liver preparations from ANIT-treated rats exert reduced aniline hydroxylase and aminopyrine demethylase activities /663/. There is a difference between the rat and mouse enzymes. Species differences in susceptibility to the cholestatic effect of ANIT and its action on drug metabolizing enzymes indicate that a metabolite rather than the parent compound is responsible for these actions /680-682/.

Impairment of drug metabolism can be measured 2 hours after administration of ANIT preceding cholestasis /679, 683-685/. Recovery of drug metabolism is slow, persisting longer than hyperbilirubinemia /663, 679/, similar to the time course of bile flow normalization. The reduction of drug metabolizing enzymes is associated with microsomal cytochrome P-450 changes /663, 666, 686-690/.

Some effects of ANIT on the activity of hepatic endoplasmic reticulum can be abolished by pretreatment with actinomycin D, puromycin, cycloheximide or ethionine; the latter substances are inhibitors of protein and RNA synthesis /522, 662, 680, 682/. These inhibitors also provide protection against hyperbilirubinemia and cholestasis. Post-treatment of animals with actinomycin D, cycloheximide or ethionine also inhibits the appearance of hyperbilirubinemia. Puromycin is only effective when given in multiple doses before and after ANIT. The protective effect is actually associated with the *de novo* synthesis of proteins and RNA.

Only doses which could block the incorporation of leucine into hepatic proteins or could inhibit the incorporation of orotic acid into RNA are effective. It seems, therefore, that the maintenance of normal protein and RNA synthesis is involved in some hepatic action of ANIT.

Several ANIT derivatives have been studied in experimental animals to produce hepatic dysfunction, which manifests as hyperbilirubinemia /691/. Isocyanates, thiocyanates and isothiocyanates have been tested in mice and it was found that their effect is associated with special structural characteristics. The active radical must contain sulfur, in combination with carbon and nitrogen; replacement with oxygen renders the compound inactive. The hydrocarbon side chain must be an aryl moiety; alkyl or cyclo-alkyl isothiocyanates exert no action. In the mouse /691/ and guinea-pig /692/ phenylisothiocyanate produces cholestasis, but phenylisothiocyanate is inac-

tive in the rat /37/. β-Naphthylisothiocyanate is inactive in the rat and mouse /684, 685/.

Some studies have shown that ANIT binds to liver microsomes *in vitro* /692, 693/. Administration of ANIT to rats and mice induces cholestasis and hyperbilirubinemia; hamsters are more resistant, and in dogs and rabbits, ANIT does not develop this effect. When the binding of ANIT to isolated microsomes obtained from the liver of different species was studied, it was found that the order of binding from the highest to the lowest was hamster > rabbit > dog > mouse > rat. This order does not correspond with the *in vivo* toxicity of ANIT, suggesting that binding of ANIT to liver microsomes does not represent an essential step in its cholestatic action.

b) Manganese - bilirubin

Intravenous administration of manganese sulfate to rats elicits characteristic morphological changes in the liver which resemble those observed in cholestasis /694/. The changes include dilatation of the bile canaliculi and Golgi apparatus, and loss and swelling of the microvilli. At early states there are no alterations of the bile ductules, but about 12 hours after manganese administration all animals show hepatocellular necrosis, randomly distributed throughout the lobule. There is no correlation between the extent of cholestatic changes and the severity of necrosis. Manganese causes a reversible decrease of bile flow /695/ and the cholestatic action is significantly enhanced by the infusion of bilirubin in a dose-dependent fashion /696-698/. The combination of subtoxic doses of manganese and bilirubin is probably responsible for the cholestasis.

Due to the abnormal appearance of the bile following manganese administration and bilirubin infusion, the probability of intracanalicular precipitation of manganese-bilirubin aggregates has been suggested as the cause of cholestasis. Ultrastructural studies confirm that more severe changes occur in the liver cell with the combined treatment than with the injection of manganese alone. The combination of the two chemicals causes an increased canalicular dilatation, formation of cytoplasmic granules and the accumulation of fibrillar electron-dense substances in the canaliculi, vacuoles and bile ducts. These substances are not present when manganese is given alone. Moreover, bilirubin administration alone does not cause any changes in the rat liver /699/.

Further studies revealed that the combined treatment is associated with alterations of the canalicular membrane /111, 700, 701/, leading to increased permeability of both the canalicular membrane and tight junctions; the latter changes seem to be more important in the progress of the condition /111, 702, 703/. As yet, however, the mechanism of the manganese-bilirubin cholestatic reaction has not been established. We still do not know which is the critical component in provoking the liver response. Some investigations suggest that the initiating step in the chain of events may be an interaction between manganese and bilirubin at the level of the hepatocyte.

c) Cobalt chloride

Cobalt is an essential trace element and is accepted as a physiologically active metal when present in vitamin B_{12}. Inorganic cobalt salts, however, are not inert to the normal cell. They can activate arginase /704/ and they also exert a variety of pathological actions in several tissues. Cobalt chloride is recognized as a cytotoxic compound; it produces polycythemia in many animals and also in man /705-707/. Some reported abnormalities after cobalt administration are associated with erythropoiesis /708-711/. Cobalt chloride given to animals or man also causes lipemia /708, 710, 712/, in which mainly triglycerides are increased in the serum /713-717/ with a slower rise of cholesterol and free fatty acids /718/. A new syndrome of cardiomyopathy characterized by fulminating heart failure has also been associated with cobalt chloride /719-721/.

Hyperglyceridemia, brought about by cobalt chloride, may develop as the consequence of increased triglyceride and lipoprotein synthesis, or decreased breakdown, or a combination of both processes. Recently, it has been reported that lipoprotein lipase, the enzyme responsible for the catabolism of circulating lipoproteins, is inhibited by cobalt /722/. Considering the hepatic action of cobalt, treatment of experimental animals with concomitant administration of cobalt chloride and phenobarbital has been proposed as an experimental model for studying the mechanism of intrahepatic cholestasis. A hypothesis was put forward that the key initiating event is the production of hypertrophic hypoactive smooth endoplasmic reticulum /30/. The impaired function of the endoplasmic reticulum is associated with reduced 7α- and 12α-hydroxylation of cholesterol, resulting in the production of excess amounts of monohydroxy bile

acids within the hepatocytes and subsequent bile stagnation due to their low micellar activity. This hypothesis however could not be confirmed /179/ or was found to be partially correct by a comprehensive analysis of liver functional responses and biochemical correlates of this model /723/.

In contrast to the toxic action of cobalt chloride, an inhibitor of cytochrome P-450 synthesis, it prevented hepatic injury and the accompanying centrilobular changes brought about by large doses of butylated hydroxytoluene /724/. Cobaltous chloride exerts a paradoxical effect on carbon disulfide-induced hepatotoxicity. In single doses, when both cobaltous chloride and carbon disulfide were administered together, the hepatic lesion was much more severe than after carbon disulfide alone. When cobaltous chloride and phenobarbital were given before carbon disulfide, hepatotoxicity was prevented /725/. This effect was specific to cobalt; other divalent metal salts (copper and zinc) neither enhanced nor inhibited carbon disulfide hepatotoxicity. Cobalt chloride administration to experimental animals also affected the biliary secretion of bilirubin and glutathione /726/.

d) Metalloporphyrins

Since the endoplasmic reticulum and the cytochrome P-450 enzyme system participate in intrahepatic cholestasis, attempts have been made to study how modifications of cytochrome synthesis affect hepatic function. The biological actions of synthetic metalloporphyrins are connected with the replacement of the central iron atom in the heme molecule. Among these compounds the effects of various substituted porphyrins have been investigated fairly widely, including cobalt, zinc, nickel, manganese and tin porphyrins. These studies have revealed that the replacement of the iron by another metal leads to novel biological and pharmacological properties.

Treatment of experimental animals with cobalt porphyrin causes an extensive and long lasting depletion of hepatic cytochrome P-450 /727-734/ and reduction of associated mixed function oxidase activity /729, 735, 752/. These effects are related to the regulation of the production of delta-aminolevulinic acid synthetase /736, 737/. A single dose of cobalt protoporphyrin produced a marked reduction in the metabolism of testosterone and 4-androstenedione /738/, and catechol estrogen synthesis /739/, and increased microsomal pro-

gesterone content and binding /740/. Pretreatment of animals with cobalt protoporphyrin prevented liver necrosis brought about by thioacetamide-S-oxide /741/.

Cobalt protoporphyrin IX and cobalt chloride caused a marked increase in the oxidative degradation of heme by hepatic microsomal enzymes through activation of delta-aminolevulinic acid synthetase /736/. In particular, the oxidation of heme by heme oxygenase is stimulated by cobalt heme /732, 733, 736, 742, 743/. Heme oxygenase is the rate limiting enzyme in heme catabolism to bile pigments. Cobalt heme (cobalt protoporphyrin IX) serves as a substrate for the enzyme and its oxidative metabolite has been identified as the natural bile pigment biliverdin IX alpha isomer /744/. Metalloporphyrins which do not bind molecular oxygen (nickel, manganese and tin protoporphyrin IX) are not substrates for heme oxygenase although they could competitively inhibit the oxidation of reactive substrates by the enzyme.

In contrast to the action of cobalt porphyrin, small doses of tin protoporphyrin or tin meso-porphyrin inhibit heme oxygenase, resulting in a significant decrease of plasma bilirubin level in physiologic conditions as well as in naturally occurring or induced forms of jaundice /745-750/. Administration of single doses of cobalt protoporphyrin to rats into the third brain ventricle or repeated parenteral doses suppressed food intake and reduced body weight related to carcass fat content /751/.

In various studies metalloporphyrins were given in different doses by various routes, mainly intravenous, intraperitoneal /737/ or subcutaneous /712, 724-726, 734/, but direct injection of cobalt protoporphyrin into the third ventricle of the brain /752/ was also reported. There is no evidence that cobaltous chloride or cobalt porphyrins exerted any irreversible pathological damage in the experimental animals. It seems that the various actions of cobalt porphyrins are associated with modification of the biochemical processes and do not cause structural alterations either at the site of administration or in the target organs where biochemical parameters were modified. The application of these metalloporphyrins results in many interesting findings, but more work needs to be done before any conclusion can be reached as to how changes in heme pigments and related activities are associated with early steps of intrahepatic cholestasis.

e) Miscellaneous chemicals

Acute administration of ethanol produces a dose-related depression of bile flow and bile acid secretion in humans, rats and dogs /753/. The acute liver response develops even if animals are given alcohol chronically. The cholestatic action of ethanol is probably associated with an inhibition of the bile acid-dependent secretion. Acute ethanol intake decreases biliary excretion of BSP and indocyanine green. Exposure to low alcohol levels depresses methylfolate transport into the bile /754/. The elimination of lorazepam /755/ and propoxyphene /756/ is reduced during ethanol infusion. Chronic exposure to ethanol stimulates both bile acid-dependent and independent fractions of canalicular bile flow. Alcohol intake modifies bile acid synthesis and metabolism, including intestinal pathways, and also affects enterohepatic circulation.

2-Ethyl-2-phenyl butyramide has been tested in dogs as a potential hypnotic agent and found to exert morphological changes characteristic of intrahepatic cholestasis /757/, and BSP retention /703/. In rats, this compound is inactive. Monochloroacetate, a by-product of water chlorination, produces hepatoxicity in rats /1001/.

Sucrose acetate isobutyrate, a food additive, also causes an impairment of hepatic function in dogs, but is inactive in rats and squirrel monkeys /758/. The effect in dogs resembles mild intrahepatic cholestasis which is reversed very quickly. There is an enhanced BSP and indocyanide green retention and an increase of serum alkaline phosphatase. The carcinostatic compound 1,3-bis(2-chloroethyl)-1-nitrosourea exerts hepatotoxic effects in patients and experimental animals /759, 760/. In the rat the symptoms include hyperbilirubinemia, pericholangitis and BSP retention leading ultimately to biliary cirrhosis. The effect of this compound in single doses shows similarity to changes brought about by chronic administration of ANIT.

In Epping, England an outbreak of jaundice occurred in 1965, due to the consumption of bread made from contaminated flour /761, 762/. Liver biopsies from many patients showed cholestasis, damage to the liver parenchyma and to the biliary tree, cellular infiltration and minimal hepatocellular necrosis. The contaminant responsible for the Epping epidemic was 4,4'-diaminodiphenylmethane, an epoxy resin hardener which got into the flour by error. Limited animal experiments with this chemical showed similar changes in mice /761/ and cats /762/. It was suggested that 4,4'-diaminodiphenylmethane

forms an insoluble salt with bile acids similarly to chlorpromazine-induced cholestasis.

The administration to rats of phalloidin, a bicyclic heptapeptide isolated from *Amanita phalloides*, induces cholestasis /136, 145, 146, 763/. Bile acid secretion is decreased and a microfilament-mediated change in junctional permeability is responsible for the efflux of biliary constituents into the intercellular space. It has been postulated that microfilaments might provide a contractile function to facilitate canalicular bile flow /505/. Rats made cholestatic due to bile duct ligation survive phalloidin intoxication, because the uptake of the major metabolite demethylphalloin is inhibited /764/. The toxic effect of phalloidin can be prevented in isolated hepatocytes by bile acids, which inhibit the uptake of the toxin /765-768/.

Dinitrophenol produced cholestasis and jaundice in some patients when given to treat obesity /637/. The liver dysfunction was due to biliary obstruction /640, 807/. In perfused rat livers this chemical exerted a cholestatic action /630, 637/. Administration of toluenediamine to experimental animals is associated with cholestasis /638/. The lesion is attributed to effects on small biliary canaliculi, although parenchymal injury and hemolysis are also present. Arsphenamine also causes cholestasis and jaundice /639/. Early stages of ethionine intoxication include bile duct obstruction and changes in the activities of several serum enzymes including alkaline phosphatase, γ-glutamyl transpeptidase, glutamate dehydrogenase and aspartate aminotransferase /996/.

6.3 Miscellaneous agents

Studies in the dog have shown that somatostatin, the tetradecapeptide hormone, inhibits basal and food-stimulated biliary secretion /769/. Studies in the rat have indicated that somatostatin decreases bile flow, bile acid secretion and the bile acid-independent fraction of the canalicular bile flow /770/. Endogenous bilirubin excretion is not affected by somatostatin. Natural plant constituents that induce intrahepatic cholestasis include sporidesmin, icterogenin and 22β-angeloyloxyoleanolic acid /771/. Aflatoxin B_1 causes a decreased bile flow and even a stoppage in isolated perfused rat liver in about 4 hours, indicating a complete cholestasis /772/.

Cholestatic jaundice is a well-known complication of gram-negative sepsis in newborns. These bacteria produce endotoxins which

affect the bile salt-independent bile flow, probably through the inhibition of Na^+/K^+-ATP-ase activity /576/. The endotoxin also impairs the metabolic activity of the liver /773/.

Jaundice has been found in man during infections with *Escherichia coli* /774/. Pathological symptoms include intracellular and intracanalicular bile stasis and several hepatocellular abnormalities /775/. It has been suggested that the circulating bacterial endotoxin is responsible for these actions. In isolated perfused rat livers, *Escherichia coli* endotoxin causes a decrease of bile flow and a reduction of Na^+/K^+-ATP-ase activity /774, 777, 778/. The endotoxin derived from these bacteria also impairs BSP and indocyanine green excretion in a dose-dependent fashion. In mice, a single dose of the hepatotoxin produces dilatation of bile canaliculi and loss of microvilli /776/.

6.4 Endogenous effects

A cholestatic factor was isolated from peripheral blood lymphocytes of patients with drug-induced allergic intrahepatic cholestasis by stimulation with a causative drug in the presence of liver soluble fractions containing a liver-specific lipoprotein /802/. Following injection of this cholestatic factor through the mesenteric vein into rats, bile flow and bile acid excretion were markedly diminished. Morphological examination of the liver of these animals shows changes similar to intrahepatic cholestasis including loss of microvilli and dilatation of bile canaliculi. The factor is only produced in hepatitis patients and it is not present in the blood of normal individuals. This novel lymphokine could be detected in the liver tissue of patients with acute intrahepatic cholestasis including drug-induced allergic hepatitis, alcohol hepatitis, lupoid hepatitis and in patients with hepatitis A type, hepatitis B type and hepatitis non-A non-B type disease. In the development of various liver diseases, this lymphokine cholestatic factor may play an important role /803/. Severe intrahepatic cholestasis could be produced by treatment of patients with recombinant interleukin-2 and lymphokine-activated killer cells /804/.

There are some other endogenous compounds that cause liver dysfunction. A patient receiving total parenteral nutrition following an emergency resection of the small intestine developed cholestatic jaundice after six months /805/. A factor was identified in the serum of this patient which produced cholestatic changes in the bile flow of

rats following an intravenous infusion. An endotoxin caused a similar impairment of the bile flow. It was proposed that the endotoxin might be an occult factor contributing to cholestasis. When an antiserum was prepared to the endotoxin isolated from a sequestered *E. coli* infection in this patient, it reduced the cholestatic effects of the patient's serum in the rats.

In another study, the effect of benign recurrent intrahepatic cholestasis was investigated on hepatic bile flow in rats *in vivo* and ^{14}C-taurocholate uptake and efflux from isolated rat hepatocytes *in vitro* /806/. Sera from patients with cholestatic disease increased significantly rat hepatic bile flow. The uptake of ^{14}C-taurocholate by isolated rat hepatocytes was decreased by the serum from the benign recurrent intrahepatic cholestasis case, but the efflux from hepatocytes remained unaltered. These data indicate that benign recurrent intrahepatic cholestasis is not mediated by a circulating cholestatic agent but rather is secondary to intrinsic factors present in the circulation or to an intrinsic abnormality in the hepatocyte bile salt secretion.

6.5 Potentiation of cholestasis

Many recent reports have demonstrated that ketones and ketogenic compounds can potentiate the adverse hepatic actions brought about by many toxicants. These include hepatonecrosis produced by chloroform and carbon tetrachloride administration to rats /808, 809, 813, 821, 822, 824, 826/, cholestasis brought about by manganese-bilirubin combination treatment /810, 811/, and taurolithocholate-induced intrahepatic cholestasis /812, 817/. Among the ketogenic agents acetone, ethanol, isopropanoyl n-hexane, methyl ethyl ketone, methyl n-butyl ketone, methylisobutylketone, 1,3-butanediol, and chlordecone, a ketonic pesticide, were the most frequently studied chemicals /814-816, 821, 823-825/.

Various chlorinated compounds potentiate each other's hepatotoxicity, such as chlordecone and bromotrichloromethane which interact at non-toxic levels. The halomethane effect is connected with cholestasis followed by a progressive liver injury. The combination treatment in experimental animals leads to extensive hepatotoxicity including cholestasis, necrosis, ballooned cells, and dilatation of rough endoplasmic reticulum /996/. Chlordecone amplifies the toxicity of other halomethane solvents /967, 970/. Several hypotheses

have been suggested for this unusually toxic interaction including: (a) induction of hepatic drug metabolizing enzymes resulting in enhanced halomethane metabolism /971, 975/; (b) ketosis /973, 974/; (c) covalent binding /968, 975/; (d) estrogenic action of chlordecone /975/; (e) insufficient availability of cellular energy required for mitosis and as a consequence unhindered progress of liver injury /966, 977, 978/.

Using 1,3-butanediol, the severity of carbon tetrachloride necrosis showed a close correlation with plasma and hepatic concentrations of β-hydroxybutyrate, the major metabolite /809/. Methyl isobutyl ketone potentiation of the cholestasis induced in rats by a manganese-bilirubin combination treatment or manganese alone, could be simulated by replacement of methyl isobutyl ketone with its metabolites 4-methyl-2-pentanol or 4-hydroxymethyl isobutyl ketone /810/. 4-Methyl-2-pentanol was a more active potentiator than 4-hydroxymethyl isobutyl ketone. Ketone potentiation of chloroform or carbon tetrachloride-induction of hepatonecrosis is connected with an increased bioactivation of these compounds by the cytochrome P-450 enzyme system /818, 820/. The 1,3-butanediol-induced increase in the cholestatic effect by sequential treatment combinations of manganese and bilirubin is reflected in a decreased bile flow /809/. It has not been reported whether any biotransformation reactions are involved in the potentiation of manganese or manganese-bilirubin-induced cholestasis in rats. Moreover, neither butanediol nor phenobarbital pretreatment potentiated tienilic acid-induced hepatotoxicity in rats /826/. However, in lithocholate and taurolithocholate-induced cholestasis and in the action of manganese-bilirubin treatment the involvement of hepatic cytosolic proteins has been reported /484, 817, 827/.

Lithocholic acid or taurolithocholic acid are both monohydroxy bile acids that produce a reversible reduction or cessation of the bile flow in rats. Both compounds cause changes in membrane lipids. Both are rapidly bound to bile canalicular membranes *in vitro*. This binding was markedly increased in a dose dependent manner when cytosolic protein was added to the incubation medium. Pretreatment with cycloheximide, an inhibitor of protein synthesis, protects rats against lithocholic acid or taurolithocholic acid-induced cholestasis. Cycloheximide treatment also reduced the duration of the cholestatic response in manganese-bilirubin treated rats. The onset of the reaction was proportional to the dose of bilirubin. Cycloheximide was

less effective in protecting animals when the action of taurolithocholic acid was potentiated with methylisobutyl ketone /817/. Administration of the ketogenic substance might increase the action of the proteins involved in cholestasis by activation or by inductive synthesis. Thus this increase might enhance the effects of taurolithocholate or manganese on bile canaliculi.

Another possibility is that these ketones act directly on the membranes due to their high lipid solubility, affecting the lipid content. It has been shown that manganese treatment increases phospholipid and cholesterol contents in the bile canalicular membrane /812/. Ketones can, therefore, compromise the fluidity of the membrane and the permeability of the biliary tree and through this action they potentiate cholestasis. In the synergistic action between carbon tetrachloride and 1,2-dibromoethane in inducing marked liver damage the potentiation of lipid peroxidation has been suggested as one of the mechanisms involved /979/.

6.6 Reversal of cholestasis

There are a number of substances that can exert a protective effect against the development of cholestasis brought about by ethinyl estradiol. These substances include S-adenosyl-L-methionine /128, 336, 341/, cholic acid /489/, phenobarbital and Triton WR-1339 /235, 412, 828-830/. S-Adenosyl-L-methionine and Triton WR-1339 reverse the abnormal membrane fluidity /291, 235, 336, 341/. On the other hand, phenobarbital treatment does not correct the abnormal membrane fluidity due to ethinyl estradiol; it increases Na^+/K^+-ATP-ase activity and normalizes bile flow /291, 418, 828/. The enhanced Na^+/K^+-ATP-ase and subsequently increased electrolyte pump may be the consequence of increased protein synthesis by phenobarbital which is a known inducer of various cytochrome P-450 dependent enzymes bound to the endoplasmic reticulum /831-833/.

a) S-Adenosylmethionine

Several investigations have proved that short-term administration of oral S-adenosylmethioinine is effective in improving clinical and laboratory measures of intrahepatic cholestasis /128, 336, 341, 834-837/. This compound reverses the impaired bile secretion induced by a wide range of hepatotoxins, including chlorpromazine, ethinyl

estradiol, ANIT and taurolithocholate. The anticholestatic action of S-adenosylmethionine may result from its role in intermediate metabolism as this molecule is involved in transsulfuration and transmethylation reactions /834/. S-Adenosylmethionine as a methyl donor may facilitate an increasing membrane fluidity and the formation of enzymatically more active endoplasmic reticulum-bound cytochrome P-450 system /838, 839/ (Fig. 6). Clinical trials have documented that intravenous administration of S-adenosylmethionine brought about a significant decrease of biochemical parameters of cholestasis such as the activity of serum aminotransferases, total and conjugated bilirubin and total bile salts. It elicited a significant improvement of pruritus in women with intrahepatic cholestasis syndrome. In addition, other studies reported that both parenteral and oral S-adenosylmethionine significantly improved several objective measures of cholestasis in patients with intrahepatic cholestasis and complicated chronic liver diseases, such as a rise of serum alkaline phosphatase, and total and conjugated bilirubin, and subjective parameters, such as general discomfort, fatigue and pruritus /835/. A recent paper described that in some cases S-adenosylmethionine may be ineffective in the therapy of benign recurrent intrahepatic cholestasis and may be hepatotoxic to some patients /836/.

b) Ursodeoxycholic acid

A bile acid was also found to reverse the symptoms of liver dysfunction. Clinical and experimental investigations have suggested that ursodeoxycholic acid may have cytoprotective or choleretic effects and therefore may be beneficial in the treatment of patients with intrahepatic cholestasis or chronic liver disease. Long-term administration of this compound brought about an improvement of biliary enzyme levels and itching /840, 841/. Clinical trials on a group of patients with severe intrahepatic jaundice, using large doses of ursodeoxycholic acid (600 mg/day), were effective in about 80% of cases /842/. In another study large doses of ursodeoxycholic acid were given to primary biliary cirrhosis patients three times daily for more than one year /843/. In almost all patients both serum alkaline phosphatase and γ-glutamyl transferase levels began to decrease significantly in the first month; alanine and aspartate aminotransferases were also reduced. Biliary enzyme levels and bilirubin levels

improved slightly after treatment. Antimitochondrial antibody titers decreased in some cases but IgM levels and other immunological parameters did not change.

In a similar study, the effect of ursodeoxycholic acid was investigated on biliary lipid secretion in primary biliary cirrhosis /844/. After four weeks of treatment significant improvement of liver function tests was achieved in primary biliary cirrhosis in stages I to III of Scheuer's classification. This treatment also affected biliary lipid secretion and serum lipoprotein levels /845/. Biliary cholesterol concentration, cholesterol output and cholesterol saturation index significantly decreased /483, 846-848/. The amounts of cholic, deoxycholic and chenodeoxycholic acids were also significantly reduced, while the amount of ursodeoxycholic acid rose. The rise of the total bile acid pool remained unaffected by ursodeoxycholic acid, but the assessment of the individual bile acids showed an enhanced proportion of ursodeoxycholic acid relative to the endogenous bile acids. Another study applying ursodeoxycholic acid for the treatment of chronic hepatitis (3 x 600 mg/day) showed significant decreases of serum γ-glutamyl transpeptidase levels /849/. Serum alanine and aspartate aminotransferase activities were also decreased; IgM levels did not change but the titer of antimitochondrial antibody decreased in most patients. It seems that the beneficial effect of ursodeoxycholic acid treatment could result not only from a reduction of intrahepatic accumulation of cytotoxic bile acids, but also from a reduction of the immunological injury /850, 851/.

Following chronic administration of ursodeoxycholic acid in concentrations of 10 mg/kg/day for a 9 month treatment period, serum aspartate and alanine aminotransferases, glutamate dehydrogenase, γ-glutamyl transpeptidase and alkaline phosphatase fell significantly after 18-24 weeks /852, 853/, and IgM was reduced. Total serum bile acid concentrations were raised, but ursodeoxycholic acid became the dominant component. Ursodeoxycholic acid feeding substantially increased urinary bile acid output and serum and urinary bile acid pattern /854/. Many ursodeoxycholic acid derivatives were identified in the urinary bile.

The application of ursodeoxycholic acid in primary biliary cirrhosis in stages I to III seems to be safe /855/. In patients in stage IV total serum bile acids are highly increased indicating some risk of toxicity. The therapeutically effective ursodeoxycholic acid dose was studied in patients with various liver dysfunctions including primary biliary

cirrhosis, primary sclerosing cholangitis and chronic hepatitis /856/. Improvements of serum enzyme levels were achieved in all groups with a 250 mg daily dose; further reductions in these parameters were found in primary biliary cirrhosis patients after 500 mg and 750 mg daily administration, but no significant difference in the other two disease categories. The rate of improvement was roughly proportional to the enrichment of conjugated biliary bile acids with ursodeoxycholic acid.

Using an experimentally-induced intrahepatic model, the choleretic effect of ursodeoxycholic acid was studied /857/. Lymphokine, a cholestatic factor injected intravenously to rats, caused a significant decrease of bile flow and bile acid excretion. When ursodeoxycholic acid was injected together with the cholestatic factor, the reduction of bile flow and excretion of bile acids were significantly suppressed. Choleretic effects were also observed when ursodeoxycholic acid was injected to normal rats. These animal experiments confirm the clinical experience that ursodeoxycholic acid reverses the symptoms of liver dysfunction and may be effective in the treatment of intrahepatic cholestasis. The beneficial effect of ursodeoxycholic acid may be due to the reduction of the hydroxylated derivatives of endogenous bile acids together with the appearance of hydroxylated derivatives of ursodeoxycholic acid, or it may be due to displacement of the more hydrophobic endogenous bile acids by the hydrophilic ursodeoxycholic acid /854/.

c) Secretin

Many animal studies have been reported on the choleretic effects of secretin /858-860/. The action of this substance was also studied in patients with prolonged jaundice due to intrahepatic cholestasis /861/. In about 80% of patients treated with secretin, serum bilirubin levels decreased in parallel with the doses applied and other liver function tests also returned to the normal range.

d) Hormonal control

There are several factors that modify bile flow, including neural effects, hormones and vascular pressure /363, 864-867/. Several hormones, such as histamine /868/ and gastrin /869/, stimulate the

production of bile flow with high chloride and bicarbonate concentrations. Gastrointestinal hormones, such as cerulein, cholecystokinin, pentagastrin and gastrin II, affect flow /870-873/. Sulfated gastrin II has a choleretic action in pharmacological doses /873, 874/. In experimental studies with dogs hydrocortisone enhances hepatocellular bile flow with elevated chloride concentration /792/. Glucagon and insulin also stimulate bile flow in dogs /872/, baboons and man /875, 876/, but not in rabbits and guinea-pigs /877, 878/.

Bile flow, concentration and excretion rates of bile acids, phospholipids and cholesterol show a circadian rhythm in rats with a peak at midnight and a minimum at noon /880-884/. Bile acid independent flow is highest at night and early morning /881, 882/. Biliary transport of large molecules is higher at night than at noon /883, 885/. These changes are associated with food intake /885, 886/ and probably the entire mechanism is regulated by the circadian rhythmical function of the pineal gland /887/.

VII. RECENT STUDIES ON CHOLESTASIS

In our laboratories we investigated the potential role of the hepatic endoplasmic reticulum in the pathogenesis of cholestasis. One of the major target sites of most foreign compounds is the endoplasmic reticulum where their metabolic transformation and elimination takes place. Our investigations were initiated to provide an answer to the following fundamental question: Why does the endoplasmic reticulum respond differently to various xenobiotics? Liver injury is among the commonest manifestations of long term toxic actions of xenobiotics and intrahepatic cholestasis is one of the most frequently occurring clinical signs of adverse effects. Testing various foreign compounds for recognition of their hepatotoxic potential, particularly of low order toxicity, often presents considerable difficulties and uncertainties. Many diverse compounds or compounds with highly similar chemical structure possess very different toxicities. Their effects on the liver cell range from slight alteration of the biochemical organization representing early manifestations of toxic damage, to abnormal morphological changes leading to pathological lesions and cell death. In most circumstances it is likely that disturbances in the function and dynamic equilibrium of various enzyme systems, result-

ing in a biochemical lesion /888/, precede the recognizable pathological damage.

There are some compounds that cause adaptive changes in the liver. These include hepatomegaly and induction of the drug metabolizing enzymes bound to the endoplasmic reticulum. These effects are associated with enhanced *de novo* synthesis of cytochrome P-450 associated functions. Normal metabolic enzymes attached to the endoplasmic reticulum or to any other subcellular particle or in cell plasma membranes generally remain unaltered by these compounds. Overall, the actions of these compounds on the liver cell are mainly connected with biochemical events and are not associated with any histopathological alterations. In contrast, there are some other compounds, defined as hepatotoxicants, that also exert liver enlargement but this is paralleled with a reduction of the cytochrome P-450 associated drug metabolizing activity, and impairment of normal metabolic enzymes bound to the endoplasmic reticulum. The impaired functional state of the cytostructural elements is also readily susceptible to structural damage leading to genuine morphological abnormalities.

Biotransformation of drugs and hydroxylation of steroids, including the conversion of cholesterol to bile acids and bile acid hydroxylation, share a common cytochrome P-450 dependent enzyme system in the hepatocyte /889-893/. These functions reside primarily in the endoplasmic reticulum and, since in intrahepatic cholestasis the activity of these enzymes is decreased /64, 894/, reduced hydroxylation of bile acids may represent the first event in this disorder /895/. According to this hypothesis the decrease of microsomal activity may co-exist with hypertrophy of the endoplasmic reticulum membranes. Such membranes have been referred to as "empty membranes" because of low cytochrome P-450 content and apparently hypertrophic structure /687/. In experimental studies using rats, phenobarbital and cobalt chloride were administered to prove this hypothesis. Both test compounds profoundly altered the rate of microsomal protein synthesis and enzyme activity by different mechanisms /896-898/. Since proliferated membranes with accompanying stimulated activity represent adaptive responses, and decreased membranes together with lowered microsomal activity indicate hepatotoxicity, we attempted to characterize the significance of proliferated membranes in the absence of elevated microsomal enzyme activity /723/.

7.1 Experimental

We applied a treatment schedule similar to earlier published reports /179, 723/. Male albino rats received phenobarbital (PB) or cobalt chloride ($CoCl_2$) alone, or in combination with lithocholic acid (LCA). Several parameters were evaluated to assess organ response to the various experimental treatments. Liver samples were taken for microsome isolation, enzymes assays, and electron microscopy.

Quantitative sterologic analyses was carried out on the livers of rats from the different treatment groups. Methods published in the literature /899-901/ were used.

7.2 Biochemical effects on hepatic microsomes

These studies indicated that cobalt chloride alone produced clear-cut signs of hepatotoxicity as manifested in significant decreases in cytochrome P-450 content, and aminopyrine N-demethylase and glucose-6-phosphatase activities. Microsomal protein content was slightly increased and there was no change in phospholipid content (Table 6). Phenobarbital treatment alone resulted in a significant rise in cytochrome P-450 content, aminopyrine N-demethylase activity and microsomal protein and phospholipid contents, but exerted no effect on glucose-6-phosphatase. Lithocholic acid given alone decreased microsomal glucose-6-phosphatase but did not affect any other parameters. When phenobarbital and cobalt chloride were administered concurrently, cytochrome P-450 content and aminopyrine N-demethylase activity returned to control levels; glucose 6-phosphatase activity was still significantly reduced, and microsomal protein and phospholipid significantly increased as compared to controls, but these values fell between the control and phenobarbital-treated levels. Combination treatment with phenobarbital, cobalt chloride and lithocholic acid resulted in a reduction of cytochrome P-450, and aminopyrine N-demethylase and glucose-6-phosphatase activities; microsomal protein and phospholipid contents were significantly raised. These data indicated that simultaneous phenobarbital and cobalt chloride treatment, whether administered alone or in combination with lithocholic acid, caused a functional impairment of the endoplasmic reticulum but did not induce a hypoactive hypertropic smooth endoplasmic reticulum /30/.

TABLE 6
Effect of experimental treatments on hepatic microsomal parameters

Treatment	Protein, mg/g liver	Phospholipid μmol P/g liver	Cytochrome P-450 nmol/mg protein	Aminopyrine N-demethylase nmol/hr/mg protein	Glucose 6-phosphatase μmol/hr/mg protein
Control	23.0±0.9	8.7±0.7	0.9±0.1	62.4±7.8	6.5±0.5
Cobalt chloride	25.4±0.6	8.2±0.3	0.3±0.1*	18.8±1.4*	1.8±0.1*
Phenobarbital	33.5±1.4*	14.1±0.7*	2.3±2.2*	162.0±18.5*	5.5±0.4
Cobalt chloride + phenobarbital	30.5±1.8*	11.2±0.5*	0.8±0.1	66.9±7.3	2.5±0.3*
Lithocholic acid	24.3±3.3	7.9±0.5	0.7±0.1	61.1±7.3	4.0±0.1*
Cobalt chloride+ phenobarbital+ lithocholic acid	35.6±3.5*	11.7±0.9*	0.7±0.0	44.6±6.4*	2.5±0.2*

Treatments: Groups of 8 animals were treated for 7 consecutive days with $CoCl_2$ (30 mg/kg, sc), PB (50 mg/kg, ip), or LCA (200 mg/kg, po) alone. Additional groups of 8 rats were administered $CoCl_2$ and PB simultaneously or received all three treatments concurrently for 7d at dose levels mentioned above. $CoCl_2$ and PB were dissolved in physiologic saline. LCA was suspended in glycerol and administered as 100 mg/kg b.i.d. 8 h apart. Control animals received volumes of physiologic saline similar to those administered to treated animals (1 ml/kg, sc and 2.5 ml/kg, ip), also for 7d. The rats were sacrified 24h after the last $CoCl_2$ and PB dosing, and 16 h after the final LCA treatment.

Values are expressed as mean ± standard error.

* Significantly different from control group. $p<0.05$.

7.3 Morphological effects on endoplasmic reticulum membranes

Cobalt chloride treatment alone brought about a significant reduction in the volume density of the smooth surfaced endoplasmic reticulum membranes (SER) and a slight increase in volume density of rough surfaced endoplasmic reticulum membranes (RER). Phenobarbital alone significantly elevated SER volume density and decreased volume density of the RER. Concurrent administration of cobalt chloride and phenobarbital, with or without lithocholic acid, resulted in no significant changes in SER or RER volume fractions (Table 7). In terms of membrane surface area availability, SER was significantly reduced and RER significantly increased by cobalt chloride alone; phenobarbital treatment resulted in increased surface area of SER without any change in RER. Combined treatment of cobalt chloride and phenobarbital, with or without lithocholic acid, resulted in significantly more surface area of RER, but no changes in SER (Table 8). Structural and functional correlates of hepatic ER membranes revealed changes predominantly within RER membranes. The combination of cobalt chloride and phenobarbital administration, with or without lithocholic acid, caused a significant decrease of glucose-6-phosphatase activity per unit surface area of RER, but no changes in cytochrome P-450 or aminopyrine N-demethylase activity per unit surface area of SER (Table 9). These quantitative morphometric findings indicated that simultaneous cobalt chloride and phenobarbital administration, with or without lithocholic acid, induced hypofunctional and relatively hypertrophic rough, but not smooth, endoplasmic reticulum membranes.

VIII. CLINICAL DIAGNOSIS OF CHOLESTASIS

The major clinical manifestations of intrahepatic and extrahepatic cholestasis are jaundice, pruritus and increased serum levels of bilirubin, bile salts, alkaline phosphatase and some other enzymes. In chronic conditions lipids are also elevated. Due to the large reserve of the liver excretory function, abnormalities usually occur when more than 2/3 of the functional capacity is disturbed. There are also some pathophysiological and histological changes and clinical features associated with the symptom of cholestasis /998/. The various markers of cholestasis represent changes correlated with the impair-

TABLE 7

Effect of experimental treatments on volume changes of hepatic endoplasmic reticulum

Parameter	Treatment	Membrane Type			
		SER	RER	GOL	Total ER
Volumetric Density (%)	Control	18.5±1.7	7.2±1.7	0.4±0.1	26.1±1.9
	$CoCl_2$	11.3±1.6*	9.6±1.2	0.6±0.2	21.6±1.8
	PB	34.1±3.9*	5.5±0.8	0.2±0.1	39.8±3.6*
	$CoCl_2$+PB	22.9±2.2	7.9±0.9	0.4±0.1	31.4±2.0
	$CoCl_2$+PB+LCA	20.4±2.9	8.5±0.6	0.5±0.1	29.4±2.9
Volume (μm^3/hepatocyte)	Control	821±87	304±69	20±6	1145±92
	$CoCl_2$	587±98	505±77	32±9	1123±13
	PB	2204±38*	344±60	12±4	2560±40*
	$CoCl_2$+PB	1610±19*	588±91	31±7	2230±21*
	$CoCl_2$+PB+LCA	1377±27*	548±75	30±8	1955±32*

Treatments as in Table 6.

Values expressed as mean ± standard error.

* Significantly different from control group, $p<0.05$

TABLE 8

Effect of experimental treatments on membrane surface changes of hepatic endoplasmic reticulum

Parameter	Treatment	Membrane Type			
		SER	RER	GOL	Total ER
Surface Density ($\mu m^2/\mu m^3$)	Control	15.1±0.7	5.0±0.4	0.4±0.1	20.5±0.8
	$CoCl_2$	9.6±0.5	6.9±0.3*	0.3±0.1	16.8±0.6
	PB	19.8±1.6	4.6±0.4	0.2±0.1*	24.6±1.4
	$CoCl_2$+PB	13.3±1.4	6.8±0.6*	0.3±0.1	20.5±1.2
	$CoCl_2$+PB+LCA	12.4±0.9	7.9±0.5*	0.4±0.1	20.7±0.7
Surface Area ($\mu m^2 x 10^3$/ hepatocyte)	Control	69.7±6.9	22.7±2.6	1.7±0.5	94.1±8.7
	$CoCl_2$	50.4±5.3	35.7±2.8	1.7±0.5	87.7±7.7
	PB	127.8±18.5*	28.8±4.1	0.9±0.3*	157.6±21.2*
	$CoCl_2$+PB	91.8±10.4	49.7±6.2*	2.6±0.6	144.1±11.6*
	$CoCl_2$+PB+LCA	81.1±10.7	50.2±5.7*	2.5±0.8	133.8±13.9*

Treatments as in Table 6.

Values expressed as mean ± standard error.

* Significantly different from control group, $p<0.05$.

TABLE 9

Effect of experimental treatments on structural and functional membrane loads of hepatic endoplasmic reticulum

Treatment	Microsomal Parameter				
	Protein (mg/m² Total ER)	Phospholipid (μmol P/m² ER)	G6P-ase (μmol/min/m² RER)	Cytochrome P450 (nmol/m² SER)	NADPH-ase (nmol/min/m² SER)
Control	3.4±0.5	1.0±0.1	0.5±0.0	2.8±0.5	1.1±1.1
$CoCl_2$	3.7±0.2	1.1±0.1	0.1±0.0*	1.8±0.6	0.8±0.1*
PB	3.1±0.3	1.4±0.2	0.6±0.2	5.2±0.5*	2.0±0.2*
$CoCl_2$+PB	3.7±0.2	1.2±0.1	0.2±0.0*	2.4±0.4	1.5±0.3
$CoCl_2$+PB+LCA	4.7±0.8	1.4±0.1*	0.2±0.0*	2.8±0.3	1.2±0.1

Treatments as in Table 6.

Values expressed as mean ± standard error.

* Significantly different from control group, $p<0.05$.

ment of bile flow. The diagnostic signs are the same whether the disorder manifests at the hepatocyte level or due to anatomic obstruction of the biliary tree. They represent changes in both intrahepatic cholestasis and any disease resulting in cholestasis.

8.1 History and physical examination

In establishing early stages of cholestasis in case histories, physical examination and preliminary laboratory tests are necessary. These are helpful in confirming that the manifestations of the disease represent cholestasis itself or a hepatobiliary disease causing cholestasis. The physical examination and the various tests give only a general diagnostic approach to cholestasis and should be adapted individually to each patient. These examinations may include a variety of investigations: ultrasonography, radionuclide imaging, percutaneous transhepatic cholangiography, biliary scintigraphy, computerized tomography, endoscopic retrograde cholangiopancreatography, liver biopsy and serologic tests /902-912/. Using these various tests, an accurate anatomical diagnosis can be made and the site of cholestasis can be determined. We cannot go into details of physical examination of the patient, as it is beyond the scope of this review to expand on the various methods required to confirm the clinical observation suspecting intrahepatic cholestasis due to various causes (Table 1).

8.2 Biochemical features

a) Alkaline phosphatase

In early stages the major indicator of cholestasis is an elevated serum alkaline phosphatase level. This effect may occur before the retention of bile acids and bilirubin. Other biochemical parameters are not suitable for the early diagnosis of this disease. Some liver enzymes, such as alanine or aspartate aminotransferase and 5'-nucleotidase, do not necessarily show a rise in cholestasis. Some enzymes, such as γ-glutamyl transpeptidase, have increased serum levels, but the release of this enzyme from the liver is influenced by many factors other than cholestasis.

Alkaline phosphatase is present in many tissues including bone, kidney, liver, intestines, leukocytes and placenta. It is important to

establish that the elevated serum alkaline phosphatase originates from the liver. The hepatic alkaline phosphatase is bound to plasma membranes and the rise of its level in the serum cannot be associated with the failure of the hepatocellular excretory function /913, 914/. Furthermore, alkaline phosphatase does not simply leak out from the damaged hepatocytes as happens with bile acids, bilirubin and aminotransferase enzymes. It is probable that due to stagnation, the retention of bile acids in the cell causes a local accumulation and through their detergent action, bile acids solubilize plasma membranes resulting in a release of bound enzymes /240/. Following the release of alkaline phosphatase into the serum, hepatocytes are stimulated to synthesize more enzyme to build up a proper balance within the cell, probably responding to a feed-back control mechanism.

In certain conditions, elevated serum alkaline phosphatase levels show variations. Besides the hepatic enzyme, fractions of bone alkaline phosphatase are also present in prolonged cholestasis. This may be connected with osteomalacia secondary to prolonged cholestasis or with bone metastases as a consequence of a liver tumor. Some hepatic malignant cells can produce ectopic alkaline phosphatase enzymes. In some instances, intestinal alkaline phosphatase is elevated in the serum; this occurs in some patients with chronic active hepatitis or cirrhosis. Occasionally, increased serum alkaline phosphatase is not associated with any intrinsic liver disease /915, 916/. Some minimal elevation of the hepatic alkaline phosphatase enzyme occurs in the serum when other disorders are present such as sepsis, myocardial infarction or Hodgkin's disease. These diseases, however, even if not shown to affect the liver, are still connected with subtle enzyme changes. In these cases, the serum alkaline phosphatase returns to normal after successful treatment of the underlying disease.

b) Bile acids

Interruption of the normal bile flow in cholestasis leads to disturbances of the bile acid transport mechanism resulting in stagnation in hepatocytes and elevated serum bile acid levels. Normally, bile acids flow through the body via the enterohepatic circulation several times daily. These compounds pass from hepatocytes to the biliary tree and finally to the lumen of the duodenum. They are absorbed

from the intestine into the portal vein and transported back to the liver. Disturbance of the bile flow and bile acid transport mechanisms leads to cholestasis. In cholestasis bile acids are not taken up by hepatocytes but they are retained in the plasma and in the skin, and severe pruritus may occur as the consequence of these high levels /917/.

c) Hyperbilirubinemia

Greater disturbance of the hepatobiliary function leads to bilirubin retention. This occurs in later stages of cholestasis. Biochemical manifestations include hyperbilirubinemia (jaundice) associated with dark urine and pale stool. These abnormalities develop only after alkaline phosphatase has been elevated in the serum for a prolonged time. Hyperpigmentation, xanthoma, malabsorption and bone disease are often associated with long standing cholestasis.

Hyperbilirubinemia can occur in certain conditions without cholestasis and without any signs of liver disease. Bile flow and other biochemical parameters are normal including serum alkaline phosphatase. The pigment retention may be due to bilirubin overproduction such as in hemolysis, or due to impaired conjugation of bilirubin such as in the Crigler-Najjar syndrome, or defective uptake by the liver such as in Gilbert's syndrome associated with increased production of unconjugated bilirubin. In some instances, the excretion of bilirubin from the hepatocytes is defective, resulting in an elevation of conjugated bilirubin in the serum. These conditions are associated with the Rotor syndrome, Dubin-Johnson syndrome, sepsis and side effects of drugs.

d) Hyperlipidemia

This condition occurs in chronic cholestasis associated with an impairment of lipid synthesis and especially cholesterol metabolism /918, 997/. Serum cholesterol and lipid contents are increased in association with the presence of lipoprotein-X. This is an abnormal lipoprotein consisting of large amounts of apo-C and albumin, and equivalent amounts of free cholesterol and phospholipids. The presence of lipoprotein-X in the serum indicates cholestasis, but does not reveal the site of impairment in the hepatocytes. Moreover, since hyperlipidemia occurs in many other conditions, lipoprotein-X

should be identified when liver disorder is suspected. In prolonged cholestasis, xanthoma and xanthelasia may develop. In obese patients the occurrence of prolonged cholestasis, fatty liver, hepatitis and cirrhosis has been reported /919, 920/.

IX. CONCLUSIONS

Cholestasis represents a disorder in the secretion of bile salt containing micelles from hepatocytes together with some other substances. This condition has many facets. A pathological survey shows that in cholestasis, bile plugs are present in the canaliculi and bile pigment is retained in hepatocytes and in Kupffer cells. The evidence of cholestasis in a clinical biochemical study consists of the presence of increased amounts of biliary substances in the circulating blood due to their regurgitation from the biliary passages. Among these substances, the measurements of alkaline phosphatase activity and bile salt concentrations can serve as a tool for the diagnosis of this disease condition. The backflow of these substances from the liver provides evidence to the clinician that cholestasis is associated with a decrease or lack of bile flow.

Our understanding of intrahepatic cholestasis and other liver damage due to either disease or to drug therapy has shown substantial recent advances, but considerable information still remains to be obtained. Numerous hypotheses have been proposed for the pathogenic mechanism of intrahepatic cholestasis, but no single one has been unequivocally proven. Since various structural and functional changes manifest during cholestasis, it is reasonable to assume that the process of intrahepatic cholestasis is most likely the resultant response to the triggering of more than one mechanism simultaneously or within extremely short intervals.

Cholestasis may be initiated by different factors; however, information from recent animal models using mechanistic or molecular approaches still leaves unelucidated the initial steps leading to hepatobiliary impairment. There is evidence that in most instances of intrahepatic cholestasis the primary event may be connected with an impairment of the endoplasmic reticulum. Accordingly, we put forward the hypothesis that cholestasis or especially drug-induced intrahepatic cholestasis represents an initial impairment of subcellular structures. Specifically, damage to the hepatic endoplasmic retic-

ulum leads to secondary disturbances of bile flow, and elevations of serum bilirubin, bile acids and alkaline phosphatase. Conditions leading to reduced function of the endoplasmic reticulum membranes are associated with decreased synthesis of membrane bound phospholipids as manifested in diminished formation of methyl group-containing bases, such as lecithin, and a reduction in the synthesis of unsaturated fatty acid side chains. Such conditions include the administration of hepatotoxicants, some progesterone derivatives, pregnancy, delayed development of membrane function in the neonate and dietary treatments using choline or methyl donor deficient diets /301, 303-306, 310, 316, 319, 331, 332, 334/. As a consequence of this impairment, the drug metabolizing activity of the endoplasmic reticulum is reduced, including hydroxylating reactions. Hydroxylation of bile acids is disturbed, which in turn causes further interference with micelle formation. This assumed mechanism is similar to the mechanism proposed by Popper and Shaffner /32/; however, we provide biochemical and morphological evidence as to how the initial steps orchestrate the further chain of events.

In some instances micelle formation may be directly impaired by drugs. Furthermore, not only drug metabolizing activities but also other functions of the endoplasmic reticulum are disturbed, such as the methylation of phospholipids, desaturation of fatty acids, activities of several membrane-bound phosphatases such as glucose-6-phosphatase, nucleoside diphosphatase and inorganic pyrophosphatase. All of these effects can lead to a disturbed secretion of bile acids, associated with morphologic changes of the bile secretory apparatus /5, 129, 132/, resulting in a back-flow of bile from the bile canaliculi between the hepatocytes into the perisinusoidal spaces and further back to the circulating blood /921-923/.

Findings in many animal models and human conditions of intrahepatic cholestasis are congruent with the suggested mechanism that the impairment of the endoplasmic reticulum function may represent one of the early events in this disorder. Reduction of hydroxylating activity of the endoplasmic reticulum in general, and the decrease of 7α- and 12α-ring hydroxylation reactions in particular, leads to the accumulation of monohydroxy bile acids in the hepatocytes as a result of the inhibited metabolism. The latter enzymes are bound to the smooth endoplasmic reticulum /30, 32/. In liver cell damage, chenodeoxycholic acid is the predominant compound /923/. Excess formation of chenodeoxycholic acid, a metabo-

lite of lithocholic acid, and disturbed hydroxylation of cholesterol related to side chain transformation, may both result from an impairment of the smooth membranes. Conjugation of bile acids with taurine or glycine is also catalyzed by microsomal enzymes, and liver injury affecting the endoplasmic reticulum leads to reduced conjugate formation.

Intrahepatic cholestasis brought about by the administration of taurolithocholate /148, 925/ is in agreement with the impaired drug metabolizing activity. The distribution of taurolithocholate or lithocholate within the micelle is different from that of dihydroxy or trihydroxy bile acids. Since the solubility of monohydroxy bile acids is relatively low as compared to other bile acids, it requires higher temperatures to remain dispersed in solution in micelles. Both in the liver and in the bile in normal concentrations, bile acids exist in polyanionic forms as spherical molecular aggregates or micelles combined with phospholipid and cholesterol, bile acids being the regulators of the secretion of these lipids /926/. With increasing concentrations of bile acids the shape of the spherical aggregates becomes elongated and furthermore the rod shaped particles form a crystalline lattice, which is finally converted to a lamellar configuration. During these changes viscosity is greatly increased /927/. Subsequently, when the increased production of monohydroxy bile acids raises their concentration, the bile acid-cholesterol-phospholipid micelle complex becomes too viscous and probably cannot cross the canalicular membrane easily. In turn, this brings about a significant reduction in the secretion of fluids from the hepatocytes. The appearance of the microvilli in the bile canaliculi is also altered. Due to the excess liquid, bile containing bile acids, pigments and other components is regurgitated from the biliary passages through the sinusoids into the circulation.

In various models of drug-induced intrahepatic cholestasis alterations of the endoplasmic reticulum are involved. Administration of ANTT causes impaired microsomal metabolism /663, 683/, compensatory increased protein synthesis and proliferation of smooth endoplasmic reticulum /928/. Several drugs inhibit the function of the endoplasmic reticulum /683, 929/. Competition may also occur between drugs and steroids, or between drugs and bile acids, for hydroxylation by drug metabolizing enzymes, resulting in impaired steroid or bile acid metabolism and leading to cholestasis.

The role of inducers of microsomal enzymes in intrahepatic cholestasis may be very complex. Phenobarbital induces proliferation of smooth endoplasmic reticulum and enhances drug metabolism and cholesterol synthesis. It also increases the bile flow in normal rats /930/. In cholestasis, phenobarbital raises serum bilirubin levels /485/ and microsomal bilirubin: UDP-glucuronyl transferase activity /931/. Phenobarbital is partially effective in protecting against the development of intrahepatic cholestasis. In contrast, however, the toxic effect of lithocholic acid in the rat could be reversed by pregnenolone-16α-carbonitrile /932/. Other steroids including estrogens and anabolic steroids also induce smooth endoplasmic reticulum membranes and hence compete with the metabolism of bile acids. Thus, these compounds may interfere with bile secretion by altering the critical concentration of bile acids in the micelles /32/.

The apparent enigma of the endoplasmic reticulum proliferation may be related to the production of different metabolites which may have different actions. Many drugs and chemicals, such as chlorpromazine and other amines, can exhaust the microsomal drug metabolizing capacity of the endoplasmic reticulum. These drugs precipitate bile salts and may impair micelle formation and bile secretion /10/. In fact, it has been shown that the relative hepatotoxic potential of chlorpromazine derivatives shows great variations /933/.

Cholestasis and recurrent jaundice of pregnancy usually develop in women in the third trimester and disappear after delivery. It also develops in women while taking oral contraceptives /934/. Cholestasis resulting from oral contraceptive steroids is more common in Scandinavia and Chile, related to a genetically modified metabolism of steroid hormones which is triggered by increasing amounts of steroids produced during pregnancy /935/. It has been demonstrated that drug metabolizing activity is reduced during pregnancy. Animal studies have established that this reduction is associated with an impairment of the endoplasmic reticulum, particularly with the production of altered membrane bound phospholipids /85, 332/. The effect of pregnancy on the endoplasmic reticulum membrane can be simulated by the administration of certain reduced progesterone derivatives /328, 337/ which are produced in great amounts by the pregnant female. It has been shown that certain metabolites of steroid hormones are responsible for neonatal jaundice in some cases, reflecting the fact that their interaction causes a delayed devel-

opment in the functioning of the endoplasmic reticulum in the newborn.

Success in the clinical use of S-adenosylmethionine for the symptomatic treatment of intrahepatic cholestasis /128, 834-836/ may provide further evidence for the feasibility of our endoplasmic reticulum function-related hypothesis. Impairment of experimental cholestasis induced by estrogens is attributed mainly to changes in lipid structures of liver plasma membranes /235, 403/: in particular, the cholesterol:phospholipid ratio and consequently membrane viscosity are increased /403, 936-939/. Administration of S-adenosyl-L-methionine decreases the cholestatic actions of estrogens in rats /336, 337, 341/. Besides estrogen cholestasis, adenosylmethionine successfully ameliorates bile flow impairment in cholestasis induced by chlorpromazine /940, 941/, ANIT /343, 942/ and lithocholic acid /938/. Adenosylmethionine exerts an anticholestatic action irrespective of the etiology of the cholestatic liver injury. Adenosylmethionine also maximizes the bile secretory rate and provides hepatocytoprotection against taurolithocholate-induced cholestasis /938/. Comprehensive studies have reported that intrahepatic cholestasis of pregnancy in women could be reversed by the administration of high doses of S-adenosyl-L-methionine /834, 943/. Increased cholesterol saturation of bile in humans brought about by ethinyl estradiol can also be prevented by adenosylmethionine /335-338/.

The ameliorating and protective action of this compound could be explained as: (a) Adenosylmethionine dependent methylation inactivates toxic metabolites /341, 944-949/. Adenosylmethionine donates its methyl group to a variety of acceptors representing the transmethylation pathway and is converted to S-adenosylhomocysteine and through the transsulfuration pathway further to cysteine. The availability of cysteine is the rate limiting step for the synthesis of glutathione and other sulfur containing compounds such as taurine sulfates. Increased levels of glutathione may affect the function of the endoplasmic reticulum /945, 947/. (b) Detoxication occurs through transsulfuration /834, 950/. (c) S-Adenosyl-L-methionine related methyl transfer modifies membrane phospholipids /316, 317, 319/ which modulate the fluidity of various membranes, particularly that of the endoplasmic reticulum where the methylating enzyme is bound, which in turn can decrease the viscosity of liver plasma membrane /951/.

In the ethinyl estradiol-induced cholestasis in the rat, the protective and reversal actions of S-adenosyl-L-methionine /940, 943, 952, 953/ may be associated with either inactivation of estrogen metabolites by sulfation or methylation of membrane phospholipids. Furthermore, in the anticholestatic activity of S-adenosyl-L-methionine, phospholipid methylation is the probable operative process; this reaction is essential in the reversal of changes occurring in membrane fluidity caused by various toxic agents such as chlorpromazine or lithocholic acid. Our studies have shown that methyl transfer from natural methyl donors to endoplasmic reticulum-bound phospholipids is impaired by hepatotoxic compounds, leading to the loss of many microsomal enzymes which function in the presence of bound phospholipid /318, 320/. Inducers of drug metabolism and several drugs that contain methyl groups can enhance the activity of S-adenosyl-L-methionine:microsomal phospholipid methyl transferase. Some methylated drugs can actually donate methyl groups to synthesize methyl group containing membrane phospholipids /301, 302/.

In summary, intrahepatic cholestasis may have many origins (Table 1). It is, therefore, very likely that various mechanisms operate in the pathogenesis and lead to the sequelae of this disorder, such as decreased bile secretion, canalicular dilatation, loss of microvilli, intracellular bile pigment accumulation, disruption of the tight junctions, regurgitation of biliary substances into the circulation, increased serum bile salt and bilirubin concentration, increased alkaline phosphatase activity, just to name the most important disturbances. All of these changes may simply represent the final manifestations of multiple abnormalities of intracellular metabolism. Experimental models that simulate human cholestasis have several limitations. The doses applied to animals to cause cholestasis are generally far greater than those given clinically to man and dose-response relationships have rarely been achieved. It is difficult to establish whether the abnormalities produced are primary events in the development, or just secondary to decreased bile flow. The various experimental procedures rarely have selective sites of action. Still, animal models are very helpful in elucidating various steps in the pathogenesis of cholestasis.

All the evidence from our studies and supporting literature data may be fragmentary, but when causes and facts are put together, the data support the assumption that changes in functioning of the endo-

plasmic reticulum may represent one of the initial steps in intrahepatic cholestasis caused by drugs or by various hepatotoxic foreign compounds, or as a result of adverse physiological and dietary conditions. In particular, the formation of methylated phospholipid species bound to endoplasmic reticulum membranes through methyl transfer reactions seems to be the first sensitive target for xenobiotics. The disturbance of synthesis of essential components of the endoplasmic reticulum membrane leads to impaired function of several membrane bound enzyme systems leading to further chain reactions and manifestations of pathological changes, and finally to the clinical expression of cholestasis.

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