CHOLESTASIS

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Cholestasis conventionally is considered a visible accumulation of bile in cells and biliary passages by the morphologist, a piling up of biliary substances in the blood by the clinician, a visualized obstruction of the bile ducts by the radiologist, and an inhibition of hepatocellular secretion of all bile constituents by the physiologist [1]. By the last, and most acceptable definition, inhibition of secretion of some bile constituents, for instance of organic anions other than bile salts but including bilirubin (as seen in the Dubin-Johnson or Rotor syndromes), is not cholestasis and does not have its consequences.

Cholestasis may be caused by demonstrable mechanical obstruction of the large bile ducts. Extrahepatic obstruction is associated with jaundice only if it involves an axis from the papilla of Vater to the bifurcation of the common duct. Otherwise, the non-obstructed part will excrete bilirubin in compensatory fashion if liver function is not severely compromised. However, other biochemical parameters, e.g. serum bile acids and alkaline phosphatase activity, may be elevated and the bile duct system in the obstructed portion of the liver may reveal hydrohepatotic dilatation. Enlarged lymph nodes on the hilus of the liver produce obstruction only exceptionally, namely if they are strategically located and invade the duct, while chronic pancreatitis is now considered a frequent cause of mechanical obstruction. Intrahepatic mechanical obstruction is seldom widespread enough to produce jaundice. Examples are sclerosing cholangitis, particularly the forms involving the liver (only rarely, suppurative cholangitis) and intrahepatic biliary atresia (infantile obstructive cholangiopathy). Cancer metastases may produce jaundice mechanically, though metabolic factors (see below) are usually more important.

Far more frequent is intrahepatic cholestasis in the absence of demonstrable bile duct obstruction and accounted for by metabolic processes [2]. Primary intrahepatic cholestasis may result from administration of drugs, intake of alcohol, viral and bacterial infections, or may be of unknown etiology. It develops in acute liver diseases as well as in chronic hepatitis or cirrhosis of any etiology. Cholestasis may be the only clinical and functional aberration, as seen in infants on hyperalimentation with pyloric obstruction or with alpha-1-antitrypsin deficiency, the most frequent cause of neonatal cholestasis. It may result from bacterial infection, particularly in childhood, endotoxins being responsible. It occurs after operations and in Hodgkin's disease and with cancer metastases, sometimes without sufficient mechanical obstruction. It is very rarely seen as the only manifestation in viral hepatitis. It follows
therapy with anabolic steroids and exceptionally intake of contraceptive steroids. Furthermore, it is the basis of recurrent jaundice of pregnancy and of idiopathic recurrent jaundice characterized by many episodes. The last three forms are sometimes familial.

In contrast to the pure form, cholestasis may be a component of hepatitis and cirrhosis of drug, viral, alcoholic, or cryptogenic etiology. The noncholestatic component is frequently obvious on clinical and laboratory examination, but sometimes, particularly in the viral and alcoholic forms, recognized only on biopsy since the cholestatic component predominates clinically and functionally.

While in previous years this intrahepatic cholestasis was explained by a primary inflammatory reaction around the bile ductules ("cholangiolitis"), electron microscopy identified in all forms of cholestasis a characteristic lesion, namely, dilatation of the bile canaliculi, not necessarily uniform throughout the liver, with disfiguration and finally disappearance of their microvilli, widening of the pericanalicular ectoplasm and alterations of hepatocytic organelles around the biliary pole of the hepatocytes. Cholestasis which is initially centrolobular represents thus an alteration of hepatocytic elements, with the dilatation of the canaliculi suggesting a local micromechanical component. The centrolobular location suggests in addition to hydromechanical pressure a preferential hepatocellular organelle lesion from low oxygen tension even in extrahepatic mechanical obstruction, where the obstacle is far distant. Thus, both in mechanical and in metabolic cholestasis the same initial targets exist and this explains the difficulty in histologic and laboratory differential diagnosis. In both forms, bile acid independent and dependent bile secretions are reduced, though only the latter is associated with a morphologic lesion and with jaundice, but impairment of either reduces the other [3].

Bile secretion depends not on hydromechanic, but on osmotic factors and consists of three components, the first two being canicular:

1) The bile salt independent fraction is regulated by the Na⁺-K⁺-ATPase ion pump. Sodium delivered between the microvilli pulls water into the canicular lumen.

2) The bile salt dependent fraction depends less on osmotic action of ionized bile salts, but more of polyanionic micellar aggregates. Most of the bile salts are first taken up by hepatocytes from the blood stream with the help of receptors and little is newly synthesized. The canicular secretion of bile salts is less efficient and thus rate limiting, but still more efficient than that of bilirubin.

3) The secretion of a carbonate-rich bile salt free fluid by bile ducts and ductules is stimulated by secretin. Some reabsorption in the system is also assumed. In normal man the amount of the three secreted components is estimated to be about 200 ml each per day.

Since bile acids are formed greatly by ring hydroxylation from cholesterol in the biotransformation system of the smooth endoplasmic reticulum (SER) and the administration of the monohydroxy bile salt lithocholate produces cholestasis, and since the SER frequently appears altered in cholestasis, a primary alteration of the SER in cholestasis was considered. Morphometry indicated an increased surface density, with normal volume density of the SER; this suggests new formation of non-enzymatic functioning membranes. Moreover, disturbances of the drug metabolism in cholestasis are reported in man, though are still controversial. However, all these manifestations appear delayed and are therefore probably the result rather than the cause
Subsequent studies of lithocholate cholestasis indicated a distortion of the canalicular membrane from lithocholate deposition, associated with excess deposition of cholesterol and release of free cholesterol into the lumen. Therefore, alterations of the canalicular membrane are now considered as the primary event in cholestasis [4]. Abnormal histochemical reactions of the apical, in contrast to the basolateral hepatocytic membrane have been known for a long time and biochemical alterations of membrane cell fractions have been established, particularly increase in cholesterol and triglycerides. A key factor is the demonstrated reduction of the activity of the membranous Na⁺-K⁺ATPase and possibly of other enzymes. They are associated with altered lipid fluidity of the cell membrane. In view of their detergent ability, bile salts influence the ATPase activity and thus not only regulate bile secretion but may have a cholestatic potential. Similar influences may be exerted by cholestatic drugs such as chlorpromazine and sex steroids, though both have additional effects contributing to cholestasis. However, the major difficulty in documentation is the preparation of pure noncontaminated canalicular membrane fractions, but available evidence indicates that plasma membranes and canalicular membranes differ in many respects [5].

Of similar importance are alterations of parts of the cellular cytoskeleton, particularly of the contractile actin microfilaments arranged in the ectoplasm around the bile canalculus and numerous at the tight junction between neighboring hepatocytes. In cholestasis these layers are widened, reflecting a defect in the normal depolymerization of the actin/myosin microfilaments. Cholestasis can be produced experimentally by agents altering microfilaments such as cytochalasin B or phalloidin. Microfilament injury reduces uptake of bile salts. The weakening of the cytoskeleton is the main explanation for the dilatation of the bile canaliculi, though other factors, such as slowing of the flow by bile plugs and ductular alterations, may contribute. Abnormal permeability of the canalicular membrane [6] is also incriminated. This possibly involves the tight junction between hepatocytes. Microvilli as expression of fluid exchange in this location are preserved even when the others have disappeared in cholestasis. Scanning electron microscopy has recently demonstrated a characteristic change on this tight junction in mechanical cholestasis [7].

The ductules also show distinct alterations in cholestasis, like proliferation and abnormalities of their epithelium, with accumulation of bile pigment, probably from reabsorption. Ductular alterations in most instances are presumably secondary to liver cell injury. Moreover, periductular inflammation interferes with bile flow and is a secondary and not, as originally assumed, a primary event.

Thus, to date, the obviously multifactorial pathogenesis of cholestasis is not fully established, while its consequences are far better documented. They are initiated by hepatocellular changes reflected first in rarification and brown pigmentation of the cytoplasm of single hepatocytes (feathery degeneration), characterized electron-microscopically by whorls of phospholipids. Prolonged effects are hydropic swelling of groups of hepatocytes, which might reflect protein retention from disturbance of the tubulin portion of the cytoskeleton as has been assumed for alcoholic liver injury [8], particularly since typical hyalin of Mallory, also explained by an antitubulin action, may be found in prolonged cholestasis. The hepatocytic change is caused by the detergent ef-
fect of retained bile acids. Dihydroxy bile acids are more detergent than trihydroxy ones in vitro and the degree of feathery degeneration parallels the hepatic dihydroxy bile salt content. Degeneration and necrosis of hepatocytes are followed by intralobular and subsequently by portal inflammation. The latter moreover leads to periductular inflammation progressing to periductular fibrosis which in turn produces periportal cholestasis in the peripheral portion of the parenchyma and thus represents a secondary mechanical component in any type of prolonged cholestasis. It accounts, for instance, for persistent interference with bile secretion after surgical correction of extrahepatic biliary obstruction or is a mechanical intrahepatic component in later cholestatic stages of primary biliary cirrhosis. The end stage is biliary cirrhosis of any type.

Cholestasis thus is a process with multiple causes and mechanisms and probably multiple sites of initiation. Any type of hepatocellular injury may be associated with cholestasis, though severe hepatocytic injury, for instance in viral hepatitis, may fail to show cholestasis, which, however, often becomes morphologically prominent in regressing stages. The basic mechanism of cholestasis in liver cell injury is impaired uptake of bile salts by the hepatocytes from the blood, presumably as result of alterations of their receptors. This interrupts the intrahepatic circulation and bile salts are excreted in the urine. Moreover, bile salts leak from the hepatocytes into the blood. Impairment of the normal sulfation increases the detergent effect, while reduction of the hepatocytic bile salt synthesis represents a minor component of the characteristic reduction of canalicular bile salt secretion in cholestasis.

Cholestasis may thus be initiated in different organelles of the hepatocytes, with the canalicular membrane being the preferred location, but endoplasmic reticulum and mitochondrial alterations contribute. These alterations reinforce each other to produce an intrahepatocellular vicious circle. This is linked to a second vicious circle on the level of the cells of the liver in that the hepatocytic injury sets in motion necro-inflammatory and fibrotic processes which interfere hydromechanically with biliary secretion.

The histologic consequences, however, distinguish only to a limited degree the mechanical large bile duct obstruction, mainly extrahepatic, from the intrahepatic metabolic cholestasis since the essential phenomena in both are identical, as are the functional manifestations recognized in hepatic tests. Variations of the basic manifestations thus depend rather on duration and extent. Only secondary histologic phenomena, mainly related to etiology, offer limited diagnostic clues [9]. As an approximation, large bile duct obstruction thus may be assumed if cholestasis is found in combination with edema of the entire portal tracts, tortuosity of bile ducts, particularly on the margin of the tracts, and prominent fibroblasts. However, none of these early criteria are really reliable, while late changes, such as bile lakes and bile infarcts, are more so. In general, significant portal inflammation without segmented leukocytes favors metabolic cholestasis in early jaundice, but after three weeks suggests large bile duct obstruction. The uncertainty of the histologic differential diagnosis points to the practical diagnostic superiority of cholangiographic methods, many of them pioneered in Japan, and other physical techniques, for instance, sonography, to establish large bile duct obstruction.

In conclusion, cholestasis is an initially hepatocytic dysfunction induced by multiple causes and mechanisms. It is associated with alterations of bile ductules, mainly secondary to liver injury from retained bile salts. Periductal and periduc-
tular inflammation and fibrosis produce a secondary mechanical biliary obstruction on the lobular periphery. The various organelle, cellular, and lobular mechanisms reinforce each other, creating a manifold process, best established by physical rather than by chemical or histologic techniques.

References