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Intrahepatic Cholestasis Induced by Drugs and Chemicals

GABRIEL L. PLAA[1,2] AND BRIAN G. PRIESTLY

Département de pharmacologie, Faculté de Médecine, Université de Montréal, Montréal, Canada, and Department of Human Physiology and Pharmacology, University of Adelaide, Adelaide, Australia

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I. Introduction

SEVERE hepatic damage related to the ingestion of drugs and chemicals has been reported in the medical literature since the beginning of this century. The major characteristics of the classical hepatotoxic response seem to be the accumulation of lipids within the hepatocytes, which leads to a fatty liver, and the degeneration of the hepatocyte, which terminates in focal, zonal or massive necrosis (112, 361). In contrast, Hanger and Gutman (187a) de-

[1] Requests for reprints should be sent to Dr. Gabriel L. Plaa, Dept. de pharmacologie, Faculté de Médecine, Université de Montreal, C.P. 6128, Montréal, Québec, Canada.
[2] Member, Medical Research Council Group in Drug Toxicology.

scribed in 1940 a series of case reports that involved the presence of jaundice after the clinical use of arsphenamine. They noted that in several hepatic biopsy specimens there was an absence of parenchymal degeneration, but bile plugs were present in many bile canaliculi, accompanied by dilatation and engorgement of the finer biliary radicles. They attributed these cases of postarsphenamine jaundice to intrahepatic obstruction of the biliary tract. In 1943, Ottenburg and Spiegel (346a) reviewed the status of nonobstructive jaundice due to chemical agents and noted that arsphenamine, cinchophen, and some sulfonamides could produce jaundice in people, but not in experimental animals, and that this reaction seemed to be allergic or idiosyncratic in origin. However, Drill's review (112) of the hepatotoxicity of drugs and chemicals published in 1952, does not mention a drug-induced lesion compatible with what is now recognized as intrahepatic cholestasis. This lesion results in the diminution or cessation of bile flow with the retention of bile salts and bilirubin, which leads to the production of jaundice; the accumulation of lipids and the presence of extensive necrosis of the hepatocytes are not prominent features. The recognition of intrahepatic cholestasis as a specific hepatic lesion did not occur until later in the 1950s. The second edition of Himsworth's monograph (197), published in 1954, and widely regarded as a text on liver injury, described a syndrome called cholangiohepatitis in which the principal feature was inflammation in and around the small bile ducts, but which could occur without jaundice or any outward sign of hepatic dysfunction. The emphasis on inflammation implied by the term cholangiolitis focused attention on the bile ducts as the primary site of the lesion and did not help to further the understanding of the etiology of this syndrome.

When Popper (367, 368, 375) popularized the term "intrahepatic cholestasis" to describe the syndrome characteristic of biliary retention but without evidence of mechanical obstruction, attention shifted to the hepatocyte, and in particular to the hepatocanalicular mechanisms involved in the elaboration of bile. This term emphasizes the functional derangement of the bile secretory system, and differentiates it from the other mechanisms which could result in plasma accumulation of biliary constituents and clinical jaundice. It was apparent that this syndrome was commonly associated with the ingestion of certain drugs, notably chlorpromazine and norethandrolone.

Although our understanding of the pathogenic mechanisms involved in drug-induced cholestasis is still quite incomplete, some hypotheses have been put forward, and there has been substantial investigation of the problem at the clinical level and in experimental animals. In this review, it is our aim to concentrate on the experimental studies, and to show how these studies complement some of the clinical observations. However, it must be acknowledged that although cholestatic lesions have been induced in animals with chemical agents, and these lesions are sometimes morphologically and functionally similar to those seen clinically, no experimental model of cholestasis has been successfully developed which duplicates all the cholestatic features produced during drug-induced cholestasis in man.

One way to illustrate some of the problems associated with the evaluation of the cholestatic potential of drugs is to observe the difference between these drugs and those which produce hepatocellular necrosis, a lesion that some have called "toxic hepatitis" (261). Some of the characteristics of the latter type of hepatic lesion are: a) a distinctive histological lesion; b) presence of a dose-response relationship; c) high incidence depending upon exposure conditions; d) reproducibility in laboratory animals; and e) a predictable latent period.

Generally speaking, cholestatic agents exhibit only the first criterion, and usually, the hepatic ultrastructural changes are observed only in those individuals who manifest the full-blown cholestatic reac-

tion. The lack of a dose-response relationship makes it very difficult to predict the risk of cholestasis associated with therapeutic dosage. The incidence of the toxic reaction is also quite different when one compares cholestatic agents with those chemicals which produce necrosis. For example, with carbon tetrachloride, it is possible to determine the dose that will produce centrolobular necrosis in virtually 100% of the population (animal or human) at risk. However, the reported incidence of drug-induced cholestatic reactions is usually very low (well below 1% in most cases), regardless of the doses administered.

Perhaps the most important difference between cholestatic and necrogenic agents is the relative ease with which the hepatic lesion seen in susceptible people can be reproduced in experimental animals. Necrotic lesions may be reproduced more easily in animals, although some knowledge of the factors which govern the threshold dose may be necessary. The demonstration of hepatocellular necrosis in animals with acetaminophen is a good case in point (90, 240, 241, 317, 318, 376, 377). However, with the possible exception of the steroids, it has been virtually impossible to reproduce in animals the drug-induced cholestatic syndrome seen in man. This aspect is very important. It is the major reason for the lack of predictability of the cholestatic potential of drugs currently on the market.

Even if the cholestatic lesion could be reproduced in laboratory animals and if the incidence is the same in both animal and human populations, then one is faced with the problem of testing enough animals to assure detection of even one susceptible subject. The number of animals required to satisfy this demand is about 300 if the true incidence is 1%, and about 300,000 if the true incidence is 0.001% (360, 519).

Some have used the lack of reproducibility in experimental animals as being an indication that drug-induced cholestasis in man is an allergic (hypersensitivity) reaction. It is recognized that allergic reactions can lead to cholestasis in man, but it does seem that, in most situations in which this mechanism has been invoked, it has been arrived at indirectly.

Finally, a predictable latent period does not seem to exist for drug-induced cholestasis. Such reactions have occurred in some cases shortly after therapy has been initiated, and in other cases it has been observed only after some weeks.

II. Other Reviews

Intrahepatic cholestasis has been extensively reviewed over the past 10 years. Virtually every reference book dealing with abnormal liver function contains at least one chapter dealing with cholestasis or impaired excretory function. Some examples of relatively recent reviews may be found in volumes such as those edited by Becker (31), Eliakim *et al.* (131), Gentilini *et al.* (164), Goresky and Fisher (176), Leevy (283), Orlandi and Jezequel (345), Schaffner *et al.* (374, 427), and Schiff (437). Popper and Schaffner have contributed a number of reviews dealing with the morphological features of cholestasis, and have discussed the possible mechanisms involved in its etiology (368, 371, 373, 420, 423–425, 429). Other reviewers include Berthelot (32), Javitt (227, 230), Plaa (358–361), and Sherlock (462).

Reviews dealing mainly with the mechanisms of bile formation have also made important contributions to the understanding of cholestatic mechanisms (*e.g.*, 10, 53, 137, 138, 260, 381, 506). The role that bile salts might play in various types of liver disease, including the cholestatic syndrome, has also been reviewed (18, 65, 228, 234, 334, 438).

III. Drugs Which Cause Cholestasis

No review of chemically induced cholestasis could be complete without some indication of which drugs are liable to produce the cholestatic syndrome during clinical use. We have not attempted to list all such

drugs, nor is it our intent to review in depth the many clinical observations dealing with drug-induced cholestasis. Other reviewers have fulfilled this onerous task more competently. In particular, Zimmerman (521–525) and Klatskin (261, 262) have devised quite useful classifications to differentiate drugs which produce cholestatic or mixed cholestatic/hepatitic lesions from those true hepatotoxins which produce primarily hepatocellular necrosis. These authors point out that it is generally accepted that most forms of drug-induced cholestasis in man seem to be due to a hypersensitivity reaction. However, they also point out that a number of these drugs can alter hepatic function without producing jaundice. Zimmerman (524) has proposed that host idiosyncracy to the drug may be mediated by allergic mechanisms or may depend upon aberrant metabolism leading to hepatotoxic metabolites (see section V H). We have concentrated on those drugs which produce clinical jaundice and for which hepatocellular necrosis does not seem to be the major feature. Furthermore, the arguments in favor of or against an allergic mechanism of action in human drug-induced cholestasis are not discussed in this review.

The differential diagnosis of cholestatic liver disease is often a difficult clinical problem. Unless biopsy material is available for histochemical or ultrastructural evaluation of the specific morphological changes which occur in the canalicular region, the differential diagnosis is usually based on profiles of elevated activity of certain serum enzymes, and the nature of the hyperbilirubinemia. It has been suggested that the recognition of abnormal bile salt retention in, or clearance from, the plasma may be a more sensitive index of cholestatic liver injury (57, 228, 342). The demonstration that an abnormal lipoprotein (LP-X) may be found in the serum of patients with cholestasis may provide a useful adjunct to the more conventional biochemical profiles, particularly now that sensitive and specific immuno- and polyanion-precipitation techniques have been developed (400, 448–454). A modification of the LP-X test is claimed to enable the differential diagnosis of extrahepatic biliary obstruction from intrahepatic cholestasis (454), but this remains to be confirmed. LP-X has also been identified in the plasma of dogs with extrahepatic cholestasis produced by bile duct ligation (88). This suggests that the test might find wider application in experimental as well as clinical studies of the cholestatic syndrome.

The problem of classifying the predominant nature of the hepatic lesions produced by drugs in clinical use is compounded by the low incidence, the variable clinicopathological signs and symptoms of the disease, the possible interaction of other drugs, and the lack of adequately standardized criteria for assessing adverse drug reactions (107, 246). Furthermore, clinicians tend to use a multiplicity of terms to describe the same syndrome.

These problems apply to even the more "notorious" cholestatic agents, and are perhaps best illustrated by reference to chlorpromazine. Some of the terms describing chlorpromazine-induced hepatic injury have included "toxic hepatitis" (412), "allergic jaundice" (199), "allergic cholangiolitis" (520), and "hypersensitivity reaction" (502). This latter report may be the origin of the oft-quoted 50% incidence for the development of mild hepatocellular dysfunction with chlorpromazine, while 0.5 to 1% is the more widely reported incidence for those patients who manifest clinical jaundice. The 50% incidence figure for chlorpromazine-related mild hepatic dysfunction has also been quoted by Zimmerman (521) on the basis of several other clinical case reports. There may be grounds for doubting this figure. Hollister *et al.* (201, 202) reported that the incidence of abnormal hepatic function tests was quite high in schizophrenic patients irrespective of the therapeutic regime, and that there was no evidence for a higher incidence of liver dysfunction in chlorpromazine-treated patients.

There are also some who believe that the

incidence of chlorpromazine-related jaundice began to fall after the first decade of its use (175,200). There is some support for this belief in the series edited by Meyler (314) and Meyler and Herxheimer (315) in which literature reports of adverse drug reactions were cataloged. Studies on the phenothiazines from the 1950s to the late 1960s confirm that cholestatic jaundice is a major source of the reports on adverse reaction to chlorpromazine. However, the 1972 edition treats hepatic dysfunction as a minor, relatively rare, adverse reaction associated with phenothiazine therapy. Some of the reasons advanced to account for a reduced incidence have included: a) a trend toward the use of newer phenothiazine derivatives with lower cholestatic potential; b) development of "desensitization" with prolonged use; c) attribution of the hepatic dysfunction to a manufacturing impurity in the case of chlorpromazine, which has since been eliminated [we found no direct evidence in the literature for this proposal and it has been refuted by the manufacturers (200; personal communication)]; d) elimination of "high risk" patients with pre-existing liver disease from chlorpromazine therapy; and e) improvement in diagnostic techniques which has allowed better discrimination between viral hepatitis and drug-related liver dysfunction. Piccinino *et al.* (356) have discussed the problems involved in the differential diagnosis of viral hepatitis from intrahepatic cholestatic jaundice of pregnancy. They found that with only biochemical and clinical data, the accuracy of their diagnosis was only 75 to 85% in cases in which the diagnosis could be confirmed by biopsy. This last point is an interesting one since it seems that the introduction of chlorpromazine into therapy in the United States coincided with an epidemic of viral hepatitis (175), and Lunel *et al.* (296) have argued that chlorpromazine may act by potentiating the effects of a latent viral hepatitis.

It is difficult to know whether coincident viral hepatitis, or poor differential diagnosis of this disease, is the reason behind the observation that chlorpromazine-induced jaundice may sometimes take the form of either pure centrolobular cholestasis or a cholestatic-hepatitic syndrome, with inflammation, elevated transaminases, *etc.* (520, 523, 524). The problem is further complicated by the fact that viral hepatitis may also occur with a cholestatic phase more prominent than that expected from the extent of the hepatocellular degeneration (55, 198, 368, 369). It has been noted (251, 368, 429) that the hepatitic component may subside during prolonged chlorpromazine-induced cholestasis, but it is not clear whether this may represent "desensitization" (459).

Another factor which has complicated the interpretation of hepatic dysfunction associated with chlorpromazine is the variability in the duration of the jaundice. In a series of 22 cases reviewed by Werther and Korelitz (504), the duration of the jaundice ranged from 7 to 122 days, whereas another report (43) has described jaundice of 17 months duration. Even longer periods of jaundice have been reported when the lesion progressed to a frank biliary cirrhosis (390, 503). No dose-response relationship could be recognized. The mean total dosage before the onset of jaundice in a group of 36 patients reviewed by Ishak and Irey (222) was 2.78 g, but doses as low as 30 to 350 mg have been reported to be sufficient to induce jaundice (518).

In table 1, some of the drugs are listed for which there have been reports linking the occurrence of cholestasis or cholestatic hepatitis with ingestion of the drug. It is very difficult to estimate the true incidence of drug-related cholestasis. The figures quoted by most reviewers are based upon case reports by individual investigators or surveys of small patient groups. There is usually no basis for estimating the number of patients taking the drug who do not manifest hepatic dysfunction. Such data could be drawn from well designed national surveys of adverse drug reactions. However, even when available in published form, these surveys are sometimes hard to interpret; there is a need to

TABLE 1
Drugs with cholestatic potential classified according to likely incidence

Drugs	References*
Common – incidence probably greater than 2%	
Erythromycin estolate	See text (sections IV E, F)
Norethandrolone	
Triacetyloeandomycin	
Less common – incidence 1% or less, but numerous cases documented	
Chlorpromazine	See text (sections III, IV E)
Methyltestosterone and related anabolic steroids	173, 308, 393, 415
Contraceptive steroids	
Iprindole	
Carbutamide, acetohexamide, chlorpropamide	
Rare – only isolated reports	
Phenothiazines: thioridazine, promazine, prochlorperazine, trifluoperazine	194, 264, 396, 469
Tricyclic antidepressants: imipramine, amitriptiline	247, 329
Anxiolytics: chlordiazepoxide, diazepam	1, 475
Antibacterials: nitrofurantoin, rifampicin, novobiocin, sulphonamides, penicillin	52, 77, 84, 116, 135, 238, 357, 365
Oral hypoglycemics: tolbutamide, tolazamide	19, 179, 497
Antirheumatics: gold salts, phenylbutazone	432
Antithyroids: carbimazole, methimazole	147, 297

* See Zimmerman (521, 524), Klatskin (261, 262), or Perez *et al.* (352) for more listings.

standardize the procedures for collecting such data, and especially a need to standardize the criteria for assessing the nature of the adverse drug reaction (107, 246).

The review of Maxwell and Williams (309) includes a table showing the number of reports of drug-induced jaundice in cases reported to the United Kingdom Committee on the Safety of Medicines over the period 1964 to 1971, along with data on the number of prescriptions written by general practitioners during 1970. These data give some indications of general incidence, although they are clearly subject to severe limitations. In order to calculate incidence, one must assume that, not only are all cases accurately diagnosed and reported, but that there has been consistency in drug use and adverse reaction reporting over the 8-year period.

Nevertheless, two interesting observations may be made on the basis of these data. Firstly, the incidence of chlorpromazine-related jaundice seems to be considerably lower than that generally acknowledged. Taking the 0.5 to 1% incidence quoted by most reviewers, and the number of chlorpromazine prescriptions dispensed in 1970 (1.5 million), one would calculate that 8,000 to 15,000 cases of jaundice might have been expected in 1970 alone, yet only 213 cases were reported for the entire 1964 to 1971 period. Of course, it is not possible to know how many patients were actually involved and how many cases were not reported, but the discrepancy in the figures is quite remarkable.

Secondly, the comparative data on the tricyclic antidepressants, which are generally regarded as causing a low incidence of cholestatic reactions in man, suggest that iprindole has a quite high cholestatic potential. When it is considered that iprindole has been in general use only since 1967 (315) and that only 240,000 prescriptions were dispensed in England in 1970, the report of 46 cases of cholestatic hepatitis for the period 1964 to 1971 infers that the cholestatic potential might exceed that of chlorpromazine. This resulted in a warning being issued by the United Kingdom Committee on the Safety of Medicines in 1971 (12). The cholestatic syndrome is

apparently reversible upon discontinuation of the drug (7, 12).

Steroid-induced cholestasis seems to differ from that of other drug classes in several important characteristics. While most forms of drug-induced cholestasis are widely regarded as being due to a hypersensitivity reaction, this does not seem to be the case in steroid-induced cholestasis. Furthermore, it seems that a structure-activity relationship has been recognized for the anabolic steroids. Alkylation of the C17 position of the steroid nucleus seems to be mandatory for cholestatic potential (92) (see section IV E). The morphological features are characteristically those of pure centrolobular cholestasis with little or no portal inflammation, and the elevation of plasma transaminases is usually not marked. It is our impression that the morphological studies of norethandrolone-induced cholestasis (*e.g.*, 426) pioneered the currently recommended morphological criteria used to differentially diagnose intrahepatic cholestasis (96, 436). For this reason, the differential diagnosis of steroid-induced cholestasis from viral hepatitis, or other forms of liver disease, seems more straightforward than that with drugs such as chlorpromazine which produce a more variegated response.

The incidence of jaundice associated with norethandrolone is difficult to estimate accurately, but it has been reported to be as high as 25% (165). The incidence of liver dysfunction characterized by sulfobromophthalein (BSP) retention is considerably higher—74% in the study of Kory *et al.* (265) and 100% in some others (189, 284, 434). Furthermore, there are strong indications that the effects are dose-related, and this contrasts strongly with the absence of clear dose-response relationships for other cholestatic agents. Furthermore, BSP retention can be produced in animals (see section IV E).

The incidence of BSP retention in women taking contraceptive steroids is also higher than the incidence of cholestatic jaundice, but interpretation of the structure-activity and dose-response relationships is more complex because of the variety of estrogen/progestogen formulations currently in use. The clinical and morphological features of cholestasis resemble those produced by the anabolic steroids (273, 274, 339, 344) but more important perhaps, is the apparent association between oral contraceptive-induced cholestasis and the benign cholestatic jaundice sometimes observed during the third trimester of pregnancy (also called idiopathic or recurrent jaundice of pregnancy). It is clear that women who experience this syndrome are highly susceptible to oral contraceptive-induced cholestasis (113). It is not clear, however, whether the estrogenic or progestational component is responsible, although some believe that the evidence favors the estrogen (339, 490). The presence of a C17 alkyl group is not mandatory in this case, and cholestatic jaundice with an incidence of about 5% has been reported with the noncontraceptive use of one of the synthetic progestogen components, norethisterone acetate (271).

One interesting feature of the cholestatic syndrome associated with oral contraceptives is the possible involvement of genetic factors. This was suggested initially by an apparently higher incidence of reports of contraceptive steroid-induced jaundice from Chile and Scandinavia (344) and subsequently supported by familial studies on the incidence of idiopathic cholestasis of pregnancy (87, 205, 272). However, there are cases where no familial correlation has been found and the supposed geographical variability in susceptibility has not been proved beyond doubt (268).

IV. Experimental Studies of Cholestasis

A. General Remarks

There are two major problems in designing and interpreting experimental studies of cholestasis.

The first problem is how to determine what constitutes a cholestatic response. Should it be based on morphological evi-

dence: *e.g.*, bile plugs; dilated bile canaliculi lacking in microvilli; a thickened pericanalicular ectoplasm? Should it be based solely on evidence of impaired biliary function: *e.g.*, reduced bile flow, even if this does not lead to complete stasis; hyperbilirubinemia; bile salt retention? Very few, if any, of the experimental studies performed with agents of known cholestatic potential in man would satisfy all of the above criteria.

The second problem is how to deal with the very low incidence, and lack of dose-response relationship seen in most instances of drug-induced cholestasis in man. If, as it has been suspected for certain drugs, the mechanism of drug-induced cholestasis in man involves a hypersensitivity reaction, is it reasonable to expect that the same response could be elicited in experimental animals? On the other hand, if the low incidence is the result of some metabolic, genetic, or nutritional idiosyncracy, is it likely that the same conditions can be reproduced in an experimental model of cholestasis? Which species should be selected for study? How does one interpret studies performed with experimental agents which produce a dose-related cholestasis (*e.g.*, as in the case of α-naphthylisothiocyanate)? How can this be related to the problem of drug-induced intrahepatic cholestasis in man?

We may not be able to answer all of these questions given the current state of our knowledge of cholestatic mechanisms. Nevertheless, the problem of chemically-induced cholestasis has been extensively studied in experimental animals and in this section, some of the studies will be reviewed which have made important contributions to our understanding of the characteristics, and perhaps the causes of the cholestatic syndrome.

B. Bile Duct Ligation

Experimental cholestasis induced by bile duct ligation (BDL) is strictly speaking not the ideal model to use to study the phenomenon of intrahepatic cholestasis, because the initiating factor is an extrahepatic obstruction. Nevertheless, BDL has been extensively studied as a cholestatic model, mainly in rats, because it is possible to delineate the changes in hepatic morphology and function which result from cholestasis as opposed to those that might cause it. Furthermore, since the time at which cholestasis occurred is known, useful information has been obtained on the rate at which hepatic changes occur during cholestasis.

In this section, we will review those studies which have dealt primarily with the morphological changes occurring in hepatic parenchymal and bile ductular cells as well as the histochemical changes, and the factors leading to the release of hepatic enzymes into the plasma. BDL-induced changes in the ultrastructure and function of the canalicular membrane are discussed more specifically in section V C, and BDL-induced changes in microsomal mixed-function oxidase activity are discussed in section V F.

In a series of papers, De Wolf-Peters *et al.* (102–104) described the morphological development of bile canaliculi in fetal, neonatal, and mature rats and compared these changes with the progressive changes which occur after BDL (100). They concluded that essentially four types of bile canaliculi could be differentiated in maturing animals. The type I canaliculi, which predominates in early fetal rat liver, is an irregular invagination of two adjacent cell membrane segments into the cytoplasm of one or two neighboring hepatocytes. Type II canaliculi seen later in fetal life have an irregular lumen, partly occupied by finger-like cytoplasmic extensions from the adjacent hepatocytes. During the perinatal period, canaliculi have progressed to type III and have a wide lumen, but few microvilli. The transition toward the normal adult canaliculus (type IV) with a lumen virtually filled with long and regular microvilli, begins approximately 1 day after birth.

In terms of histochemical staining for

alkaline phosphatase activity, the type IV canaliculus is characterized by a weak enzyme activity while the type III seems to be devoid of enzyme activity. However, intense staining for enzymic activity is observed during the type III to IV transition period. With morphometric analysis, it was shown that type I and IV canaliculi possess the greatest surface/volume ratio, while the type III has the lowest.

De Wolf-Peters *et al.* (102–104) concluded that the type III canaliculi, with dilated lumen and lacking microvilli or alkaline phosphatase activity, would also be devoid of bile secretory function. They speculated that there should be a brief cholestatic phase during the perinatal period before the type III to IV transition, and that the immaturity of the bile secretory apparatus should be considered along with the immaturity of metabolic systems in some types of neonatal jaundice.

Changes in the nature of bile canaliculi have been noted during BDL-induced cholestasis in the adult rat (100). One day after BDL, many transition type III to IV canaliculi were observed along with normal type IV. By 2 days, type III canaliculi predominated, but some type I canaliculi also began to appear. Increasing numbers of type I and II canaliculi had appeared by day 3. The question arises whether the emergence of type I canaliculi resulted from the collapse of type III canaliculi, or whether this represented the formation of new structural units in an attempt to regain normal excretory function. The authors favored the latter hypothesis.

The application of scanning electron microscopy by Vial *et al.* (500) has tended to confirm the existence of canaliculi with structures reminiscent of type III and type IV after 3 days BDL. This newer microscopic technique brought to light another interesting finding. It seemed that at the junctional zone between two hepatocytes, an overlapping "marginal ridge" was formed which tended to seal off the distended canaliculi and prevented their rupturing into the intercellular space. The increasing tortuosity of the canaliculi, and expansion of the distending canalicular walls below the marginal ridge could have given rise to structures which would resemble the type I canaliculi described by De Wolf-Peters *et al.* (100), thus casting some doubt on their hypothesis concerning the formation of new excretory units.

The changes which occur at the canalicular level after BDL occur more rapidly than the gross changes in hepatic structure. Johnstone and Lee (237) have analyzed the progressive changes in hepatocytes, bile ductular cells, and biliary stroma 1 to 40 days after BDL. The rate of proliferation of hepatocytes reaches a maximum of 24 times normal 4 days after BDL, while bile duct epithelium proliferation peaks at 50 times normal 1 day after BDL. However, it is not until approximately 8 to 12 days after BDL that increasing proportion of ductular cells and supporting tissue make any appreciable change in the relative percentage of liver volume for each cell type. The total number of hepatocytes fell marginally toward the end of the 40-day study period.

There has been considerable interest in determining the pattern of serum enzyme changes after BDL. Two enzymes, alkaline phosphatase (AP; EC 3.1.3.1) and 5′-nucleotidase (5′N; EC 3.1.3.5), have been the focus of attention, although some interest has been shown in γ-glutamyltranspeptidase (γ-GGT; EC 2.3.2.1) and leucine aminopeptidase (LAP; EC 3.4.1.1). The serum activity of these enzymes, which are localized in the membranes of hepatocyte and bile ductular cells, is increased during extrahepatic cholestasis in man (27, 80, 507a) and in experimental animals (270).

Kryszewski *et al.* (270) studied the temporal changes in both liver and serum of the first three of these enzymes. They found that significant serum elevation with all three could be detected 12 hr after BDL in the rat, but that AP and 5′N peaked at 24 hr, then gradually declined, while γ-GGT peaked at 48 hr, and remained elevated even 192 hr after BDL.

The temporal pattern seemed to be different in the liver. Hepatic 5′N and γ-GGT underwent a lag phase of 24 to 48 hr, then increased steadily while AP activity rose sharply, with no lag phase, and began to decline again between 12 and 24 hr after BDL. These patterns were modified by inhibitors of protein synthesis. In particular, the rise in both serum and hepatic AP was reduced, although part of the reduced AP response may have been due to inhibition of intestinal phosphatase activity since this is probably the more significant source of serum AP activity in the rat (398). The effects of cycloheximide and actinomycin D pretreatment on γ-GGT and 5′N were more complex. Actinomycin D enhanced the effects of BDL on 5′N, but not γ-GGT and cycloheximide did the opposite.

It is clear that different factors regulate these enzyme changes. A number of authors have suggested that the increase in serum AP results mainly from the *de novo* synthesis of hepatic AP. Results consistent with this hypothesis have been obtained in the rat (242, 243, 399), dog (366), and cat (447). The studies by Kryszewski *et al.* (270) confirm this earlier hypothesis, but suggest that different mechanisms may operate for 5′N and γ-GGT. In particular, they observed that the increase in 5′N and γ-GGT seemed to coincide with the phase of bile duct proliferation.

Kaplan and Righetti (243), who also showed that cycloheximide prevented AP induction, do point out, however, that another interpretation can be made of these data. They state that prevention of increased enzyme activity by agents which inhibit ribonuleic acid (RNA) synthesis cannot be taken as unequivocal proof that such increases are synonymous with true enzyme induction. They cite the work of Griffin and Cox (182) who have demonstrated that the increase in AP in HeLa cells by adrenocorticoids was actually due to activation of existing enzyme rather than *de novo* synthesis. They further cite the work of Schimke *et al.* (439) and Kenney (250) who showed that an apparent induction of tryptophan pyrrolase by tryptophan was due to a decreased degradation by rapidly turning over proteolytic enzymes.

Ronchi and Desmet (413) studied the changes in the intrahepatic distribution of these and other enzymes. In normal rat liver, γ-GGT is localized at the apical border of the epithelial cells of bile ducts and ductules. The activity seemed to be greater in both original and newly proliferating ductular cells after BDL. The early rise in hepatic AP activity seemed to represent a widening of its distribution, from localization in periportal canaliculi in controls to more widespread activity in hepatocyte membranes generally 24 hr after BDL. Hepatic LAP activity in canalicular membranes declined rapidly while a strong increase in activity was seen in ductular cells.

Relatively few studies have been performed in species other than the rat. However, Aronsen *et al.* (14) have studied the dog, while Hagerstrand (186) compared changes in the dog, rat, and man in extrahepatic obstruction. The times after obstruction at which these studies were made makes comparison difficult with the rat studies. However, there did not seem to be any marked species differences in BDL-induced changes in hepatic enzymes.

Some effects of BDL on intralobular distribution of enzymes have been noted as well. The increase in AP activity in dog liver was found to occur more markedly in cells in the central half of the lobule (14). In the rat, BDL altered the zonal distribution of another enzyme, succinic dehydrogenase (76). A marked gradient from periportal to centrolobular regions was noted in the controls, while BDL led to a gradual increase in the middle and central regions, until, by 18 to 19 days, a high, diffuse level of activity was seen in all surviving cells within the lobe. The authors speculated that these changes were linked to changes in the oxygen demand of the cells.

A particularly interesting approach to

the study of regional BDL-induced changes has been that of Cooper *et al.* (81) and later Roze *et al.* (416). This technique of partial, or selective biliary obstruction (SBO) involves ligation of the bile ducts from only part of the liver, enabling a comparison of obstructed and unobstructed lobes within the same liver. Roze *et al.* (416) showed that the unobstructed lobes had a marked adaptive capacity to compensate for lost biliary output of bile lipid and bilirubin. Cooper *et al.* (81) confirmed that rats with SBO had minimal hyperbilirubinemia, although serum AP and serum lipids were markedly increased. Histochemical examination of the unobstructed *versus* obstructed lobes was not done, so it is not known whether the plasma accumulation of AP was accompanied by the same induction of hepatic AP seen in completely obstructed animals.

Perhaps of greater interest were the morphological changes which occurred. Light microscopy revealed that after 48-hr SBO, there was a marked increase in the prominence of the portal triads, with evidence of inflammatory cell infiltration and bile duct proliferation in the obstructed lobe compared with the unobstructed. The hepatic parenchyma remained relatively normal, and there was no evidence of bile stasis or necrosis. Most surprising was the difference seen under the electron microscope. The obstructed lobe showed most of the ultrastructural features of cholestasis, except that the canaliculi, which contained osmiophilic material, were normal in size, with intact microvilli. In contrast, canaliculi from the unobstructed lobe, were dilated and had blunted microvilli. This, combined with the finding of a more prominent Golgi apparatus in the unobstructed lobe, led the authors to speculate that these changes might reflect a "work hypertrophy" to the increased secretory load.

The fact that these dilated canaliculi could be found in what might be described as a "hyperfunctional" part of the liver, casts some doubt on the current interpretation that dilated canaliculi are nonfunctional units.

C. Bile Salts

The toxic effects of the monohydroxy bile salt, lithocholate, on the hepatobiliary system have been known for some time (65, 347). The early reports of Holsti (203, 204) described a ductular cell reaction in rabbits fed desiccated hog bile, with the eventual development of a condition resembling cirrhosis. The toxic component of the bile was found to be lithocholic acid. Subsequently, lithocholic acid has been shown to be capable of producing bile duct hyperplasia in other species, including reptiles, amphibia, birds, rodents, and primates (210, 347).

The rat is not as sensitive to the cirrhogenic effects of lithocholic acid, presumably due to its ability to metabolize lithocholate by further hydroxylation (487). However, rats fed 0.75 to 1% lithocholic acid in a low protein diet did show an inflammatory reaction with ductular cell proliferation and finally portal fibrosis. In addition the bile ducts were filled with yellow-green concretions. The importance of the dietary protein content in modifying the lithocholic acid dose-response relationship was emphasized by the negative findings of Lidberg (288).

Selye (455) has shown that the catatoxic steroid pregnenolone-16α-carbonitrile (PCN) reverses these toxic effects of lithocholic acid in the rat. Other steroid and nonsteroid hormones were partially effective. The role of microsomal enzyme induction in this protective effect was not clear since other enzyme inducers tested were either partially protective (phenobarbital) or ineffective (diphenylhydantoin). Lithocholic acid-induced biliary cirrhosis develops rather slowly, and the histological manifestations are quite different from those seen in intrahepatic cholestasis. Therefore the relevance of monohydroxy bile acid toxicity in the etiology of cholestasis was not appreciated until Javitt (225) reported the rapid onset of cholestasis in

rats infused intravenously with the taurine conjugate of lithocholate. A similar cholestatic response was also reported in the hamster (231, 254). Subsequently, the taurolithocholate-induced cholestatic model became the subject of intensive study.

The aqueous solubility of sodium taurolithocholate is lower than that of the potassium taurolithocholate, and unconjugated lithocholate salts are even less soluble. It has been suggested that secretion of the conjugate across the bile canaliculus from a high K^+/low Na^+ intracellular environment to a high Na^+/low K^+ biliary environment could result in intracanalicular precipitation of sodium taurolithocholate (231, 467). Abolition of the cholestatic response by simultaneous infusion of primary bile salts is consistent with this hypothesis (231, 383). Javitt and Emerman (231) stressed the importance of infusing at least a 1:1 molar ratio of micelle-forming bile salts to taurolithocholate to reverse the cholestatic effect in rats, and 2:1 in hamsters. The more soluble 3α-sulphate esters of tauro- and glycolithocholate are much less potent as cholestatic agents (149) and it has been shown that this is not due to a reduced hepatic clearance or biliary excretion of these metabolites (290).

An alternative hypothesis has been suggested by King and Schoenfield (254). In the isolated perfused hamster liver, they showed that the dose-dependent reduction in bile flow, which resulted from the addition of sodium taurolithocholate to the perfusate, did not interfere with the ability of the liver to secrete BSP. Furthermore, the bile salt concentration in bile was actually increased. The taurolithocholate had been extensively metabolized during the experiment and since during the period of reduced bile flow, the ratio of solubilizing bile salts to taurolithocholate was greater than 3:1, these authors discounted the idea that intracanalicular precipitation had occurred. By extrapolating the plot of bile flow versus bile salt excretion to zero bile salt excretion, they suggested that the bile salt-independent fraction (BSIF) represented 75% of basal bile flow. It was therefore implied that taurolithocholate-induced reduction of the BSIF was the more important of the possible mechanisms. This hypothesis has been disputed by Javitt (229) who points out that the slope of the regression of bile flow on bile salt concentration was altered during taurolithocholate infusion. This would be consistent with an increase in the relative proportionof dihydroxy bile salts resulting from the metabolism of taurolithocholate and a change in the osmotic activity rather than a decrease in BSIF.

Irrespective of the mechanism involved in taurolithocholate-induced cholestasis, the morphological changes seen with electronmicroscopy are similar to those seen in other forms of cholestasis (324, 325, 383, 422). These include dilatation of canaliculi and loss of microvilli; the appearance of bile plugs, sometimes with lamellar inclusions; and dilatation and fragmentation of the endoplasmic reticulum. Thickening of pericanalicular ectoplasm and dilatation of the Golgi apparatus were noted in some studies (323, 422) but not in others (325, 383). The changes were reversible within 24 to 48 hr.

The application of more advanced electronmicroscopic techniques has emphasized the importance of the changes in the bile canaliculi and pericanalicular regions. Miyai *et al.* (323, 324), with freeze-fractured replicates, showed that the canalicular microvilli widen and flatten, forming multilamellar foldings. They also detected crystalline material within the canaliculi which they postulated could have been precipitated lithocholic acid. The observation that the amount of these crystalline deposits seemed to be related to the dose of lithocholate infused lends support to the hypothesis that intracanalicular precipitation is involved in the mechanism of lithocholate-induced cholestasis. Scanning electronmicroscopy (279) also confirmed the changes in canalicular membranes and microvilli. More impor-

tantly this technique demonstrated differences between taurolithocholate-induced cholestasis and ethinylestradiol-induced cholestasis and BDL. Specific membrane changes produced by taurolithocholate were interpreted to be caused by a direct effect on the membrane. Significantly, these changes were absent when noncholestatic forms of lithocholate (lithocholate sulfate or taurolithocholate/taurocholate) were infused.

The ability of bile salts to induce cholestasis in the rat is not limited to the poorly soluble lithocholates. Fisher *et al.* (149) have reported on the ability of the dihydroxy bile salt, chenodeoxycholate (CDC), to produce cholestasis in isolated perfused liver preparations from female Wistar rats. Subsequent comparison of the morphological changes produced by lithocholate and chenodeoxycholate showed that CDC-induced cholestasis was more probably due to a generalized hepatocellular necrosis rather than the specific canalicular membrane effect of lithocholate (325).

It was also shown that perfused livers of female rats are more susceptible than the male to the cytotoxic and cholestatic effects of CDC (150). Female rat livers were found to accumulate more CDC and the sex difference is probably related to the lower capacity of the female rat liver to metabolize CDC to the less toxic trihydroxy bile salt β-muricholate (516).

It seems that the 0.3 mM concentration of CDC in the perfusate used by Fisher's group must be close to the critical concentration required to demonstrate a sex difference. Liersh and Hesse (289) were unable to find a cytotoxic or cholestatic response with either male or female rats with a perfusate CDC concentration of 0.25 mM, whereas CDC-induced cholestasis has been demonstrated in the male rat *in vivo* by intravenous injection of 0.1 mM CDC/kg (111). In an abstract published in 1976, Fisher *et al.* (151) showed that concentrations of CDC in the perfusate of the isolated perfused rat liver (sex unspecified) up to 1 mM did not produce cholestasis although CDC did accumulate in the hepatocytes. Most of the CDC was metabolized to muricholic acid. An interesting observation was that when similar concentrations of the taurine conjugate of CDC were used, metabolism to muricholic acid was not as extensive, and cholestasis did occur.

Lithocholate and chenodeoxycholate clearly differ in the mechanism by which they produce cholestasis. On the one hand, CDC is cytotoxic and cholestasis results from a generalized hepatocellular dysfunction. On the other hand, lithocholate seems to interact directly with the bile secretory function of the canalicular membrane, and therefore should be a more appropriate model to study the phenomenon of intrahepatic cholestasis. One observation which is difficult to explain in view of the different mechanisms, is the reversibility of both types of cholestasis by concurrent cholate administration (149). Whether the protective effect is due to the choleretic effects of cholate, or its ability to reduce hepatic uptake of the more toxic bile salts is not apparent from the data.

The taurolithocholate model in the rat is complicated since it is clear that metabolism of lithocholate to less toxic bile salts and the interaction with micelle-forming bile salts may both be factors which can ameliorate the cholestatic response. Furthermore, taurolithocholate-induced cholestasis in the rat is dose-dependent and lasts for only a matter of a few hours (383). In order to produce a taurolithocholate-induced cholestasis of longer duration, which would be more comparable to the duration of drug-induced cholestasis in man, would require either repetitive bolus intravenous injections, or long-term infusions. Neither of these techniques is particularly convenient. Unfortunately, chronic oral administration of lithocholate in the rat fails to produce the cholestatic response, although a different type of hepatic lesion (biliary cirrhosis and lithogenesis) may be demonstrated.

The rabbit has a poor capacity to bio-

transform lithocholate to more polar metabolites (235). The cirrhogenic effects of lithocholate are more easily demonstrable in this species (347), although the development of the lesion is slow. A hepatic lesion consistent with cholestasis, and characterized by hyperbilirubinemia, mild elevation of serum aspartate aminotransferase, and impaired microsomal mixed-function oxidase activity has been reported to develop within 2 to 6 days in rabbits receiving 50 mg/kg of lithocholic acid orally twice daily along with 50 mg/kg of erythromycin or neomycin (108, 384). The morphological similarity of this lesion to intrahepatic cholestasis remains to be evaluated, but since man is more like the rabbit than the rat in ability to detoxify lithocholate by hydroxylation, the rabbit is potentially a more useful species in which to elaborate the experimental model of lithocholate-induced cholestasis.

D. α-Naphthylisothiocyanate (ANIT)

ANIT has interested pathologists for some time because of the bile duct hyperplasia and biliary cirrhosis seen with chronic administration. However, the cholestatic response induced by acute administration of ANIT is more germane to the present discussion. The cholestasis and hyperbilirubinemia produced by a single dose of ANIT in susceptible species such as the rat and mouse, is dose-dependent and quite reproducible (28, 29, 218, 358, 359). Therefore this compound has been studied most extensively with a view to understanding the mechanisms of chemically-induced cholestasis.

1. Morphological aspects. Studies have been initiated to determine the morphological alterations induced by ANIT. A good many of the earlier studies were more interested in the chronic effects produced by this substance which eventually leads to biliary cirrhosis. However, we will limit our discussion to the acute morphological changes that have been described.

Eliakim *et al.* (130) were among the first to describe the morphological alterations seen in rats after acute administration of ANIT; these observations were carried out by light microscopy. One day after administration the hepatocytes were devoid of glycogen and showed mild fatty changes; small foci of necrosis were also observed, mainly near the larger portal spaces. After the 4th day, fatty changes were no longer seen and glycogen reappeared after 1 week. By 2 weeks the hepatocytes appeared entirely normal. The bile ducts showed the most pronounced changes. After 24 hr there was widespread necrosis and desquamation of the epithelium in some of the larger bile ducts; the lumen contained amorphous material composed of cellular debris and fatty material. By 48 hr the small bile ducts contained plugs of mucus, occasionally mixed with desquamated cells. Increased granulocyte activity was evident. The bile ducts seemed dilated and well lined by epithelium of a regular size which occasionally showed mitotic figures; bile ductules started proliferating and after 4 days the number of mitotic figures in the ductule epithelium increased. After 4 days the periportal edema and granulocyte infiltration gradually subsided; although the lumen of the large bile duct was somewhat dilated, the epithelium lining was entirely regenerated but irregular in thickness. After the 2nd week the changes were almost stationary. The authors concluded that the acute administration of ANIT resulted in intrahepatic biliary obstruction due to cholangiolitis. Others have studied the effects of acute ANIT administration in rodents (24, 170, 280, 312, 326–328, 494). Leduc (280) reported the formation of new bile ducts in mice 24 hr after ANIT.

McLean and Rees (312) studied the histological effects of ANIT at periods shorter than 1 day. At 6 hr, portal tracts were mildly edematous and infiltrated with inflammatory cells. At that time the bile duct epithelium was normal. At 12 hr inflammatory cell infiltration was seen around interlobular bile ducts, the epithe-

lium of which was found to be swollen. By 24 hr the changes were more advanced.

Desmet *et al.* (97) and Krstulovic *et al.* (269) performed a histochemical study of rat liver after ANIT intoxication. At 12 and 24 hr after the initial dose of ANIT only a slight leukocytic infiltration could be observed around the smaller bile ducts. Twenty-four hours after ANIT there was a gradual depletion of glycogen which was more pronounced in the periportal areas. After 36 hr the canalicular adenosine triphosphatase (ATPase) activity decreased particularly around the necrotic foci. Alkaline phosphatase increased at 36 and 48 hr particularly in the area of the canaliculi. The enzyme activity was not homogeneously distributed throughout the liver tissue. Areas with very strong activity alternated with zones of less pronounced activity. Thirty-six hours after the administration of ANIT, 5′-nucleotidase activity declined in the area of the canaliculi. At 48 hr the lining cells of the interlobular bile ducts were necrotic and desquamated. These authors suggested that ANIT induces two types of changes in the liver tissue. During the early period it seemed that there was a gradual and progressive alteration in the parenchymal cells. Later, however, the signs of acute obstruction and changes in the bile ductular cells appeared.

Up to this time, most pathologists seemed to think that the major morphological alterations caused by the acute administration of ANIT were due to effects on bile ductular cells and not the hepatocytes. However, Steiner and Baglio (478) studied the cytoplasm of the hepatocytes in rats by electron microscopy and found that indeed serious alterations occurred after the administration of this substance in the feed for 5 or 6 days. These authors also reported work performed during the acute intrahepatic cholestatic phase (479). Alterations in the bile canaliculi were observed in all animals killed after 5 or more days. Although dilatation of the lumen of the bile canaliculi was always accompanied by some reduction of microvilli, there were some canaliculi in which the villi were reduced but the lumen was not obviously dilated. The Golgi networks were generally more elaborate in these animals and occupied a greater area of the liver cells than normally seen. Lysosomes in the vicinity of the Golgi apparatus were considerably increased in number. Pericanalicular vacuoles, separated from the canalicular lumen, were observed. These authors pointed out that these alterations were not specific to ANIT poisoning and that they are seen in cholestasis induced by ligation. They concluded that the pathogenesis of the canalicular changes was similar and probably due to the interferance with bilirubin transport in the hepatic cells. Furthermore, they concluded that these observations indicated a disturbance in the excretory function of the liver in ANIT poisoning and that the site of action was probably the hepatocyte rather than the bile ducts.

Moran and Ungar (328) made an interesting observation when treating rats with intermittent doses of ANIT. The cholangiotoxic effect on the interlobular bile ducts was observed only after the first two courses of treatment. The regenerated epithelium of the interlobular bile ducts was found to be resistant to the subsequent treatments of ANIT. However, hyperbilirubinemia occurred each time the ANIT was administred, regardless of the presence of resistant bile ducts.

In 1973, Schaffner *et al.* (430) studied the acute effects of ANIT on rat microsomol function and also on hepatic ultrastructure. Three hours after the administration of ANIT dilatation of the lamellae of the Golgi zone and alterations in mitochondria were observed. Small osmiophilic droplets were observed in the dilated vesicles and cisternae and the Golgi appartus. The mitochondrial changes consisted of breaks in the altered membranes, swelling variations in size, and apparent fission into two mitochondria. The changes were more apparent in the lobular periphery than in the

central zone, although many central cells had abnormal mitochondria. The endoplasmic reticulum, glycogen content, and bile canaliculi were normal. Changes were also seen in the small bile ducts in the portal tracts. The outer membranes of the mitochondria of bile ductular cells showed breaks and some of these organelles were swollen. The luminar surface contained numerous large blebs; the lateral cell border of these cells were straight instead of interdigitated as normally seen. The ductal basement membrane was normal and single. The sinusoidal endothelium cells were swollen and focally missing or disrupted. The endothelium in the portal blood vessels and especially in the arterioles was also swollen and vacuolated and the smooth muscle cells contained pinocytotic vesicles on all surfaces. After 1 day the structural changes that were observed at 3 hr were also seen but were more pronounced with the exception of the sinusoidal epithelium which had returned to normal. Mitochondrial division was more striking at this time. In addition the bile canaliculi were dilated more in the periphery than in the lobular center in contrast to the changes in the Golgi zone. The smooth endoplasmic reticulum appeared increased throughout the lobule, while rough endoplasmic reticulum was decreased only peripherally. Glycogen was virtually absent from all cells. The hepatocytes in the central zone showed some variations in the density from cell to cell. The hepatocytes had extensive hyaloplasmic blebs extending into the perisinusoidal space of Disse or the lumen of the bile canaliculi. These studies indicated that the hepatocyte is affected more quickly than the bile ductular cells in the acute phase of ANIT intoxication. Furthermore, it showed that complete destruction of ductular cells with mechanical obstruction to bile flow was not observed. Consequently it seems that the acute effects of ANIT are due to its effects on the hepatocytes and not the bile ductular cells.

Rüttner *et al.* (418) and Spycher and Rüttner (474) demonstrated that in the rat ANIT could damage the cell membranes. Their studies were carried out 24 and 48 hr after the administration of ANIT. By electron microscopy, they were able to demonstrate that vesicles appeared in the space of Disse. In 1975, studies were carried out with ANIT to determine whether the enzymes contained in the liver plasma membranes are altered (195). Liver plasma membranes were isolated and their purity was confirmed by phase contrast microscopy, electron microscopy, and estimation of enzyme markers for possible contamination by organelles. The activities of three plasma enzymes were estimated: Mg^{++}-ATPase, 5′-nucleotidase, and Na^{+}-K^{+}-ATPase. These activities were assessed 1, 2, 3, and 7 days after administration of ANIT. The hyperbilirubinemia in these animals was evident after 1 day and reached its peak 48 hr after ANIT, but had returned to normal by 7 days. The activities of the three enzymes in the liver plasma membranes were all found to decrease after the administration of ANIT. The peak reduction with MG^{++}-ATPase occurred at 2 days. With Na^{+}-K^{+}-ATPase, the peak depression occurred sometimes between 1 and 2 days. With all three enzymes, the activities had returned to normal by 7 days. These investigators also performed histochemical studies on sections of liver obtained from rats killed 1, 2, or 3 days after ANIT treatment. There was a widespread decrease in ATPase activity in the area of the biliary canaliculi. Other investigators have shown that icterogenin and 17α-ethinyl substituted steroids can cause similar decreases in the activity of these plasma membrane enzymes (125, 192, 464). Hertzog *et al.* (195) believed that the changes seen after administration of ANIT are part of the pathogenesis of the lesion. However, it must be pointed out that their studies were done after cessation of bile flow had occurred. Therefore, it is difficult to state that the changes observed caused the diminution in bile flow and are not the result of the cholestasis.

These authors do point out that Na^+-K^+-ATPase is predominantly localized in junctional complexes and not in microvilli of bile canaliculi, whereas Mg^{++}-ATPase and 5′-nucleotidase are located in both of these sites; they feel that the observation that ANIT depresses all three enzymes suggests that this substance has a more generalized injurious effect on the plasma membrane than had been realized.

Balazs (22) studied hepatic ultrastructural changes in rats receiving only one dose of ANIT. Within the first 24 hr the biliary canaliculi became dilated and reached their peak dilatation on the 3rd or 4th day. The microvilli were greatly decreased. The organelles of the pericanalicular cytoplasmic portions were found to be normal. By the 5th or 6th days, the canalicular dilatation tended to decrease and normal structures were observed at 7 to 10 days. The changes observed in the biliary canaliculi at 24 and 48 hr were similar to those induced by ligation of the bile duct.

Although most of the studies with ANIT have been performed in rodents, a few have been done in nonrodent species. Gopinath and Ford (174) studied the effects of ANIT in sheep and calves. They found that the acute administration of this substance resulted in hyperbilirubinemia and in the increase of several cytoplasmic enzymes. With histochemical procedures they demonstrated a marked hepatocellular response to ANIT consisting of swelling, vacuolation, and loss of particular staining of the periportal cells; the histochemical changes were reduced activity of glutamate dehydrogenase, succinic tetrazolium reductase, and nonspecific esterases throughout the liver lobule. They found a centrolobular increase in sinusoidal alkaline phosphatase and a reduction in BSP clearance. They stressed that many of the earlier morphological studies tended to focus on the bile duct epithelium as the site of action of ANIT. However, in the sheep and the calf, it seems that it is primarily the hepatocyte which is affected, and their opinion was that the bile duct epithelium findings had overshadowed the effects of ANIT on hepatic cells. Sheep and calves seemed to be resistant to the proliferative effects of this compound. This study also suggests that the acute cholestatic effect of ANIT is different from the chronic proliferative effect on bile ductular cells.

Ungar and Popp (495) in 1976 reported that acute ANIT administration can cause edema, distension, and necrosis of the gall bladder, but that in mice this response is more delayed in its appearance than that of the intrahepatic bile ducts.

When one takes into account all of these morphological studies, it is obvious that the acute administration of ANIT affects the plasma membrane, a number of different hepatocyte organelles, the biliary canaliculi, the bile ductular cells, and the gall bladder. There is a temporal dissociation of these various effects, and it is not clear whether one or more leads to the cholestatic response observed.

2. Functional aspects. It has been shown that various species respond differently to the chronic administration of ANIT (355). This is also true regarding the acute cholestatic response (218). In the rat (109, 218) the onset of hyperbilirubinemia occurs between 12 and 24 hr, reaching a maximum by about 5 days, and returning to normal values by about 7 days. The decrease in bile flow occurs more abruptly, between 16 and 24 hr (109, 218, 292), and in doses in excess of 150 mg/kg, the stasis is complete. Cholestasis lasts for about 5 days. In the mouse, the sequence of events is quite similar, but occurs with a lower dose (29, 218). In both rats and mice, bilirubinuria is detectable during the cholestatic period. Hamsters are more resistant, but larger doses provoke cholestasis and hyperbilirubinemia. In contrast to the species differences seen with such cholestatic agents such as 2-ethyl-2-phenyl butyramide (see section IV H), dogs are resistant to the cholestatic effects of acute ANIT administration (218).

In addition to the hyperbilirubinemia, which is predominantly due to the accu-

mulation of conjugated bilirubin, ANIT can produce other alterations in the function of the hepatic cells. In mice and rats, BSP retention occurs within 2 to 4 hr after the acute administration of this substance (29, 218). This abnormal BSP retention occurs at a time when bile flow has not been diminished. Since the conjugation of BSP with glutathione does not seem to be affected it seems that this early BSP retention is due to an alteration in the uptake of BSP by the hepatocyte. This also seems to occur with bilirubin, since ANIT can cause a prolongation in plasma bilirubin disapperance when exogenous amounts of bilirubin are given to animals whose biliary excretion has been eliminated by bile duct ligation (406).

In rats (109) and dogs (218), ANIT produces an increase in plasma alanine aminotransferase activity, although the increase is mild in comparison to that produced by a necrogenic agent. In the rat, this increase may be detected as early as 2 hr after ANIT; *i.e.*, well before the onset of cholestasis. Plasma 5′-nucleotidase also rises sharply in rats (109), but follows a time course similar to that of the bile flow changes.

Goldfarb *et al.* (170, 171) and Garay *et al.* (162) reported that an increase in bilirubin excretion was observed when ANIT was fed to rats for 3 or more weeks. Roberts and Plaa (407) demonstrated that an increase in biliary bilirubin excretion also occurred after the acute administration of ANIT, before the eventual onset of cholestasis. These studies demonstrated that a nonerythropoietic source of bilirubin was probably the cause of the increase in bilirubin production. In rats, it was demonstrated that the amount of radioactivity incorporated into bilirubin from δ-aminolevulinic acid was increased in a dose-related manner. These data suggested that enhanced bilirubin synthesis may also be involved in ANIT-induced hyperbilirubinemia. It has been established by other investigators that the incorporation of δ-aminolevulinic acid into bilirubin is accomplished largely independently of erythropoiesis (223, 411, 444).

Finally, ANIT has been demonstrated to cause an impairment of microsomal mixed-function oxidase activity (29, 56, 93, 95, 109, 364, 385). This effect lasts for several days and will be discussed in more detail in section IV d, 3.

Therefore, we see that ANIT produces a cessation of bile flow which leads to hyperbilirubinemia, can also increase the synthesis of bilirubin from nonerythropoietic sources, affects BSP retention at a time when bile stasis is not evident, and can also depress the activity of drug metabolizing enzymes located in the endoplasmic reticulum. Some of these effects can be abolished by pretreating rats with inhibitors of protein and RNA synthesis (220, 221, 493). Pretreatment of rats with cycloheximide, actinomycin D, ethionine or puromycin resulted in a significant protection against the appearance of hyperbilirubinemia and cholestasis. Cycloheximide and ethionine prevented completely the appearance of ANIT-induced hyperbilirubinemia even when administered 24 hr before ANIT; actinomycin D offered only a partial protection and this only occurred up to 12 hr pretreatment; puromycin was effective if administered in multiple doses before and after ANIT. Posttreatment with actinomycin D, cycloheximide, or ethionine also resulted in an inhibition of the hyperbilirubinemic response. This protection occurred with doses of the inhibitors which could block the incorporation of leucine into hepatic protein and also block the incorporation of orotic acid into hepatic RNA. These data suggest that unimpaired protein synthesis may be involved in some of the hepatotoxic effects of ANIT. However, a direct effect of these inhibitors on the enzymes involved in the biotransformation of ANIT, independent of an inhibition of protein or RNA synthesis, is possible (61, 293, 465); its role in the observed protection remains to be established.

Pretreatment of rats with these inhibitors also blocks the ANIT-induced increase

in bilirubin formation from nonerythropoietic sources (493). Cycloheximide and ethionine pretreatment inhibited the increase in biliary bilirubin excretion and the incorporation of δ-aminolevulinic acid into bilirubin; cycloheximide was found to be more effective than ethionine. Actinomycin D prevented the ANIT-induced increase in biliary bilirubin excretion and produced a decrease but irregular incorporation of δ-aminolevulinic acid into bilirubin. On the other hand, pretreatment of rats with these inhibitors had no effect on the early BSP retention induced by ANIT; also none of the inhibitors studied had an effect on the prolongation of pentobarbital-sleeping time produced by ANIT (221). Therefore the studies effected with the use of inhibitors of protein and RNA synthesis indicate that the four major effects of ANIT on the rat hepatocyte can be differentiated from each other. The production of cholestasis, with the resulting hyperbilirubinemia, and the increased synthesis of bilirubin seem to be due to the same toxic moiety and seem to be related to each other; the early increase in BSP retention and the prolongation of pentobarbital sleeping time seem to be due to some other moiety and do not seem to be related to the cholestatic response induced by ANIT.

The hepatic clearance of exogenously administered bilirubin was reduced in ANIT-treated rats and mice at a time when cessation of bile flow had not occurred (406). Moreover, the maximal rate of bilirubin excretion into bile and bile bilirubin concentration were diminished significantly. The storage and uptake of bilirubin also seem to be affected, in that ANIT-treated mice whose bile ducts had been acutely ligated demonstrated a decrease in the disappearance rate of exogenously administered bilirubin from the plasma and a reduction in hepatic bilirubin concentration. No effect of bilirubin conjugation could be demonstrated. Therefore, ANIT seems to have a direct effect on hepatic uptake, storage, and excretion of an exogenous bilirubin load. Whether bilirubin retention after ANIT treatment is a consequence of any single effect remains uncertain. However, it is clear that the mechanism of the acute action of ANIT, although indeed complex, is largely an effect on hepatocyte function.

Because of the importance of bile salts in the formation of bile and also the presence of a bile salt-independent fraction in normal animals, there has been some interest in determining whether ANIT can affect either of these parameters and lead to the cessation of bile flow. The effect of ANIT on bile salt synthesis has not been determined. Schaffner *et al.* (430) measured bile concentration in the liver 48 hr aftr ANIT (*i.e.,* after cholestasis had been established for some time). The concentration of the dihydroxy bile salts was decreased, but the concentration of trihydroxy bile salts was markedly elevated. Lock *et al.* (292) demonstrated that canalicular bile flow (as measured by erythritol clearance), bile acid excretion, and endogenous bilirubin excretion were normal within the first 12 hr after ANIT administration. These authors concluded that cholestasis is a late event in ANIT-induced hepatotoxicity and furthermore cannot be explained by a gradual alteration in bile formation.

The hepatic dysfunction produced by ANIT congeners has also been investigated (30). A number of commercially available isothiocyanates, thiocyanates, and isocyanates were studied in mice, with hyperbilirubinemia as the parameter of hepatic dysfunction. It was found that particular structural features seemed necessary for production of acute hyperbilirubinemia in this species: *Condition 1*—the active radical must contain sulfur rather than oxygen in combination with carbon and nitrogen; α-naphthylisocyanate, in which the sulfur is replaced by oxygen was inactive. *Condition 2*—the hydrocarbon moiety must be aryl; alkyl isothiocyanates were inactive. The contribution of the aryl group could be due to three possible fac-

tors: steric effects, inductive effects, *i.e.*, electron shifts, or planar configuration. Corresponding isothiocyanates with a bulky alkyl group or cyclo-alkyl group were inactive. Hence, steric effects do not seem to be the responsible factor. The allyl group has inductive effects which are similar to phenyl or naphthyl groups, but allyl isothiocyanate was inactive. Therefore, planar configuration seems to be a necessary feature. This is consistent with the lack of activity of fluorescein isothiocyanate which is another compound of about the same size and inductive effect as ANIT but does not possess planar configuration. ANIT, β-naphthylisothiocyanate and phenylisothiocyanate are the only compounds which fit these conditions. Of these three compounds only ANIT and phenylisothiocyanate were found to be active in mice. There is no obvious explanation for the lack of effect with β-naphthylisothiocyanate.

In mice, the effects of phenylisothiocyanate resemble very closely the effects of ANIT; hyperbilirubinemia and BSP retention occur and the effects persist for about 10 days (30). Mazzanti (310) has reported that phenylisothiocyanate produces cholestasis in the guinea pig. However, recent studies conducted in our laboratories (unpublished observations) indicate that phenylisothiocyanate does not produce hyperbilirubinemia and cholestasis in the rat whereas it does so in the mouse. There is no apparent explanation for this species difference with this compound. β-Naphthylisothiocyanate is also inactive in the rat.

3. Metabolic aspects. The interaction of ANIT with the cytochrome P-450-dependent microsomal mixed-function oxidases (MMFO) system has been investigated from two points of interest:

a) How does ANIT inhibit MMFO activity, and is this inhibition related to the mechanism of its cholestatic effects (see sect. V F)?

b) Are the cholestatic effects of ANIT due to the parent compound, or to a metabolite resulting from microsomal oxidation?

It was discovered quite early in studies with ANIT that the effects of drugs metabolized by the hepatic MMFO could be prolonged by acute ANIT administration in rats and mice. Pentobarbital sleeping time was prolonged in mice, and the ED50 for this effect was similar to the ED50 for the production of hyperbilirubinemia (29). Pentobarbital- and hexobarbital-sleeping time, and zoxazolamine-paralysis time were all prolonged in the rat by cholestatic doses of ANIT (56, 109). These indications of MMFO impairment could be detected as early as 2 hr after ANIT, and clearly preceded the cholestasis. The recovery of the MMFO system was slow, persisting beyond the period of hyperbilirubinemia (29, 109), but bearing a closer relationship to the recovery of bile flow (109). Impairment of MMFO activity seemed to be associated with changes in the amount of microsomal cytochrome P-450 (85, 93, 109, 161, 430).

When assessment of MMFO activity was made *in vitro*, the effects of ANIT treatment were more difficult to assess. It was found that, not only were there differences between substrates in their sensitivity to inhibition (93, 364, 430), but that the time course of the inhibitory effect was related to the type of microsomal preparation used. The inhibitory effect during the precholestatic period (*e.g.*, at 2 hr) could only be demonstrated when a postmitochondrial supernatant of a rat liver was used as a source of enzyme (56, 364) or a 105,000 × *g* microsomal pellet was used in conjunction with low substrate concentration (93). Drew and Priestly (109) showed that during the precholestatic period, 10,000 × *g* supernatant preparations from ANIT-treated rats exhibited reduced aminopyrine demethylase and aniline hydroxylase activities, while 105,000 × *g* microsomal pellet suspensions from the same livers were unaffected. Aminopyrine demethylase and aniline hydroxylase activities were equally affected in both types of microsomal preparations after cholestasis had set in. These authors concluded that the precholestatic impairment was due to a direct inhibitory effect of residual ANIT

and/or its metabolites in the 10,000 $\times g$ supernatant, but that this inhibitor could be washed out during preparation of the microsomal pellet. The postcholestatic impairment was probably the result of the destruction of cytochrome P-450 by the retained biliary products.

Further experiments should be done to confirm this hypothesis. For example, it would be necessary to demonstrate that the differential inhibitory effect is related to the *in vivo* ANIT dose, or more precisely, the concentration of ANIT retained in the microsomal preparations. Also, the effects of a noncholestatic dose of ANIT should be studied in order to exclude the possibility that the postcholestatic inhibition of MMFO is not related to some persistent ANIT metabolite. The inhibition of microsomal puromycin N-demethylation demonstrated both *in vivo* and *in vitro* by Derr *et al.* (95) occurred 2 hr after a noncholestatic dose of ANIT was administered. Plaa *et al.* (364) showed inhibition of hexobarbital and aniline oxidation 2 hr after the administration of a noncholestatic dose to mice. Neither of these studies were extended beyond 2 hr. In 1976, Mitchell *et al.* (318a) reported that in mice hepatic cytocrome P-450 is decreased 2 hr after ANIT administration, while other preliminary studies (129a) in rats show that cytochrome P-450 is decreased at 12 hr but not at 2 hr. These data suggest a species difference exists. In contrast to the experiments performed by Drew and Priestly (109), these studies (129a, 318a) were performed in animals given ANIT by the intraperitoneal route. The effect of ANIT on cytochrome P-450 requires clarification.

Studies with ANIT added *in vitro* to microsomal preparations confirm that ANIT is a potent inhibitor of the MMFO system (85, 364, 385). The studies also show that ANIT varies in its K_i for various substrates; approximately 8 μM for hexobarbital, >1 mM for chlorpromazine (364), and approximately 0.1 mM for both aminopyrine and aniline (385).

ANIT has been compared to SKF 525-A in both spectrum and potency of its inhibitory effects, although it has not been shown that the effects on microsomal enzymes are diphasic like those of SKF 525-A (456). In a further analogy to SKF 525-A (433) it has been shown that ANIT too can convert the nature of the inhibition of aniline hydroxylation from competitive to noncompetitive (385).

It does not seem likely that the inhibition of MMFO activity is fundamental to the cholestatic mechanism of ANIT. If it were, one would have to explain how other potent MMFO inhibitors lack cholestatic potential. The ANIT congeners, β-naphthylisothiocyanate and phenylisothiocyanate are weaker enzyme inhibitors in the mouse (364), but their inhibitory potency does not seem to correlate well with their cholestatic potency.

However, it now seems clear that interaction of ANIT with the MMFO resulting in its own metabolism does play a fundamental role in the cholestatic mechanism. The first clue to this was the finding that microsomal enzyme inducers and inhibitors could potentiate or ameliorate the hyperbilirubinemic response to ANIT (208, 403, 404), and that the potentiation by enzyme inducers could be diminished by simultaneous treatment with actinomycin D or ethionine (403).

Species differences in susceptibility to the cholestatic effects of ANIT lend support to the hypothesis that a metabolite, rather than the parent substance, is the agent responsible. More recently, it has been shown that ANIT is metabolized by microsomal enzymes in the rat, and its subsequent disposition is critical in the development of the cholestatic lesion.

Capizzo and Roberts (59–61) have studied the disposition of ANIT in the rat. Approximately 70% of the radioactive material derived from (isothiocyanate-^{14}C)-ANIT was absorbed from the gastrointestinal tract within 24 hr and was found widely distributed in all body tissues. The liver, kidney, adipose tissue, and blood

contained relatively greater accumulation of the ^{14}C label when compared with the remaining tissues. Differential centrifugation of liver homogenates failed to demonstrate any preponderance of radioactivity in a single fraction. Radioactivity was found in all excretion products examined and approximately 80% of the administered dose was recovered 72 hr later. Of this, 40% was in the urine, 30% in the expired gases, and 10% in the feces. The findings of ^{14}C in expired gases, derived from the isothiocyanate moiety, indicated that ANIT could undergo biotransformation. In other species (60) evaluation of urinary ^{14}C metabolites by thin-layer chromatography showed definite qualitative and quantitative differences in the hamster and rabbit compared to the remaining species. While the distribution of ANIT in rats, mice, and guinea pigs was not found to be markedly different, the amount of ^{14}C excreted in the expired air was significantly greater in hamsters and rabbits than that found in rats. The urinary ^{14}C content was significantly lower in the hamsters but not in the rabbits at 24 hr when compared to rats. The hamsters and rabbits had a significantly lower liver ^{14}C content when compared to the rats. Rats were found to excrete at least five metabolites in the urine; the hamster and guinea pig excreted at least four metabolites and the mice and rabbits excreted three metabolites. In this study, it was demonstrated that the hyperbilirubinemic response could be elucidated in mice, rats, guinea pigs, and hamsters, but not in the rabbit. Although the urinary metabolites separated by thin-layer chromatography were not characterized other than by R_f value, the data reported by Capizzo and Roberts (60) indicated that the four species which responded to ANIT each had two metabolites which migrated in a comparable fashion. One was a relatively nonpolar metabolite which had an R_f value slightly less than 0.75 and the other was a polar metabolite with an R_f of about 0.10. Neither of these metabolites was found in the urine of rabbits.

The expired ^{14}C in the various species by comparison did not correlate with the extent of the ANIT-induced hyperbilirubinemia. The rabbits and hamsters had significantly increased amounts of expired ^{14}C and a significantly lesser degree of hyperbilirubinemia, whereas mice and guinea pigs had both greater amounts of expired ^{14}C and hyperbilirubinemia. The authors proposed that one possible explanation for the species variation of ANIT disposition is that the urine and expired ^{14}C excretion concentrations may represent competitive metabolic pathways involved in ANIT biotransformation and that hamsters may form less of the toxic metabolites (urinary ^{14}C) because of a predominant second competitive metabolic pathway (expired ^{14}C). Capizzo and Roberts (61) have shown that the potentiating effects of enzyme inducers, and modification of this response by protein synthesis inhibitors, correlates well with changes in metabolite excretion. The chronic administration of ANIT was also found to influence the acute response to a subsequent high dose of ANIT (61). ANIT administration for a period of 60 days blocked the hyperbilirubinemic response to a large dose of ANIT; this too was correlated with an increase in ANIT-derived ^{14}C excreted in the expired air.

Roberts (401) reported that approximately 2 to 3% of (isothiocyanate-^{14}C) ANIT added to rat hepatic microsomes was converted to $^{14}CO_2$ after a 15-min incubation *in vitro* and trapping of the gaseous phase in NaOH. When the microsomal supernatant was boiled before incubation only trace amounts of $^{14}CO_2$ were recovered after the incubation period. When microsomal enzyme preparations were obtained from animals treated with phenobarbital or chlorpromazine there was a 2- to 5-fold increase in the amount of $^{14}CO_2$ recovered, and the simultaneous administration of actinomycin D and phenobarbital prevented the increase in $^{14}CO_2$ production. The ^{14}C remaining in the incubation mixture was extracted with methanol and analyzed chromatographically. The majority of the ^{14}C obtained from these extracts

failed to migrate as ANIT. The major component was a single peak at the origin with less than 10% of the radioactivity remaining as free ANIT. Chromatography of extracts from incubated samples revealed several small peaks in addition to the major peak near the origin. These data indicate that the liver microsomal fraction seems capable of converting ANIT added *in vitro* to CO_2 and other metabolites. They also indicate that it is possible to induce metabolic pathways leading to CO_2 production *in vitro* by the same treatments which do so *in vivo*. Others (87a) have shown that CO_2 can be formed *in vitro* during incubations of CS_2 or its metabolite COS with rat hepatic microsomes.

Hypothermia can also modify the response of the rat to the acute effects of ANIT. Both the hyperbilirubinemic and the cholestatic responses have been shown to be markedly dependent on the body temperature of the rat (405). When the body temperature was lower than 30°C, no hyperbilirubinemia was observed and only 20% of the animals exhibited cholestasis, whereas when body temperature was elevated to 38°C marked hyperbilirubinemia was observed and 100% of the animals exhibited cholestasis. These observations indirectly suggest that ANIT is metabolized before it exerts its acute effects.

Distribution studies have also been performed with dually labeled ANIT (293, 465). In these studies, the ^{14}C appeared in the isothiocyanate portion of the molecule and the ^{3}H appeared in position 4 of the naphthalene ring. It was expected that if ANIT did not undergo biotransformation one would expect the ratio of $^{3}H/^{14}C$ activity in the blood and bile at any given time point would be identical with the 6.3:1 ratio administered. If on the other hand loss of the isothiocyanate moiety from the naphthalene ring or a carbon-nitrogen cleavage would be a prominent feature in ANIT metabolism, the ratio should change. In blood the ratio was closer to 6.3 only a few minutes after administration and dropped within 50 to 60 min toward a constant value near 5.8 which was maintained for the duration of the 8-hr experiment. The differences were even more dramatic in bile. The $^{3}H/^{14}C$ ratios stayed close to the value of 6.3 (amount administered) during the first 50 min but then rose to a value of 7.5 thus indicating that proportionally more ^{3}H was excreted than ^{14}C. The differences were even more striking when these ratios were compared in cycloheximide-treated animals. Previous studies (220, 221) had shown that pretreatment of animals with cycloheximide could abolish the hyperbilirubinemic and cholestatic response to ANIT. With dually labeled ANIT (293, 465) it was demonstrated that cycloheximide had a very marked effect on the ratio of $^{3}H/^{14}C$ excreted in the bile. In those animals treated with cycloheximide the ratio obtained in the bile for the first 8 hr of observation was identical with the ratio of ANIT administered to the animal and this contrasted to the increasing ratio observed in normal rats. The simplest interpretation of these data would be as follows: In normal rats within 1 hr or so after administration of ANIT, one or several metabolites appear in the bile; these metabolites contain a greater amount of ring labeled material which could mean that normal naimals converted some ANIT into a compound consisting of the modified naphthalene ring only. This unidentified and still hypothetical "toxic metabolite" could exert its cholestatic effects in a number of ways. It might act directly on the mechanisms of canalicular bile formation, either during biliary excretion, or subsequent to its enterohepatic recirculation. The need for an intact enterohepatic recirculation for the response was emphasized by the studies of Roberts and Plaa (405). On the other hand, cycloheximide-protected animals excreted material in their bile in which the $^{3}H/^{14}C$ ratio did not differ from the originally administered material. In other words, protected animals might excrete only unaltered ANIT in their bile. Furthermore, it was demonstrated that cycloheximide affected the ratios in a dose-related manner comparable to the dose-response relationship exhibited in its pro-

tection against ANIT responses *in vivo* (220, 221). Skelton *et al.* (465) extended the studies and concluded that other inhibitors of protein synthesis (actinomycin D and ethionine), which also protect against ANIT-induced hyperbilirubinemia and cholestasis (220) effected similar changes in the $^{3}H/^{14}C$ ratio of ANIT excreted in bile after the administration of dually labeled ANIT. With these two inhibitors it was found that they had to be given 4 hr before ANIT administration in order to effect the decrease within 8 hr after ANIT. All of these data are consistent with the hypothesis that the hyperbilirubinemia and cholestasis induced by ANIT is due to the formation of a toxic metabolite and that protecting agents such as cycloheximide, actinomycin D, and ethionine may act by altering the formation of this toxic moiety.

While the bulk of the evidence indicates that the acute cholestatic response to ANIT is due to a biotransformation product, Williams (508a) reported that the addition of ANIT *in vitro* to rat liver-derived cells in culture (ARL 3 line) is cytotoxic. The author interpreted this to indicate that ANIT, and not a metabolite, was involved in the toxic response. However, several congeners of ANIT, which have been shown to be devoid of cholestatic activity *in vivo* (30), were also found to be cytotoxic in this tissue culture system. Furthermore, the concurrent addition of cycloheximide, which protects against ANIT-induced cholestasis *in vivo* (220, 221), failed to alter the cytotoxic effect of ANIT in culture. These inconsistencies cast some doubt on the appropriateness of this experimental system to establish whether metabolic products are involved in ANIT-induced cholestasis.

Within the last few years there has been considerable interest in the irreversible binding of the hepatotoxic substances to microsomal protein or microsomal lipids as a mechanism of action of hepatotoxicity (see section V G). Preliminary reports have been published concerning the irreversible binding of ANIT to rat liver microsomes and an attempt has been made to correlate this binding with the cholestatic effect (129, 362). The binding of an active metabolite of ANIT to rat liver microsomes was examined *in vitro*. (4-Naphthyl-^{3}H)ANIT or (isothiocyanate-^{14}C)ANIT binds extensively nonenzymically, but this binding increases in the presence of reduced nicotine adenine dinucleotide phosphate (NADPH) and oxygen. Enzymic binding was inhibited by carbon monoxide, SKF 525-A, and piperonyl butoxide, thus indicating that the cytochrome P-450-dependent mixed-function oxidase mediated the binding Pretreatment of rats with SKF 525-A also reduced toxicity *in vivo* and decreased the *in vitro* enzymatic binding whereas pretreatment with phenobarbital, which enhances toxicity *in vivo*, increased binding. However, pretreatment with cycloheximide, which abolishes toxicity *in vivo*, did not affect ANIT binding to liver microsomes. Furthermore, pretreatment with pregnenolone-16α-carbonitrile, which reduces toxicity *in vivo* (219), actually increased the enzymic binding of ANIT to liver microsomes. These results indicate a lack of correlation between *in vivo* toxicity and *in vitro* microsomal binding in rats.

A subsequent study (362) compared the effect of irreversible binding of ANIT to liver microsomes *in vitro* in different species. ANIT in mice and rats results in hyperbilirubinemia and cholestasis; hamsters are more resistant, whereas rabbits and dogs fail to develop these effects. When binding of ANIT to microsomes from different species was examined, it was found that binding to microsomes from hamsters was the highest followed by rabbits > dogs > mice > rats. These quantitative differences in these species do not correlate with the toxicity of ANIT *in vivo* in the same species. The results of these preliminary studies suggest that irreversible binding of ANIT to liver microsomal protein is not involved in its cholestatic effect.

However, they do demonstrate that a reactive metabolite can be formed from ANIT by the MMFO system and further studies should be performed to see whether binding to other hepatocytic macromolecules could correlate better with *in vivo* toxicity. Before the possible role of irreversible binding of ANIT to proteins or other macromolecules can be resolved it will be essential that *in vivo* binding studies also be performed and compared with *in vivo* toxicity.

E. Steroids

The cholestatic reaction associated with the clinical use of anabolic and contraceptive steroids has already been discussed briefly in section III. Furthermore, the interaction of sex hormones with the liver has been the subject of a number of reviews (6, 113, 339, 351, 471). In this section, we wish to concentrate on the studies, in people and laboratory animals, which have sought to characterize the nature of the hepatic dysfunction, and have shed some light on the possible mechanisms.

In man, the functional impairment which has been most often demonstrated, is the plasma retention of BSP. Estradiol and estriol were shown to provoke BSP retention in almost all patients receiving the steroids for periods ranging from 4 to 41 days (266, 331). In some patients, BSP retention was evident within 24 hr of initiating steroid treatment; however in all cases, the hepatic disposal of BSP reverted to normal within 1 to 4 weeks after ceasing the estrogen therapy. An increase in serum alkaline phosphatase in approximately 50% of the treated group was the only other index of hepatic dysfunction. Oral contraceptives have been reported to cause similar effects on BSP clearance (273, 339).

The anabolic steroids, methyltestosterone and norethandrolone, have been shown to cause marked BSP retention and impairment of BSP hepatic clearance in all patients provided the dose is sufficiently high (189, 284, 434). A high incidence of BSP retention has also been reported for methandrostenolone (498), but testosterone propionate, in either equimolar, or equivalent anabolic doses, does not produce BSP retention (189). Jaundice was not seen, nor were other indices of hepatic function abnormal in these studies. Maximum depression of BSP clearance was found 7 to 10 days after initiation of therapy. It was concluded that the defect involved hepatic excretory mechanisms rather than hepatic uptake.

Schaffner *et al.* (428) have shown that dilatation of bile canaliculi and loss of microvilli, which are characteristics of the cholestatic syndrome, also occurred in non jaundiced patients treated with doses of norethandrolone sufficient to produce BSP retention. Approximately 30% of the canaliculi were abnormal, although no other abnormalities were found in the parenchymal cells. These authors concluded that the bile canalicular alterations reflected a quantitative rather than qualitative difference from those seen in frankly jaundiced patients. This implies that the cholestatic jaundice which sporadically occurs with the administration of substituted testosterone preparations is a regular response, with variations in intensity, and is not the result of a hypersensitivity reaction.

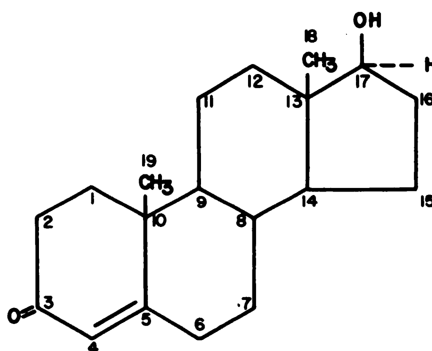
The structure-activity relationships in steroid-induced hepatic dysfunction in man have been studied by de Lorimier *et al.* (92). The incidence of BSP retention was highest (100%) with normethandrone and norethindrone, moderate (43–85%) with methyltestosterone, fluoxymestrone and methandriol, and low (20%) with ethisterone. It was concluded that the 3-ketone group predisposed to a greater potential for BSP retention than the 3-hydroxyl group, and that the nature of the C17-alkyl group could modify the response. The BSP retention was found to be unre-

lated to the anabolic, androgenic, or progestational activity of the steroid. The structural analogs of these, and other steroids with cholestatic potential are shown in table 2.

The effect of estrogens on bile formation in rats has been the subject of a number of reports. In 1969, Forker (154) reported that the administration of seven daily subcutaneous injections of estrone resulted in a bile flow decrease of approximately 30% in virgin female rats. This decrease was evident for basal bile flow, and also during dehydrocholate-induced choleresis. At the same time, it was observed that the steady state BSP excretion was reduced by more than 50%, and these data indicated that estrone can also affect the active transport of BSP into bile. The oral administration of ethinylestradiol for 5 to 9 days has also been shown to produce a 40 to 60% decrease in bile flow in female rats (185, 267).

There is some controversy of the mechanism of estrogen-induced bile flow impairment. Forker (154) has argued that estrone promotes an increased permeability of the biliary tree that results in excessive water and solute reabsorption from the bile. He analyzed the biliary clearances of mannitol and sucrose in terms of theoreti-

TABLE 2
Structural formulae of the potentially cholestatic steroids



Common names	Structural modifications		
	C17 substitution	Ring A changes	Other changes
Methyltestosterone	α-methyl		
Methandriol	α-methyl	3 β-hydroxy No 4, 5 double bond	5, 6 double bond
Oxandrolone	α-methyl	O replaces C_2 No 4, 5 double bond	
Methandrostenolone	α-methyl	Extra 1, 2 double bond	
Stanozolol	α-methyl	3, 2-c pyrazole No 4, 5 double bond	
Oxymestrone	α-methyl	4 β-hydroxy	
Bolasterone	α-methyl		7-methyl
Fluoxymesterone	α-methyl		11 β-hydroxy 9α-fluoro
Normethandrone	α-methyl		Lacks 19 methyl
Norethandrolone	α-ethyl		Lacks 19 methyl
Ethylestrenol	α-ethyl	Lacks 3-keto	Lacks 19 methyl
Ethisterone	α-ethynyl		
Norethindrone	α-ethynyl		Lacks 19 methyl
Norethynodrel	α-ethynyl	No 4, 5 double bond	5, 10 double bond
Ethinylestradiol	α-ethynyl	Aromatized 3 β-hydroxy	

cal models which permit differentiation of the influence of convection and diffusion, and concluded that the data were consistent with increased permeability. Gumucio and Valdivieso (185), with ^{14}C-erythritol clearance as an index of canalicular bile flow, concluded that the ethinylestradiol-induced reduction in bile flow was due to inhibition of the bile salt-independent fraction of bile water secretion. They further showed (184) that phenobarbital, which stimulates the bile salt-independent fraction of bile flow, could reverse the cholestatic effects of ethinylestradiol. The present state of knowledge of the mechanisms by which this fraction of bile flow is elaborated is still somewhat limited (see section V B). However, the finding that cholestatic steroids can affect Na^+-K^+-ATPase activity *in vitro* (192) lends some support to the hypothesis of Gumucio and coworkers.

Gumucio's group has also investigated the effects of ethinylestradiol on bile salt excretion (185). They showed a slight, but insignificant decrease in bile salt output (partially compensated for reduced bile flow by an increased bile salt concentration). However, the clearance of infused taurocholate was markedly reduced in ethinylestradiol-treated rats. Furthermore, phenobarbital pretreatment did not abolish the cholestatic effects of ethinylestradiol in rats subjected to bile salt-induced choleresis. These data suggest that estrogen can affect several parameters of bile productions, and it is still not clear which of these is of major importance in producing the cholestatic reaction.

Anabolic steroids are also capable of altering biliary function in animals. The oral administration of methyltestosterone and norethandrolone were found to produce dose-related increases in plasma BSP retention. Oxandrolone seemed to be less effective and testosterone propionate had no effect on BSP retention (285). Other investigators have reported BSP retention occurring in rabbit with certain anabolic steroids (69, 190, 286). Lennon (286) showed that 17α-alkylation was a specific requirement in order to demonstrate BSP retention in steroid-treated rabbits. The magnitude of effects on BSP retention varied among the various steroids and did not seem to be related to myotroplaic or androgenic activities. The 17α-alkylated steroids studied were: norbolethone, bolasterone, methyltestosterone, ethylestrenol, norethandrolone, methandrostenolone, fluoxymesterone, oxymetholone, oxandrolone, stanozolol, oxymesterone, methylandrostenediol. Norbolethone can also impair BSP and indocyanine green clearance from the perfusate of an isolated perfused rat liver preparation (26). This was accompanied by a decrease in the excretion rate of BSP and indocyanine green in the bile. Higher concentrations of norbolethone added to the perfusate also caused a decrease in the rate of bile formation.

Arias (13) reported that the administration of norethandrolone and methyltestosterone to rats resulted in a decrease in hepatic capacity of these animals to excrete bilirubin. This was manifested by a decrease in the bilirubin Tm observed in the animals pretreated with these 17α-alkylated anabolic steroids. However, subsequent work (408) has cast some doubt upon this observation. Roberts *et al.* (408) carried out extensive investigations in the excretion of bilirubin in norethandrolone-treated rats. They demonstrated that norethandrolone-treated rats would produce a diminution in bilirubin Tm only if the body temperature of the rat during the bilirubin infusion was uncontrolled and allowed to drop to hypothermic levels. When the bilirubin Tm infusions were performed in normothermic norethandrolone-treated rats there was no diminution in bilirubin Tm. Since many of the earlier reports involving excretion of substances into the bile have not taken account of the susceptibility of the rat to hypothermia and the importance of temperature on biliary excretion (402) it is difficult to come to defini-

tive conclusions regarding the effects of these agents on the biliary excretion of exogenous substances.

Gallagher *et al.* (159, 160) have studied the effect of estradiol and related steroids on estrogen-induced impairment of liver function in rats. These authors found that estradiol affected plasma BSP retention after 5 days of administration in a dose-related manner. They further showed that the BSP retention produced by estradiol became evident by the 4th day and returned to normal 4 days after cessation of treatment. The steroid was found to not affect the BSP conjugating capacity of hepatic tissue *in vitro* nor did it affect hepatic glutathione content. The alteration in plasma dye clearance was also demonstrated with the use of phenol-3,6-dibromophthalein sulfonate, a chemical analog of BSP which is not conjugated (224) before its excretion into bile. These observations indicate that estradiol is not affecting conjugation of BSP. In addition, these authors studied various other steroids to determine whether they exhibited an effect similar to estradiol. The compounds capable of producing BSP retention included: estrone, α-estradiol, estriol, epi-estriol, estradiol-3-acetate, estradiol-17-acetate, estradiol-3-methyl ether, mestranol, 11β-hydroxyestrone, 16-ketoestrone, equilenin,17-dioxyestrone, 2,17α-dimethylestradiol, and diethylstilbestrol. Twenty-three steroids were inactive in this study; five of these were actually bile acids. The authors concluded that a specific oxygenated group in the C or D ring of the estratriene nucleus was not an essential requirement for inducing BSP retention. However certain structural substitutions in the A ring diminished or abolished the capacity of these steroids to impair BSP disappearance. The only natural steroids which regularly impaired BSP disappearance were C-18 compounds of phenolic A ring type. The metabolites of estradiol which retain activity were all compounds which contained a 17-deoxysteroid configuration. The presence or absence of specific oxygenated substituents in the C or D of the estratriene nucleus did not seem an essential requirement for retention of this biological activity. A double bond between C-6 and C-7 diminished or eliminated activity, and an additional bond between C-8 and C-9 restored activity. Hydroxylation of the steroid nucleus at the C-2 position diminished the capacity of these estrogens to impair hepatic clearance of BSP, and methoxylation had the same effect. Methylation at C-2 or C-1 seemed to abolish the activity whereas methylation at the C-17 position restored activity. Chemical substitution on the oxidant group at C-3 did not abolish the capacity of the estrogens to impair BSP clearance, although the oxygen function itself at this position seemed essential for activity. The authors concluded that with the contraceptive steroids the determination of possible BSP impairment should be based primarily on the molecular characteristics of the individual steroid components rather than on the relative roles as progestins or estrogens. The authors also point out a very important aspect: the structural characteristics which have been used for determining structure-activity relationships are based on the structural characteristics of the administered compounds and it is evident that the biological potency of the steroids may be significantly affected by their *in vivo* biotransformation.

Heikel and Lathe (191) have investigated the effects of various oral contraceptive steroids on bile formation and on bilirubin Tm in rats. Both 17α-ethynyl substituted estrogens and progestins reduced basal bile flow between 12 and 48 hr after administration. The parent compounds 17α-estradiol and 19-nortestosterone had little effect. A larger dose of progestogen was required than estrogen. Bilirubin Tm was little affected by mestranol; however, an elevation of serum conjugated bilirubin after infusion of bilirubin was produced by both 17α-ethynylestradiol and mestranol but not by the progestogens. These effects were not due to hypothermia since body

temperature was maintained during the determination of bilirubin Tm.

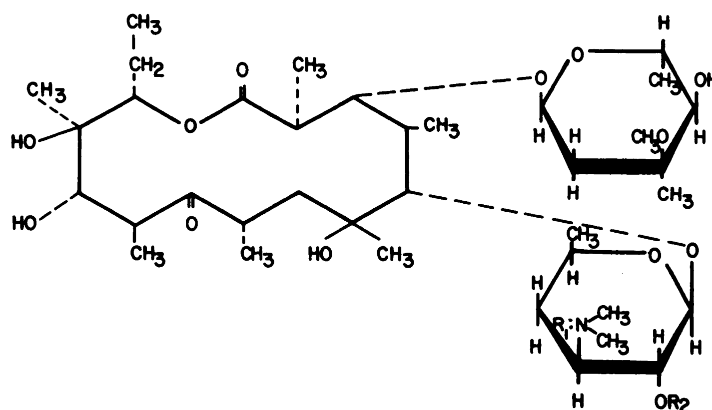
Although most authors have interpreted the results obtained in animals as an indication of cholestasis, it has not been possible to demonstrate the fully developed intrahepatic cholestatic lesion in the usual type of laboratory animal. With electron microscopy, dilatation of the bile canaliculi has been described in rats after the administration of norethandrolone (428). However, neither hyperbilirubinemia nor canalicular bile plug formation have been demonstrated in rats. In 1970, Imai and Hayashi (217) reported that they could produce steroid-induced intrahepatic cholestasis in certain selected species of mice. In their studies, male DS mice were employed and the steroid was administered orally for 5 consecutive days. A number of steroids were employed but most of the work was done with norethisterone. The livers of these treated animals were enlarged and colored dark brown. Jaundice was evident and histological examination of the liver invariably revealed occurrence of bile plugs in the bile canaliculi as well as in interlobular biliary ducts. The hepatocytes appeared swollen, exhibited a decrease of cytoplasmic basophilia, and occasionally contained bile pigment granules. Single cell necrosis of the parenchymal cells was occasionally noted along with cellular infiltration at the periportal area. Dilatation of the bile canaliculi and reduction of microvilli were frequently noted in electron micrographs. An amorphous fine granular or fibrillar material was occasionally seen in the dilated bile canaliculi. Plasma bilirubin concentrations were significantly elevated in the norethisterone-treated mice. The activity of plasma alkaline phosphatase and transaminases was also increased in these animals. These observations are very similar to those reported in man after intrahepatic cholestasis induced by anabolic steroids. Other strains of mice were investigated, but the DS mice and C57BL strain seemed to be the most sensitive. The lesion was also produced in CBA and C3H mice, but ICR mice were the least sensitive. In this study it was not possible to produce intrahepatic cholestasis in Sprague-Dawley rats. Other steroids found to produce this effect in the DS strain of mice were methyltestosterone, oxymetholone, mestranol, and norethandrolone. On the other hand, the lesion could not be produced with testosterone propionate, progesterone, or 17β-estradiol. This study is a very important one since it indicates that certain species, and particularly certain strains of mice, may be more susceptible to the cholestatic effects of steroids. It has yet to be determined whether this is an inherent increase in sensitivity of the hepatic tissue or whether this reflects a species difference in biotransformation. However, the studies should be pursued in depth by other investigators to determine whether these strains of mice are better for uncovering the cholestatic potential of drugs.

Estrogens and progestins have other effects on the biochemical function of the hepatocyte, many of which are sex related. These effects have been extensively reviewed by Song *et al.* (471) but do not seem closely related to the cholestatic response. ALA-synthetase, the first enzyme in the bile synthetic sequence that converts glycine and succinyl coenzyme A to porphyrins is affected by estrogens. Much interest has been given to this particular phenomenon since the activity of ALA-synthetase is considered to be the rate-limiting step in this biosynthetic scheme; in acute intermittent porphyria, the genetic defect is thought to result in an overproduction of ALA-synthetase. It seems likely that steroids may be involved in the pathogenesis of acute intermittent porphyria in certain patients.

F. Erythromycins

The macrolide antibiotic erythromycin and its derivatives (table 3) provide another useful model for studying the importance of structure-activity relationships in cholestasis. There have been a number of

TABLE 3
Structures of erythromycin derivatives



Erythromycin derivative	R_1 substitution	R_2 substitution
Base		H
Stearate	$C_{17}H_{35}COOH$	H
Propionate*		CH_3CH_2COO-
Estolate*	$C_{12}H_{25}OSO_3H$	CH_3CH_2COO-
Acetate		CH_3COO-
Ethylsuccinate		$C_2H_5OOC(CH_2)_2COO$-
Gluceptate	$C_7H_{14}O_8$	H
Lactobionate	$C_{12}H_{22}O_{12}$	H
Cetylsulfate	$C_{12}H_{33}OSO_3H$	H

* Cholestatic.

clinical reports (50, 54, 145, 166, 236, 298, 311) of mild reversible cholestasis associated with the use of the lauryl sulfate salt of erythromycin propionate [erythromycin estolate (EE)]. The biochemical and morphological features of EE-induced hepatic dysfunction are primarily cholestatic, but since there have also been reactions with the character of hepatocellular necrosis, the reaction has also been classified as mixed cholestatic-hepatitic (262). Hepatic dysfunction has been clearly established in susceptible individuals after rechallenge with EE (409). This has led to the suggestion that hypersensitivity subsequent to prior exposure is important in the pathogenesis of the cholestatic reaction. Estimates of the incidence of cholestasis during clinical use have been as high as 12% (410) which seems high for a hypersensitivity drug reaction.

A number of erythromycin esters and salts are in current clinical use; however, the propionyl ester, and its lauryl sulfate salt (EE) seem to be the only forms of erythromycin with cholestatic potential. It is possible that this might be due to the fact that EE has advantages in gastric acid stability and absorption resulting in higher blood levels than with other orally administered derivatives (40). However, the most conclusive evidence for the low cholestatic potential of the other derivatives is found in the report of Tolman *et al.* (491).

They described a patient with a history of EE-induced cholestasis who was challenged with erythromycin base, stearate, propionate, estolate, acetate, and the soluble parenteral formulations erythromycin ethylsuccinate and gluceptate. A cholestatic reaction characterized by hyperbilirubinemia, elevated leukocyte count, serum glutamic-oxaloacetic transaminase (SGOT) and alkaline phosphatase, and fever, occurred only during challenge with erythromycin propionate or EE. The lauryl sulphate component is relatively unimportant since it could not induce cholestasis when combined with either erythro-

mycin base, or the analog 2'ester with acetate instead of propionate. Plasma levels of all erythromycin derivatives administered were greater than 1 μg/ml, and no correlation was observed between plasma level and toxicity.

Studies with *in vitro* preparations such as the isolated perfused rat liver (248), cultured mouse liver cells (117) isolated rat hepatocytes (526), and cultured human hepatocytes (Chang cells) (115, 120) have confirmed the greater toxicity of erythromycin propionate and EE compared with other derivatives. In these *in vitro* experiments, the relative cytotoxicity of erythromycin derivatives correlated with their relative ability to lower surface tension. The most cytotoxic derivative in the Chang cell study of Dujovne (115) was an unmarketed derivative, erythromycin cetyl sulfate, and this also had the highest surfactant activity. A similar correlation between surfactant activity and cytotoxicity in *in vitro* liver preparations has also been noted for the phenothiazines, bile salts (119), and the laxative dioctylsulfosuccinate (121) (see section IV, G).

Since lauryl sulfate, which is added to erythromycin propionate to form an insoluble tasteless pharmaceutical preparation, is in itself strongly surfactant, it was of interest to determine the contribution it would make to cytotoxicity in these experiments. Lauryl sulfate cytotoxicity in Chang cell cultures was only slightly less than that of erythromycin propionate, but the combination was synergistic. An interesting but unexplained observation was that erythromycin propionate plus lauryl sulfate was significantly more cytotoxic than the combination of these two chemicals as EE. The *in vivo* toxicity of lauryl sulfate administered orally is reported to be quite low, although it is well absorbed and extensively metabolized in man and in the rat (120, 332). Therefore, the *in vitro* studies point to a significant role for lauryl sulfate, although the *in vivo* study of Tolman *et al.* (491) implies that it is insignificant in the clinical manifestation of EE-induced hepatic dysfunction.

In spite of the fact that cytotoxicity of erythromycin derivatives *in vitro* is a good predictor of cholestatic potential in man, an erythromycin-induced cholestatic reaction in experimental animals *in vivo* has yet to be demonstrated. The nearest to a cholestatic response that has been demonstrated in animals was the ability of erythromycin propionate and EE to reduce bile flow in the isolated perfused rat liver (248). The lack of response *in vivo* may be related to species differences in metabolism. In the rat, Murphy *et al.* (333) have shown that erythromycin propionate is metabolized by hydrolysis to the base, and also by N-demethylation. The biological half-lives of the propionate and the N-demethylated metabolite were $5^1/_2$ and $1^1/_2$ hr, respectively. Biliary excretion is a significant route of elimination in the rat, but the importance of this route in man is equivocal. Probably a more important difference between man and the rat is the extent of hydrolysis of the proprionate to the base. Man hydrolyses EE relatively slowly, and a consistent 3.5:1 ratio of ester:base has been reported in plasma after oral administration of EE (480).

There seem to have been no systematic studies on the related antibiotic triacetyloleandomycin, although its cholestatic potential in man seems to be similar to that EE (410). In the study of Ticktin and Zimmerman (489), only 2 of 50 patients developed jaundice but more than 50% developed abnormal liver function, which led to the discontinuation of the drug during the 3rd or 4th week of the study.

G. Phenothiazines and Tricyclic Antidepressants

Although the incidence of cholestasis associated with the clinical use of phenothiazines and tricyclic antidepressants varies considerably with the modification of chemical structure, this group of drugs represents one of the major problems in drug-induced intrahepatic cholestasis. There is still considerable controversy as to whether the mechanism of action is by a direct toxic effect of the drugs or their metabolites, or whether a hypersensitivity reaction is involved. Furthermore, the his-

tological features of the hepatic lesion resemble biliary obstruction in some cases, and hepatocellular necrosis/hepatitis in others (see section III). For these reasons, the phenothiazines, in particular chlorpromazine (CPZ), and more recently the tricyclic antidepressants and thioxanthenes, have been the subject of extensive investigation in animals in order to elucidate the possible mechanisms of hepatotoxicity.

There seem to be important species variations in the ability to demonstrate CPZ-induced hepatobiliary dysfunction. In the dog, a reduction in bile flow after intravenous injection of CPZ has been reported in two studies. Sharma and Prasad (457) showed that the reduced bile flow was accompanied by a decrease in bile viscosity (contradictory to what had been suggested as a possible mechanism) and an increase in bilirubin concentration. Stefko and Zbinden (477) showed that the decrease in bile flow (which could also be seen with much higher doses of chlordiazepoxide and diazepam) was associated with an increase in intrabiliary pressure. It is not known whether these CPZ-induced changes in bile flow might have been mediated by neurohumoral interaction, although it is known that these factors play a role in regulating bile flow (476).

In an abstract in 1975, Ros *et al.* (414) reported CPZ-induced cholestasis in the rhesus monkey after intravenous infusion. After an initial 3-hr period of complete cholestasis, the rate of bile flow recovery was related to the dose of CPZ injected, but the cholestasis was completely reversible within 24 hr. Biliary lipid secretion paralleled bile flow, and the authors tentatively explained the cholestasis on the basis of a CPZ-induced reduction in both bile-salt independent and bile lipid-dependent flow. However, these authors may have overlooked possible hemodynamic effects which could have altered bile flow.

Neither chronic nor acute administration of CPZ to the rat has resulted in the development of cholestasis. Popper *et al.* (370) reported no growth retardation or altered hepatic histology in rats receiving various diets supplemented with CPZ for up to 187 days. Balazs and Juhazs (23) examined the ultrastructure of rats of both sexes receiving CPZ by subcutaneous injection for up to 8 weeks and found dilatation and vacuolization of the pericanalicular cytoplasm, but not the dilatation of bile canaliculi and shortening of canalicular microvilli characteristic of the cholestatic lesion. Knodell (263) reported hemolysis-stimulated changes in bilirubin output, but no changes in bile flow, or bile salt/phospholipid/cholesterol output in rats after intravenous injection of CPZ.

Schnack (440) reported BSP retention in rats treated with CPZ. However, since conjugation of BSP with glutathione is a critical factor in its biliary clearance, and CPZ reduces hepatic glutathione concentration (313), this report does not necessarily mean that hepatic function was impaired. Eckhardt and Plaa (126) observed plasma BSP retention in mice 2 hr after oral treatment with CPZ or 13 other phenothiazine derivatives. However, the high dose of BSP employed in this study was selected to negate effects on BSP biliary excretion and to measure indirectly the acute decrease in hepatic blood flow produced by phenothiazine derivatives. This decrease in hepatic blood flow after treatment with CPZ or thioridazine was observed directly in isolated perfused rat livers (363) and in anesthetized dogs (128) and is due to an increased hepatic resistance to blood flow. Other studies showed that acute CPZ administration did not affect BSP metabolism and biliary excretion in mice and rats (127). The authors concluded that the acute effect of phenothiazine derivatives on plasma BSP retention were due to a decrease in hepatic blood flow and not to hepatic dysfunction.

Therefore, in spite of the fact that some of the *in vivo* studies reported altered hepatic function, the morphological features which characterize phenothiazine-induced cholestasis in people have not been reproduced in experimental animals. This lends some credence to the hypersensitivity hypothesis.

Hepatotoxicity has also been demonstrated in some *in vitro* experiments. A reduction in the ability of isolated perfused rat livers to secrete bile and to excrete BSP has been demonstrated when CPZ (249), other phenothiazines (492), or chlordiazepoxide (3) were added to the perfusion medium. Phenothiazines (118, 526), thioxanthenes (4), and tricyclic antidepressants (2) resulted in substantial dose-related leakage of intracellular enzymes from isolated rat hepatocyte suspensions. These *in vitro* results are probably manifestations of a direct toxic effect of these surfactant substances on the hepatocyte cell membrane. The relative potency of the phenothiazines correlates well with their critical micelle concentrations (15), and a similar correlation between *in vitro* toxicity and surfactant activity has been demonstrated for the erythromycins, bile salts and oxyphenisatin (see section IV, F). One cannot rule out the possibility that these surfactant effects might be a significant part of the mechanism by which some of these drugs produce intrahepatic cholestasis. However, at this stage there is insufficient information available to speculate.

H. Manganese

In 1968, Witzleben *et al.* (515) described the morphological changes occurring in the liver after the acute intravenous administration of manganese sulfate. This substance was found to induce characteristic ultrastructural alterations in the hepatocyte which resemble those observed in cholestasis. In particular, it was found that the bile canaliculi became dilated with loss and swelling of the microvilli. In addition, increased prominence and dilatation of the Golgi apparatus were observed. These initial studies were performed in rats 20 hr after the administration of manganese sulfate. In the same study, it was observed that the Tm for bilirubin excretion into bile was very markedly reduced in rats 5 hr after the administration of manganese.

Although the hepatocytes showed ultrastructural changes with the administration of manganese alone, no alterations in the ultrastructure of bile ductules were observed at this time. Six hours after administration of the manganese, cytoplasmic basophilia was evident by light microscopy and multiple small foci of hepatocellular necrosis were observed; by 12 hr all animals exhibited hepatocellular necrosis which involved either single cells or more commonly patchy areas. The necrotic cells were randomly scattered throughout the lobule, with no marked zonal distribution. However, a predilection for midzonal localization of necrosis was sometimes evident. There was no clear correlation between the severity of the necrosis observed and the extent of the cholestatic morphological changes observed.

In subsequent work, Witzleben reported that manganese caused a brief reversible decrease in the flow of bile (509), and that this cholestatic effect was markedly potentiated in a dose-related manner by infusion of bilirubin (45, 510, 511). The bile collected from animals given manganese followed by bilirubin infusion was markedly abnormal in appearance. It became turbid after collection and tended to adhere to the sides of the collecting tubes. This turbidity was not observed in bile collected from rats given manganese alone, but it could be reproduced *in vitro* by adding manganese sulfate to bile collected from normal rats infused with bilirubin, or to a concentrated bilirubin solution.

This suggested that the cholestatic mechanism might have involved intracanalicular precipitation of a manganese/bilirubin aggregate. This hypothesis was supported by the finding of yellow acellular material in the bile ducts, although canalicular plugs were not evident. With the electron microscope, it was possible to demonstrate that the infusion of bilirubin 4 hr after the injection of manganese resulted in more severe ultrastructural alterations than those observed with manganese alone. These abnormalities included increased canalicular dilatation, the presence of prominent cytoplasmic

vacuoles and swollen areas of pericanalicular cytoplasm. In addition, fibrillar electron-dense material was observed within the canaliculi, vacuoles, pericanalicular ectoplasm, and bile ducts; these findings were not observed in animals given manganese alone. These ultrastructural alterations are consistent with an obstructive process. An interesting observation was that if the infusion of bilirubin followed the injection of manganese by 24 hr, the effects on bile flow and hepatic ultrastructure were very mild, and not different from that of rats given manganese alone.

It seems that the infusion of bilirubin itself does not result in the cholestatic picture that has been described thus far. Bilirubin infused at rates high enough to saturate the hepatic excretory capacity has resulted in diminution of bile flow in the rhesus monkey (163), but not in the isolated perfused rat liver (38). But it seems that the addition of bilirubin to the manganese-loaded animals results in very profound cholestatic effects, the extent of which depends both on the dose of bilirubin and the interval after manganese loading. Bile flow had partially recovered by 24 hr and appeared to be normal by 48 hr (511). The ultrastructural changes in the canalicular region seemed to be more extensive 24 hr after the injection of manganese than at the onset of the cholestasis (511). The cytoplasm of the bile duct epithelial cells also showed prominent alterations, with inclusions of amorphous or fibrillar material. By 48 hr the canaliculi remained dilated and with loss of microvilli, although the presence of intraluminal material was greatly reduced. Fibrillar material was still evident at this time although less so than at 24 hr. The bile duct cells contained less osmiophilic material than that observed at 24 hr, but the epithelium itself was even more abnormal.

Thus, there was a marked disparity between the ultrastructural changes which occurred at the peak of the cholestatic response (4 hr) and at later times. It seemed that the reversibility of the cholestasis was out of phase with the alterations in ultrastructure. This difference seems to be more noticeable in the bile ductular cells, but it is also evident in the canalicular alterations. Witzleben concluded (511) that ductular and canalicular alterations, at least at the onset of cholestasis, were not causative events but were either resultant effects or the reflection of a more slowly developing response to the same insult that initiates cholestasis.

Witzleben postulated several mechanisms to explain the bilirubin enhancement of cholestasis in the manganese-treated animals. The first was that the manganese and bilirubin do in fact interact within the biliary tree to form an insoluble precipitate. A second possibility was that manganese and bilirubin acted synergistically on the hepatocyte directly to affect the formation of bile. This author however was unable to demonstrate any alterations in bile salt composition in animals treated with manganese.

In 1975, Witzleben and Boyce (513, 514) attempted to determine whether the concentration of bilirubin in the liver, blood, or bile is critical to the degree of cholestasis produced or whether the biliary concentration of manganese is a critical feature. Unfortunately, the results shed little light on this particular situation. They used BSP as the substance to modify bilirubin concentration in the bile. In animals simultaneously infused with bilirubin and BSP, they showed that BSP infusion protected against the cholestatic response. This was dramatically demonstrated in the bile flow rates obtained 4 hr after the initial injection of manganese. BSP infusion in animals which had also received bilirubin exhibited a bile flow rate which was indistinguishable from the control animals. Ten minutes after the administration of bilirubin it was possible to demonstrate that BSP infusion resulted in a diminution in the concentration of bilirubin in the bile; however, 30 to 60 min after the infusion of bilirubin the concentration of this substance in the bile was identical in

the animals infused with or without BSP. Yet, the animals which had not been infused with BSP exhibited a diminished bile flow rate. These data indicate that it is not the concentration of bilirubin in the bile which seems to be the determinant factor. The subsequent study (514) on the role of manganese also failed to show a direct relationship between the concentration of manganese in the bile and the degree of cholestasis. Bilirubin infusion itself was found to increase the manganese concentration in the bile; however, the total amount of manganese excreted in these animals was not altered when compared to nonbilirubin infused controls. The increased concentration of manganese seemed to be due to the decrease in bile flow induced by the bilirubin infusion. When BSP was infused into the animals to prevent the manganese-bilirubin cholestatic response, it was observed that manganese excretion in the bile increased significantly both in concentration and in total manganese content. These experiments therefore demonstrated that in the situation in which BSP protects against cholestasis, manganese excretion in the bile is actually increased rather than decreased. These data suggest that manganese concentration itself in the bile is not a determinant factor.

Unfortunately, the mechanisms of the manganese-bilirubin cholestatic reaction are not well understood and the experiments with BSP infusion have failed to determine which component is critical to the response. However, the experiments do suggest that the initiating event may be an interaction between bilirubin and manganese at the level of the hepatocyte. However attractive this hypothesis is, it must also be admitted that intracanalicular interaction has not been completely eliminated. If one assumes that manganese and bilirubin interact in the canaliculi to produce an insoluble aggregate, one could explain the protective effect of BSP as being mainly an inhibition of this interaction. To date, no one has investigated the effect of BSP-glutathione on the solubilization of bilirubin in bile and on the precipitating effect of manganese in bile containing high concentrations of bilirubin. The data of Witzleben and Boyce (513) suggest that bilirubin may be excreted in different forms depending upon the presence of BSP. They found that the protective effect of BSP infusion on the diminution in bile flow could be overcome if a higher dose of bilirubin were infused. Yet, the final concentration of bilirubin obtained in bile in these experiments was similar regardless of the concentration of bilirubin infused.

There are other disparities in the model which are still difficult to explain. Klaassen (258) found that the intermittent administration of manganese chloride to rats, rabbits, and dogs had very little effect on the rate of bile production in rats and dogs over a period of 2 hr. Manganese, however, had a marked effect on the production of bile in the rabbit. Klaassen confirmed the observation of Witzleben that manganese followed by an intravenous dose of bilirubin resulted in a marked diminution in bile flow in the rat. However, he observed that the cholestatic effect no longer occurred if the bilirubin was administered before the manganese. No explanation is available for this interesting observation. Klaassen also observed a fine precipitate in the bile of rats given manganese, which became especially evident after the bile has been allowed to stand for a few minutes. He further noted that rabbit bile which is normally green due to biliverdin content lost its green color after manganese administration.

Regardless of the mechanisms involved, the discovery that manganese and bilirubin can interact to cause a physiological and a morphological cholestasis has produced an interesting experimental model. The fact that bilirubin alone can induce cholestasis in the primate under certain circumstances is of interest, too (163). It is also worth noting that other organic compounds that are efficiently and selectively

excreted by the liver can become cholestatic with increasing dosage (see sections V, B; V, E).

I. Miscellaneous Agents

There are other isolated observations of chemically induced cholestasis in animals in which there has been little or no follow-up. Also there have been studies on drugs which are reputedly cholestatic in man, but for which other mechanisms for producing jaundice have been established in animal experiments.

The compound 2-ethyl-2-phenyl butyramide, while being evaluated as a potential hypnotic agent, was found to produce bile plugs and other morphological features of intrahepatic cholestasis in dogs (252), and evidence of BSP retention (378). Administration of this compound to rats failed to produce any evidence of hepatic dysfunction. The food additive, sucrose acetate isobutyrate, was also shown to alter hepatic function in dogs, but not rats or squirrel monkeys (388). The response in dogs was characterized morphologically as a readily reversible mild intrahepatic cholestasis, with BSP and indocyanine green retention and elevated serum alkaline phosphatase. There seem to have been no follow-up studies to characterize these cholestatic responses.

The carcinostatic nitrosourea derivative, 1, 3-bis(2-chloroethyl)1-nitrosourea (BCNU), produces an interesting hepatotoxic effect in people and experimental animals (99, 488). The response, which is characterized in the rat by BSP retention, hyperbilirubinemia, pericholangitis, and ultimately biliary cirrhosis, may be considerably delayed in onset after the administration of a single dose. The response has been compared with that of ANIT, although the time course of the onset of cholestasis differs markedly for the two agents. Furthermore the biliary cirrhosis seen after a single dose of BCNU resembles the effects of chronic ANIT administration rather than the intrahepatic cholestasis of hepatocytic origin seen after a single dose of ANIT.

The antibiotics novobiocin and rifampicin have been classified (262) as drugs with cholestatic potential, but there is little or no evidence that they produce features of cholestasis other than jaundice and BSP retention (52, 77, 84, 282, 357, 365). Both of these effects may be explained by selective actions on the hepatic uptake of organic anions, and inhibition of glucuronyl transferase activity (188, 468). Similar observations have been made for cholecystographic agents (262). The combination of rifampicin with isoniazid, itself a hepatotoxic drug, produces a much higher incidence of clinical jaundice with both cholestatic and cytotoxic features (287, 365) and furthermore, rifampicin and isoniazid exhibit synergistic hepatotoxic properties in rats (209).

Nitrofurantoin is another example of a drug which occasionally produces hepatic dysfunction (262). The features have been described as cholestatic (135), although one report (172), in which lightmicroscopic and ultrastructural studies on biopsy specimens were reported, described a lesion with more of a hepatocellular necrotic than intrahepatic cholestatic character. There seem to have been relatively few studies on the hepatic effects of nitrofurantoin in animals. Conklin *et al.* (78, 79) reported that nitrofurantoin is excreted in the bile of dogs, undergoes enterohepatic recirculation, and its excretion is accompanied by a marked choleresis.

In 1965 an outbreak of jaundice occurred in Epping, England; at least 84 people, who had consumed bread baked from a sack of contaminated flour, were affected (264a, 264b). Liver biopsies indicated cellular infiltration, cholestasis, and damage both to the liver parenchyma and to the biliary tree, but hepatocellular necrosis was minimal. The contaminant responsible for "Epping jaundice" was identified as 4, 4′-diaminodiphenylmethane, a hardener for epoxy resins. Only limited animal studies have been done with this substance. In mice, hepatic changes were observed, but these were not identical with those observed in people (264a).Im-

pairment of hepatic function was said to occur in cats, but no details were given (264b). Smith (468a) has hypothesized that perhaps 4, 4′-diaminodiphenylmethane forms insoluble salts with bile acids, but to our knowledge this has been studied experimentally with this particular compound, although such a mechanism has also been proposed for chlorpromazine-induced cholestasis (see section V, G).

Jaundice has been observed in man during nonhepatic infections with *Escherichia coli* (495a). Histologically, intracellular and intracanalicular bile stasis, along with hepatocellular abnormalities, have been described (31a). It has been shown that *E. coli* endotoxin causes a diminution in bile flow in the isolated perfused rat liver (495a). The endotoxin also caused a dose-dependent impairment of BSP and indocyanine green excretion. The authors hypothesized that circulating endotoxin may contribute to the production of intrahepatic cholestasis seen during bacterial infection. A single dose of endotoxin has been shown to produce hepatic changes in mice (287a); dilated bile canaliculi with loss of microvilli were among the hepatocytic alterations observed.

V. Hypotheses on Mechanisms Leading to Cholestasis

A. General Remarks

In this section, some of the mechanisms which have been proposed to account for the production of the cholestatic syndrome will be discussed. However, at the outset, we wish to make it clear that the search for a single cholestatic mechanism which could explain all types of cholestasis (chemically induced and idiopathic) may be too simplistic.

By way of analogy, if we look at the extensive work that has gone into the study of fatty liver production (294), it is clear that at least five different mechanisms can be postulated. These are: a) increased mobilization of fatty acids; b) decreased oxidation of fatty acids; c) increased synthesis of fatty acids; d) increased conversion to triglycerides; and e) decreased hepatic secretion of triglycerides. With some hepatotoxic chemicals, one or two of the above mechanisms may be more important than others; with some agents, only one mechanism may be involved. Yet all of these mechanisms lead to the same final response; that is, the production of a fatty liver. Is it not reasonable that cholestasis may also be the resultant response to the activation of one or more mechanisms? Furthermore, is it not reasonable that chemically unrelated drugs may produce a similar cholestatic response, but which may involve different, or complex multiple mechanisms?

B. Altered Canalicular Bile Flow

There are a number of sites along the biliary tract where alteration of the flux of water and electrolytes could be a prime factor leading to cholestasis. Of these sites, interference with canalicular bile formation seems to be the most likely factor involved in intrahepatic cholestasis.

The mechanisms involved in canalicular bile formation and regulation of canalicular flow have been reviewed in detail by others (10, 136–138, 226, 260, 381, 506). It seems reasonable that in order to have some understanding of the mechanisms involved in intrahepatic cholestasis, we should first understand the mechanisms involved in the formation of bile by the hepatocyte. Since it is apparent that our knowledge of the relationships between bile salt, electrolyte, and water flux across the canalicular membrane is still rudimentary, it is perhaps not surprising that our knowledge of cholestatic mechanisms is quite limited. Part of the reason why our knowledge of biliary secretory mechanisms is less than that of comparable renal secretory mechanisms is the inability to sample canalicular bile. Changes in bile composition which occur during passage of bile along the biliary tract complicate the issue. The finding that certain neutral sugars, such as erythritol and mannitol, seem to be passively secreted across the canalicular membrane along with water, and are neither secreted nor reabsorbed by

the biliary ductules, has provided a useful tool for measuring canalicular bile flow (48, 71, 152, 153, 155, 156, 431, 507).

The ability to measure canalicular bile flow soon led to the elaboration of a new and important concept. It had long been assumed that secretion of bile salts provided the major osmotic drive for the inward flux of water into the canaliculus. This was based upon the known choleretic effects of infused bile salts, and the linear regression that could be shown for bile flow and bile salt excretion. The discovery that the linear regression for bile salt excretion on canalicular bile flow did not extrapolate to zero led to the concept that part of the canalicular bile flow was independent of bile salt excretion. This component, variously called bile salt (acid)-independent fraction (BSIF, BAIF), or bile acid-independent formation of canalicular origin (BAIFC), was shown to undergo marked species variation, but generally constituted 40 to 70% of the basal canalicular flow rate depending on the experimental conditions employed (47, 49, 138, 380, 507).

The methods currently employed to quantify BSIF are indirect, and subject to possibly unwarranted assumptions about the meaningful extrapolation of bile salt excretion to zero. Indeed, in the rat (21, 259), during bile salt depletion and repletion, the bile flow may remain relatively constant in the face of marked changes in bile salt output, and in some cases the linear regression of bile salt excretion on bile flow can have a negative slope. One interpretation of these data is that BSIF is not constant during altered bile salt excretion, and that there may well be an interaction between bile salts and the mechanism responsible for elaborating the BSIF.

Much of the evidence points to the probability that active sodium secretion, mediated by canalicular membrane Na^+-K^+-ATPase is an important feature in the elaboration of the BSIF. This hypothesis is based mainly on studies on the effects of inhibitors of this enzyme on bile flow. However, two facts which are consistent with an interdependency of bile salt-dependent and bile salt-independent canalicular flow should be mentioned here. Firstly, bile salts and certain nonionic detergents activate Na^+-K^+-ATPase (133). The mechanism is complex but it seems to involve, at least partly, the "exposure" of latent enzymic active sites (443). This could mean that bile salts secreted across the canalicular membrane could cooperatively stimulate the secretion of Na^+ by a Na^+-K^+-ATPase pump. There is evidence, at least for some tissues, that the ouabain-sensitive active sites of this Na^+-pump are accessible from only one side of the membrane. Secondly, the active transport of bile salts across the hepatocyte membrane has been shown to be Na^+-dependent (106, 394, 446). One factor which seems to be at variance with the concept of cooperative transport is that inhibition of bile flow in the rat has been demonstrated both *in vivo* (111, 445) and in the isolated perfused liver (349, 445) under conditions of taurocholate overload. Furthermore, Accatino and Simon (5), in 1976, isolated from hepatocyte surface membranes a purportedly specific bile acid receptor, which reversibly binds cholate and taurocholate, follows Michaelis-Menten kinetics, but which is independent of Na^+, Ca^{2+} and Mg^{++}, and is not energy-dependent.

Since the BSIF seems to play such an important role in determining canalicular bile flow, there has been considerable interest in the effect of drugs and hormonal factors on this fraction. Substances which function as choleretics by virtue of an increase in BSIF include cyclic adenosine monophosphate (cAMP) (330), hydrocortisone (122, 299, 527), thyroid hormone (278), prostaglandin A_1 (275), and possibly vasopressin (382).

Phenobarbital and some other barbiturates enhance bile flow and the biliary clearance of drugs and endogenous metabolites (62). Increased liver weight associated with the inductive properties of phenobarbital may account for a part of the increased bile flow, although the poor correlation between the time course of bile

flow enhancement and increased liver weight or microsomal enzyme activity makes it unlikely that this is the only mechanism. Furthermore, other enzyme inducers, while producing comparable increases in liver weight, fail to stimulate bile flow (255, 260). Enhancement of bile salt output seems to be a rather late response (184, 392). Therefore, the evidence suggests strongly that phenobarbital enhances the BSIF, and this has been demonstrated in the rat (33, 256, 350) and rhesus monkey (392) although in the primate, part of the increased bile flow may be due to enhanced bile salt synthesis and biliary lipid output (392). Phenobarbital may enhance BSIF in man, although there is no evidence available as yet. It has not been established whether the phenobarbital-enhanced plasma clearance of dyes such as rose bengal and BSP during cholestasis in man is due to enhanced hepatic uptake or enhanced biliary excretion (37, 484). Other choleretic agents for which enhanced BSIF has been implicated include: carbutamide (496), diazepam (187), the catatoxic steroids pregnenolone-16α-carbonitrile (PCN), and spironolactone (527), theophylline, and 1-ethyl-4-(isopropylidenehydrazino)-1H-pyrazolo-3, 4-b)-pyridine-5-carboxylic acid (SO-20009); the last two probably act by elevating cAMP (25).

Of greater interest, in terms of the possible involvement of impaired canalicular bile flow in intrahepatic cholestasis are those drugs for which experimental evidence has suggested an inhibition of BSIF. Such agents include: chlorpromazine (414); estrogens, in particular ethinyl-estradiol (185); anabolic steroids (191); dyes which undergo extensive biliary excretion such as indocyanine green (207), rose bengal (105), and possibly the unconjugated fraction of BSP (442); and finally, Na^+-K^+-ATPase inhibitors such as ouabain, scillaren, ethacrynic acid, and amiloride (39, 47, 137, 139, 140).

The mechanism by which these agents inhibit BSIF is unknown, although inhibition of canalicular membrane Na^+-K^+-ATPase could well be a factor. Unfortunately, the relationship between these experimental findings and drug-induced intrahepatic cholestasis is not clear. Of the agents listed above, only chlorpromazine and the steroids (see sections III, IV E, and IV G) have been clearly implicated as cholestatic agents in man. Also, in view of the supposed importance of Na^+-K^+-ATPase in elaborating the BSIF, the ambivalent results with potent inhibitors of this enzyme are disturbing.

With ethacrynic acid the results of Erlinger *et al.* (139, 140) were quite clearcut, and showed reduction of bile flow, independent of any effect on bile salt output in the rabbit. However, Shaw *et al.* (458) could demonstrate a decreased bile flow in only three of eight rabbits given three intravenous injections of ethacrynic acid; the bile flow reduction was correlated with reduced arterial blood pressure. The *in vivo* bile flow response to ethacrynic acid in the rat, guinea pig, dog, and sheep was a choleresis rather than cholestasis. Chenderovitch *et al.* (72) also showed that ethacrynic acid produces choleresis in the rat, and this choleresis seemed to be associated with an increase in the BSIF, possibly due to the osmotic effect of ethacrynic acid and/or its cysteine adjunct excreted in the bile, or an increase in electrolyte concentration in the bile. Both ouabain and ethacrynic acid produced a choleresis in the isolated perfused rat liver (177).

Several ideas have been put forward to explain these discrepancies. There are species differences in the sensitivity of Na^+-K^+-ATPase to inhibitors, and there is a poor correlation between Na^+-K^+-ATPase inhibition *in vitro* and diuretic activity for ethacrynic acid (443). This could possibly explain the unusual effects on bile flow in the rat, which has relatively low canalicular membrane Na^+-K^+-ATPase activity (133), and is also resistent to the diuretic effects of ethacrynic acid (34, 206). It is clear that more information is required on the inhibitory effect of these compounds on the specific hepatic canalicular membrane Na^+-K^+-ATPase in order to resolve this point. Furthermore, the concentration of

the inhibitory agent in relation to the location of the enzyme may be important. The ouabain-sensitive Na^+-K^+-ATPase of a number of secretory cells seems to be localized on one side of the membrane (443). It is therefore possible that inhibitors have to achieve an effective inhibitory concentration within the bile canaliculi if the Na^+-K^+-ATPase is localized on the luminal side. It will be quite difficult to prove this localization. The studies of Wachstein and Meisel (501) and Essner *et al.* (141) suggest that the ATPase measured histochemically might be on the luminal side of the membrane. However, the techniques are technically difficult and subject to diffusion artifacts, and the Wachstein-Meisel stain does not specifically measure Na^+-K^+-ATPase (419). Furthermore, more than one type of ATPase seems to exist in the canalicular membrane. Emmelot and Bos (133) have demonstrated a marked variability in the relative activity of Mg^{++} ATPase (EC 3.6.1.4) and Na^+-K^+-ATPase (EC 3.1.3.5) in plasma membranes isolated from rat hepatocytes.

Graf *et al.* (178) have suggested that the Na^+-K^+-ATPase-dependent mechanism regulating bile flow might be located on the sinusoidal side of the liver cell. Bile flow would then be regulated by the effects of this pump on intracellular Na^+. Evidence which supports this is the finding that uptake of taurocholate into isolated rat hepatocytes is Na^+-dependent (446).

Another mechanism of regulating BSIF which requires further study is the relationship between BSIF and hepatic blood flow. Portacaval shunt in the rat reduces both bile flow and liver mass by a roughly equal amount (196, 379). This was interpreted as a reduction in BSIF since bile salt output was maintained at normal levels (bile salt concentration was increased), and there was no evidence to suggest that the osmotic effects of the bile salts were altered. Electrical stimulation of the posterior hypothalamus increases bile flow in cats (36); an indirect effect due to an increase in hepatic blood flow could not be ruled out.

It is clear that we need more insight into the nature of BSIF, and the factors which regulate it. Reduced BSIF may or may not be an important mechanism for initiating intrahepatic cholestasis, but it is perhaps significant that most, if not all, agents which are able to reduce canalicular bile flow other than by producing hepatocellular necrosis, seem to act specifically on the BSIF. Lindblad *et al.* (291) made an interesting observation in patients who had surgical relief of extrahepatic cholestasis. They found that the BSIF was quite variable in this group, but did not seem to be significantly different from a group of noncholestatic cholecystectomized patients. We will need to find a more acceptable method of measuring, or defining BSIF. The present method, involving extrapolation of bile salt output regression lines, assumes a far greater knowledge of the complex relationship between micellar bile salt, lipid, and water excretion than we have at present.

It is noted that most of these studies concern inhibition of BSIF as a possible mechanism of cholestasis. However, inhibition of bile salt-dependent bile flow has received relatively little attention as an alternate mechanism. This component may be involved in monohydroxy bile salt-induced cholestasis (337, 338) (see section V F). Ros *et al.* (414) have suggested that inhibition of bile lipid excretion might be a component of the cholestatic reaction induced in rhesus monkeys infused with chlorpromazine. Despopoulos has proposed (98) that steroid-induced cholestasis may involve impairment of bile salt secretion, resulting from an altered conversion of cholate to taurocholate. He believes that both passive and active secretion of bile salts occurs in the kidney and the liver, but that only conjugated bile salts may be actively secreted. The amount of supporting evidence for this novel hypothesis is limited, but it warrants further investigation.

C. Canalicular Membrane Function

Although most studies dealing with the

mechanisms of canalicular bile formation have concentrated on determining the relationships between bile salt, electrolyte, and water flux, until recently little attention has been given to the role played by protein and lipid elements within the canalcular membrane. The canalicular membrane is essentially a specialized portion of the membrane which surrounds the entire hepatocyte. This membrane, which is often called the liver plasma membrane (LPM), seems to have a functional polarity, in that histochemical staining has revealed the different localization of phosphatase enzymes on the sinusoidal, canalicular, and lateral regions (133, 419, 463, 464, 501). Alkaline phosphatase (EC 3.1.3.1) and Mg^{++}-activated ATPase (EC 3.6.3.5) predominate in the canalicular region; Co^{++}-activated cytidine monophosphatase (EC 3.1.3.1) in the sinusoidal region; and 5′-nucleotidase (EC 3.1.3.5) is prominent at both sites.

Studies on the composition and function of the LPM have been hampered by the difficulty of preparing membrane preparations of sufficiently high purity. The methods were pioneered by Neville (335) and later, by Emmelot and coworkers (133, 134). Modifications of the technique have aimed toward enrichment of the bile canalicular fragments in the LPM preparations (472), and Fisher *et al.* (148), in 1975, reported the preparation of canalicular and noncanalicular membrane fragments of high purity. Another modification of the technique enables the isolation of a bile ductular cell fraction from liver homogenates (341).

The protein, lipid, and carbohydrate composition of the LPM is quite heterogeneous, and the problem of assessing turnover and functional significance of the individual components may well be insoluble. Nevertheless, Fisher *et al.* (148) have shown canalicular membrane fragments are comparatively rich in phospholipid and cholesterol, although their ratio is similar to that in the LPM as a whole. Furthermore, polyacrylamide gel electrophoretic separation of the proteins reveal marked differences between the two fractions as well.

Simon and Arias (463, 464) have studied the turnover of proteins with pulse-labeling techniques in LPM fragments enriched with bile canaliculi, as well as the factors influencing the activities of 5′-nucleotidase, Mg^{++}-ATPase, and Co^{++}-cytosine monophosphatase (CMPase). Furthermore, they compared control preparations with those from rats in which severe (BDL) or mild (ethinylestradiol) cholestasis had been established. Both forms of cholestasis resulted in increased alkaline phosphatase activity, and reduced activities of 5′-nucleotidase and Mg^{++}-ATPase; Co^{++}-CMPase was not affected. The reduced 5′-nucleotidase and Mg^{++}-ATPase activities were due to a decrease in the V_{max} but the K_m was unchanged, except for a reduced K_m for Mg^{++}-ATPase in the BDL model. Since neither taurocholate, taurochenodeoxycholate nor ethinylestradiol altered the activities of these enzymes in a pattern similar to that induced by cholestasis, it was concluded that none of these steroids were involved in regulating the activities of these enzymes during cholestasis. Neither form of cholestasis altered the turnover or relative composition of the major protein bands separated by SDS-polyacrylamide gel electrophoresis, although some qualitative differences were noted with some of the minor bands. It was not possible to determine whether these minor band changes were related to the enzyme changes. Similar studies of LPM enzyme changes during ANIT-induced cholestasis have been discussed in section IV D.

The importance of these studies is that they show that changes which occur in canalicular membrane composition during cholestasis may well be quite subtle, and involve quite specific proteins. The analytical problems which will be encountered in trying to unravel this complex problem will be formidable.

Evans *et al.* (143) compared the protein and lipid components of a canalicular-rich LPM preparation with the proteins and

lipids of normal rat bile. They found that neither protein nor lipid components were comparable, although they did find in bile a small amount of glycoprotein which could have originated from the outer face of the canalicular membrane as the result of the action of bile salts secreted into the bile. They concluded that in normal animals, the canalicular membrane is refractory to the solubilizing effects of bile salts.

These assumptions may not hold true for at least one form of cholestasis. Miyai *et al.* (323) concluded that the ultrastructural changes seen in the canaliculi and associated structures in lithocholate-induced cholestasis were probably due to the accumulation of high concentrations of lithocholate in the immediate vicinity. Layden *et al.* (279) reached a similar conclusion in the case of taurolithocholate-induced cholestasis, but showed that canalicular changes did not occur in ethinylestradiol-induced cholestasis. It has been suggested that canalicular membrane fragments are major constituents of the lamellar profiles commonly seen in bile plugs associated with intrahepatic cholestasis (35).

De Broe *et al.* (91) have obtained evidence which supports the idea that the LPM becomes fragmented during cholestasis. They isolated vesicles from the serum of patients with cholestasis, and these vesicles were rich in alkaline phosphatase, of a type found associated with the liver, as well as 5′-nucleotidase, γ-glutamyltranspeptidase, and leucine aminopeptidase. Perhaps, the most significant observation was that these membrane fragments could be detected in the early stages of cholestasis.

The studies discussed so far have been useful in that they have shown what changes occur during cholestasis, but they have not indicated which if any of the changes might be involved as an initiating step. However, some recent studies on the structure and function of microfilaments have brought to light a new, and intriguing hypothesis for a possible initiating factor in cholestasis.

In the last several years, there has been a growing amount of interest in microfilaments. These structures have been found by electron microscopy to have a diameter which ranges from about 50 to 100 Å. Microfilaments have been described in a wide variety of cell types and suggestions have been made that these filaments represent a contractile element for cells and may be involved in cell secretory processes (9, 20, 142, 146, 343, 505). Oda *et al.* (340) demonstrated that a rich microfilament network is present around the bile canaliculi. This network corresponds to the ectoplasmic zone observed in conventional electron micrographs of normal liver. The thin microfilaments have the ultrastructural characteristics of actin-containing microfilaments. These authors suggest that these microfilaments may have a contractile function and that their location and disposition are such that they may provide a system for the contraction and dilatation of bile canaliculi. The suggestion is that microfilaments maintain the tone of the canalicular system by alteration of their contractile state. These microfilaments have been demonstrated in isolated bile canaliculi, and in isolated hepatocytes.

Cytochalasin B, a fungal alkaloid, disrupts microfilaments and inhibits their contractile function. This has been demonstrated in a number of different cell types and this alkaloid has been shown to inhibit diverse cellular contractile movements (9, 142, 307, 343, 461, 505).

Phillips and coworkers (353, 354) have performed an interesting series of studies with cytochalasin B aimed at determining the role that impaired microfilament function might play in intrahepatic cholestasis. They have infused cytochalasin B into rat liver, perfused both *in situ* and in the isolated perfused liver preparation. The morphological changes induced by cytochalasin B were similar in both preparations. Bile canalicular dilatation and fine vacuolation of the hepatocytes were seen; however, bile ducts and ductules were normal. By electron microscopy, 30 min after the addition of cytochalasin B, dilated bile canaliculi were recognized especially in

periportal and midzonal regions. The dilated canaliculi showed loss of microvilli. After 2 to 3 hr the changes were more evident. While the Golgi complex was prominent, the cytoplasmic organelles all appeared normal. Electron-dense amorphous and fibrillar substances were evident in some of the dilated bile canaliculi.

Dose-related inhibition of bile flow was noted in both preparations, but was more marked in the liver perfused *in situ*. One hour after the addition of cytochalasin B, bile flow was reduced more than 50% and was completely arrested after 2 hr.

When isolated hepatocytes were incubated in the presence of cytochalasin B, the microfilaments lost their filamentous structure and became granular and amorphous. These data suggest that in hepatocytes, cytochalasin B altered structural conformation of the pericanalicular microfilaments which could then result in dilatation of the bile canaliculi, and loss of their microvilli. Since it is known that microfilaments extend into the canalicular microvilli (340), Phillips *et al.* (353, 354) speculated that normal pericanalicular microfilament function would tend to reduce stagnation of bile and facilitate the flow of bile from the canaliculi to the ducts. On the basis of their data, they state that a relationship exists between cytochalasin B-induced cholestasis and microfilament dysfunction. Cytochalasin B has also been shown to inhibit Mg^{++}-ATPase of reconstituted actomyosin and this suggests an effect on the interaction between actin and myosin (336). Another possibility is that this compound may alter the properties of plasma membranes by reacting with a membrane component (142). A third possibility is that cytochalasin B might release microfilaments from their attachment to the membranes (473). While Phillips *et al.* (353, 354) recognized that it would be premature to suggest that microfilament dysfunction underlies a wide variety of cholestatic disorders, they feel that in the cytochalasin B model it is quite likely that microfilament dysfunction is the primary mechanism of cholestasis. In 1976, de Vos *et al.* (101) reported a case of progressive intrahepatic cholestasis (Byler's disease) in which the microfilament structures of the pericanalicular ectoplasm were markedly hypertrophied. They were also cautious about ascribing functional significance to this striking observation. As pointed out by French (156a) the cAMP system seems to be involved in bile flow, and can be influenced by hormones. Theoretically cholestasis could result by interference with the hormone receptor-stimulated contraction of the pericanalicular microfilaments. Direct evidence is needed which links hormonal receptor-mediated contraction of microfilaments to bile flow.

D. Altered Ductular Cell Permeability

Transport of water and solutes across the ductular cell epithelium plays some part in regulating bile flow, but there is marked species variation in the extent of ductular reabsorption and the ductular secretory stimulation by secretin (138). Enhanced ductular reabsorption or biliary regurgitation, which is thought to occur during cholestasis, undoubtedly contributes to formation of a hepatocellular-ductular cell short circuit, which exacerbates the hepatocellular damage and leads to periductular inflammation and fibrosis (96, 425). In common with difficulties encountered when trying to interpret morphological alterations in cholestasis, is the difficulty of assessing whether enhanced ductular reabsorption of bile is the result of cholestasis, or whether it might be an initiating event. The bulk of the evidence favors the former interpretation, although there remains the possibility that it may be an initiating mechanism when the hepatic lesion takes the form of primary biliary cirrhosis leading subsequently to cholestasis (425). Enhanced ductular reabsorption has been proposed to account for ANIT-induced cholestasis, but it has been largely discounted in favor of a hepatocytic site of attack (see section IV D).

E. Impaired Mitochondrial Function

Curling of mitochondrial cristae is one of the morphological features commonly

seen in the cholestatic syndrome (372, 425). There has been some speculation that the impairment of oxidative phosphorylation which should accompany these mitochondrial abnormalities might deprive the cell of energy needed for the secretion of bile. It is likely that these mitochondrial changes may be due in part to the retention of bile. Both bilirubin and bile salts are uncouplers of oxidative phosphorylation and seem to be capable of altering the morphology and oxidative function of mitochondria (281, 435, 512). Therefore, the more likely explanation is that the mitochondrial changes are the result of, rather than the cause of the cholestasis.

There are many agents which have been shown to modify oxidative phosphorylation *in vitro*, but not all of them exert clearcut effects on bile flow. There is a particularly interesting correlation in the case of BSP. Intravenous administration of BSP, or the preformed conjugate BSP-glutathione (GSH), which is the major biliary metabolite of BSP, usually results in a slight choleresis. However, under conditions where the conjugation of BSP is impaired, the excretion of larger amounts of unconjugated BSP is accompanied by a fall in bile flow (386, 387, 441, 442). BSP also depresses bile flow in the isolated perfused rat liver (183). Killenberg and Hoppel (253) have shown that BSP is an inhibitor of oxidative phosphorylation in isolated rat liver mitochondria, but that BSP-GSH does not inhibit, nor can it reverse the inhibitory effects of BSP.

It would be unwise to generalize on the importance of these findings. Salicylate and 2, 4-dinitrophenol are potent uncouplers of oxidative phosphorylation, yet their effect on bile flow is either to produce a choleresis in the dog (389, 417) or little or no effect in the rat (466). When combined these two agents decrease bile flow in the rat and this is associated with a decrease in hepatic ATP concentration (124). 2, 4-Dinitrophenol produces a decrease in bile flow in the isolated perfused rat liver (466). Bilirubin, which is also an uncoupler, added in amounts sufficient to saturate the hepatic uptake system, failed to produce any effects on bile flow in the isolated rat liver (38).

Therefore, there seems to be insufficient evidence at this time to implicate disordered mitochondrial function as an initiating step in cholestasis.

F. Smooth Endoplasmic Reticulum (SER) Function and Monohydroxy Bile Salts

Impaired hydroxylation of bile salts in the hypertrophic hypoactive smooth endoplasmic reticulum (HHSER) of the hepatocyte was first postulated to be a prime factor in the etiology of cholestasis by Schaffner and Popper in 1969 (424). Their hypothesis has been elaborated in some of their subsequent review articles (214, 373, 425) and a series of papers with experimental evidence on the mechanism and significance of HHSER have been published (85, 86, 94, 180, 181, 211–213, 421, 430). The reader is referred to these articles for a more expansive review of the hypothesis. A somewhat similar hypothesis, in which an unsaturated monohydroxy bile salt (3β-hydroxycholenoate) has been implicated as a possible causative factor in neonatal cholestasis and biliary atresia, has been described by Javitt (229) and Jenner and Howard (234).

The basic premise of the Schaffner-Popper hypothesis is that impaired hydroxylation of the steroid nucleus occurs during biosynthesis of the primary bile salts cholate and chenodeoxycholate from cholesterol. This shift toward less hydroxylated derivatives would result in a reduction of the osmotic drive provided by the secretion of bile salts across the canalicular membrane. The significance of bile salt secretion as a factor in regulating bile flow may be seen in the reviews by Wheeler (506) or Javitt (226), and articles by Klaassen (257, 259). The hydroxylation of the steroid nucleus during biosynthesis of the bile salts is catalyzed by enzymes of the endoplasmic reticulum (46, 132). The involvement of cytochrome P-450 in the initial, and appar-

ently rate-limiting step (7-α-hydroxylation of cholesterol), and in other hydroxylation reactions involving the primary and secondary bile salts, is important since much of the experimental evidence shows that, during cholestasis, the binding capacity and catalytic activity of cytochrome P-450 is impaired.

The biosynthetic pathway of the bile salts is rather complicated and a simplified sequence is shown in Figure 1 (see 46, 132, 438 for more detail). It had been assumed that oxidation of the side chain precluded further hydroxylation of the steroid ring (132). Therefore, it was implicit in the Schaffner-Popper hypothesis that hypoactivity of microsomal hydroxylases could result in premature side chain oxidation and a shift in bile salt synthesis in favor of less-hydroxylated derivatives. Recently, a biosynthetic pathway which bypasses the initial 7-α-hydroxylation of cholesterol and substitutes 26-hydroxylation on the side chain as an initial step has been shown in the rat (232, 322), and also in man (11). Two monohydroxy bile salts, 3-β-hydroxy-5-cholanoate and 3-α-hydroxy-5β-cholanoate (lithocholate) have been identified as intermediary metabolites derived from 26-hydroxycholesterol in the rat. These metabolites possibly occur in man as well, but the evidence is not conclusive. The significance of this secondary pathway is that it provides for the overproduction of lithocholic acid by a primary biosynthetic pathway, rather than by the secondary metabolic effects of the bacterial microflora. Studies published in 1976 (460) show that some of the intermediary reactions which occur during side chain oxidation, are catalyzed by microsomal rather than mitochondrial enzymes, but it is not known how these reactions are affected during cholestasis.

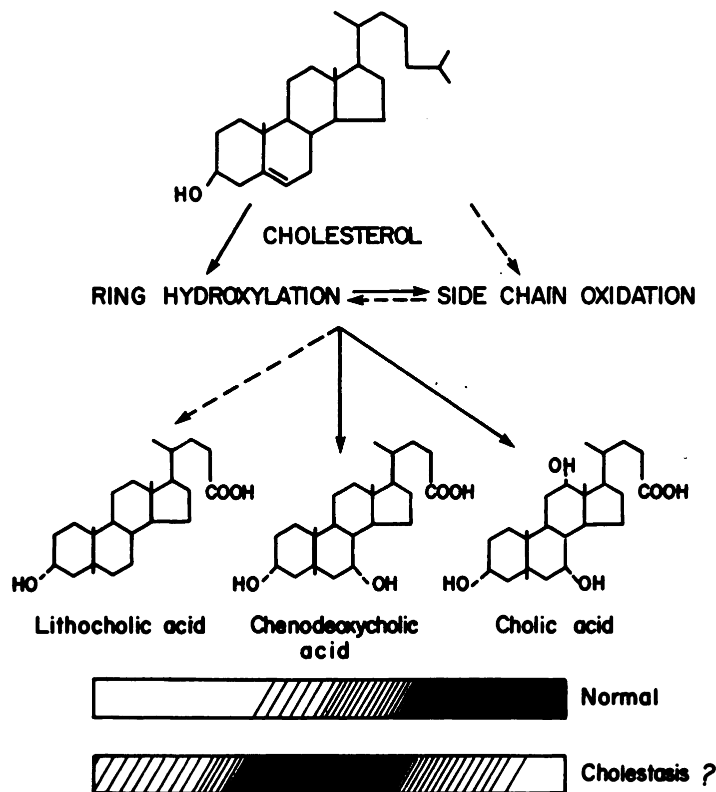


FIG. 1. Simplified pathways of bile salt biosynthesis from cholesterol. Dashed arrows represent minor pathways which may take on greater prominence in cholestasis, leading to a shift toward less-hydroxylated bile salt production.

Schaffner and Popper speculated in their original hypothesis that the overproduction of lithocholate could also be due to a shift from cholate to chenodeoxycholate as the major primary bile salt. Lithocholate is the secondary bile salt derived by bacterial 7-α-dehydroxylation of chenodeoxycholate, but it normally makes only a very small contribution to the circulating bile acid pool due to subsequent sulfation and excretion (83).

Two possible mechanisms by which excess monohydroxy bile salt secretion could cause cholestasis have been proposed. Schaffner and Popper suggested (424) that the physicochemical properties of monohydroxy bile salts would result in their forming mixed micelles with cholesterol, phospholipids, and other bile salts which would have the character of liquid crystals. These abnormal micelles would result in either reduced bile salt-dependent flow of water, or the formation of bile plugs. Studies published in 1975 (279) have suggested, as an alternative, that monohydroxy bile salts may be cholestatic by virtue of a direct effect on the canalicular membrane (see sections IV C; V C).

An important aspect of the Schaffner-Popper hypothesis is that it implies that the retention of bile constituents, notably the more surfactant di- and trihydroxy bile salts, would exacerbate the cholestasis by further impairing the activity of the microsomal cytochrome P-450-dependent oxidases. It was suggested too, that a periductular inflammatory response to monohydroxy bile salts might also exacerbate the cholestatic reaction, by constricting the biliary tract or allowing excessive ductular reabsorption of bile.

In the evaluation of the monohydroxy bile salt/HHSER hypothesis we concentrated on two questions: 1) Does excess monohydroxy bile salt synthesis occur, and if so, does this cause the cholestasis? 2) Does a HHSER occur in cholestasis, and if so, does it initiate cholestasis, or is it merely a consequence of the cholestatic syndrome?

In answer to the first question, it seems that there is no direct evidence to show that monohydroxy bile salts are increased during cholestasis. Total serum bile salts rise in most types of liver disease, particularly obstructive disease (158, 305, 346). In fact it has been suggested that elevation of serum bile salts may occur prior to, or in the absence of, hyperbilirubinemia in neonatal cholestasis and may therefore provide a more sensitive indicator of the onset of cholestasis (228, 342). Lithocholic acid has been difficult to quantify because of analytical problems. However, advances in the specificity and sensitivity of gas liquid chromatography (GLC) techniques of bile salt analysis (58) have allowed the quantification of the very low lithocholate levels found in both normal and pathological states. There is no clear indication that unconjugated lithocholate concentrations are elevated in liver disease.

This is partly because lithocholate is extensively metabolized by conjugation with sulphate and subsequently excreted in the urine and bile (82, 304, 348, 481, 482). As well as limiting the enterohepatic recirculation (83, 295), this conjugation reaction is potentially of great importance in limiting the toxicity of lithocholate. Animal studies have clearly shown that sulfated derivatives of lithocholate have little or no cholestatic potency (see section IV C). Furthermore, sulfate conjugating activity seems to be enhanced during extrahepatic cholestasis (304) and during intrahepatic cholestasis of pregnancy (169, 490). Chenodeoxycholate treatment for the dissolution of gallstones would be expected to result in excessive lithocholate *via* secondary metabolism by the gut microflora, but there have been conflicting reports as to whether lithocholate sulfates are to be found in increased amounts (51, 89, 483). It is less clear whether sulfation is enhanced in other types of hepatic disease or whether this detoxification reaction can occur in all species. Studies in primates treated chronically with chenodeoxycholate revealed hepatic lesions (123) and the evidence sug-

gests that these lesions might be caused by lithocholate for which the rhesus monkey lacks an adequate sulfating capacity (8).

Although sulfation is probably the more important detoxifying mechanism, there is also some evidence that lithocholate may be detoxified by further hydroxylation. The ability of human liver microsomes to 6α-hydroxylate lithocholate *in vitro* has been reported (86). In the rat, 6β- and 7α-hydroxylation reactions are quite active (487). Glucuronidation is another metabolic pathway commonly utilized by steroids to promote excretion, although bile salts do not normally form glucuronides *in vivo*. The identification of bile salt glucuronides in the plasma and urine of human beings with intrahepatic cholestasis, reported in 1976 (16, 17, 157), suggests that yet another metabolic detoxification pathway may be available for toxic bile salts.

It is clear then that the overproduction of monohydroxy bile salts by a hypoactive microsomal enzyme system is still at best speculative. However, a real possibility of monohydroxy bile salt toxicity exists if the absorption from the gut is enhanced and detoxifying conjugation and hydroxylation reactions are impaired. The estimated lithocholate synthesis rate in man (3–4 mg/kg/day) exceeds the dietary lithocholate intake (0.7 mg/kg/day) required to produce cirrhosis in a susceptible species, the rabbit (64). The amount of monohydroxy bile salt needed to precipitate a toxic reaction in man is not known, but animal studies with lithocholate and taurolithocholate show that the cholestatic effects are dose-related (see section IV C). It is reasonable to assume that the endogenous rate of lithocholate production by the bacterial microflora could constitute a significant hazard if all of the lithocholate were to be absorbed and reach the liver in an unchanged form.

The evidence for the involvement of a monohydroxy bile salt in neonatal cholestasis and biliary atresia is stronger. Both 3-β-monohydroxycholenoate and its probable precursor, 26-hydroxycholesterol have been found in human meconium (277), and substantial amounts of 3-β-monohydroxycholenoate sulfate have been found in the urine and serum of infants which manifest these syndromes (233, 276, 306). This abnormal bile salt has not been found in normal neonates.

In answer to the second question, it is necessary to assess both the extent of SER proliferation, and its specific activity in order to determine whether during cholestasis it corresponds with the HHSER defined by Popper and Schaffner. Proliferation of the SER is a common adaptive change seen after exposure to many lipophilic chemicals (215, 216), and it is also seen commonly as a morphological feature of cholestasis in people and experimental animals (96, 373). On the other hand, this is not invariably so, and in an abstract published in 1975, Capurso *et al.* (63) report a study of eight cholestatic patients in whom morphometric analysis of the SER showed no significant proliferation, although the changes in mitochondrial cristae and canalicular membrane proliferation characteristic of cholestasis were noted.

In order to demonstrate that the SER is hypoactive, one usually assesses the activity of the cytochrome P-450-dependent MMFO system. In animals, this may be achieved by determining *in vivo* the kinetics of plasma disappearance or pharmacological activity of substrates for the MMFO or by determining the rate of substrate oxidation *in vitro* by microsomal preparations. In man, the problem is somewhat more difficult, because methods used to assess MMFO activity are indirect, and furthermore subject to considerable variability due to the influence of environmental and genetic factors (499).

Hypoactivity of microsomal enzymes has been demonstrated in most types of liver disease (*e.g.*, cirrhosis, hepatitis) in man but in cholestatic liver disease, the evidence is far from conclusive (508). Two reports illustrate this point. Hepner *et al.*

(193) reported that in 16 out of 24 patients with cholestasis not caused by malignant disease, microsomal function measured by $^{14}CO_2$ production from ^{14}C-aminopyrine, was normal. Carulli *et al.* (70) reported that the plasma half-lives of drugs metabolized by the hepatic SER were moderately elevated in patients with cholestatic hepatitis and extrahepatic biliary obstruction, but not in three patients with intrahepatic recurrent cholestasis. Furthermore, the half-life of some of the drugs was normal in some cholestatic patients who demonstrated prolonged half-life to one or more of the other drugs. One interesting point was that the half-life of tolbutamide was decreased in the most severe cases suggesting that metabolism might be enhanced. However, the shortened half-life could be reversed by lowering the bile salt concentration (*e.g.*, with cholestyramine). The authors speculated that these changes may have been related to interaction with plasma protein binding rather than hepatic metabolism.

Studies with animal models of cholestasis have also failed to establish that HHSER is a critical feature in the etiology of cholestasis. The *in vitro* activity of the MMFO has been studied in both chemically induced cholestasis, or in BDL-induced cholestasis. The latter technique has been particularly useful, since it has permitted an analysis of the changes in the MMFO which are the result of cholestasis as opposed to those changes which might cause it.

Cholestasis induced by BDL has been shown to result in a decrease in the activity of aminopyrine demethylase, while the content of cytochrome P-450, and the activities of aniline hydroxylase, NADPH-cytochrome *c* reductase, and cytochrome P-450 reductase underwent more moderate decreases (110, 212, 421). The binding of type I substrates to cytochrome P-450 was markedly decreased, the apparent spectral dissociation constant for hexobarbital being increased by more than two orders of magnitude, and the positive modifier effect of type I substrates on cytochrome P-450 reductase was abolished. BDL did not seem to alter the binding of type II substrates, nor was the negative modifier effect of type II substrates on cytochrome P-450 reductase affected. These data suggested that the effect of cholestasis on the hepatocellular smooth endoplasmic reticulum was specifically an alteration of the type I binding site.

The addition of whole bile, bile salts, or detergents to microsomes *in vitro* produced changes in cytochrome P-450 function similar to those seen in BDL rats (94, 211, 213). At low concentrations, taurochenodeoxycholate, which is itself a type I substrate, produced competitive inhibition of type I, but not type II metabolism. Modification of the type I binding site occurred at concentrations in the range 0.6 to 1 mM and at concentrations in excess of 1 mM, taurochenodeoxycholate destroyed the type I binding site, converting cytochrome P-450 to cytochrome P-420.

The major difficulty in reconciling the *in vitro* effects of bile salts with *in vivo* effects is that the dihydroxy bile salts and their conjugates are much more potent than the trihydroxy bile salts in their detergent and inhibitory effects on cytochrome P-450. In the rat, hydroxylation of retained bile salts continues during BDL-induced cholestasis, and the concentration of dihydroxy bile salts increases only slightly in comparison to the marked increase in trihydroxy metabolites (180). In man, these mechanisms are absent, or less efficient, and concentrations of dihydroxy bile salts similar to those producing destruction of P-450 *in vitro* have been found in cholestatic human livers (181).

Cholestasis has also been shown to alter the turnover of cytochrome P-450. With SDS-polyacrylamide gel electrophoresis, MacKinnon and Simon (301) showed that the half-life for degradation of the P-450 apoprotein was similar to that previously obtained for the P-450 prosthetic group, indicating that the heme and protein moieties are degraded as a single unit.

Cholestasis produced by 3 days BDL increased the half-life for degradation of the cytochrome P-450 apoprotein from 24 to 50 hr. They concluded that the reduction in cytochrome P-450 content may therefore be due to reduced synthesis, and data obtained from double-isotope pulse labeling studies tended to confirm this. They also showed that ethinylestradiol-induced cholestasis produced essentially the same effects on cytochrome P-450 turnover (303) and that enzyme inducers such as phenobarbital and 3-methylcholanthrene could partially reverse the changes in microsomal cytochrome content induced by both forms of cholestasis (302). PCN seems to be even more potent in reversing BDL-induced changes in cytochrome P-450 function (470).

These studies suggest that pharmacological reversal of changes in microsomal enzyme activity after cholestasis can occur, but that the effects on the turnover of various microsomal proteins are relatively specific. This point is emphasized in the studies by Solymoss and Zsigmond (470) who showed that prolonged BDL (5 weeks) produced HHSER, but that treatment with PCN during this period reversed the decrease in microsomal protein and cytochrome P-450 content, and the activities of NADPH-cytochrome *c* reductase, bilirubin uridine diphosphate (UDP) glucuronyl transferase, and ethylmorphine demethylase. However, the related catatoxic steroid, spironolactone, while reversing the effects of BDL on enzyme activity, failed to increase the cytochrome P-450 content. None of the inducers significantly reduced the hyperbilirubinemia which accompanied the cholestasis.

While it is clear that HHSER can develop during experimental cholestasis, the time course of this development is critical in relationship to the Schaffner-Popper hypothesis. Their own studies with BDL- and ANIT-induced cholestasis (85, 421, 430) show that the impairment of the microsomal enzymes is progressive. Studies by Drew and Priestly (109, 110) show that with both BDL- and ANIT-induced cholestasis, there is a period of approximately 24 hr after cholestasis has been established when washed microsomes, but not 10,000 × *g* supernatants, derived from SER are normoactive. Therefore, the bulk of the evidence supports the contention that when HHSER occurs during cholestasis, it is a consequence of the hepatic dysfunction, rather than a causative factor.

One other aspect which should be mentioned here is the interaction of drugs with the MMFO during cholestasis. There is no evidence to suggest that drug-induced intrahepatic cholestasis could be caused by interactions with the MMFO leading to production of HHSER. Attempts to demonstrate potentiation of the cholestatic effects of lithocholate in animals by using drugs with clinically demonstrable cholestatic potential either have been unsuccessful, as in the case of chlorpromazine (111) or have caused nonspecific potentiating effects, as in the case of erythromycin estolate (108, 384).

Enzyme inducers have been shown to reverse some of the SER changes caused by extrahepatic cholestasis in rats, but they do not seem to be able to reduce the amount of jaundice. On the other hand, there are indications that phenobarbital therapy may be of some value in reducing the hyperbilirubinemia and bile salt retention and improving liver function in some forms of intrahepatic cholestasis in man (37, 484–486).

G. Intracanalicular Precipitation

Biliary concrements (also called biliary thrombi, or bile plugs) are a common morphological feature observed in cholestasis irrespective of whether the cholestasis is intrahepatic or extrahepatic in origin (96). According to Biava (35), these bile plugs consist mainly of fibrillar material derived from pericanalicular ectoplasm and fragmented membranes. In studies by Popper and Schaffner in 1970 (373) and Desmet in 1972 (96), it has been suggested that the lamellar components of bile plugs are

probably liquid crystals of bile salt-phospholipid-cholesterol mixtures.

Physiologists have long been interested in the physicochemical properties of bile salt mixed micelles (67, 68), but mainly from the point of view of the importance of these factors in the etiology of gallstone formation. With the possible exception of the monohydroxy bile salt hypothesis (see section V F) it had not been suggested that bile plug formation is an initiative step in intrahepatic cholestasis. Rather it had been assumed that thrombus formation, seen initially in the centrolobular region, was a sequel to cholestasis, resulting from either the reduced flow of water (425) or fluid reabsorption associated with altered permeability or fragmentation of pericanalicular membranes (96).

It is of interest to note that in the rat, the species used most widely in experimental studies of cholestasis, canalicular bile plug formation can rarely be demonstrated (96, 425). The exceptions seem to be in taurolithocholate-induced cholestasis (see section V C) and possibly in manganese-bilirubin-induced cholestasis (see section IV H), although plugs were seen in the ductules rather than in the canaliculi.

Clarke and Denborough in 1971 (73) put forward the hypothesis that cholestasis associated with the clinical use of chlorpromazine, other phenothiazines and tricyclic antidepressants (74), and erythromycin (75) might be due to intracanalicular precipitation due to the formation of insoluble complexes of the drugs with normal bile components. These authors demonstrated that phenothiazines formed precipitates when mixed *in vitro* with diluted human gallbladder bile, irrespective of the pathological status of the gallbladder of the donor. They further demonstrated that the primary interaction was between positively charged drug molecules and negatively charged protein carboxyl groups, although the extent of nonionic binding was also a factor. In the case of glycoprotein fractions, isolated by density-gradient centrifugation, the interaction was shown to be specific for sialic acid residues on the protein since precipitation was abolished by removal of the sialic acid residues with neuraminidase. However, the interaction was not specific to bile, since sialic acid-glycoprotein rich fractions from other fluids (saliva, gastric juice, ovarian cyst fluid) also formed precipitates with chlorpromazine (74). The chlorpromazine concentration range required to produce a precipitate was 1 to 10 mM for low density protein fractions, but > 25 mM for glycoproteins.

A point of criticism is that the data imply that thrombus formation and therefore the incidence of cholestasis, should be dose-related. While the authors acknowledge that this is not the case in clinical experience with phenothiazine-induced cholestasis, their explanation (74) of the anomaly is based on the fact that precipitation with low-density proteins was reversible with higher drug concentration. Unfortunately, the insolubility of chlorpromazine/glycoprotein complexes was shown to be irreversible, and the same explanation does not apply.

The authors did comment on the fact that the glycoprotein in bile contained a large amount of "blood-group material," which they failed to define. Since almost all of their studies were done with bile from group B donors, one cannot exclude the possibility that the interaction might be specific for blood group B patients. While this might be a factor worthy of further investigation, there are no data in the clinical literature to suggest that sensitivity to drug-induced cholestasis is related to blood group.

Carey *et al.* (66) also discussed the possibility that chlorpromazine-induced cholestasis might result from an intracanalicular precipitation mechanism. *In vitro* they showed that electrostatic interaction of chlorpromazine with bile salts produced insoluble 1:1 complexes. The precipitation of these complexes was markedly influenced by the stoichiometry of lipid-bile salt mixed micelles, and relatively small

changes in the ratio of chlorpromazine/bile salt/phospholipid resulted in solubilization of the complexes.

The complexity of this interaction, and the requirement of a critical concentration ratio make it easier to explain why a dose-response relationship for chlorpromazine-induced cholestasis would be difficult to establish. However, further experiments carried out by this group cast doubt on the significance of intracanalicular precipitation as a mechanism for chlorpromazine. They reported (414) that a reversible cholestasis could be induced in the rhesus monkey by intravenous infusion of chlorpromazine. Extensive biliary excretion of chlorpromazine occurred, but at no time during the experiments was there any evidence seen of insoluble bile salt/chlorpromazine complex formation in the canaliculi (see section IV H).

The interaction between erythromycin lactobionate and bile also seems to involve complex formation with the bile salt components of bile rather than interactions with proteins and glycoproteins (75). Turbidity was maximal when erythromycin lactobionate (25 mM) was added to a 1/100 dilution of whole bile, but was negligible when more diluted or more concentrated bile samples, or bile fractionated on a cesium chloride gradient were used. The turbidity was probably due to a specific interaction between erythromycin lactobionate and trihydroxy bile salts resulting in the formation of stable emulsion.

The significance of this mechanism in erythromycin-induced cholestasis remains highly speculative. On the one hand, the stoichiometry of the complex formation is quite specific, requiring a 2:1 erythromycin:bile salt molar ratio. Solubilization of the emulsion occurred with relatively small changes in this ratio and could be accomplished by exposure to a glass surface, which was presumed to absorb the positively charged drug. Therefore, precipitation *in vivo* would occur only when the critical concentrations coincided. On the other hand, the interaction has only been demonstrated with erythromycin lactobionate. The only other erythromycin derivative tested was a new synthetic derivative, erythromycylamine, which did not interact. There is no evidence that erythromycin lactobionate causes cholestasis with clinical use.

These studies have been criticized (300) on the basis that extrapolation from *in vitro* studies which used drug and bile component concentrations in the nonphysiological range to speculate on mechanisms of jaundice seen with clinical use of these drugs is unwarranted. Another point of criticism which we could make is that these *in vitro* studies have considered only the interaction between bile components and the parent compounds. Since most compounds appear in bile as metabolites, it would be reasonable to expect that these would have different binding characteristics than the parent compound.

One potential aspect of bile secretion, which may be associated with intracanalicular precipitation, and which has received little attention as a potential cholestatic mechanism is altered bile viscosity. The viscosity of bile is largely determined by its content of mucous substances (44) (probably hexosamines in the main) and variation in mucin content is thought to play an important role in the formation of gallstones. Increased bile viscosity could be expected to increase resistance to flow, but to our knowledge, no study has attempted to correlate cholestasis with changes in hexosamine content.

H. Reactive Metabolites

It has been well established that the formation of a reactive intermediary metabolite is a vital step in the hepatotoxicity caused by such diverse chemical agents as bromobenzene (239, 319, 395, 517), acetaminophen (90, 240, 241, 317, 318, 376, 377), carbon tetrachloride (391), furosemide (318a), and isoniazid (320, 321). A similar mechanism is thought to occur in chemically induced hepatic carcinogenesis (144). The extent of reactive metabolite forma-

tion, which may be very smallin relation to total metabolite formation, varies with the interaction of inducers and inhibitors of microsomal enzyme activity, as well as the activity of detoxification reactions, such as those involving glutathione conjugation. However, the correlation between the amount of irreversible protein binding, used as an index of reactive metabolite formation, and the extent of the cellular damage produced, has been quite good in most cases (167, 168, 316).

The mechanism by which reactive metabolites produce biochemical lesions which result in hepatotoxicity is unknown. Nor is it known why the manifestation of that toxicity should be cell necrosis on the one hand, or cellular proliferation on the other. It is reasonable that cholestasis might be an alternative manifestation of liver injury produced by the reactive metabolites of certain drugs. This general concept has been discussed by Zimmerman (524) and Remmer (397).

So far only a few drugs with suspected cholestatic potential have been shown to be capable of forming reactive metabolites which can bind irreversibly to proteins in *in vitro* experiments. These include imipramine (244), norethisterone (245), and ethinylestradiol (41, 42). The possibility that other drugs with similar cholestatic potential might form reactive metabolites is yet to be investigated.

It has been observed that cholestasis is primarily centrolobular in origin. An early explanation was based on the anatomical organization of the biliary system. It was suggested that bile secreted in the centrolobular region would need a greater energy expenditure to get it to the collecting system, but there would be a lower oxygen tension available and such cells would be more vulnerable to toxic drug actions (114). This concept, however, is not supported by the lobular distribution of histochemical and ultrastructural abnormalities in cholestasis of different etiology (425). An alternative explanation would be one based on the greater content of the MMFO in the centrolobular region (437) and a greater propensity for reactive metabolite formation. Such a hypothesis has been used to explain the centrolobular necrosis produced by a number of hepatotoxic agents for which there is good evidence indicating the necessity of an intermediary metabolite (361).

The concept that formation of a reactive metabolite may be a prerequisite in cholestasis as well as other forms of liver injury may be a useful one in explaining some of the unique features of drug-induced cholestasis in man. For example, the low incidence in man, and the inability to reproduce the lesion in laboratory animals may be due to host-idiosyncracy in metabolic activity. Both of these could be due to individual variation in not only the activity of the enzymes involved in the formation of the toxic metabolite, but also the activity of, and substrate availability for, subsequent detoxification pathways. The studies done on acetaminophen hepatotoxicity, and its relationship to glutathione availability (90, 241, 318, 377) suggest that a threshold dose is inherent for this mechanism of toxicity. The species variability to ANIT (see section IV D) and the strain differences in susceptibility to steroids (see section IV E) are compatible with such a concept.

Other aspects of the cholestatic syndrome may be explained on the basis of a toxic metabolite concept. Drug reactions which are classified as mixed hepatitic-cholestatic (352, 524) may in fact be manifestations of different types of toxic effects caused by the same toxic metabolite, or conversely, specific toxic syndromes resulting from different metabolites. Drug-induced cholestasis, described clinically as a hypersensitivity reaction and having the character of an immune response, may be due to antigenic material formed by irreversibly protein-bound metabolites (244).

One of the more attractive facets of this hypothesis is that it might be possible to study the factors influencing toxicity of cholestatic drugs with covalent binding of

metabolite(s) as an index of reactive metabolite formation and dynamics, in much the same way as has been done with acetaminophen. However, there are risks inherent in this approach. There is already some doubt that the relatively nonspecific binding measured by this approach is a valid measure of the specific metabolite-macromolecule interaction which might initiate the toxic reaction (167, 168). Preliminary studies (129, 362) also suggest that in the case of the experimental cholestatic agent ANIT, there is a poor correlation between irreversible microsomal protein binding and the cholestatic potency of ANIT and/or its metabolite(s) (see section IV D). The study of Bolt and Kappus (41) with ethinylestradiol illustrates another pitfall. They showed that different enzyme systems (rat microsomes or mushroom tyrosinase) caused the formation of different types of reactive metabolites with different covalent binding characteristics.

At the present time, there is little evidence on which to speculate the mechanisms by which reactive metabolites could initiate cholestasis. If we are permitted to be provocative, such a speculation could be made for chlorpromazine. Many possible mechanisms have been proposed for chlorpromazine-induced cholestasis (see section IV G, V G), including the possibility that it inhibits the bile salt-independent fraction of canalicular bile production (414). If inhibition of Na^+-K^+-ATPase is the fundamental factor leading to reduced BSIF (see section V B), then it is worth noting that, although chlorpromazine itself is a relatively weak inhibitor of Na^+-K^+-ATPase, chlorpromazine free radicals (possible reactive intermediary metabolites) are quite potent inhibitors of this enzyme (443). In any event, the concept of reactive metabolites is one which deserves more attention in chemically induced cholestasis.

VI. Concluding Remarks

In the last 25 years, our concepts regarding chemically induced intrahepatic cholestatis have evolved rather remarkably. What was once thought to be a form of liver injury relatively unique to man is now known to occur in animals, although the causative agents may not be the same in the different species. A lesion which was first described primarily in morphological terms is now being studied biochemically. Even at the morphological level we find that the rudimentary description of the presence of bile plugs in histological sections has now evolved to the point that serious consideration is being given to the ultrastructural alterations and the role of the microfilaments that surround the bile canaliculus.

Yet there are a number of very important unanswered questions. Is the cholestatic lesion induced by drugs in a very small percentage of the human population truly a reflection of an individualized allergic reaction, as most authors suggest; is it a resultant of a mixed event (*e.g.*, direct toxicity followed by an allergic phenomenon), or is it a direct toxicity which depends upon host (organ) susceptibility to attain the fully developed lesion? Why the apparent lack of a clear dose-dependent relationship even in man? Could it be that the susceptible subpopulation does exhibit a dose-dependent relationship but that we are unable to discern this relationship because the overall incidence is too small (the responders are diluted by the nonresponders)?

The fundamental problem is our lack of knowledge of how bile is formed. Neither the biochemical events which lead to the normal formation of bile, nor the morphological components of the secretory process involved in this key event are well enough understood. The biologist knows that in order to study a normal complex process he must be able to alter it in a controlled manner. In this sense it may be that studies performed with chemically induced intrahepatic cholestasis in animals can contribute to our overall understanding of the various events involved in the normal formation of bile. It would be desirable that the chemicals used in animals also be the

drugs that elicit the response in man, but this condition is not absolutely essential. Studies with controlled experimentally induced intrahepatic cholestasis in animals seem essential to a better understanding of bile formation and this lesion should be envisioned as an experimental tool for studying normal bile formation. Hopefully, the results of such studies will permit us to better understand drug-induced intrahepatic cholestasis in man and to develop better methods for the detection of this potential in new drugs.

REFERENCES

1. ABBRUZZESE, A. AND SWANSON, J.: Jaundice after therapy with chlordiazepoxide hydrochloride. New Engl. J. Med. 273: 321-322, 1965.
2. ABERNATHY, C. O., LUKACS, L. AND ZIMMERMAN, H. J.: Toxicity of tricyclic antidepressants to isolated rat hepatocytes. Biochem. Pharmacol. 24: 347-350, 1975.
3. ABERNATHY, C. O., SMITH, S. AND ZIMMERMAN, H. J.: The effect of chlordiazepoxide hydrochloride on the isolated perfused rat liver. Proc. Soc. Exp. Biol. Med. 149: 271-274, 1975.
4. ABERNATHY, C. O. AND ZIMMERMAN, H. J.: The toxicity of thioxanthene neuroleptics to isolated rat liver cells. Proc. Soc. Exp. Biol. Med. 150: 385-389, 1975.
5. ACCATINO, L. AND SIMON, F. R.: Identification and characterization of a bile acid receptor in isolated liver surface membranes. J. Clin. Invest. 57: 496-508, 1976.
6. ADLERCREUTZ, H. AND TENHUNEN, R.: Some aspects of the interaction between natural and synthetic female sex hormones and the liver. Amer. J. Med. 49: 630-648, 1970.
7. AJDUKIEWICZ, A. B., GRAINGER, J., SCHWER, P. J. AND SHERLOCK, S.: Jaundice due to iprindole. Gut 12: 705-708, 1971.
8. ALLAN, R. N., GADACZ, T. R., MACK, E. AND HOFMANN, A. F.: Impaired lithocholate sulfation in the rhesus monkey: A mechanism for chenodeoxycholate toxicity. Gastroenterology 69: 802, 1975.
9. ALLISON, A. C., DAVIES, P AND DEPETRIS, S.: Role of contractile microfilaments in macrophage movement and endocytosis. Nature (London) 232: 153-155, 1971.
10. ANDERSON, K. E. AND JAVITT, N. B.: Bile formation. *In* The Liver: Normal and Abnormal Functions, part A., ed. by F. F. Becker, pp. 371-400, Marcel Dekker, New York, 1974.
11. ANDERSON, K. E., KOK, E. AND JAVITT, N. B.: Bile acid synthesis in man: Metabolism of 7α-hydroxycholesterol-^{14}C and 26-hydroxycholesterol-^{3}H. J. Clin. Invest. 51: 112-117, 1972.
12. ANONYMOUS: Jaundice from iprindole (Prondol). Drug Ther. Bull. 9: 10-11, 1971.
13. ARIAS, I. M.: Effects of a plant acid (icterogenin) and certain anabolic steroids on the hepatic metabolism of bilirubin and sulfobromophthalein (BSP). Ann. N.Y. Acad. Sci. 104: 1014-1025, 1963.
14. ARONSEN, K. F., HAGERSTRAND, I. AND NORDEN, J. G.: Enzyme studies in dogs with extra-hepatic biliary obstruction. Scand. J. Gastroenterol. 3: 355-368, 1968.
15. ATTWOOD, D., FLORENCE, A. T. AND GILLAN, J. M. N.: Micellar properties of drugs. Properties of micellar aggregates of phenothiazines and their aqueous solutions. J. Pharm. Sci. 63: 988-993, 1974.
16. BACK, P.: Bile acid glucuronides. II. Isolation and identification of a chenodeoxycholic acid glucuronide from human plasma in intrahepatic cholestasis. Hoppe-Seyler's Z. Physiol. Chem. 357: 213-218, 1976.
17. BACK, P AND BOWEN, D. V.: Bile acid glucuronides. III. Chemical synthesis and characterization of glucuronic acid coupled mono-di- and tri-hydroxy bile acids. Hoppe-Seyler's Z. Physiol. Chem. 357: 219-224, 1976.
18. BACK, P. AND GEROCK, W.: Bile Acids in Human Diseases, F. K. Schattauer Verlag, Stuttgart, 1972.
19. BAIRD, R. W., AND HULL, J. G.: Cholestatic jaundice from tolbutamide. Ann. Intern. Med. 53: 194-196, 1960.
20. BAKER, P. C. AND SCHROEDER, T. E.: Cytoplasmic filaments and morphogenetic movement in the amphibian neural tube. Develop. Biol. 15: 432-450, 1967.
21. BALABAUD, CH., KRON, K. AND GUMUCIO, J. J.: The bile salt non-dependent fraction (BSNDF) of canalicular bile water in the rat. Gastroenterology 69: 805, 1975.
22. BALAZS, M.: Liver injury induced by alpha-naphthylisothiocyanate. Acta Morphol. Acad. Sci. Hung. 19: 213-231, 1971.
23. BALAZS, M AND JUHAZS, J.: Electron microscopic study of the injurious effects of chlorpromazine on rat liver cells. Exp. Path. 11: 25-37, 1975.
24. BALAZS, M. AND MEDVECZKY, E.: Acute, subacute and chronic effect of alpha-naphthyl-isothiocyanate on the liver of the rat. Acta Morphol. Acad. Sci. Hung. 16: 413-424, 1968.
25. BARNHART, J. L. AND COMBES, B.: Characteristics common to choleretic increments of bile induced by theophylline, glucagon and SQ-20009 in the dog. Proc. Soc. Exp. Biol. Med. 150: 591-596, 1975.
26. BASSAN, H., KENDLER, J., HARINASUTA, U. AND ZIMMERMAN, H. J.: Effects of an anabolic steroid (Norbolethone) on the function of the isolated perfused rat liver. Biochem. Pharmacol. 20: 1429-1435, 1971.
27. BATSAKIS, J. G., KREMERS, B. J., THIESSEN, M. M. AND SHILLING, J. M.: Biliary tract enzymology—A clinical comparison of serum alkaline phosphatase, leucine aminopeptidase, and 5′-nucleotidase. Amer. J. Clin. Pathol. 50: 485-490, 1968.
28. BECKER, B. A. AND PLAA, G. L.: The nature of α-naphthylisothiocyanate-induced cholestasis. Toxicol. Appl. Pharmacol. 7: 680-685, 1965.
29. BECKER, B. A. AND PLAA, G. L.: Quantitative and temporal delineation of various parameters of liver dysfunction due to α-naphthylisothiocyanate. Toxicol. Appl. Pharmacol. 7: 708-718, 1965.
30. BECKER, B. A. AND PLAA, G. L.: Hepatotoxicity of α-naphthylisothiocyanate congeners with particular emphasis on phenylisothiocyanate. Toxicol. Appl. Pharmacol. 7: 804-811, 1965.
31. BECKER, F. F.: The liver: Normal and abnormal functions, Part A, Marcel Dekker, New York, 1974.

31a. BERNSTEIN, J. AND BROWN, A. K.: Sepsis and jaundice in early infancy. Pediatrics 29: 873-882, 1962.

32. BERTHELOT, P.: Mechanisms and prediction of drug-induced liver disease. Gut 14: 332-339, 1973.
33. BERTHELOT, P., ERLINGER, S., DHUMEAUX, D. AND PREAUX, A.-M.: Mechanism of phenobarbital-induced hypercholeresis in the rat. Amer. J. Physiol. 219: 809-813, 1970.
34. BEYER, K. H., BAER, J. E., MICHAELSON, J. K. AND RUSSO, H. F.: Renotropic characteristics of ethacrynic acid: A phenoxyacetic saluretic-diuretic agent. J. Pharmacol. Exp. Ther. 147: 1-22, 1965.
35. BIAVA, C.: Studies on cholestasis. The fine structure and morphogenesis of hepatocellular and canalicular bile pigment. Lab. Invest. 13: 1099-1123, 1964.

36. Birnbaum, D., Wajsbort, J. and Feldman, S.: Changes in bile secretion produced by hippocampal and hypothalamic stimulation. Exp. Neurol. 24: 265–271, 1969.
37. Bloomer, J. R. and Boyer, J. L.: Phenobarbital effects in cholestatic liver disease. Ann. Intern. Med. 82: 310–317, 1975.
38. Bloomer, J. R. and Zaccaria, J.: Effect of graded bilirubin loads on bilirubin transport by perfused rat liver. Amer. J. Physiol. 230: 736–742, 1976.
39. Bloomer. J. R., Zaccaria, J. and Klatskin, G.: Inhibitory effects of scillaren and dinitrophenol on bilirubin excretion by the isolated perfused rat liver. Proc. Soc. Exp. Biol. Med. 151: 539–542, 1976.
40. Blough, H. A., Hall, W. H. and Hong, L.: Serum levels and clinical results with erythromycin propionate. Amer. J. Med. Sci. 239: 539–547, 1960.
41. Bolt, H. M. and Kappus, H.: Irreversible binding of ethinylestradiol metabolites to protein and nucleic acids as catalyzed by rat liver microsomes and mushroom tyrosinase. J. Steroid Biochem. 5: 179–184, 1974.
42. Bolt, H. M., Kappus, H. and Kasbohrer, R.: Metabolism of 17α-ethinylestradiol by human liver microsomes *in vitro*. Aromatic hydroxylation and irreversible protein binding of metabolites. J. Clin. Endocrinol. Metab. 39: 1072–1080, 1974.
43. Bolton, B. H.: Prolonged chlorpromazine jaundice. Amer. J. Gastroenterol 48: 497–503, 1967.
44. Bouchier, I. A. D., Cooperband, S. R. and El Kodsi, B. M.: Mucous substances and viscosity of normal and pathological human bile. Gastroenterology 49: 343–353, 1965.
45. Boyce, W. and Witzleben, C. L.: Bilirubin as a cholestatic agent. II. Effect of variable doses of bilirubin on the severity of mangenese-bilirubin cholestasis. Amer. J. Pathol. 72: 427–432, 1973.
46. Boyd, G. S. and Percy-Robb, I. W.: Enzymatic regulation of bile acid synthesis. Amer. J. Med. 51: 580–587, 1971.
47. Boyer, J. L.: Canalicular bile formation in the isolated perfused rat liver. Amer. J. Physiol. 221: 1156–1163, 1971.
48. Boyer, J. L. and Bloomer, J. R.: Canalicular bile secretion in man – Studies utilizing biliary clearance of (C-14) mannitol. J. Clin. Invest. 54: 773–781, 1974.
49. Boyer, J. L. and Klatskin, G.: Canalicular bile flow and bile secretory pressure. Evidence for non-bile salt dependent fraction in the isolated perfused rat liver. Gastroenterology 59: 853–859, 1970.
50. Braun, P.: Hepatotoxicity of erythromycin. J. Infect. Dis. 119: 300–306, 1969.
51. Bremmelgaard, A. and Pedersen, L.: Bile acids during long-term chenodeoxycholic acid treatment. Scand. J. Gastroenterol. 11: 161–165, 1976.
52. Bridges, R. A., Berendes, H. and Good, R. A.: Serious reactions to novobiocin. J. Pediat. 50: 579–585, 1957.
53. Brooks, F. P.: The secretion of bile. Amer. J. Dig. Dis. 14: 343–349, 1969.
54. Brown, A. R.: Two cases of untoward raction after "Ilosone". Brit. Med. J. 2: 913–915, 1963.
55. Burke, J. A.: The cholestatic form of viral hepatitis. Clin. Pediat. 13: 636–639, 1974.
56. Buxton, B. H., Witschi, H. and Plaa, G. L.: Biochemical changes provoked in rat liver by cholestatic doses of α-naphthylisothiocyanate. Toxicol. Appl. Pharmacol. 24: 60–72, 1973.
57. Calcraft, B. Larusso, N. F. and Hofmann, A. F.: Development of a simple, safe, bile acid clearance test. Gastroenterology 69: 812, 1975.
58. Campbell, C. B., McGuffie, C. and Powell, L. W.: The measurement of sulphated and non-sulphated bile acids in serum using gas-liquid chromatography. Clin. Chim. Acta 63: 249–262, 1975.
59. Capizzo, F. and Roberts, R. J.: Disposition of the hepatotoxin α-naphthylisothiocyanate (ANIT) in the rat. Toxicol. Appl. Pharmacol. 17: 262–271, 1970.
60. Capizzo, F. and Roberts, R. J.: α-Naphthylisothiocyanate (ANIT)-induced hepatotoxicity and disposition in various species. Toxicol. Appl. Pharmacol. 19: 176–187, 1971.
61. Capizzo, F. and Roberts, R. J.: Effect of phenobarbital, chlorpromazine, actinomycin D and chronic α-naphthylisothiocyanate-administration on α-naphthylisothiocyanate-^{14}C disposition and α-naphthylisothiocyanate-induced hyperbilirubinemia. J. Pharmacol. Exp. Ther. 179: 455–464, 1971.
62. Capron, J. P. and Erlinger, S.: Barbiturates and biliary function. Digestion 12: 43–56, 1975.
63. Capurso, L., Koch, M., Freddara, U., Lorenzini, I., Jesequel, A. M. and Orlandi, F.: Contribution of morphometry to the study of cholestasis in man. Digestion 12: 294, 1975.
64. Carey, J. B., Jr.: Bile salt metabolism in man. *In* The Bile Acids. Chemistry and Metabolism, vol. 2, Physiology and Metabolism, ed. by P. P. Nair and D. Kritchevsky, pp. 55–82, Plenum Press, New York, 1973.
65. Carey, J. B., Jr., Wilson, I. D., Zaki, F. G. and Hanson, R. F.: The metabolism of bile acids with special reference to liver injury. Medicine 45: 461–470, 1966.
66. Carey, M. C., Hirom, P. C. and Small, D. M.: A study of the physiological interactions between biliary lipids and chlorpromazine hydrochloride. Bile-salt precipitation as a mechanism of phenothiazine-induced bile secretory failure. Biochem. J. Pharmacol. 153: 519–531, 1976.
67. Carey, M. C. and Small, D. M.: The characteristics of mixed micellar solutions with particular reference to bile. Amer. J. Med. 49: 590–608, 1970.
68. Carey, M. C. and Small, D. M.: Micelle formation by bile salts. Arch. Intern. Med. 130: 506–527, 1972.
69. Carmichael, R. H., Wilson, C. and Martz, B. L.: Effect of anabolic steroids on liver function tests in rabbits. Proc. Soc. Exp. Biol. Med. 113: 1006–1008, 1963.
70. Carulli, N., Manenti, F., Ponz de Leon, M., Ferrari, A. Salviolo, G. and Gallo, M.: Alteration of drug metabolism during cholestasis in man. Eur. J. Clin. Invest. 5: 455–462, 1975.
71. Chenderovitch, J., Phocas, E. and Rautureau, M.: Effects of hypertonic solutions on bile formation. Amer. J. Physiol. 205: 863–867, 1963.
72. Chenderovitch, J., Raizman, A. and Infante, R.: Mechanism of ethacrynic acid-induced choleresis in the rat. Amer. J. Physiol. 229: 1180–1187, 1975.
73. Clarke, A. E. and Denborough, M. A.: Interaction of chlorpromazine with bile. Clin. Chem. 17: 998–1001, 1971.
74. Clarke, A. E., Maritz, V. M. and Denborough, M. A.: Interaction of of tricyclic drugs and human bile. Chem. Biol. Interact. 5: 265–277, 1972.
75. Clarke, A. E., Maritz, V. M. and Denborough, M. A.: Interaction of tricyclic drugs and human bile. ate *in vitro*. Aust. N. Z. J. Med. 5: 25–31, 1975.
76. Cohen, P. J.: Change in the distribution of succinic dehydrogenase within the rat hepatic lobule after ligation of the common bile duct. Anat. Rec. 153: 429–444, 1965.
77. Cohn, H. D.: Clinical studies with a new rifamycin derivative. J. Clin. Pharmacol. J. New Drugs 9: 118–125, 1969.
78. Conklin, J. D., Sobers, R. J. and Wagner, D. L.: Further studies on nitrofurantoin excretion in dog hepatic bile. Brit. J. Pharmacol. 48: 273–277, 1973.
79. Conklin, J. D. and Wagner, D. L.: Excretion of nitrofurantoin in dog hepatic bile. Brit. J. Pharmacol. 43:

140–150, 1971.
80. COODLEY, E. L.: Enzyme diagnosis in hepatobiliary disease. Amer. J. Gastroenterol. 52: 189–202, 1969.
81. COOPER, A. D., JONES, A. L., KOLDINGER, R. E. AND OCKNER, R. K.: Selective biliary obstruction: A model for the study of lipid metabolism in cholestasis. Gastroenterology 66: 574–585, 1974.
82. COWEN, A. E., KORMAN, M. G., HOFMANN, A. F. AND CASS, O. W.: Metabolism of lithocholate in healthy man. I. Biotransformation and biliary excretion of intravenously administered lithocholate, lithocholylglycine, and their sulfates. Gastroenterology 69: 59–66, 1975.
83. COWEN, A. E., KORMAN, M. G., HOFMANN, A. F., CASS, O. W. AND COFFIN, S. B.: Metabolism of lithocholate in healthy man. 2. Enterohepatic circulation. Gastroenterology 69: 67–76, 1975.
84. COX, R. P., FOLTZ, E. L., RAYMOND, S. AND DREWYER, R.: Novobiocin jaundice. New Engl. J. Med. 261: 139–141, 1959.
85. CZYGAN, P., GREIM, H., HUTTERER, F., SCHAFFNER, F. AND POPPER, H.: Comparison of two types of intrahepatic jaundice in rats with bile duct ligation. Acta Hepatogastroenterol. 21: 339–345, 1974.
86. CZYGAN, P., GREIM, H., TRÜLZSCH, D., RUDICK, J., HUTTERER, F., SCHAFFNER, F., POPPER, H., ROSENTHAL, O. AND COOPER, D. Y.: Hydroxylation of taurolithocholate by isolated human liver microsomes. II. Cytochrome P-450 dependency. Biochim. Biophys. Acta 35: 168–171, 1974.
87. DALEN, E. AND WESTERHOLM, B.: Occurrence of hepatic impairment in women jaundiced by oral contraceptives and in their mothers and sisters. Acta Med. Scand. 195: 459–463, 1974.
87a. DALVI, R. R., HUNTER, A. L. AND NEAL, R. A.: Toxicological implications of the mixed-function oxidase catalyzed metabolism of carbon disulfide. Chem.-Biol. Interact. 10: 347–361, 1975.
88. DANIELSSON, B., EKMAN, R., JOHANSSON, B. G. AND PETERSSON, B. G.: Abnormal low density plasma lipoproteins occurring in dogs with obstructive jaundice. Fed. Eur. Biochem. Soc. Lett. 63: 33–36, 1976.
89. DANZIGER, R. G., HOFMANN, A. F., THISTLE, J. L. AND SCHOENFIELD, L. J.: Effect of oral chenodeoxycholic acid on bile acid kinetics and biliary lipid composition in women with cholelithiasis. J. Clin. Invest. 52: 2809–2821, 1973.
90. DAVIS, D. C., POTTER, W. Z., JOLLOW, D. J. AND MITCHELL, J. R.: Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. Life Sci. 14: 2099–2109, 1974.
91. DEBROE, M. E., BORGERS, M. AND WIEME, R. J.: The separation and characterization of liver plasma membrane fragments circulating in the blood of patients with cholestasis. Clin. Chim. Acta 59: 369–372, 1975.
92. DELORIMIER, A. A., GORDAN, G. S., LOWE, R. C. AND CARBONE, J. V.: Methyltestosterone, related steroids, and liver function. Arch. Intern. Med. 116: 289–294, 1965.
93. DENK, H.: Die chemische Struktur des endoplasmatischen Retikuliums und die Funktion des mikrosomalen Biotransformationssystems der Leberzelle der Ratte bei experimenteller Cholestase. Pathol. Eur. 7: 43–65, 1972.
94. DENK, H., SCHENKMAN, J. B., BACCHIN, P. G., HUTTERER, F., SCHAFFNER, F. AND POPPER, H.: Mechanism of cholestasis. III. Interaction of synthetic detergents with the microsomal cytochrome P-450 dependent biotransformation system *in vitro*. Exp. Mol. Pathol. 14: 263–275, 1971.
95. DERR, R. F., LOECHLER, D. K., ALEXANDER, C. S. AND NAGASAWA, H. T.: Inhibition of rat liver microsomal N-demethylase by α-naphthylisothiocyanate: Studies with puromycin aminocleoside. Proc. Soc. Exp. Biol. Med. 126: 844–845, 1967.
96. DESMET, V. J.: Morphologic and histochemical aspects of cholestasis. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 97–132, Grune & Stratton, New York, 1972.
97. DESMET, V. J., KRSTULOVIC, B. AND VAN DAMME, B.: Histochemical study of rat liver in alpha-naphthyl isothiocyanate (ANIT) induced cholestasis. Amer. J. Pathol. 52: 401–421, 1968.
98. DESPOPOULOS, A.: Hepatic and renal excretory metabolism of bile salts: A background for understanding steroid-induced cholestasis. J. Pharmacol. Exp. Ther. 176: 273–283, 1971.
99. DE VITA, V. T., CARBONE, P. P., OWENS, A. H., GOLD, G. L., KRANT, M. J. AND EDMONSON, J.: Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea NSC-409962. Cancer Res. 25: 1876–1881, 1965.
100. DE VOS, DE WOLF-PEETERS, C., DESMET, V., BIANCHI, L. AND ROHR, H. P.: Significance of liver canalicular changes after experimental bile duct ligation. Exp. Mol. Pathol. 23: 12–34, 1975.
101. DE VOS, R., DE WOLF-PEETERS, C., DESMET, V., EGGERMONT, E. AND VAN ACKER, K.: Progressive intrahepatic cholestasis (Byler's disease): Case report. Gut 16: 943–950, 1976.
102. DE WOLF-PEETERS, C., DE VOS, R. AND DESMET, V.: Histochemical evidence of a cholestatic period in neonatal rats. Pediat. Res. 5: 704–709, 1971.
103. DE WOLF-PEETERS, C., DE VOS, R. AND DESMET, V.: Electron microscopy and histochemistry of canalicular differentiation in fetal and neonatal rat liver. Tissue Cell 4: 379–388, 1972.
104. DE WOLF-PEETERS, C., DE VOS, R AND DESMET, V.: Electron microscopy and morphometry of canalicular differentiation in fetal and neonatal rat liver. Exp. Mol. Pathol. 21: 339–350, 1974.
105. DHUMEAUX, D., ERLINGER, S., BENHAMOU, J.-P. AND FAUVERT, R.: Effects of rose bengal on bile secretion in the rabbit: Inhibition of a bile salt-independent fraction. Gut 11: 134–140, 1970.
106. DIETMAIER, A., GASSER, R., GRAF, J. AND PETERLIK, M.: Sodium-dependent transport of bile acids in the isolated perfused rat liver. Digestion 12: 273, 1975.
107. DOLL. R.: Recognition of unwanted drug effects. Brit. Med. J. 2: 69–76, 1969.
108. DREW, R. AND PRIESTLY, B. G.: Hepatic microsomal drug metabolism in rabbits treated with lithocholic acid and erythromycin estolate/stearate. Clin. Exp. Physiol. Pharmacol. 2: 441–442, 1975.
109. DREW, R. AND PRIESTLY, B. G.: Drug metabolism during α-naphthylisothiocyanate-induced cholestasis. Toxicol. Appl. Pharmacol. 35: 491–499, 1976.
110. DREW, R. AND PRIESTLY, B. G.: Hexobarbital sleeping time and drug metabolism in rats with ligated bile ducts – a lack of correlation. Biochem. Pharmacol. 25: 1659–1663, 1976.
111. DREW, R. AND PRIESTLY, B. G.: Unpublished observations.
112. DRILL, V. A.: Hepatotoxic agents: Mechanism of action and dietary interrelationship. Pharmacol. Rev. 4: 1–42, 1952.
113. DRILL, V. A.: Benign cholestatic jaundice of pregnancy and benign cholestatic jaundice from oral contraceptives. Amer. J. Obstet. Gynecol. 119: 165–174, 1974.
114. DUBIN, I. N. AND PETERSON, L. H.: An explanation for the centrolobular localization of intrahepatic bile stasis in acute liver diseases. Amer. J. Med. Sci. 236: 45–52, 1958.
115. DUJOVNE, C. A.: Liver cell culture toxicity and surfactant potency of erythromycin derivatives. Toxicol. Appl. Pharmacol. 32: 11–20, 1975.
116. DUJOVNE, C. A., CHAN, C. H. AND ZIMMERMAN, H. J.: Sulfonamide hepatic injury. Review of the literature and report of a case due to sulfamethoxazole. New

Engl. J. Med. 277: 785–788, 1967.

117. DUJOVNE, C. A., LAVELLE, G., WEISS, P. BIANCHINE, J. R. AND LASAGNA, L.: Toxicity of hepatotoxic drugs on mouse liver tissue culture. Arch. Int. Pharmacodyn. Thér. 186: 84–93, 1970.

118. DUJOVNE, C. A., LEVY, R. AND ZIMMERMAN, H. J.: Hepatotoxicity of phenothiazines *in vitro* as measured by loss of aminotransferases to surrounding media. Proc. Soc. Exp. Biol. Med. 128: 561–563, 1968.

119. DUJOVNE, C. A. AND MARDIAT, J.: Is the hepatotoxic potential of bile acids, chlorpromazine and erythromycin estolate related to their detergent potency? Gastroenterology 69: 818, 1975.

120. DUJOVNE, C. A., SHOEMAN, D. AND BIANCHINE, J.: Experimental bases for the different hepatotoxicity of erythromycin preparations in man. J. Lab. Clin. Med. 79: 832–844, 1972.

121. DUJOVNE, C. A. AND SHOEMAN, D. W.: Toxicity of a hepatotoxic laxative preparation in tissue culture and excretion in bile in man. Clin. Pharmacol. Ther. 13: 602–660, 1972.

122. DUMONT, M. AND ERLINGER. S.: Influence of hydrocortisone on bile formation in the rat. Biol. Gastroenterol. (Paris) 6: 197–203, 1973.

123. DYRSZKA, H., SALEN, G., ZAKI, F. G., CHEN, T. AND MOSBACH, E. H.: Hepatic toxicity in the rhesus monkey treated with chenodeoxycholic acid for 6 months: Biochemical and ultrastructural studies. Gastroenterology 70: 93–104, 1976.

124. EAKINS, M. N., SLATER, T. F. AND DELANEY, V. B.: Bile secretion in relation to decreases in liver adenosine triphosphate concentration in the rat. Biochem. J. 115: 62–63P, 1969.

125. EAKINS, M. N., SLATER, T. F., SAWYER, B. AND BULLOCK, G.: The effects of icterogenin and sporidesmin on the isolated perfused rat liver and on the adenosine triphosphatases of rat liver plasma membranes. Biochem. Soc. Trans. 1: 170–172, 1973.

126. ECKHARDT, E. T. AND PLAA, G. L.: The effect of phenothiazine derivatives on the disappearance of sulfobromophthalein from mouse plasma. J. Pharmacol. Exp. Ther. 138: 387–391, 1962.

127. ECKHARDT, E. T. AND PLAA, G. L.: Role of biotransformation, biliary excretion and circulatory changes in chlorpromazine-induced sulfobromophthalein retention. J. Pharmacol. Exp. Ther. 139: 383–389, 1963.

128. ECKHARDT, E. T., PLAA, G. L. AND DARBY, T. B.: The effect of thioridazine on hepatic blood flow. Arch. Int. Pharmacodyn. Thér. 145: 109–122, 1963.

129. EL-HAWARI, A. M. AND PLAA, G. L.: *In vitro* irreversible binding of α-naphthylisothiocyanate to liver microsomes: Correlation with hepatotoxicity. Fed. Proc. 35: 551, 1976.

129a. EL-HAWARI, A. M. AND PLAA, G. L.: Changes in the composition and function of the hepatic endoplasmic reticulum after α-naphthylisothiocyanate: Its role in hepatotoxicity. Proc. First Int. Congr. Toxicol. (in press).

130. ELIAKIM, M., EISNER, M. AND UNGAR, H.: Experimental intrahepatic obstructive jaundice following ingestion of alpha-naphthyl-isothiocyanate. Bull Res. Counc. Israel 8E: 7–17, 1959.

131. ELIAKIM, M., ESCHAR, J. AND ZIMMERMAN, H. J.: International Symposium on Hepatotoxicity, Academic Press, New York, 1974.

132. ELLIOTT, W. H. AND HYDE, P. M.: Metabolic pathways of bile acid synthesis. Amer. J. Med. 51: 568–579, 1971.

133. EMMELOT, P. AND BOS, C. J.: Studies on plasma membranes. III. Mg^{2+}-ATPase (Na^+-K^+-Mg^{2+})-ATPase and 5′-nucleotidase activity of plasma membranes isolated from rat liver. Biochim. Biophys. Acta 120: 369–382, 1966.

134. EMMELOT, P., BOS, C. J., VAN HOEVEN, R. P. AND VAN BLITTERSWIJK, W. J.: Isolation of plasma membranes from rat and mouse livers and hepatomas. *In* Methods in Enzymology, vol. 31, Biomembranes, part A, ed. by S. Fleischer and L. Packer, pp. 75–90, Academic Press, New York, 1974.

135. ENGEL, J. J., VOGT, T. R. AND WILSON, D. E.: Cholestatic hepatitis after administration of furan derivatives. Arch. Intern. Med. 135: 733–735, 1975.

136. ERLINGER, S.: Bile secretion and multiplicity of hepatic excretory mechansims. Biol. Gastroenterol. (Paris) 2: 105–112, 1971.

137. ERLINGER, S.: Physiology of bile flow. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 63–82, Grune & Stratton, New York, 1972.

138. ERLINGER, S. AND DHUMEAUX, D.: Mechanisms and control of secretion of bile water and electrolytes. Gastrenterology 66: 281–304, 1974.

139. ERLINGER, S., DHUMEAUX, D. AND BENHAMOU, J.-P.: Effect on bile formation of inhibitors of sodium transport. Nature (London) 223: 1276–1277, 1969.

140. ERLINGER, S., DHUMEAUX, D., BERTHELOT, P. AND DUMONT, M.: Effect of inhibitors of sodium transport on bile formation in the rabbit. Amer. J. Physiol. 219: 416–422, 1970.

141. ESSNER, E., NOVIKOFF, A. G. AND MASEK, B.: Adenosinetriphosphatase and 5′-nucleotidase activities in the plasma membrane of liver cells as revealed by electron microscopy. J. Biophys. Biochem. Cytol. 4: 711–715, 1958.

142. ESTENSEN, R. D., ROSENBERG, M. AND SHERIDAN, J. D.: Cytochalasin B: Microfilaments and "contractile" processes. Science 173: 356–358, 1971.

143. EVANS, W. H., KREMMER, T. AND CULVENOR, J. B.: Role of membranes in bile formation. Comparison of the composition of bile and a liver bile-canalicular plasma-membrane subfraction. Biochem. J. 154: 589–595, 1976.

144. FARBER, E.: Hepatic carcinogenesis. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 173–182, Grune & Stratton, New York, 1972.

145. FARMER, C. D., HOFFMAN, H. N. AND SHORTER, R. G.: Intrahepatic cholestasis associated with the ingestion of erythromycin estolate (Ilosone). Gastroenterology 45: 157–160, 1963.

146. FELDMANN, G. AND MAURICE, M.: Microtubules, microfilaments et sécrétion cellulaire. Biol. Gastroenterol. (Paris) 8: 269–274, 1975.

147. FISCHER, M. G., NAYER, H. R. AND MILLER, A.: Methimazole-induced jaundice. J. Amer. Med. Ass. 223: 1028–1029, 1973.

148. FISHER, M. M., BLOXAM, D. L., ODA, M., PHILLIPS, M. J. AND YOUSEF, I. M.: Characterization of rat liver cell plasma membranes. Proc. Soc. Exp. Biol. Med. 150: 177–184, 1975.

149. FISHER, M. M., MAGNUSSON, R. AND MIYAI, K.: Bile acid metabolism in mammals. I. Bile acid-induced intrahepatic cholestasis. Lab. Invest. 25: 88–91, 1971.

150. FISHER, M. M., MAGNUSSON, R., PHILLIPS, M. J. AND MIYAI, K.: Bile acid metabolism in mammals. IV. Sex difference in chenodeoxycholic acid metabolism in the rat. Lab. Invest. 27: 254–262, 1972.

151. FISHER, M. M. YOUSEF, I. M. AND BLOXAM, D. L.: Biliary secretion of chendeoxycholic acid (CDCA) by the isolated perfused rat liver (IPRL). Can. Fed. Biol. Soc. 19: 16, 1976.

152. FORKER, E. L.: Two sites of bile formation as determined by mannitol and erythritol clearance in the guinea pig. J. Clin. Invest. 46: 1189–1195, 1967.

153. FORKER, E. L.: Bile formation in guinea pigs: Analysis with inert solutes of graded molecular radius. Amer. J. Physiol. 215: 56–62, 1968.

154. FORKER, E. L.: The effect of estrogen on bile formation in the rat. J. Clin. Invest. 48: 654–663, 1969.

155. FORKER, E. L.: Hepatocellular uptake of inulin, su-

crose, and mannitol in rats. Amer. J. Physiol. 219: 1568-1573, 1970.

156. FORKER, E. L., HICKLIN, T. AND SORNSON, H.: The clearance of mannitol and erythritol in rat bile. Proc. Soc. Exp. Biol. Med. 126: 115-119, 1967.

156a. FRENCH, S. W.: Is cholestasis due to microfilament failure? Hum. Pathol. 7: 243-244, 1976.

157. FROHLING, W. AND STIEHL, A.: Bile salt glucuronides: Identification and quantitative analysis of the urine of patients with cholestasis. Eur. J. Clin. Invest. 6: 67-74, 1976.

158. FROSCH, B., WAGENER, H. AND IMMICH, H.: Serum conjugated bile acids in various liver diseases. Clin. Chim. Acta 22: 294-295, 1968.

159. GALLAGHER, T. F., JR., MUELLER, M. N. AND KAPPAS, A.: Studies on the mechanism and structural specificity of the estrogen effect on BSP metabolism. Trans. Ass. Amer. Physicians Philadelphia 27: 187-195, 1965.

160. GALLAGHER, T. F., JR., MUELLER, M. N. AND KAPPAS, A.: Estrogen pharmacology. IV. Studies on the structural basis for estrogen-induced impairment of liver function. Medicine 45: 471-479, 1966.

161. GALLENKAMP, H. AND RICHTER, E.: Influence of α-naphthylisothiocyanate (ANIT) on microsomal cytochrome P-450, protein and phospholipid content in rat liver. Biochem. Pharmacol. 23: 2431-2435, 1974.

162. GARAY, E. R., PEREZ, V., NOIR, B. AND ROYER, M.: Chronic intoxication induced by alpha-naphthylisothiocyanate in the rat. Arch. Pathol. 80: 127-134, 1965.

163. GARTNER, L. M., LANE, D. L. AND CORNELIUS, C. E.: Bilirubin transport by liver in adult *Macaca mulatta*. Amer. J. Physiol. 220: 1528-1535, 1971.

164. GENTILINI, P., TEODORI, U., GORINI, S. AND POPPER, H.: Intrahepatic cholestasis, Raven Press, New York, 1975.

165. GILBERT, E. F., DASILVA, A. Q. AND QUEEN, D. M.: Intrahepatic cholestasis with fatal termination following norethandrolone therapy. J. Amer. Med. Ass. 185: 538-539, 1963.

166. GILBERT, F. I.: Cholestatic hepatitis caused by esters of erythromycin and oleandomycin. J. Amer. Med. Ass. 182: 1048-1050, 1962.

167. GILLETTE, J. R.: A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity. I. Correlation of changes in covalent binding of reactivity metabolites with changes in incidence and severity of toxicity. Biochem. Pharmacol. 23: 2785-2794, 1974.

168. GILLETTE, J. R.: A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity. II. Alterations in the kinetics of covalent binding. Biochem. Pharmacol. 23: 2927-2938, 1974.

169. GIUSTI, G., PICCININO, F., RICCIARDI, I., DEL RIO, G., SAGNELLI, E. AND MANZILLO, G.: Steroids sulphates in plasma of women with intrahepatic cholestasis of pregnancy. Digestion 12: 305-306, 1975.

170. GOLDFARB, S., SINGER, E. J. AND POPPER, H.: Experimental cholangitis due to alpha-naphtyl-isothiocyanate (ANIT). Amer. J. Pathol. 40: 685-698, 1962.

171. GOLDFARB, S., SINGER, E. J. AND POPPER, H.: Biliary ductules and bile secretion. J. Lab. Clin. Med. 62: 608-615, 1963.

172. GOLDSTEIN, L. I., ISHAK, K. G. AND BURNS, W.: Hepatic injury associated with nitrofurantoin therapy. Amer. J. Dig Dis. 19: 987-998, 1974.

173. GOLDSTEIN, M. J. AND ROTHENBERG, A. J.: Jaundice in a patient receiving acetohexamide. A case report. New Engl. J. Med. 275: 97-99, 1966.

174. GOPINATH, C. AND FORD, E. J. H.: The effect of alpha-naphthylisothiocyanate on the liver of sheep and calves. J. Pathol. 100: 269-280, 1970.

175. GORDON, M.: Phenothiazines. *In* Psychopharmacological Agents, vol. II, ed. by M. Gordon, pp. 156-157, Academic Press, New York, 1967.

176. GORESKY, C. A. AND FISHER, M. M. (eds.): Jaundice, Plenum Press, New York, 1975.

177. GRAF, J., KORN, P. AND PETERLIK, M.: Choleretic effects of ouabain and ethacrynic acid in the isolated perfused rat liver. Naunyn-Schmiederg's Arch. Pharmakol. Exp. Pathol. 272: 230-233, 1972.

178. GRAF, J., KORN, P. AND PETERLIK, M.: Mechanism of bile formation in the isolated perfused rat liver: Influence of changes in the ionic composition of the perfusion medium. *In* Isolated Liver Perfusion and Its Applications, pp. 271-276, Raven Press, New York, 1973.

179. GREGORY, D. H., ZAKI, G. F., SARCOSI, G. A., AND CAREY, J. B.: Chronic cholestasis following prolonged tolbutamide administration. Arch. Pathol. 84: 194-201, 1967.

180. GREIM, H., TRULZSCH, D., ROBOZ, , J., DRESSLER, K., CZYGAN, P., HUTTERER, F., SCHAFFNER, F. AND POPPER, H.: Mechanism of cholestasis. 5. Bile acids in normal rat livers and in those after bile duct ligation. Gastroenterology 63: 837-846, 1972.

181. GREIM, H., TRULZSCH, D., CZYGAN, P., RUDICK, J., HUTTERER, F., SCHAFFNER, F. AND POPPER, H.: Mechansim of cholestasis. 6. Bile acids in human livers with or without biliary obstruction. Gastroenterology 63: 846-850, 1972.

182. GRIFFIN, M. J. AND COX, R. P.: Studies on the mechanism of hormone induction of alkaline phosphatase in human cell cultures. II. Rate of enzyme synthesis and properties of base level and induced enzymes. Proc. Nat. Acad. Sci. U.S.A. 56: 946-953, 1966.

183. GROSZMANN, R. J., KOTELANSKI, B., KENDLER, J. AND ZIMMERMAN, H. J.: Effect of sulfobromophthalein and indocyanine green on bile excretion. Proc. Soc. Exp. Biol. Med. 132: 712-714, 1969.

184. GUMUCIO, J. J., ACCATINO, L., MACHO, A. M. AND CONTRERAS, A.: Effect of phenobarbital on the ethynyl estradiol-inducec cholestasis in the rat. Gastroenterology 65: 651-657, 1973.

185. GUMUCIO, J. J. AND VALDIVIESO, V. D.: Studies on the mechanism of the ethynylestradiol impairment of bile flow and bile salt excretion in the rat. Gastroenterology 61: 339-344, 1971.

186. HAGERSTRAND, I.: Enzyme histochemistry of the liver in extrahepatic biliary obstruction. A comparison between man, dog and rat. Acta Pathol. Microbiol. Scand. A 81A: 737-750, 1973.

187. HANASONO, G. K., DE REPENTIGNY, L., PRIESTLY, B. G. AND PLAA, G. L.: The effects of oral diazepam pretreatment on the biliary excretion of sulfobromophthalein in rats. Can. J. Physiol. Pharmacol. (in press).

187a. HANGER, F. M. JR. AND GUTMAN, A. B.: Postarsphenamine jaundice—apparently due to obstruction of intrahepatic biliary tract. J. Amer. Med. Ass. 115: 263-271, 1940.

188. HARGREAVES, T. AND LATHE, G. H.: Inhibitory aspects of bile secretion. Nature (London) 200: 1172-1176, 1963.

189. HEANEY, R. P. AND WHEDON, G. D.: Impairment of hepatic bromsulphalein clearance by two 17-substituted testosterones. J. Lab. Clin. Med. 52: 169-175, 1958.

190. HEIKEL, T. A. J.: Effect of steroid drugs on biliary secretion: Intrahepatic cholestatis. Biochem. J. 103: 63P-64P, 1967.

191. HEIKEL, T. A. J. AND LATHE, G. H.: The effect of oral contraceptive steroids on bile secretion and bilirubin Tm in rats. Brit. J. Pharmacol. 38: 593-601, 1970.

192. HEIKEL, T. A. J. AND LATHE, G. H.: The effect of 17α-ethinyl-substituted steroids on adenosine triphosphatases of rat liver plasma membrane. Biochem. J.

118: 187-189, 1970.

193. HEPNER, G. W. AND VESSELL, E. S.: Quantitative assessment of hepatic function by breath analysis after oral administration of ^{14}C-aminopyrine. Ann. Intern. Med. 83: 623-638, 1975.

194. HERRON, G. AND BOUDRO, S.: Jaundice secondary to promazine, and an analysis of possible cross sensitivities between phenothiazine derivatives. Gastroenterology 38: 87-90, 1960.

195. HERTZOG, P. J., BHATHAL, P. S., DORLING, P. R. AND LE PAGE, R. N.: α-Naphthylisothiocyanate-induced cholestasis in the rat: Studies of liver plasma membrane enzymes. Pathology 7: 13-23, 1975.

196. HERZ, R., PAUMGARTNER, G. AND PREISIG, R.: Bile salt metabolism and bile formation in the rat with a portacaval shunt. Eur. J. Clin. Invest. 4: 223-228, 1974.

197. HIMSWORTH, H. P.: The Liver and Its Diseases, Harvard University Press, Cambridge, Mass., 1954.

198. HOFFBAUER, F. W.: Clinical aspects of jaundice resulting from intrahepatic obstruction. J. Amer. Med. Ass. 169: 1453-1461, 1959.

199. HOLLISTER, L. E.: Allergy to chlorpromazine manifested by jaundice. Amer. J. Med. 23: 870-879, 1957.

200. HOLLISTER, L.: Chlorpromazine jaundice. J. Amer. Med. Ass. 169: 1235-1236, 1959.

201. HOLLISTER, L. E., CAFFEY, E. M., JR. AND KLETT, C. J.: Abnormal symptoms, signs, and laboratory tests during treatment with phenothiazine derivatives. Clin. Pharmacol. Ther. 1: 284-293, 1960.

202. HOLLISTER, L. E. AND HALL, R. A.: Phenothiazine derivatives and morphologic changes in the liver. Amer. J. Psychiat. 123: 211-212, 1966.

203. HOLSTI, P.: Experimental cirrhosis of the liver in rabbits induced by gastric instillation of dessicated whole bile. Acta Pathol. Microbiol. Scand. Suppl. 113: 1-67, 1956.

204. HOLSTI, P.: Cirrhosis of the liver induced in rabbits by gastric instillation of 3-monohydroxycholanic acid. Nature (London) 186: 250, 1960.

205. HOLZBACH, R. T. AND SANDERS. J. H.: Recurrent intrahepatic cholestasis of pregnancy. J. Amer. Med. Ass. 193: 542-543, 1965.

206. HOOK, J. B. AND WILLIAMSON, H. E.: Lack of correlation between natriuretic activity and inhibition of renal Na-K-activated ATPase. Proc. Soc. Exp. Biol. Med. 120: 358-360, 1965.

207. HORAK, W., GRABNER, G. AND PAUMGARTNER, G.: Inhibition of bile salt-independent bile formation by indocyanine green. Gastroenterology 64: 1005-1012, 1973.

208. HORKY, J., GRASSO, P., GOLBERG, L.: Influence of phenobarbital on histological and biochemical changes in experimental intrahepatic cholestasis. Exp. Pathol. 5: 200-211, 1971.

209. HUGUES, F. C., MARCHE, C. AND MARCHE, J.: Effets hépato-biliaires de l'association rifampicine-isoniazide. 1. Etude histologique chez le rat. Thérapie 24: 899-906, 1960.

210. HUNT, R. D., LEVEILLE, G. A. AND SANBERLICH, H. E.: Dietary bile acids and lipid metabolism. III. Effects of lithocholic acid in mammalian species. Proc. Soc. Exp. Biol. Med. 115: 277-280, 1964.

211. HUTTERER, F., BACCHIN, P. G., DENK, H., SCHENKMAN, J. B., SCHAFFNER, F. AND POPPER, H.: Mechanism of cholestasis. 2. Effect of bile acids on the microsomal electron transfer system *in vitro*. Life Sci. 9: 1159-1166, 1970.

212. HUTTERER, F., BACCHIN, P. G., RAISFELD, I. H., SCHENKMAN, J. B., SCHAFFNER, F. AND POPPER, H.: Alteration of microsomal biotransformation in the liver in cholestasis. Proc. Soc. Exp. Biol. Med. 133: 702-706, 1970.

213. HUTTERER, F., DENK, H., BACCHIN, P. G., SCHENKMAN, J. B., SCHAFFNER, F. AND POPPER, H.: Mechanism of cholestasis. 1. Effect of bile acids on microsomal cytochrome P-450 dependent biotransformation system *in vitro*. Life Sci. 9: 877-887, 1970.

214. HUTTERER, F., GREIM, H., TRULZSCH, D., CZYGAN, P. AND SCHENKMAN, J. B.: Microsomal biotransformation system in cholestasis. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 151-171, Grune & Stratton, New York, 1972.

215. HUTTERER, F., KLION, F. M., WENGRAF, A., SCHAFFNER, F. AND POPPER, H.: Hepatocellular adaptation and injury. Structural and biochemical changes following dieldrin and methyl butter yellow. Lab. Invest. 20: 455-464, 1969.

216. HUTTERER, F., SCHAFFNER, F. AND POPPER, H.: Hypertrophic, hypoactive smooth endoplasmic reticulum. A sensitive indicator of hepatotoxicity exemplified by dieldrin. Science 161: 1017-1019, 1968.

217. IMAI, K. AND HAYASHI, Y.: Steroid-induced intrahepatic cholestasis in mice. Jap. J. Pharmacol. 20: 473-481, 1970.

218. INDACOCHEA-REDMOND, N. AND PLAA, G. L.: Functional effects of α-naphthylisothiocyanate in various species. Toxicol. Appl. Pharmacol. 19: 71-80, 1971.

219. INDACOCHEA-REDMOND, N. AND PLAA, G. L.: Effect of 16α-pregnenolone carbonitrile (PCN) on α-naphthylisothiocyanate (ANIT)-induced hepatotoxicity. Can. Fed. Biol. Soc. 17: 53, 1974.

220. INDACOCHEA-REDMOND, N., WITSCHI, H. AND PLAA, G. L.: Effect of inhibitors of protein and ribonucleic acid synthesis on the hyperbilirubinemia and cholestasis produced by α-naphthylisothiocyanate. J. Pharmacol. Exp. Ther. 184: 780-786, 1973.

221. INDACOCHEA-REDMOND, N., WITSCHI, H. AND PLAA, G. L.: Effects of inhibitors of protein and ribonucleic acid synthesis on α-naphthylisothiocyanate-induced hyperbilirubinemia, sulfobromophthalein retention and prolongation of pentobarbital hypnosis. J. Pharmacol. Exp. Ther. 189: 278-284, 1974.

222. ISHAK, K. G. AND IREY, N. S.: Hepatic injury associated with the phenothiazines. Clinicopathologic and follow-up study of 36 patients. Arch. Pathol. 93: 283-304, 1972.

223. ISRAELS, L. G., LEVITT, M., NOVAK, W. AND ZIPURSKY, A.: The early bilirubin. Medicine 45: 517-521, 1966.

224. JAVITT, N. B.: Phenol 3,6-dibromphthalein disulfonate, a new compound for the study of liver disease. Proc. Soc. Exp. Biol. Med. 177: 254-257, 1964.

225. JAVITT, N. B.: Cholestasis in rats induced by taurolithocholate. Nature (Lodon) 210: 1262-1263, 1966.

226. JAVITT, N. B.: Bile salt regulation of hepatic excretory function. Gastroenterology 56: 622-625, 1969.

227. JAVITT, N. B.: The cholestatic syndrome. Amer. J. Med. 51: 637-641, 1971.

228. JAVITT, N. B.: Bile salts and liver disease in childhood. Postgrad. Med. J. 50: 354-361, 1974.

229. JAVITT, N. B.: Current status of cholestasis induced by monohydroxy bile acids. *In* Jaundice, ed. by C. A. Goresky and M. M. Fisher, pp. 401-409, Plenum Press, New York, 1975.

230. JAVITT, N. B.: Cholestatic jaundice. Med. Clin. N. Amer. 59: 817-821, 1975.

231. JAVITT, N. B. AND EMERMAN, S.: Effect of sodium taurolithocholate on bile flow and bile acid excretion. J. Clin. Invest. 47: 1002-1014, 1968.

232. JAVITT, N. B. AND EMERMAN, S.: Metabolic pathways of bile acid formation in the rat. Mt. Sinai J. Med. 37: 477-481, 1970.

233. JAVITT, N. B., MORRISSEY, K. P., SIEGEL, E., GOLDBERG, H., GARTNER, L. M., HOLLANDER, M. AND KOK, E.: Cholestatic syndromes in infancy: Diagnostic value of serum bile acid patterns and cholestyramine administration. Pediat. Res. 7: 119-125, 1973.

234. JENNER, R. E. AND HOWARD, E. R.: Unsaturated monohydroxy bile acids as a cause of idiopathic obstructive cholangiopathy. Lancet ii: 1073-1075, 1975.

235. JOHANSSON, G.: On the metabolism of lithocholic acid in the chicken and the rabbit. Acta Chem. Scand. 20: 240-244, 1966.
236. JOHNSON, D. F. AND HALL, W. H.: Allergic hepatitis caused by propionyl erythromycin ester of lauryl sulphate. New Engl. J. Med. 265: 1200-1202, 1961.
237. JOHNSTONE, J. M. S. AND LEE, E. G.: A quantitative assessment of the structural changes in the rat's liver following obstruction of the common bile duct. Brit. J. Exp. Pathol. 57: 85-94, 1976.
238. JOKELA, S.: Liver disease due to nitrofurantoin. Gastroenterology 53: 306-311, 1967.
239. JOLLOW, D. J., MITCHELL, J. R., ZAMPAGLIONE, N. AND GILLETTE, J. R.: Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11: 151-169, 1974.
240. JOLLOW, D. J., MITCHELL, J. R., POTTER, W. Z., DAVIS, D. C., GILLETTE, J. R. AND BRODIE, B. B.: Acetaminophen-induced hepatic necrosis. II. Role of covalent binding *in vivo*. J. Pharmacol Exp. Ther. 187: 195-202, 1973.
241. JOLLOW, D. J., THORGEIRSSON, S. S., POTTER, W. Z., HASHIMOTO, M. AND MITCHELL, J. R.: Acetaminophen-induced hepatic necrosis. IV. Metabolic disposition of toxic and non-toxic doses of acetaminophen. Pharmacology 12: 251-271, 1074.
242. KAPLAN, M. M. AND RIGHETTI, A.: Induction of liver alkaline phosphatase by bile duct ligation. Biochim. Biophys. Acta 184: 667-669, 1969.
243. KAPLAN, M. M. AND RIGHETTI, A.: Induction of rat liver alkaline phosphatase: The mechanism of the serum elevation in bile duct obstruction. J. Clin. Invest. 49: 508-516, 1970.
244. KAPPUS, H. AND REMMER, H.: Irreversible protein binding of (^{14}C) imipramine with rat and human liver microsomes. Biochem. Pharmacol. 24: 1079-1084, 1975.
245. KAPPUS, H. AND REMMER, H.: Metabolic activation of norethisterone (norethindrone) to an irreversibly protein-bound derivative by rat liver microsomes. Drug Metab. Disposit. 3: 338-344, 1975.
246. KARCH, F. E. AND LASAGNA, L.: Adverse drug reactions. A crticial review. J. Amer. Med. Ass. 234: 1236-1241, 1975.
247. KARKALAS, Y. AND LAL, H.: Jaundice following therapy with imipramine and cyproheptadine. Clin. Toxicol. 4: 47-53, 1971.
248. KENDLER, J., ANURAS, S., LABORDA, O. AND ZIMMERMAN, H. J.: Perfusion of the isolated rat liver with erythromycin estolate and other derivatives. Proc. Soc. Exp. Biol. Med. 139: 1272-1275, 1972.
249. KENDLER, J., BOWRY, S., SEEFF, L. B. AND ZIMMERMAN, H. J.: Effect of chlorpromazine on the function of the perfused isolated liver. Biochem. Pharmacol. 20: 2439-2445, 1971.
250. KENEY, F. T.: Turnover of rat liver tyrosine transaminase: Stabilization after inhibition of protein synthesis. Science 156: 525-528, 1967.
251. KESSLER, G., HALPERN, P. AND BRODY, H.: Serial serum glutamic oxalacetic transaminase (SGOT) and electrophoresis studies in thorazine-induced jaundice. J. Lab. Clin. Med. 50: 250-256, 1957.
252. KEYSSER, C. H., WILLIAMS, J. A., VAN PETTEN, L. E. AND COY, N.: Experimental production by 2-ethyl-2-phenyl butyramide of intrahepatic cholestasis with bile plugs in dogs. Nature (London) 199: 498-499, 1963.
253. KILLENBERG, P. G. AND HOPPEL, C. L.: Inhibition of rat liver mitochondrial oxidative phosphorylation by sulfobromophthalein. Mol. Pharmacol. 10: 108-118, 1973.
254. KING, J. E. AND SCHOENFIELD, L. J.: Cholestasis induced by sodium taurolithocholate in isolated hamster liver. J. Clin. Invest. 50: 2305-2312, 1971.
255. KLAASSEN, C. D.: Biliary flow after microsomal enzyme induction. J. Pharmacol. Exp. Ther. 168: 218-223, 1969.
256. KLAASSEN, C. D.: Studies on the increased biliary flow produced by phenobarbital in rats. J. Pharmacol. Exp. Ther. 176: 743-751, 1970.
257. KLAASSEN, C. D.: Does bile acid secretion determine canalicular bile production in rats? Amer. J. Physiol. 220: 667-673, 1971.
258. KLAASSEN, C. D.: Biliary excretion of manganese in rats, rabbits and dogs. Toxicol. Appl. Pharmacol. 29: 458-468, 1974.
259. KLAASSEN, C. D.: Bile flow and composition during bile acid depletion and administration. Can. J. Physiol. Pharmacol. 52: 334-348, 1974.
260. KLAASSEN, C. D.: Biliary excretion of xenobiotics. CRC Crit. Rev. Toxicol. 4: 1-29, 1975.
261. KLATSKIN, G.: Toxic and drug-induced hepatitis. *In* Diseases of the Liver, pp. 498-609, 3rd ed. ed. by L. Schiff, J. B. Lippincott, Philadelphia, 1969.
262. KLATSKIN, G.: Drug-induced hepatic injury. *In* The liver and Its Diseases, ed. by F. Schaffner, S. Sherlock and C. M. Leevy, pp. 163-178, Intercontinental Medical Book Corp., New York, 1974.
263. KNODELL, R. G.: Effects of chlorpromazine on bilirubin metabolism and biliary secretion in the rat. Gastroenterology 69: 965-972, 1975.
264. KOHN, N. AND MYERSON, R. M.: Cholestatic hepatitis associated with trifluoperazine. New Engl. J. Med. 264: 549-550, 1961.
264a. KOPELMAN, H., ROBERTSON, M. H. AND SANDERS, P. G.: The Epping jaundice. Brit. ed. J. 1: 514-516, 1966.
264b. KOPELMAN, H., SCHEUER, P. J. AND WILLIAMS, R.: The liver lesion of the Epping jaundice. Quart. J. Med. N.S. 35: 553-564, 1966.
265. KORY, R. C., BRADLEY, M. H., WATSON, R. N., CALLAHAN, R. AND PETERS, B. J.: A six-month evaluation of an anabolic drug, norethandrolone, in underweight persons. II. Bromsulphalein (BSP) retention and liver function. Amer. J. Med. 26: 243-248, 1959.
266. KOTTRA, L. L. AND KAPPAS, A.: Estrogen pharmacology. III. Effect of estradiol on plasma disappearance rate of sulfobromophthalein in man. Arch. Intern. Med. 117: 373-376, 1966.
267. KREEK, M. J., PETERSON, R. E., SLEISENGER, M. H. AND JEFFRIES, G. H.: Effects of ethinylestradiol-induced cholestasis on bile flow and biliary excretion of estradiol and estradiol glucuronide by the rat. Proc. Soc. Exp. Biol. Med. 131: 646-650, 1969.
268. KREEK, M. J., SLEISENGER, M. H. AND JEFFRIES, G. H.: Recurrent cholestatic jaundice of pregnancy with demonstrated estrogen sensitivity. Amer. J. Med. 43: 795-803, 1967.
269. KRSTULOVIC, B., VAN DAMME, B. AND DESMET, V. J.: Comparative histochemical study of rat liver in bile-duct ligation and in alphanaphthyl isothiocyanate (ANIT) intoxication. Amer. J. Pathol. 52: 423-436, 1968.
270. KRYSZEWSKI, A. J., NEALE, G., WHITFIELD, J. B. AND MOSS, D. W.: Enzyme changes in experimental biliary obstruction. Clin. Chim. Acta 47: 175-182, 1973.
271. LANGLANDS, A. O. AND CRAIG MARTIN, W. M.: Jaundice associated with norethisterone-acetate treatment of breast cancer. Lancet i: 584-585, 1975.
272. LARSSON-COHN, U.: Jaundice and oral contraceptives. Lancet i: 679, 1967.
273. LARSSON-COHN, U. AND STENRAM. U.: Jaundice during treatment with oral contraceptive agents. J. Amer. Med. Ass. 193: 422-426, 1965.
274. LARSSON-COHN, U. AND STENRAM. U.: Liver ultrastructure and function in icteric and non-icteric women using oral contraceptive agents. Acta Med. Scand. 181: 257-264, 1967.
275. LAUTERBURG, B., PAUMGARTNER, G. AND PREISIG, R.:

Prostaglandin-induced choleresis in the rat. Experientia 31: 1191–1193, 1975.

276. LAVY, U., BURSTEIN, S. AND JAVITT, N. B.: Fetal bile acid metabolism: Quantitation of 26-hydroxy-cholesterol and 7α-hydroxy-cholesterol in human meconium. Gastroenterology 65: 556, 1973.
277. LAVY, U. AND JAVITT, N. B.: Cholestatic syndromes in childhood: Quantitative estimation of bile acid excretion in urine and feces. Gastroenterology 66: 849, 1974.
278. LAYDEN, T. J. AND BOYER, J. L.: The effect of thyroid hormone on bile salt-independent bile flow and Na^+-K^+-ATPase activity in liver plasma membranes enriched in bile canaliculi. J. Clin. Invest. 57: 1009–1018, 1976.
279. LAYDEN, T. J., SCHWARZ, J. AND BOYER, J. L.: Scanning electron microscopy of the rat liver: Studies of the effect of taurolithocholate and other models of cholestasis. Gastroenterology 69: 724–738, 1975.
280. LEDUC, E. H.: Cell modulation in liver pathology. J. Histochem. Cytochem. 7: 253–255, 1959.
281. LEE, M. J. AND WHITEHOUSE, M. W.: Inhibition of electron transport and coupled phosphorylation in liver mitochondria by cholanic (bile) acids and their conjugates. Biochem. Biophys. Acta 100: 317–328, 1965.
282. LEES, A. W., ASGHER, B., HASHEM, M. A. AND SINHA, B. N.: Jaundice after rifampicin. Brit. J. Dis. Chest. 64: 90–95, 1970.
283. LEEVY, C. M.: Evaluation of Liver Function, Eli Lilly & Co., Indianapolis, Ind., 1974.
284. LEEVY, C. M., CHERRICK, G. R. AND DAVIDSON, C. S.: Observations on norethandrolone-induced abnormalities in plasma decay of sulfobromophthalein and indocyanine green. J. Lab. Clin. Med. 57: 918–926, 1961.
285. LENNON, H. D.: Effect of several anabolic steroids on sulfobromophthalein (BSP) retention in rabbits. Steroids 5: 361–373, 1965.
286. LENNON, H. D.: Relative effects of 17α-alkylated anabolic steroids on sulfobromophthalein (BSP) retention in rabbits. J. Pharmacol. Exp. Ther. 151: 143–150, 1966.
287. LESOBRE, R., RUFFINO, J., TEYSSIER, L., ACHARD, F. AND BREFORT, G.: Les ictères au cours du traitement par la rifampicine. Rev. Tuberc. Pneumol. 33: 393–403, 1969.
287a. LEVY, E., SLUSSER, R. J. AND RUEBNER, B. H.: Hepatic changes produced by a single dose of endotoxin in the mouse. Amer. J. Pathol. 52: 477–502, 1968.
288. LIDBERG, L.: Toxicity to rats and guinea pigs of 3-monohydroxycholanic acid (Lithocholic acid). Exp. Pathol. 4: 182–184, 1970.
289. LIERSCH, M. A. AND HESSE, W.: Synthetic capactiy and cell metabolites of bile duct obstructed rat livers. Effect of free and conjugated dehydroxybile acids. Acta Hepatogastroenterol. 22: 281–289, 1975.
290. LIERSCH, M., CZYGAN, P. AND STIEHL, A.: Studies on hepatic uptake and secretion of bile salt sulfates by the isolated perfused rat liver. Digestion 12: 326–327, 1975.
291. LINDBLAD, L., HAMMARSTEN, J. AND SCHERSTEN, T.: Bile flow and biliary lipid secretion following release of biliary obstruction in man. Scand. J. Gastroenterol. 10: 633–639, 1975.
292. LOCK, S., WITSCHI, H. P. AND PLAA, G. L.: Bile production and flow in rats treated with α-naphthylisothiocyanate (ANIT). Can. Fed. Biol. Soc. 19: 102, 1976.
293. LOCK, S., WITSCHI, H. P., SKELTON, F. S., HANASONO, G. AND PLAA, G. L.: Effect of cycloheximide on the distribution of α-naphthylisothiocyanate in rats. Exp. Mol. Pathol. 21: 237–245, 1974.
294. LOMBARDI, B.: Considerations on the pathogenesis of fatty liver. Lab. Invest. 15: 1–20, 1966.
295. LOW-BEER, T. S., TYOR, M. P. AND LACK, L.: Effects of sulfation of taurolithocholate and glycolithocholic acids on their intestinal transport. Gastroenterology 56: 721–726, 1969.
296. LUNEL, J., ALBOT, G. AND PAGNIEZ, R.: Etude critique des ictéres à la chlorpromazine. Sem. Hôp. Paris 29: 1791–1807, 1966.
297. LUNZER, M., HUANG, S. N., GINSBURG, J., AHMED, M. AND SHERLOCK, S.: Jaundice due to carbimazole. Gut 16: 913–917, 1975.
298. LUNZER, M. R., HUANG, A. N., WARD, K. M. AND SHERLOCK, S.: Jaundice due to erythromycin estolate. Gastroenterology 68: 1284–1291, 1975.
299. MACAROL, V., MORRIS, T. Q., BAKER, K. J. AND BRADLEY, S. E.: Hydrocortisone choleresis in the dog. J. Clin. Invest. 49: 1714–1723, 1970.
300. MACKINNON, A. M.: The problem of intrahepatic cholestasis. Aust. N. Z. J. Med. 5: 578–579, 1975.
301. MACKINNON, A. M. AND SIMON, F. R.: Reduced synthesis of hepatic microsomal cytochrome P_{450} in the bile duct ligated rat. Biochem. Biophys. Res. Commun. 56: 437–443, 1974.
302. MACKINNON, A. M. AND SIMON, F.: Pharmacological reversal of cholestasis-associated decrease in hepatic cytochrome P-450. Biochem. Pharmacol. 24: 748–749, 1975.
303. MACKINNON, A. M., SUTHERLAND, E. AND SIMON, F.: The effect of cholestasis on microsomal protein turnover and the mixed function oxidase system. Gastroenterology 65: 558, 1973.
304. MAKINO, I., HASHIMOTI, H., SHINOZAKI, K., YOSHINO, K. AND NAKAGAWA, S.: Sulfated and nonsulfated bile acids in urine, serum, and bile of patients with hepatobiliary diseases. Gastroenterology 68: 545–553, 1975.
305. MAKINO, I., NAKAGAWA, S. AND MASHINO, K.: Conjugated and unconjugated serum bile acid levels in patients with hepatobiliary diseases. Gastroenterology 56: 1033–1039, 1969.
306. MAKINO, I., SJOVALL, J., NORMAN, A. AND STANDUIK, B.: Excretion of 3β-hydroxy-5-cholenoic acid and 3β-hydroxy-5α-cholanoic acids in the urine of infants with biliary atresia. Fed. Eur. Biochem. Soc. Lett. 15: 161–164, 1971.
307. MANASEK, F. J., BURNSIDE, B. AND STROMAN, J.: The sensitivity of developing cardiac myofibrils to cytochalasin-B. Proc. Nat. Acad. Sci. U.S.A. 69: 308–312, 1972.
308. MARBLE, A. AND CAMERINI-DAVALOS, R.: Clinical experience with sulfonylurea compounds in diabetes. Ann. N. Y. Acad. Sci. 71: 239–248, 1957.
309. MAXWELL, J. D. AND WILLIAMS, R.: Drug-induced jaundice. Brit. J. Hosp. Med. 9: 193–200, 1973.
310. MAZZANTI, L.: The effect produced on the guinea pig liver by some substances related to 1-naphthylisothiocyanate. III. Phenylisothiocyanate. Boll. Soc. Ital. Biol. Sper. 31: 628–629, 1956 per Chem. Abstr. 50: 7319e, 1956.
311. MCKENZIE, I. AND DOYLE, A.: Two cases of jaundice following "Ilosone." Med. J. Aust. 1: 349–351, 1966.
312. MCLEAN, M. R. AND REES, K. R.: Hyperplasia of bile ducts induced by *alpha*-naphthyl-*iso*-thiocyanate: Experimental biliary cirrhosis free from biliary obstruction. J. Pathol. Bacteriol. 76: 175–188, 1958.
313. MENNEAR, J. H., BRADLEY, C. A. AND MIYA, T. S.: A study of the chlorpromazine induced decreased liver glutathione reaction. Proc. Soc. Exp. Biol. Med. 112: 199–202, 1963.
314. MEYLER, L. (ed.): Side Effects of Drugs. Excerpta Medica Foundation, Amsterdam, vols. I–V, 1957–1966.
315. MEYLER, L. AND HERXHEIMER, A. (eds.): Side Effects of Drugs. Excerpta Medica Foundation, Amsterdam, vols. VI, VII, 1968, 1972.
316. MITCHELL, J. R., JOLLOW, D. J., GILLETTE, J. R. AND BRODIE, B. B.: Drug metabolism as a cause of drug toxicity. Drug Metab. Disposit. 1: 418–423, 1973.
317. MITCHELL, J. R., JOLLOW, D. J., POTTER, W. Z., DAVIS,

D. C., Gillette, J. R. and Brodie, B. B.: Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J. Pharmacol. Exp. Ther. 187: 185-194, 1973.

318. Mitchell, J. R., Jollow, D. J., Potter, W. Z., Gillette, J. R. and Brodie, B. B.: Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther. 187: 211-217, 1973.

318a. Mitchell, J. R., Nelson, W. L. Potter, W. Z., Sasame, H. A. and Jollow, D. J.: Metabolic activation of furosemide to a chemically reactive, hepatotoxic metabolite. J. Pharmacol. Exp. Ther. 199: 41-52, 1976.

319. Mitchell, J. R., Reid, W. D., Christie, B., Moskowitz, J., Krishna, G. and Brodie, B. B.: Bromobenzene-induced hepatic necrosis: Species differences and protection by SKF-525-A. Res. Commun. Chem. Pathol. Pharmacol. 2: 877-888, 1971.

320. Mitchell, J. R., Thorgeirsson, U. P., Black, M., Timbrell, J. A., Snodgrass, W. R., Potter, W. Z., Jollow, D. J. and Keiser, H. R.: Increased incidence of isomazid hepatitis in rapid acetylators: Possible relation to hydrazine metabolites. Clin. Pharmacol. Ther 18: 70-79, 1975.

321. Mitchell, J. R., Zimmerman, H. J., Ishak, K. G., Thorgeirsson, U. P., Timbrell, J. A., Snodgrass, W. R. and Nelson, S. D.: Isoniazid liver injury: Clinical spectrum. Pathology and probable pathogenesis. Ann. Intern. Med. 84: 181-192, 1976.

322. Mitropoulos, K. A. and Myant, N. G.: The formation of lithocholic acid, chenodeoxycholic acid and other bile acids from 3β-hydroxychol-5-enoic acid *in vitro* and *in vivo*. Biochem. Biophys. Acta. 144: 430-439, 1967.

323. Miyai, K., Mayr, W. W. and Richardson, A. L.: Acute cholestasis induced by lithocholic acid in the rat: A freeze-fracture replica and thin section study. Lab. Invest. 32: 527-535, 1975.

324. Miyai, K., Mayr, W., Richardson, A and Fisher, M. M.: An ultrastructural look at intrahepatic cholestasis. *In* Jaundice, ed. by C. A. Goresky and M. M. Fisher, pp. 383-400, Plenum Press, New York, 1975.

325. Miyai, K., Price, V. M. and Fisher, M. M.: Bile acid metabolism in mammals: Ultrastructural studies on the intrahepatic cholestasis induced by lithocholic and chenodeoxycholic acids in the rat. Lab. Invest. 24: 292-302, 1971.

326. Moran, E., Eliakim, M., Suchowolski, A. and Ungar, H.: Serum vitamin B_{12} and glutamic-oxalacetic transaminase in experimental intrahepatic obstructive jaundice. Gastroenterology 49: 408-415, 1961.

327. Moran, E., Eliakim, M. and Ungar, H.: The effect of cortisone on cholangitis induced by α-naphthylisothiocyanate in rats. Gastroenterology 48: 773-783, 1965.

328. Moran, E. and Ungar, H.: The effect of intermittent administration of alpha-naphthyl isothiocyanate to rats. Amer. J. Pathol. 44: 947-960, 1964.

329. Morgan, D. H.: Jaundice associated with amitriptyline. Brit. J. Psychiat. 115: 105-106, 1969.

330. Morris, T. Q.: Choleretic responses to cyclic AMP and theophylline in the dog. Gastroenterology 62: 187, 1972.

331. Mueller, M. N. and Kappas, A.: Estrogen pharmacology. I. The influence of estradiol and estriol on hepatic disposal of sulfobromophthalein (BSP) in man. J. Clin. Invest. 43: 1905-1914, 1964.

332. Murphy, P. J., Williams, T. L., McMahon, R. E., Crabtree, R. E. and Ridolfo, A. S.: Metabolism of propionyl erythromycin lauryl sulfate. II. Fate of the lauryl sulfate moiety in the rat and man. Drug Metab. Disposit. 3: 164-170, 1975.

333. Murphy, P. J., Williams, T. L., McMahon, R. E. and Marshall, F. J.: Metabolism of propionyl erythromycin lauryl sulfate. I. Fate of the proprionyl erythromycin moiety in the rat. Drug. Metab. Disposit. 3: 155-163, 1975.

334. Nair, P. P. and Kritchevsky, D.: The Bile Acids Chemistry, Physiology and Metabolism, vol. 2, Physiology and Metabolism, Plenum Press, New York, 1973.

335. Neville, D. M.: The isolation of a cell membrane fraction from rat liver. J. Biophys. Biochem. Cytol. 8: 413-422, 1960.

336. Nicklas, W. J. and Berl, S.: Effects of cytochalasin B on uptake and release of putative transmitters by synaptosomes and on brain actomyosin-like protein. Nature (London) 247: 471-473, 1974.

337. Norman, A. and Strandvik, B.: Excretion of bile acids in extrahepatic biliary atresia and intrahepatic cholestasis of infancy. Acta Pediat. Scand. 62: 253-263, 1973.

338. Norman, A. and Strandvik, B.: Bile acid excretion after disappearance of jaundice in intrahepatic cholestasis of infancy. Acta Pediat. Scand. 62: 264-268, 1973.

339. Ockner, R. K. and Davidons, C. S.: Hepatic effects of oral contraceptives. New Engl. J. Med. 276: 331-334, 1967.

340. Oda, M., Price, V. M., Fisher, M. M. and Phillips, M. J.: Ultrastructure of bile canaliculi, with special reference to the surface coat, and the pericanalicular web. Lab. Invest. 31: 314-323, 1974.

341. Oda, M., Yousef, I. M. and Phillips, M. J.: Isolation of bile ducts from rat liver: Technique and preliminary ultrastructural characterization. Exp. Mol. Pathol. 23: 214-219, 1975.

342. Odell, G. B., Javitt, N. B., Cukier, J. O., Maglalang, A. and Kok. E.: Neonatal jaundice and cholestasis. Gastroenterology 69: 851, 1975.

343. Orci, L., Gabbay, K. H. and Malaisse, W. J.: Pancreatic beta-cell web: Its possible role in insulin secretion. Science 175: 1128-1130, 1972.

344. Orellana-Alcalde, J. M. and Dominguez, J. P.: Jaundice and oral contraceptive drugs. Lancet i: 1278-1280, 1966.

345. Orlandi, F. and Jezequel, A. M. (eds.): Liver and Drugs. Academic Press, New York, 1972.

346. Osborn, E. C., Wootton, I. D. P. Da Silva, L. C. and Sherlock, S.: Serum-bile acid levels in liver disease. The Lancet ii: 1049-1053, 1959.

346a. Ottenberg, R. and Spiegel, R.: The present status of non-obstructive jaundice due to infectious and chemical agents. Medicine 22: 27-71, 1943.

347. Palmer, R. H.: Toxic effects of lithocholic acid and related 5-β-H-steroids. *In* Bile Salt Metabolism, ed. by L. Schiff, J. B. Carey, Jr. and J. M. Dietschy, pp. 184-204, Charles C Thomas, Springfield, Ill. 1969.

348. Palmer, R. H. and Bolt, M. G.: Bile acid sulfates. I. Synthesis of lithocholic acid sulfates and their identification in human bile. J. Lipid Res. 12: 671-679, 1971.

349. Paumgartner, G., Herz, R., Sauter, K. and Schwarz, H. P.: Taurocholate excretion and bile formation in the isolated perfused rat liver. An in vitro-in vivo comparison. Naunyn-Schmiedebergs Arch. Pharmakol. Exp. Pathol. 285: 165-174, 1974.

350. Paumgartner, G., Horak, W., Probst, P. and Grabner, G.: Effect of phenobarbital on bile flow and bile salt excretion in the rat. Naunyn-Schmeidebergs Arch. Phamakol. Exp. Pathol. 270: 98-101, 1971.

351. Perez, V. and Gorodisch, S.: Female sex hormones and the liver. *In* The Liver and Its Diseases, ed. by F. Schaffner, S. Sherlock and C. M. Leevy, pp. 179-190, Intercontinental Medical Book Corp., New York, 1974.

352. Perez, V., Schaffner, F. and Popper, H.: Hepatic drug reactions. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 597-625,

Grune & Stratton, New York, 1972.

353. PHILLIPS, M. J., ODA, M., MAK, E. AND FISHER, M. M.: Bile canalicular structure and function. *In* Jaundice, ed. by C. A. Goresky and M. M. Fisher, pp. 367–382, Plenum Press, New York, 1975.
354. PHILLIPS, M. J., ODA, M., MAK, E., FISCHER, M. M. AND JEEJEEBHOY, K. N.: Microfilament dysfunction as a possible cause of intrahepatic cholestasis. Gastroenterology 69: 48–58, 1975.
355. PHILLIPS, M. J. AND STEINER, J. W.: Comparative study of α-naphthylisothiocyanate-induced liver injury. Lab. Invest. 13: 779–793, 1964.
356. PICCININO, F., MANZILLO, G. AND SAGNELLI, E.: The differential diagnosis between intrahepatic cholestatic jaundice and viral hepatitis during pregnancy. Acta Hepatogastroenterol. 22: 144–150, 1975.
357. PIERON, R., MARIEN, CL. AND JAGUEUX, M.: Ictères et rifampicine. Sem. Hôp. Paris 47: 1286–1295, 1971.
358. PLAA, G. L.: Functional aspects of the cholestatic response induced by α-naphthylioslthiocyanate in mice and rats. Agents Actions 1: 22–27, 1969.
359. PLAA, G. L.: Hyperbilirubinemia and cholestasis, a different form of liver injury produced in animals. *In* Essays in Toxicology, vol. 2, ed. by F. R. Blood, pp. 137–153, Academic Press, New York, 1970.
360. PLAA, G. L.: Nonsteroid drug-induced cholestasis and experimental cholestasis. *In* Jaundice, ed. by C. A. Goresky and M. M. Fisher, pp. 351–366, Plenum Press, New York, 1975.
361. PLAA, G. L.: Toxicology of the liver. *In* Toxicology: The Basic Science of Poisons, ed. by L. J. Casarett and J. Doull, pp. 170–189, Macmillan, New York, 1975.
362. PLAA, G. L. AND EL-HAWARI, A. M.: Species differences in hepatotoxicity and *in vitro* irreversible binding of α-naphthylisothiocyanate to liver microsomes. Pharmacologist 18: 171, 1976.
363. PLAA, G. L., MCGOUGH, E. C., BLACKER, G. J. AND FUJIMOTO, J. M.: Effect of thioridazine and chlorpromazine on rat liver hemodynamics. Amer. J. Physiol. 199: 793–796, 1960.
364. PLAA, G. L., ROGERS, L. A. AND FOUTS, J. R.: Effect of acute alpha-naphthylisothiocyanate administration on hepatic microsomal drug metabolism in the mouse. Proc. Soc. Exp. Biol. Med. 119: 1045–1048, 1965.
365. PLOMPTEUX, G., TOULET, J., VITEAU, J. M., ABIVEN, M. AND BONFANTE, N.: Tolérance hépatique chez l'homme de l'association rifampicine-isoniazide. Ann. Gastroent. Hépatol. 7: 1–24, 1971.
366. POLIN, S. G., SPELLBERG, M. A., TEITELMAN, L. AND OKUMURA, M.: The origin of elevaltion of serum alkaline phosphatase in hepatic disease. Gastroenterology 42: 431–438, 1962.
367. POPPER, H.: Liver disease—morphologic considerations. Amer. J. Med. 16: 98–117, 1954.
368. POPPER, H.: Cholestasis. Ann. Rev. Med. 19: 39–56, 1968.
369. POPPER, H.: The problem of hepatitis. Amer. J. Gastroenterol. 55: 335–346, 1971.
370. POPPER, H., DUBIN, A., BRUCE, C., KENT, G. AND KUSHNER, D.: Effect of chlorpromazine upon experimental hepatic injury. J. Lab. Clin. Med. 49: 767–773, 1957.
371. POPPER, H. AND SCHAFFNER, F.: Drug-induced hepatic injury. Ann. Intern. Med. 51: 1230–1252, 1959.
372. POPPER, H. AND SCHAFFNER, F.: Fine structural changes of the liver. Ann. Intern. Med. 59: 674–691, 1963.
373. POPPER, H. AND SCHAFFNER, F.: Pathophysiology of cholestasis. Human Pathol. 1: 1–24, 1970.
374. POPPER, H. AND SCHAFFNER, F. (eds.): Progress in Liver Disease, vol. IV, Grune & Stratton, New York, 1972.
375. POPPER, H. AND SZANTO, P. B.: Intrahepatic cholestasis (cholangiolitis). Gastroenterology 31: 683–700, 1956.
376. POTTER, W. Z., DAVIS, D. C., MITCHELL, J. R., JOLLOW, D. J., GILLETTE, J. R. AND BRODIE, B. B.: Acetaminophen-induced hepatic necrosis. III. Cytochrome P_{450}-mediated covalent binding *in vitro*. J. Pharmacol. Exp. Ther. 187: 203–210, 1973.
377. POTTER, W. Z., THORGEIRSSON, S. S., JOLLOW, D. J. AND MITCHELL, J. R.: Acetaminophen-induced hepatic necrosis. V. Correlation of hepatic necrosis, covalent binding and glutathione depletion in hamsters. Pharmacology 12: 129–143, 1974.
378. POUTSIAKA, J. W., KEYSSER, C. H., THOMAS, B. G. H. AND LINEGAR, C. R.: Simultaneous determination in dogs of liver and kidney functions with bromosulfalein and phenolsulfonephthalein. Toxicol. Appl. Pharmacol. 4: 55–69, 1962.
379. PRANDI, D., DUMONT, M. AND ERLINGER, S.: Influence of portacaval shunt on bile formation in the rat. Eur. J. Clin. Invest. 4: 197–200, 1974.
380. PRANDI, D., ERLINGER, S., GLASINOVIC, J.-C. AND DUMONT, M.: Canalicular bile production in man. Eur. J. Clin. Invest. 5: 1–6, 1975.
381. PREISIG, R.: Evaluation of the action of foreign compounds on biliary excretion. *In* Liver and Drugs, ed. by F. Orlandi and A. M. Jezequel, Academic Press, New York, 1972.
382. PREISIG, R., STREBEL, H, EGGR, G AND MACAROL, V.: Effect of vasopressin on hepatocytic and ductal bile formation in the dog. Experientia 28: 1436–1437, 1972.
383. PRIESTLY, B. G., CÔTÉ, M. G. AND PLAA, G. L.: Biochemical and morphological parameters of taurolithocholate-induced cholestasis. Canad. J. Physiol. Pharmacol. 49: 1078–1091, 1971.
384. PRIESTLY, B. G. AND DREW, R.: Interaction between lithocholic acid and erythromycin estolate/stearate producing hepatotoxicity in rabbits. Clin. Exp. Physiol. Pharmacol. 2: 441, 1975.
385. PRIESTLY, B. G. AND DREW, R.: *In vivo* and *in vitro* effects of α-naphthylisothiocyanate on hepatic drug metabolism. Toxicol. Appl. Pharmacol. 37: 000–000, 1976 (in press).
386. PRIESTLY, B. G. AND PLAA, G. L.: Reduced bile flow after sulfobromophthalein administration in the rat. Proc. Soc. Exp. Biol. Med. 135: 373–376, 1970.
387. PRIESTLY, B. G. AND PLAA G. L.: Sulfobromophthalein metabolism and excretion in rats with iodomethane-induced depletion of hepatic glutathione. J. Pharmacol. Exp. Ther. 174: 221–231, 1970.
388. PROCTER, B. G., DUSSAULT, P. AND CHAPPEL, C. I.: Biochemical effects of sucrose acetate isobutyrate (SAIB) on the liver. Proc. Soc. Exp. Biol. Med. 142: 595–599, 1973.
389. PUGH, P. M. AND STONE, S. L.: The effect of 2,4-dinitrophenol and related compounds on bile secretion. J. Physiol. 198: 39–49, 1968.
390. READ, A. E., HARRISON, C. V. AND SHERLOCK, S.: Chronic chlorpromazine jaundice. Amer. J. Med. 31: 249–258, 1961.
391. RECKNAGEL, R. O. AND GLENDE, E. A.: Carbon tetrachloride hepatotoxicity: An example of lethal cleavage. CRC Crit. Rev. Toxicol. 2: 263–297, 1973.
392. REDINGER, R. N. AND SMALL, D. M.: Primate biliary physiology. VIII. The effect of phenobarbital upon bile salt synthesis and pool size, biliary lipid secretion, and bile composition. J. Clin. Invest. 52: 161–172, 1973.
393. REICHEL, J., GOLDBERG, S. B., ELLENBERG, M. AND SCHAFFNER, F.: Intrahepatic cholestasis following administration of chlorpropamide. Amer. J. Med. 28: 654–660, 1960.
394. REICHEN, J. AND PAUMGARTNER, G.: Sodium-dependent membrane transport responsible for hepatocellular uptake of taurocholate. Gastroenterology 69: 855, 1975.

395. REID, W. D., CHRISTIE, B., KRISHNA, G., MITCHELL, J. R., MOSKOWITZ, J. AND BRODIE, B. B.: Bromobenzene metabolism and hepatic necrosis. Pharmacology 6: 41-55, 1972.
396. REINHART, M. J., BENSON, R. M., KWASS, S. K. AND STOREY, W. F.: Suggestive evidence of hepatotoxicity concomitant with thioridazine hydrochloride use. J. Amer. Med. Ass. 197: 767-769, 1966.
397. REMMER, H.: Experimental intrahepatic cholestasis. *In* Intrahepatic cholestasis, ed. by P. Gentilini, U. Teodori, S. Gorini and H. Popper, pp. 165-177, Raven Press, New York, 1975.
398. RIGHETTI, A. B. B. AND KAPLAN, M. M.: The origin of the serum alkaline phosphatase in normal rats. Biochim. Biophys. Acta 230: 504-509, 1971.
399. RIGHETTI, A. B. B. AND KAPLAN, M. M.: Properties of rat liver alkaline phosphatase before and after bile duct ligation. Proc. Soc. Exp. Biol. Med. 145: 726-728, 1974.
400. RITLAND, S.: Demonstration of the abnormal lipoprotein of cholestasis, LPX, by precipitation with polyanion. Scand. J. Gastroenterol. 9: 507-510, 1974.
401. ROBERTS, R. J.: Microsomal metabolism of the hepatotoxin α-naphthylisothiocyanate (ANIT) following phenobarbital, chlorpromazine, or actinomycin D treatment. Proc. Soc. Exp. Biol. Med. 142: 365-367, 1973.
402. ROBERTS, R. J., KLAASSEN, C. D. AND PLAA, G. L.: Maximum biliary excretion of bilirubin and sulfobromophthalein during anesthesia-induced alteration of rectal temperature. Proc. Soc. Exp. Biol. Med. 125: 313-316, 1967.
403. ROBERTS, R. J. AND PLAA, G. L.: Potentiation and inhibition of α-naphthylisothiocyanate-induced hyperbilirubinemia and cholestasis. J. Pharmacol. Exp. Ther. 150: 499-506. 1965.
404. ROBERTS, R. J. AND PLAA, G. L.: Effect of norethandrolone, acetohexamide, and enovid on α-naphthylisothiocyanate-induced hyperbilirubinemia and cholestasis. Biochem. Pharmacol. 15: 333-341, 1966.
405. ROBERTS, R. J. AND PLAA, G. L.: The effect of bile duct ligation, bile duct cannulation, and hypothermia on α-naphthylisothiocyanate-induced hyperbilirubinemia and cholestasis in rats. Gastroenterology 50: 768-774, 1966.
406. ROBERTS, R. J. AND PLAA, G. L.: Alteration of the plasma disappearance and biliary excretion patterns of exogenously administered bilirubin by α-naphthylisothiocyanate. J. Pharmacol. Exp. Ther. 155: 330-336, 1966.
407. ROBERTS, R. J. AND PLAA, G. L.: Alteration in biliary bilirubin content and non-erythropoietically derived bilirubin synthesis in rats after α-naphthylisothiocyanate administration. J. Pharmacol. Exp. Ther. 161: 382-388, 1968.
408. ROBERTS, R. J., SHRIVER, S. L. AND PLAA, G. L.: Effect of norethandrolone on the biliary excretion of bilirubin in the mouse and rat. Biochem. Pharmacol. 17: 1261-1268, 1968.
409. ROBINSON, M. M.: Demonstration by "challenge" of hepatic dysfunction associated with propionyl erythromycin ester lauryl sulfate. Antibiot. Chemother. 12: 147-151, 1962.
410. ROBINSON, M. M.: Hepatic dysfunction associated with triacetyloleandomycin and propionyl erythromycin ester lauryl sulfate. Amer. J. Med. Sci. 243: 502-509, 1962.
411. ROBINSON, S. H., OWEN, C. A., FLOCK, E. V. AND SCHMID. R.: Bilirubin formation in the liver from nonhemoglobin sources. Experiments with isolated perfused rat liver. Blood 26: 823-829, 1965.
412. RODIN, A. E. AND ROBERTSON, D. M.: Fatal toxic hepatitis following chlorpromazine therapy. Arch. Pathol. 66: 170-175, 1958.
413. RONCHI, G. AND DESMET, V.: Histochemical study of so-called "marker enzymes of cholestasis" during extrahepatic bile duct observation in the rat. Beitr. Pathol. Anat. Allg. Pathol. 149: 213-226, 1973.
414. ROS, E. R., SMALL, D. M. AND CAREY, M. C.: The effects of chlorpromazine hydrochloride (CPZ) on bile formation and biliary lipid secretion in the primate. Gastroenterology 68: 957, 1975.
415. ROTHFELD, E. L., GOLDMAN, J. GOLDBERG, H. H. EINHORN, S. AND EINHORN, S.: Severe chlorpropamide toxicity. J. Amer. Med. Ass. 172: 54-56, 1960.
416. ROZE, C., SOUCHARD, M., DE LA TOUR, J., VAILLE, C. AND DEBRAY, C.: Effets de l'obstruction sélective des voies biliaires sur la sécrétion biliaire chez le rat. Biol. Gastroenterol. 8: 21-31, 1975.
417. RUTISHAUSER, S. C. B. AND STONE, S. L.: The effect of sodium salicylate on bile secretion in the dog. J. Physiol. 245: 549-565, 1975.
418. RÜTTNER, J. R., SPYCHER, M. A. AND KUENZLE, C.: Zur Pathologie der Ikterns. Der ANIT-induzierte Ikterns der Ratte, ein Modell einer durch Zellmembranschädigung bedingten toxischen Hepatose. Pathol. Microbiol. 27: 403-409, 1964.
419. SANDSTRÖM, B.: On the specificity of histochemically demonstrable bile canalicular phosphatase activities. Histochemie 22: 316-323, 1970.
420. SCHAFFNER, F.: Morphologic studies on bile secretion. Amer. J. Dig. Dis. 10: 99-115, 1965.
421. SCHAFFNER, F., BACCHIN, P. G., HUTTERER, F., SCHARNBECK, H. H., SARKOZI, L. L., DENK, H. AND POPPER, H.: Mechanism of cholestasis. 4. Structural and biochemical changes in the liver and serum in rats after bile duct ligation. Gastroenterology 60: 888-897, 1971.
422. SCHAFFNER, F. AND JAVITT, N. B.: Morphologic changes in hamster liver during intrahepatic cholestasis induced by taurolithocholate. Lab. Invest. 15: 1783-1792, 1966.
423. SCHAFFNER, F. AND POPPER, H.: Morphologic studies of cholestasis. Gastroenterology 37: 565-573, 1959.
424. SCHAFFNER, F. AND POPPER, H.: Cholestasis is the result of hypoactive hypertrophic smooth endoplasmic reticulum in the hepatocyte Lancet ii: 355-359, , 1969.
425. SCHAFFNER, F. AND POPPER, H.: Causation and consequences of cholestasis: An overview. *In* Jaundice, ed. by C. A. Goresky and M. M. Fisher, pp. 329-349, Plenum Press, New York, 1975.
426. SCHAFFNER, F., POPPER, H. AND CHESROW, E.: Cholestasis produced by the administration of norethandrolone. Amer. J. Med. 26: 249-254, 1959.
427. SCHAFFNER, F., POPPER H. AND LEEVY, C. (eds.): The Liver and its Diseases, Intercontinental Book Corp., New York, 1974.
428. SCHAFFNER, F., POPPER, H. AND PEREZ, V.: Changes in bile canaliculi produced by norethandrolone: Electron microscopic study of human and rat liver. J. Lab. Clin. Med. 56: 623-628, 1960.
429. SCHAFFNER, F. AND RAISFELD, I. H.: Drugs and the liver: A review of metabolism and adverse reactions. *In* Advances in Internal Medicine, vol. 5, ed. by G. H. Stollerman, pp. 221-251, Year Book Publishers, Chicago, 1969.
430. SCHAFFNER, F., SCHARNBECK, H. H., HUTTERER, F., DENK, H., GREIM, H. A. AND POPPER, H.: Mechanism of cholestasis. VII. α-Naphthylisothiocyanate-induced jaundice. Lab. Invest. 28: 321-331, 1973.
431. SCHANKER, L. S. AND HOGBEN, C. A. M.: Biliary excretion of inulin, sucrose, and mannitol: Analysis of bile formation. Amer. J. Physiol. 200: 1087-1090, 1961.
432. SCHENKER, S., OLSON, K. N., DUNN, D., BREEN, K. J. AND COMBES, B.: Intrahepatic cholestasis due to therapy of rheumatoid arthritis. Gastroenterology 64: 622-629, 1973.
433. SCHENKMAN, J. B., WILSON, B. J. AND CINTI, D. L.:

Diethylaminoethyl 2,2-diphenylvalerate HCl (SKF-525-A)—*in vivo* and *in vitro* effects of metabolism by rat liver microsomes—formation of an oxygenated complex. Biochem. Pharmacol. 21: 2372–2383, 1972.

434. SCHERB, J., KIRSCHNER, J. AND ARIAS, I. Studies of hepatic excretory function. The effect of 17α-ethyl-19-nortestosterone on sulfobromophthalein sodium (BSP) metabolism in man. J. Clin. Invest. 42: 404–408, 1962.

435. SCHERSTEN, T.: Metabolic differences between hepatitis and cholestasis on the liver. *In* Progress in Liver Diseases, ed. by H. Popper and F. Schaffner, vol. 4, pp. 133–150, Grune & Stratton, New York, 1972.

436. SCHEUR, P. H. AND BIANCHI, L. and an International group: Guidelines for diagnosis of therapeutic drug-induced liver injury in liver biopsies. Lancet i: 854–857, 1974.

437. SCHIFF, L. (ed.): Diseases of the Liver, 3rd ed., J. B. Lippincott, Philadelphia, 1969.

438. SCHIFF, L., CAREY, J. B., JR. AND DIETSCHY, J. M. (eds.): Bile Salt Metabolism. Charles C Thomas, Springfield, Ill. 1969.

439. SCHIMKE, R. T., SWEENEY, E. W. AND BERLIN, C. M.: The role of synthesis and degradation in the control of rat liver tryptophan pyrrolase. J. Biol. Chem. 240: 322–331, 1965.

440. SCHNACK, H.: Zur Aetiologie des Chlorpromazin-Ikterus: Tierexperimentelle und Klinische Beobachtungen Pathol. Microbiol. 27: 419–428, 1964.

441. SCHULZE, P.-J. AND CZOK, G.: Studies on the decrease in bile flow produced by sulfobromophthalein. Toxicol. Appl. Pharmacol. 28: 406–417, 1974.

442. SCHULZE, P.-J. AND CZOK, G.: Reduced bile flow in rats during sulfobromophthalein infusion. Toxicol. Appl. Pharmacol. 32: 213–224, 1975.

443. SCHWARTZ, A., LINDENMAYER, G. E. AND ALLEN, J. C.: The sodium-potassium adenosine triphosphatase: Pharmacological physiological and biochemical aspects. Pharmacol. Rev. 27: 1–134, 1975.

444. SCHWARTZ, S. AND CARDINAL, R.: Non-hemoglobin heme intermediates in the biosynthesis of bile pigments. Medicine 46: 73–81, 1967.

445. SCHWARZ, H. P., HERZ, R., SAUTER, K. AND PAUMGARTNER, G.: Taurocholate induced anticholeresis in the rat. Eur. J. Clin. Invest. 3: 268, 1973.

446. SCHWARZ, L. R., BURR, R., SCHWENK, M., PFAFF, E. AND GREIM, H.: Uptake of taurocholic acid into isolated rat-liver cells. Eur. J. Biochem. 55: 617–623, 1975.

447. SEBESTA, D. G., BRADSHAW, F. J. AND PROCKOP, D. J.: Source of elevated serum alkaline phosphatase activity in biliary obstruction: Studies utilizing isolated liver perfusion. Gastroenterology 47: 166–170, 1964.

448. SEIDEL, D.: A new immunochemical technique for a rapid, semi-quantitative determination of the abnormal lipoprotein (LP-X) characterizing cholestasis. Clin. Chim. Acta 31: 225–229, 1972.

449. SEIDEL, D., AGOSTINI, B. AND MÜLLER, P.: Structure of an abnormal plasma lipoprotein (LP-X) characterizing obstructive jaundice. Biochem. Biophys. Acta 260: 146–152, 1972.

450. SEIDEL, D., ALAUPOVIC, P AND FURMAN, R. H.: A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. J. Clin. Invest. 48: 1211–1223, 1969.

451. SEIDEL, D., ALANPOVIC, P., FURMAN, R. H. AND MCCONATHY, W. J.: A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. J. Clin. Invest. 49: 2396–2407, 1970.

452. SEIDEL, D., BUFF, H. U., FAUSER, U. AND BLEYL, U.: On the metabolism of lipoprotein-X (LP-X). Clin. Chem. Acta 66: 195–207, 1976.

453. SEIDEL, D. AND FELLIN, R.: Behaviour of serum lipoproteins in cholestasis. *In* Intrahepatic Cholestasis, ed. by P. Gentilini, U. Teodori, S. Gorini and H. Popper, pp. 131–140, Raven Press, New York, 1975.

454. SEIDEL, D., GRETZ, H. AND RUPPERT, C.: Significance of the LP-X test in differential diagnosis of jaundice. Clin. Chem. 19: 86–91, 1973.

455. SELYE, H.: Prevention by catatoxic steroids of lithocholic acid-induced biliary concrements in the rat. Proc. Soc. Exp. Biol. Med. 141: 555–558, 1972.

456. SERRONE, D. M. AND FUJIMOTO, J. M.: The effect of certain inhibitors in producing shortening of hexobarbital action. Biochem. Pharmacol. 11: 609–615, 1962.

457. SHARMA, J. N. AND PRASAD, C. R.: Effect of chlorpromazine on the bile secretion in dogs. Indian. J. Med. Res. 55: 258–260, 1967.

458. SHAW, H., CAPLE, I. AND HEATH, T.: Effect of ethacrynic acid on bile formation in sheep, dogs, rats, guinea pigs and rabbits. J. Pharmacol. Exp. Ther. 182: 27, 1972.

459. SHAY, H. AND SIPLET, H.: Relationship of chemical structure of chlorpromazine to its liver-sensitizing action. Gastroenterology 35: 16–24, 1958.

460. SHEFFER, S., CHENG, F. W., DAYAL, B., HAUSER, S., TINT, G. S., SALEN, G. AND MOSBACH, E. H.: A 25-hydroxylation pathway of cholic acid biosynthesis in man and rat. J. Clin. Invest. 57: 897–903, 1976.

461. SHEPRO, D., BELLAMARICH, F. A. ROBBLEE, L. AND CHAO, F. C.: Antimotility effect of cytochalasin B observed in mammalian clot retraction. J. Cell Biol. 47: 544–547, 1970.

462. SHERLOCK, S.: Biliary secretory failure in man. Ann. Intern. Med. 65: 397–408, 1966.

463. SIMON, F. R. AND ARIAS, I. M.: Alterations in liver plasma membranes and their possible role in cholestasis. Gastroenterology 62: 342–345, 1972.

464. SIMON, F. R. AND ARIAS, I. M.: Alteration of bile canalicular enzymes in cholestasis. A possible cause of bile secretory failure. J. Clin. Invest. 52: 765–775, 1973.

465. SKELTON, F. S., WITSCHI, H. AND PLAA, G. L.: Effects of cycloheximide, actinomycin D and ethionine on the biliary excretion of labelled alpha-naphthylisothiocyanate in rats. Exp. Mol. Pathol. 23: 171–180, 1975.

466. SLATER, T. F. AND DELANEY, V. B.: The effects of various drugs and toxic agents on bile flow rate and composition in the rat. Toxicol. Appl. Pharmacol. 20: 157–174, 1971.

467. SMALL, D. M. AND ADMIRAND, W.: Bile salts—Solubility of lithocholates. Nature (London) 221: 265–267, 1969.

468. SMITH, D. S. AND FUJIMOTO, J. M.: alterations produced by novobiocin during biliary excretion of morphine, morphine-3-glucuronide and other compounds. J. Pharmacol. Exp. Ther. 188: 504–515, 1973.

468a. SMITH, R. L.: The Excretory Function of Bile, Chapman and Hall, Ltd., London, 1973.

469. SOLOMON, F. A., JR. AND CAMPAGNA, F. A.: Jaundice due to prochlorperazine (Compazine). Amer. J. Med. 27: 840–843, 1959.

470. SOLYMOSS, B AND ZSIGMOND, G.: Effect of microsomal enzyme inducers on the liver changes produced by common bile-duct ligature. Proc. Soc. Exp. Biol. Med. 147: 430–433, 1974.

471. SONG, C. S., RIFKING, A. B., GILLETTE, P. N. AND KAPPAS, A.: Hormones and the liver. The effect of estrogens, progestins, and pregnancy on hepatic function. Amer. J. Obstet. Gynecol. 105: 813–848, 1969.

472. SONG, C. S., RUBIN, W., RIFKIND, A. B. AND KAPPAS, A.: Plasma membranes of the rat liver. Isolation and enzymatic characterization of a fraction rich in bile

canaliculi. J. Cell Biol. 41: 124–132, 1969.
473. SPUDICH, J. A.: Effects of cytochalasin B on actin filaments. Cold Spring Harbor Symp. Quant. Biol. 37: 585–593, 1972.
474. SPYCHER, M. A. AND RÜTTNER, J. R.: Zur Pathologie des Ikterns. Elektronenmikroskopische Untersuchungen über die Fruhveränderungen der Leberparenchymzellen beim alpha naphthyl-isothiocyanat induzierten Ikterus des Ratte. Pathol. Microbiol. 27: 387–402, 1964.
475. STACHER, G.: Intrahepatische Cholestase nach einer hochdosierten Kombinationstherapie mit Diazepam und Barbituraten bei Tetnuspatientun. Wien. Klin. Wochenschr. 85: 401–406, 1973.
476. STASIEWICZ, J. AND WORMSLEY, K. G.: Functional control of the biliary tract. Acta Hepatogastroenterol. 21: 450–468, 1974.
477. STEFKO, P. L. AND ZBINDEN, G.: Effect of chlorpromazine, chlordiazepoxide, diazepam and chlorprothixene on bile flow and intrabiliary pressure in cholecystectomized dogs. Amer. J. Gastroenterol. 39: 410–417, 1963.
478. STEINER, J. W. AND BAGLIO, C. M.: Electron microscopy of the cytoplasm of parenchymal liver cells in α-naphthyl-isothiocyanate-induced cirrhosis. Lab. Invest. 12: 765–790, 1963.
479. STEINER, J. W., PHILLIPS, M. J. AND BAGLIO, C. M.: Electron microscopy of the excretory pathways in the liver in alpha-naphthyl isothiocyanate intoxication. Amer. J. Pathol. 43: 677–696, 1963.
480. STEPHENS, V. C., PUGH, C. T., DAVIS, N. E., HOEHN, M. M., RALSTON, S., SPARKS, M. C. AND THOMPKINS, L.: A study of the behavior of propionyl erythromycin in blood by a new chromatographic method. J. Antibiot. 22: 551–557, 1969.
481. STIEHL, A.: Bile salt sulphates in cholestasis. Eur. J. Clin. Invest. 4: 59–63, 1974.
482. STIEHL, A., EARNEST, D. L. AND ADMIRAND, W. H.: Sulfation and renal excretion of bile salts in patients with cirrhosis of the liver. Gastroenterology 68: 534–544, 1975.
483. STIEHL, A., RAEDSCH, R. AND KOMMERELL, B.: Increased sulfation of lithocholate in patients with cholesterol gallstones during chenodeoxycholate treatment. Digestion 12: 105–110, 1975.
484. STIEHL, A., THALER, M. M. AND ADMIRAND, W. H.: Effects of phenobarbital on bile salts and bilirubin in patients with intrahepatic and extrahepatic cholestasis. New Engl. J. Med. 286: 858–861, 1972.
485. STIEHL, A., THALER, M. M. AND ADMIRAND, W. H.: Effects of phenobarbital on bile salt metabolism in cholestasis due to intrahepatic bile duct hypoplasia. Pediatrics 51: 992–997, 1973.
486. THALER, M. M.: Effect of phenobarbital on hepatic transport and excretion of ^{131}I-rose bengal in children with cholestasis. Pediat. Res. 6: 100–110, 1972.
487. THOMAS, P. J., HSIA, S. L., MATSCHINER, J. T., DOISY, E. A., JR., ELLIOTT, W. H., THAYER, S. A. AND DAISY, E. A.: Bile acids XIX: Metabolism of lithocholic acid-24-C^{14} in the rat. J. Biol. Chem. 239: 102–105, 1964.
488. THOMPSON, G. R. AND LARSON, R. E.: The hepatotoxicity of 1,3-bis(α-chloroethyl)-1-nitrosourea (BCNU) in rats. J. Pharmacol. Exp. Ther. 166: 104–112, 1969.
489. TICKTIN, H. E. AND ZIMMERMAN, H. J.: Hepatic dysfunction and jaundice in patients receiving triacetyloleandomycin. New Engl. J. Med. 267: 964–968, 1962.
490. TIKKANEN, M. J. AND ADLERCREUTZ, H.: Recurrent jaundice in pregnancy. III. Quantitative determination of urinary estriol conjugates including studies in pruritis gravidarum. Amer. J. Med. 54: 600–604, 1973.
491. TOLMAN, K. G., SANELLA, J. J. AND FRESTON, J. W.: Chemical structure of erythromycin and hepatotoxicity. Ann. Intern. Med. 81: 58–60, 1974.
492. TOTH, I., KENDLER, J., NAGPAUL, C AND ZIMMERMAN, H. J.: Perfusion of the isolated rat liver with phenothiazines. Proc. Soc. Exp. Biol. Med. 140: 1467–1471, 1972.
493. TRAIGER, G. J., DE REPENTIGNY, L. AND PLAA, G. L.: Effect of inhibitors of protein and ribonucleic acid synthesis on the alteration in biliary bilirubin excretion and non-erythropoietically derived bilirubin synthesis in rats after α-naphthylisothiocyanate administration. Biochem. Pharmacol. 23: 2845–2856, 1974.
494. UNGAR, H., MORAN, E., EISNER, M AND ELIAKIM, M.: Rat intrahepatic biliary tract lesions from alphanaphthyl isothiocyanate. Arch Pathol. 73: 427–435, 1962.
495. UNGAR, H. AND POPP, J. A.: Acute cholecystitis and persistent liver necrosis in mice provoked by isothiocyanate. Arch. Pathol. Lab. Med. 100: 127–131, 1976.
495a. UTILI, R., ABERNATHY, C. O. AND ZIMMERMAN, H. J.: Cholestatic effects of *Escherichia coli* endotoxin on the isolated perfused rat liver. Gastroenterology 70: 248–253, 1976.
496. VAILLE, CH., DEBRAY, CH., ROZE, C. AND SOUCHARD, M.: Action of carbutamide upon the biliary flow of the rat. J. Pharmacol. (Paris) 3: 449–458, 1972.
497. VAN THIEL, D. H., DE BELLE, R., MELLOW, M., WIDERLITE, L. AND PHILIPPS, E.: Tolazamide hepatotoxicity: A case report. Gastroenterology 67: 506–510, 1974.
498. VERDY, M., TETREAULT, L., MURPHY, W. AND PERRON, L.: Effect of methandrostenolone on blood lipids and liver function tests. Can. Med. Ass. J. 98: 397–401, 1968.
499. VESSELL, E.: Relationship between drug distribution and therapeutic effects in man. Annu. Rev. Pharmacol. 14: 249–270, 1974.
500. VIAL, J. D., SIMON, F. R. AND MACKINNON, A. M.: Effect of bile duct ligation on the ultrastructural morphology of hepatocytes. Gastroenterology 70: 85–92, 1976.
501. WACHSTEIN, M. AND MEISEL, E.: Histochemistry of hepatic phosphatases at a physiologic pH. Amer. J. Clin. Path. 27: 13–23, 1957.
502. WAITZKIN, L.: Hepatic dysfunction due to chlorpromazine hypersensitivity. Ann. Intern. Med. 49: 607–619, 1958.
503. WALKER, C. O. AND COMBES, B.: Biliary cirrhosis induced by chlorpromazine. Gastroenterology 51: 631–640, 1966.
504. WERTHER, J. L. AND KORELITZ, B. I.: Chlorpromazine jaundice. Analysis of twenty-two cases. Amer. J. Med. 22: 351–366, 1957.
505. WESSELLS, N. K., SPOONER, B. S., ASH, J. F., BRADLEY, M. O., LUDUENA, M. A., TAYLOR, E. L., WRENN, J. T. AND YAMADA, K. M.: Microfilaments in cellular and developmental processes. Science 171: 135–143, 1971.
506. WHEELER, H. O: Secretion of bile acids by the liver and their role in the formation of hepatic bile. Arch. Intern. Med. 130: 533–541, 1972.
507. WHEELER, H. O., ROSS, E. D. AND BRADLEY, S. E.: Canalicular bile production in dogs. Amer. J. Physiol. 214: 866–874, 1968.
507a. WHITFIELD, J. B., POUNDER, R. E., NEALE, G. AND MOSS, D. W.: Serum γ-glutamyl transpeptidase activity in liver disease. Gut 13: 702–708, 1972.
508. WILKINSON, G. R. AND SCHENKER, S.: Drug disposition and liver disease. Drug Metab. Rev. 4: 139–175, 1975.
508a. WILLIAMS, G. M.: The direct toxicity of alpha-naphthylisothiocyanate in cell culture. Chem.-Biol. Interact. 8: 363–369, 1974.

509. WITZLEBEN, C. L.: Manganese-induced cholestasis: Concurrent observations on bile flow rate and hepatic ultrastructure. Amer. J. Pathol. 57: 617-625, 1969.

510. WITZLEBEN, C. L.: Bilirubin as a cholestatic agent: Physiologic and morphologic observations. Amer. J. Pathol. 62: 181-194, 1971.

511. WITZLEBEN, C. L.: Physiologic and morphologic natural history of a model of intrahepatic cholestasis. Amer. J. Pathol. 66: 577-588, 1972.

512. WITZLEBEN, C. L.: Hepatic ultrastructural effects of cholic acid overload. Exp. Mol. Pathol. 16: 47-53, 1972.

513. WITZLEBEN, C. L. AND BOYCE, W. H.: Bilirubin as a cholestatic agent. III. Prevention of bilirubin-related cholestasis by sulfobromophthalein. Arch. Pathol. 99: 492-495, 1975.

514. WITZLEBEN, C. L. AND BOYCE, W. H.: Bilirubin as a cholestatic agent. IV. Effect of bilirubin and sulfobromophthalein (BSP) on biliary manganese excretion. Arch. Pathol. 99: 496-498, 1975.

515. WITZLEBEN, C. L., PITLICK, P., BERGMEYER, J. AND BENOIT. R.: Acute manganese overload. A new experimental model of intrahepatic cholestasis. Amer. J. Pathol. 53: 409-423, 1968.

516. YOUSEF, I. M., MAGNUSSON, R., PRICE, V. M. AND FISHER, M. M.: Bile acid metabolism in mammals. V. Studies on the sex difference in the response of the isolated perfused rat liver to chenodeoxycholic acid. Can. J. Physiol. Pharmacol. 51: 418-423, 1973.

517. ZAMPAGLIONE, N., JOLLOW, D. J., MITCHELL, J. R., STRIPP, B., HAMRICK, M. AND GILLETTE, J. R.: Role of detoxifying enzymes in bromobenzene-induced liver necrosis. J. Pharmacol. Exp. Ther. 187: 218-227, 1973.

518. ZAROWITZ, H. AND FRIEDMAN, I. S.: Jaundice following small amounts of chlorpromazine. New York State J. Med. 57: 1922-1924, 1957.

519. ZBINDEN, G.: Progress in Toxicology. Special Topics, Springer Verlag, New York, 1974.

520. ZELMAN, S.: Liver cell necrosis in chlorpromazine jaundice (Allergic cholangiolitis). Amer. J. Med. 27: 708-729, 1959.

521. ZIMMERMAN, H. J.: Clinical and laboratory manifestations of hepatotoxicity. Ann. N. Y. Acad. Sci. 104: 954-987, 1963.

522. ZIMMERMAN, H. J.: Toxic hepatophathy. Amer. J. Gastroenterol. 49: 39-56, 1968.

523. ZIMMERMAN, H. J.: Drug-induced hepatic disease. Israel J. Med. Sci. 10: 386-397, 1974.

524. ZIMMERMAN, H. J.: Hepatic injury caused by therapeutic agents. *In* The Liver: Normal and Abnormal Functions, part A, ed. by F. F. Becker, pp. 225-302, Marcel Dekker, New York, 1974.

525. ZIMMERMAN, H. J.: Liver disease caused by medicinal agents. Med. Clin. N. Amer. 59: 897-909, 1975.

526. ZIMMERMAN, H. J., KENDLER, J., LIBBER, S. AND LUKACS, L.: Hepatocyte suspensions as a model for demonstration of drug hepatotoxicity. Biochem. Pharmacol. 23: 2187-2189, 1974.

527. ZSIGMOND, G. AND SOLYMOSS, B.: Increased canalicular bile production induced by pregnenolone-16α-carbonitrile, spironolactone and cortisol in rats. Proc. Soc. Exp. Biol. Med. 145: 631-635, 1974.

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