INTRAHEPATIC CHOLESTASIS

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Preface

Intrahepatic cholestasis represents one of the most challenging problems both in clinical medicine and in the understanding of liver function in general. Indeed, the complex history of the concept of this disorder has not only challenged the clinical acumen of the physician at the bedside, but, moreover, the attempt to explain it has led to significant progress in many aspects of hepatology. Far beyond the importance of its incidence in clinical medicine, the study of this disorder serves as an experiment of nature. It has thus fertilized the investigation of jaundice, bile secretion, bile acid metabolism, metabolic alterations of the liver, hepatic immunology, and the radiologic and radionuclide study of the diseased liver. For these reasons the Giovanni Lorenzini Foundation, in cooperation with the Gastroenterological Center of the University of Florence, convened an international symposium in Florence dealing with this problem, bringing together clinical and basic scientists from divergent fields and from many countries.

Over 100 years ago the German pathologist Virchow was puzzled, even with the techniques available then, by the appearance of jaundice in the absence of a demonstrable obstacle in the biliary passages. He hit upon the idea that a catarrhal inflammation in the duodenum may extend into the common duct. A mucous plug was incriminated by him to explain the phenomenon of intrahepatic cholestasis, which was then termed catarrhal jaundice. Although this concept was soon discarded, we are still arguing about the basis of intrahepatic cholestasis, how it should be treated, and what the outcome is for the patient. Modern clinical laboratory techniques have further advanced our considerations, but as of today the full answer is not available.

It has become clear that many key motives have recurred, such as the question as to the elusive mechanical cause of cholestasis at either the microscopic or the ultrastructural level, the function of the biliary excretion of water and bile acids, the role of lipid metabolism, the importance of the drug-handling enzyme system of the liver involved in microsomal biotransformation, and the possibility that crippling cirrhosis may result even if no obstacle is present—in short, the unpredictability of the disease. When we thus review what this book contains, we admire the farsightedness of the organizers to speak of a first volume, implying that there may be another one in years to come to find answers for the questions that have not been

answered and, even more so, to resolve the problems that have been created by giving some preliminary answers. At that future time, advances in physical methods of radiology and radioisotope studies may have taken precedence over biochemical, immunologic, and histologic studies in distinguishing intrahepatic cholestasis from the extrahepatic form. At that time the physician may have a rational therapy at his disposal to replace the merely supportive management available today.

The organizing foundation led by Dr. Gorini, and the organizing clinical scientists Professors Teodori and Gentilini, deserve the sincere thanks of the international medical community for permitting us to focus on one of the most thorny problems in the clinical practice of hepatology.

Hans Popper

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Introduction to the Problem of Intrahepatic Cholestasis

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Intrahepatic cholestasis has now become one of the most important and complex problems in the field of liver disease, from both a pathogenetic and a clinical point of view. Clinically, two difficulties may be encountered: (a) differentiating between intrahepatic and extrahepatic cholestasis, which is often easy but may be very difficult, and (b) distinguishing within the area of intrahepatic cholestasis different anatomoclinical pictures, justified by the different etiology, and, above all, different prognoses.

The pathogenetic problems inherent in intrahepatic cholestasis are even more open to queries. It should be pointed out that according to the most renowned hepatologists, and among these particularly Popper (4), the pathogenetic mechanism of intrahepatic cholestasis tends to shift from a prevalently anatomic to a functional concept. In the past, stress was laid particularly on the obstruction, or at least reduction, of the intrahepatic bile ways, both at the level of the cholangioles and at that of the interlobular ducts. This concept derived mainly from the well-known histologic findings of the so-called primary biliary cirrhosis in which, from the second stage onward, there are findings showing inflammation first and then ductular destruction although accompanied by considerable but inefficient ductular proliferation. Today, however, we know that in numerous diseases characterized by intrahepatic cholestasis this obstructive mechanism either does not intervene at all or intervenes with some delay. Pathogenesis therefore shifts toward a prevalently functional concept, which involves the biligenic activity of the liver cells, also taking into account a possible ductular reabsorption and, consequently, a reduction in bile flow.

A preliminary consideration is necessary: the question of whether cholestasis denotes a more or less marked incapacity to excrete bile *in toto* or whether a "dissociated" cholestasis could be considered to indicate a defect in the excretion of only some of the components. A classic example is the Dubin-Johnson syndrome in which the excretion of conjugated bilirubin is blocked, the latter regurgitating into the sinusoids, whereas the excretion of bile salts, cholesterol, and alkaline phosphatase is perfectly normal. In accordance with Coppo (2), we tend to separate this syndrome from intra-

hepatic cholestasis because it is not actually bile retention that is involved but, rather, bilirubin retention. On the other hand, if we consider the fundamental role played by bile acids, particularly the di- and trihydroxy ones in micelle form, in determining the flow of water and electrolytes in the bile, we then clearly realize that without their retention the bile flow reduction—which today logically appears as the most important functional aspect of intrahepatic cholestasis—in reality does not take place.

Therefore, if a functional change in the liver cells is to be regarded as the pathogenetic basis, it seems logical to consider both bile salt formation and excretion.

It is worth recalling that although only trace amounts of monohydroxy cholic acids are normally present in the bile, in pathologic conditions there may be a considerable increase. This may be due essentially to a hydroxylation defect in the cyclic structure of cholesterol in the smooth endoplasmic reticulum. Excess amounts of mono- or even dihydroxy (chenodeoxycholic acid) bile acids are then formed at the expense of cholic acid, the trihydroxy substance. On the other hand, the prevalence of chenodeoxycholic acid entails excess formation in the intestine of its derivative lithocholic acid, which is then reabsorbed in abnormally high amounts via the enterohepatic circulation. It is also suggested that in some cases changes in the intestinal wall may favor abnormal reabsorption.

The increase in monohydroxy acids entails lower bile flow at cell level, but above all it is the toxic action exerted by lithocholic acid which should be stressed since it causes a cholestatic syndrome both at cell level (causing degenerative changes in the microvilli) and at ductular level (inducing inflammatory and proliferative phenomena). Schaffner and Popper (6) stress that this qualitative change in bile salt secretion causes a cholestatic syndrome, and point out particularly the hydroxylation defect in the smooth endoplasmic reticulum, which seems paradoxically hypertrophic. This hypertrophy does not entail hydroxylation of the bile acids, but rather an increase in the synthesis of cholesterol, phospholipids, and proteins. The increment in cholesterol synthesis may aggravate the situation by providing a greater amount of the precursor for the formation of bile acids which, owing to the hydroxylation defect, are prevalently monohydroxy acids, thus bringing about the aggravation of the situation mentioned above.

I should like to stress that functional changes in liver cells can cause lesions in the canaliculi and ductules through an alteration in bile acid composition, which means that in the genesis of intrahepatic cholestasis interactions are often present between hepatocellular, canalicular, and ductular factors.

The same interactions can again be seen in the genesis of a morphologic finding frequently observed in intrahepatic cholestasis, consisting of intracanalicular bile thrombi, located primarily in the centrilobular zone. The role, if any, of these thrombi in causing bile stasis is probably a very modest

one since they occupy only a limited portion of the vast canalicular bed. It is, rather, the genesis of these bile thrombi which is a problem yet to be solved. A brief mention of the many aspects which this problem presents will suffice in order to fully evaluate its complexity.

One aspect emerges from observations under the light and electron microscopes. The fundamental studies by Biava (1) were carried out both on the material forming the intracanalicular thrombi and on the accumulation of bile pigment within the liver cells in these circumstances. Biava observed formations in the liver cells which sometimes consisted of amorphous material and sometimes had finely fibrillar structures (presumably bilirubin). Other formations contained in juxtacanalicular vesicles had irregular lamellated structures and derived either from the Golgi apparatus or from the smooth endoplasmic reticulum. These were a mixture of lecithin, cholesterol, and bile salts, mainly in the form of liquid crystals. Biava observed both these types of structure in the intracanalicular thrombi. This led him to conclude that bile thrombi result from the intracanalicular precipitation of bilirubin and altered cytoplasmic constituents of the liver cells, following the fragmentation of the microvilli and pericanalicular cell membranes.

A canalicular or, more precisely, a "hepatocellular" factor can therefore be added to the purely mechanical concept of a distal stasis. I use the verb to add and not to substitute because Biava (1) reported the same findings both in the cholestatic forms with no overt obstacle and in forms deriving from extrahepatic obstruction. This would seem to suggest that this hepatocellular process does not play the primary role but only one of the roles in maintaining the cholestatic syndrome. In this connection it should be recalled that cholestasis, whatever its genesis, induces the above-mentioned functional changes in the smooth endoplasmic reticulum: decrease in hydroxylating activity and hypertrophy with excess cholesterol synthesis, giving rise to a vicious cycle which accounts for the persistence of many cholestatic syndromes. Popper and co-workers (5) recently presented a series of experimental contributions showing that bile salt retention in liver cells (even induced by obstructive cholestasis) entails a functional imbalance in the microsomal system.

The same applies to those changes in the protein and enzymatic constituents of the hepatocellular cytoplasmic membranes located near the biliary pole. These changes have been found both in experimentally induced extrahepatic obstruction and in intrahepatic cholestasis caused by ethinyl estradiol (7).

Still in connection with the genesis of bile thrombi, there is the possibility of an abnormal reabsorption of water and electrolytes both at bile canaliculus and at cholangiole level owing to a change in the osmotic pump entailing an abnormal concentration of bile which tends to precipitate under the form of bile thrombi. In addition, because of the altered permeability of the ductules,

the bile salts and other solutes may diffuse into the surrounding tissues also giving rise to a reduction in water flow. Javitt (3) termed this phenomenon "barrier failure." I need hardly mention that, instead, at ductular level we normally have hydrocholeresis, stimulated by secretin and characterized by bicarbonate secretion.

This abnormal reabsorption of water, electrolytes, and bile salts is of interest in the genesis of bile thrombi; it also may play an important role, according to the different forms involved, in the pathogenesis itself of cholestasis.

The evidence presented corroborates the consideration made at the beginning of this chapter concerning the growing importance of functional factors which, through different mechanisms, cause a reduction in bile canaliculus flow.

However, we cannot deny that, at least in some forms, obstructive or destructive phenomena occurring in the cholangioles, in the ductules located in the portal tracts, and finally in the largest septal ducts may actually be of importance. This is the case of primary biliary cirrhosis and above all primary sclerosing cholangitis. I should add that the obstructive mechanism operating in the beginning is then followed by the above-mentioned morphologic and functional changes in the liver cells, so that even in these cases a vicious cycle arises.

Notwithstanding this interaction between the various factors, which makes a clear division difficult, a list of the various mechanisms which may be responsible for intrahepatic cholestasis is given below, following a topographic criterium.

1. Primary hepatocellular changes, causing a qualitatively altered bile secretion and, in particular, a defect in bile acid oxidation.
2. Changes in the intracellular structures (cell membranes, microvilli) which may (a) induce a defect in the excretion of conjugated bilirubin which then regurgitates into the sinusoids and (b) concur to the formation of bile thrombi when these structures fall into the canaliculi.
3. Increased permeability to water and electrolytes of cell membranes and extralobular ductules, consequently leading to the concentration of canalicular bile (changes in the osmotic pump) or even increased permeability to bile salts and other solutes (barrier failure).
4. Obstruction or destruction of the canals of Hering and intralobular bile ductules.
5. Obstruction and/or destruction of the interlobular ductules with periductular inflammatory reaction.
6. Obstruction of the septal ducts depending, above all, on a sclerosing process.

The various anatomoclinical forms of intrahepatic cholestasis are caused mainly by one of the above mechanisms, but this does not necessarily rule

out the possibility that more than one mechanism may be involved in the same clinical form.

SUMMARY

In studies on intrahepatic cholestasis, attention has shifted from the old concept of bile tract obstruction to a functional concept involving both cells and canaliculi and bile ductules.

Functional changes in the liver cells particularly consist of bile acid hydroxylation deficiency. The production of monohydroxy bile acids reduces bile secretion. The excretion of lithocholic acid may damage the entire excretory apparatus from the canalicular microvilli to the ductules.

Functional changes in the canaliculi and cholangioles may entail excess reabsorption of water and electrolytes. Changes in the ductules may determine the diffusion of bile salts into the interstitial spaces, ductular hydrocholeresis therefore not occurring.

Obstructive and/or destructive phenomena occurring in the ductules and bile ducts should, however, still be considered in the pathogenesis of certain types of intrahepatic cholestasis, particularly in primary biliary cirrhosis and sclerosing cholangitis, even though the secondary effects of these phenomena – the morphologic and functional changes in the liver cells – aggravate cholestasis owing to the formation of a vicious cycle.

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Morphologic Aspects of Intrahepatic Cholestasis

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The term cholestasis will be used here in its morphologic sense, i.e., the microscopically demonstrable accumulation of bile pigment in liver tissue (20). Intrahepatic cholestasis is cholestasis caused by a lesion located within the anatomic confines of the liver. The morphologic alterations observed in liver tissue in intrahepatic cholestasis may be subdivided into three groups: (1) features of cholestasis in general, (2) features specific to intrahepatic cholestasis, and (3) features related to the cause of cholestasis.

FEATURES OF CHOLESTASIS IN GENERAL

These can be categorized into essential and secondary features of cholestasis which have been the subject of several extensive recent reviews (20, 46, 47). Figure 1 shows a schematic diagram of the main essential features of cholestasis. Since this subject is largely covered in the next chapter by Popper and Schaffner, the present discussion is limited to a brief review of the possible morphologic pathways of bile regurgitation in cholestasis.

The precise anatomic pathway of bile regurgitation is still hypothetical. The previously held concept of a direct communication between canaliculus and sinusoid (51) has been excluded (39), except in the case of hepatocellular necrosis and rupture of the canaliculi. Such features occur only after cessation of the cell's secretory activity and hence cannot play an important role in the pathogenesis of bile regurgitation and jaundice (54). Ductular reabsorption of bile components has been definitely established (53), although its quantitative importance remains to be elucidated. The major problem still lies around the hepatocyte itself and its intercellular junctions. It remains an open question to what extent the canalicular tight junctions might be or become permeable to water, electrolytes, and other small molecules, allowing an escape of bile solutes from the intercellular canaliculi toward the blood (Fig. 2). In addition to intercellular escape, transhepatocytic regurgitation offers two further possibilities. A first pathway could be a diacytotic process (Fig. 3) with vacuoles pinching off from the bile canaliculus and then fusing with the sinusoidal cell border. Studies with retrograde injection of peroxidase into the bile duct suggest that such a