REVIEW ARTICLE

Biochemistry of bile secretion

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Introduction

Secretion of bile is a unique function of the liver and represents one of its major activities. Bile is both a secretory and an excretory fluid and, as such, its composition is complex and varies according to the nutritional state of the individual.

The secretory functions most prominently include: (a) the delivery to the intestinal tract of bile salts and their associated lipids to aid fat digestion and absorption, and (b) the secretion, in some species, of polymeric IgA (pIgA) to help protect the biliary and upper intestinal tracts from infection. The excretory functions notably include the excretion of liver-derived metabolites of potentially toxic endogenous (e.g. steroid hormones, bilirubin) or exogenous (e.g. drugs, environmental chemicals) materials.

Composition of bile

Hepatic bile, i.e. that draining down through the liver, is essentially an iso-osmotic fluid, its osmoticity reflecting that of the fluid perfusing the organ. The predominant cation is Na^+ and the inorganic anions are Cl^- and HCO_3^- . Organic anions contribute up to about 40 mM, largely bile salts, but also including varying amounts of endogenous or exogenous anions made by liver metabolism; the content of xenobiotic anionic metabolites, if present to any extent, may result in an increase in bile volume (cholerisis). Bile also contains appreciable amounts of lipids, i.e. phosphatidylcholine and cholesterol, and proteins. The proteins include plasma proteins (e.g. albumin), liver-specific proteins (e.g. secretory component, 5'-nucleotidase, lysosomal enzymes) and polymeric IgA. Gall bladder bile has been concentrated and many of the components have risen in concentration accordingly. Other changes also may have occurred during storage in the gall bladder, e.g. addition of mucin, enzymic action, selective reabsorption of ions, etc. (For earlier reviews on general aspects of bile composition and formation, see Javitt, 1976; Forker, 1977; Boyer, 1980; Jones *et al.*, 1980a; Reichen & Paumgartner, 1980; Erlinger, 1981; Klaasen & Watkins, 1984.)

Functional anatomy of the hepatobiliary system

Hepatic portal blood is responsible for about 75% of the afferent supply to the liver, the other 25% being derived from the hepatic artery; both streams mix in the hepatic sinusoids and leave via the hepatic vein. The sinusoids are lined by endothelial, Kupffer, fat and stellate cells; gaps ('fenestrations') between some of these allow passage of plasma, but not erythrocytes, into the space of Disse. Plasma therefore directly bathes the sinusoidal plasma membranes of the hepatocytes which are responsible for the initial stages of bile formation,

and which use mainly plasma-derived materials (see Jones *et al.*, 1980a; Klaasen & Watkins, 1984).

Hepatocytes closest to the afferent blood supply are termed Zone 1 or periportal cells and are exposed to the highest levels of nutrients (from the hepatic portal stream) and oxygen (from the hepatic artery), whereas the cells closest to the efferent outflow, termed Zone 3, perivenous, or centrilobular cells, are exposed to a depleted plasma. Zone 2 cells lie between the two extremes but are less well defined, due to anastomoses in the sinusoids which tend to blur the zones, except at the extremes (see Jones *et al.*, 1980a; Klaasen & Watkins, 1984).

Periportal cells appear to be most active in terms of gluconeogenesis, oxidative metabolism and bile salt transport, whereas centrilobular cells are most active in glycolytic, ketogenic and biotransformation reactions (Gumucio & Miller, 1981; Jungermann & Katz, 1982). Many of these differences result from the direction of the nutrient supply rather than phenotypic gene expression, since they will change in balance if the blood supply is reversed or the cells are isolated and cultured (Hausinger, 1983; Chen *et al.*, 1984; Thurman & Kauffman, 1985). The physiological direction of the blood supply provides for an interception of the incoming stream of xenobiotic materials from the intestine with any endogenous, potentially toxic, materials arriving by the hepatic artery (see Klaasen & Watkins, 1984). Liver-derived metabolites of these substances are usually more polar than their precursor molecules and, after excretion in bile (Smith, 1973), will then be eliminated from the body providing they are not absorbed by the intestines. Where intestinal absorption occurs, the biliary metabolites then undergo an enterohepatic circulation and may result in toxicity (see Chipman, 1982; Klaasen & Watkins, 1984). In the case of the bile salts, an enterohepatic circulation is a physiological necessity and a specific intestinal transporter therefore exists. The absorption of toxic metabolites may be via: (i) simple diffusion, (ii) transporters for normal dietary constituents, or (iii) following their conversion to other (usually less polar) metabolites due to the action of the gut microflora (see Chipman, 1982; Klaasen & Watkins, 1984).

Hepatocyte plasma membranes are differentiated into three domains: (i) sinusoidal, bathed by plasma; (ii) contiguous, the main area of cell-cell contact; (iii) bile canalicular, rich in microvilli and limited by the tight junction. All three domains have been isolated in a relatively pure form and their comparative biochemistry is now emerging (see Evans, 1980). The secretory lumen, the bile canaliculus, is formed between two or more adjacent hepatocytes by an apparent 'zipping together' of the lipoprotein membranes at the tight junction and the structure is stabilized by associated microfilaments and the proximity of desmosomes. Bile canaliculi are

larger and contain more microvilli in periportal than in centrilobular areas (see Jones *et al.*, 1980a; Klaasen & Watkins, 1984); they sometimes appear to penetrate quite deeply into the cell (Motta *et al.*, 1978). Microvilli undergo movement and this may be important in determining bile flow; movement is probably determined by their subjacent cytoskeleton, since interference with the actin network causes cessation of microvillar contractions and reduction in bile flow (cholestasis) (Oshio & Phillips, 1981).

Bile canaliculi are extracellular channels leading, via bile ductules, to ducts in each liver lobe and hence to the common bile duct. This then opens into the duodenum. In some species (e.g. the rat) it receives contributions from small pancreatic ducts along its lower length. Hepatic bile represents canalicular bile modified by ductular processes; canalicular bile is impossible to sample with current techniques.

Techniques for studying bile secretion

Hepatic bile can be obtained from an intact animal by a cannula inserted high up in the common bile duct (Mulder *et al.*, 1981): (i) this fistula bile represents the sum total of whole animal metabolism including liver function; (ii) if the cannula is not tied in correctly, pancreatic juice may contaminate and modify this bile (Billington *et al.*, 1986); (iii) substantial depletion of the enterohepatic circulation may modify biliary composition; (iv) anaesthesia may affect secretory processes. Special techniques, however, allow unsampled bile to be recycled and can also be used to study the effects of anaesthetics (Kuipers *et al.*, 1985).

Collection of bile from an isolated perfused liver allows greater control and standardization of conditions to liver-only functions (Meijer *et al.*, 1981). An important modification is the single-pass perfusion, which allows uptake and secretion kinetics to be studied with greater precision than recycling perfusion (Lowe *et al.*, 1985). Since (rat) livers are normally perfused via the hepatic portal vein only, the isolated liver produces a bile with little ductular modification, as duct cells are normally supplied via the hepatic artery (see Jones *et al.*, 1980a). By reversing the direction of perfusion (orthograde $\rightarrow$ retrograde) the influence of zonation of hepatocyte function can then be studied.

Isolated primary hepatocytes have been much used for cellular uptake and metabolism studies (Fry & Bridges, 1979; Fry, 1981; Forte, 1984), but since bile formation is a function of polarized cells, isolated hepatocytes are of less use in studies of bile secretion *per se*. Recently, however, hepatocyte primary cultures have been maintained under conditions under which tight junctions, and therefore bile canaliculi, reform (see Reid & Jefferson, 1984). Hepatocyte doublets have been used, for example, to demonstrate the secretion of fluorescent cholephiles (Gebhardt & Jung, 1982), the structure of tight junctions (Gebhardt *et al.*, 1982) and the magnitude and direction of the transmembrane potential of the canaliculus membrane (Graf *et al.*, 1984, 1985).

Hepatocyte plasma membrane preparations have made useful contributions to understanding some phenomena, but open or fragmented preparations are of little use for transport studies. Preparations of closed vesicles of defined orientation have recently been obtained from the individual domains of the membrane and these have been particularly useful in defining the

transmembrane driving forces of several transport systems (Inoue *et al.*, 1983; Meier *et al.*, 1984a).

Overall aspects of the processes of bile formation

Although most components originate from plasma, their different compositional balance in bile and plasma indicates that more specific processes have been involved than simple ultrafiltrations.

A sheet of hepatocytes stands as the main barrier between the two fluids. Transcellular transport of single molecules will involve sinusoidal and canalicular transport systems, and cytosolic movement in solution. Where the transcellular movement occurs rather in vesicles, then uptake may involve receptor-mediated or non-specific endocytosis, followed by guided movement of the vesicles across the cell, and exocytosis of vesicle contents at the canalicular pole (see Blitzer & Boyer, 1982; Lowe *et al.*, 1985). During their passage through the cell, by either route, molecules destined for bile may be modified or be joined by others synthesized by the cell.

The barrier properties of the hepatocyte sheet depend largely on the performance of the tight junctions sealing the bile canaliculi (and bile ductules). These not only restrict passage from plasma to bile but also the leak-back of biliary components into the circulation. Tight junctions appear to act as a 'molecular sieve', increasingly restricting molecules of increasing molecular size. In terms both of permeability characteristics and morphology, hepatic junctions appear to be intermediate between tight (e.g. frog skin) and loose (e.g. avian salt gland) epithelia.

Bile is an iso-osmotic fluid, suggesting that the junctions allow passage of water and, to some extent, small ions ($\text{Li}^+ > \text{Na}^+ > \text{K}^+$ and $\text{NO}_3^- > \text{Cl}^- > \text{CH}_3\text{COO}^- > \text{SO}_4^{2-}$). Paracellular permeability of size-matched molecules, e.g. carboxy-inulin (negative) versus methoxy-inulin (neutral), suggests that the tight junctions form a more restricting barrier to negatively charged molecules. This is of physiological importance in that it serves to retain in the biliary tract high- M_r anions (xenobiotic conjugates), bile salt micelles and mixed micelles, and a distinct protein population (see Blitzer & Boyer, 1982; Boyer, 1983; Klaasen & Watkins, 1984; Lowe *et al.*, 1985; Kan & Coleman, 1986).

Bile salt transport

Bile acids are derived from cholesterol. The carboxy group on the side chain is usually conjugated with glycine or taurine. Under physiological conditions they are partly, or wholly, unprotonated and are often referred to as bile salts; conjugated bile salts have the lowest pK values. The properties of these molecules are a balance between the hydrophobic steroid ring structure and the hydrophilic region which results from the juxtaposition of ring hydroxy groups and the charge on the carboxylate or conjugate anion. It is the number, locations and orientations of the ring hydroxy groups which have the most profound effect on the properties of the molecule (Strange, 1981; Hofmann & Roda, 1984; Carey, 1985).

Although (primary) bile salts are made in the liver, the efficiency of the enterohepatic circulation means that most of the molecules in the bile salt pool have been reabsorbed from the intestines, wherein some have undergone partial dehydroxylation (to secondary bile salts) and may also have become deconjugated. In their

next pass through the liver these products are often reconstituted and rehydroxylated; man can readily conjugate, but is less efficient at rehydroxylation than is the rat (Strange, 1981; Heaton, 1972; Schölmerich *et al.*, 1983).

Returning from the intestine to the liver, bile salts are largely bound to albumin; this reduces their loss through the kidney. Under normal loads, hepatic uptake is largely periportal (Jones *et al.*, 1980b; Suchy *et al.*, 1983) but, when periportal cells are selectively damaged with allyl alcohol, bile salts are then effectively secreted by centrilobular cells (Gumucio *et al.*, 1978). The K_m and V_{max} of both groups of cells are in the same range, but centrilobular cells have a lower K_m (Stacey & Klaasen, 1981; Buscher *et al.*, 1987; but see Ugele *et al.*, 1987). This may allow them to exhaust the lower bile salt concentrations following periportal perfusion and to be progressively recruited to work at V_{max} if the bile salt load increases.

Approx. 90% of the taurocholate (cholytaurine) is removed from the perfusing fluid in a single pass through rat, or dog, liver; the proportion of unconjugated salts is lower, 40–55% (O'Maille *et al.*, 1967; Hoffman *et al.*, 1975; Iga & Klaasen, 1981). In different species, the highest uptake rate is often that for the bile salt predominating in the particular species (Aldini *et al.*, 1983). The secretory rate maximum (SR_m) for an individual bile salt is the peak output; after this has been reached output usually declines. It is not therefore a conventional T_{max} , which should remain largely constant (Hardison *et al.*, 1981). The magnitude of SR_m is usually considerably smaller than that of the V_{max} of uptake, showing that sinusoidal uptake phenomena are not the rate-limiting steps in overall secretion (Reichen & Paumgartner, 1976).

Uptake into the hepatocyte against an unfavourable gradient of bile salts is predominantly due to a Na^+ :bile salt electroneutral cotransport system located in the sinusoidal membrane. In vectorial systems [e.g. isolated cells (Van Dyke *et al.*, 1982) or sealed o:o sinusoidal membrane vesicles (Inoue *et al.*, 1982; Duffy *et al.*, 1983; Meier *et al.*, 1984b)], the setting up of an electrochemical gradient of Na^+ will drive the bile salt transporter; reduction of the Na^+ gradient will reduce bile salt transport. The energy input *in vivo* for the creation of the Na^+ gradient is derived from the activity of an Na^+ + K^+ -ATPase in the plasma membrane (Blitzer & Boyer, 1982; Boyer, 1982, 1986; Arias, 1986).

Different bile salts show both saturation kinetics and competition for uptake with one another and it is likely, therefore, that they share the same transport protein(s) (see Strange, 1981; Klaasen & Watkins, 1984; Hardison & Bellentani, 1986). By using radiolabelled and photo-affinity analogues of bile salts, two polypeptides of 56 kDa and 48 kDa have been identified in hepatocytes and plasma membrane preparations; these probably represent the membrane transporter(s) in the sinusoidal membrane (Accatino & Simon, 1976; Anwer *et al.*, 1977; Kramer *et al.*, 1982; von Dippel *et al.*, 1983; Buscher *et al.*, 1987; Levy *et al.*, 1987). Antibody to the 56 kDa polypeptide inhibited Na^+ -linked cotransport (Levy *et al.*, 1987). The 48 kDa polypeptide was labelled with the bile salt derivatives and a number of other compounds, and showed competitive phenomena. This polypeptide may represent a second transporter of wider specificity than for bile salts only; it is independent of

the Na^+ gradient but its driving force is not known (Buscher *et al.*, 1987; Petzinger *et al.*, 1987).

By using bile salt analogues for labelling, a large number of bile salt binding proteins have been identified in liver cytosol (Strange, 1981; Abberger *et al.*, 1983; Sugiyama *et al.*, 1983; Sionim *et al.*, 1984; Henderson *et al.*, 1986). Some of these proteins may be involved in transcytosolic transport, in bile salt metabolism, or in binding other hydrophobic molecules (see Strange, 1981; Blitzer & Boyer, 1984; Klaasen & Watkins, 1984; Stolz *et al.*, 1987). Such protein (and organelle) binding may serve to reduce the effective cytosolic free concentration of bile salts to quite low levels (Strange, 1981; Blitzer & Boyer, 1982).

Transcellular transit time for bile salts is very short, e.g. of the order of 2 min (Lowe *et al.*, 1984), and is compatible with movement in free solution. Such diffusional movement would present the bile salt molecule to the monomolecular transport system in the bile canaliculus membrane. Transport of bile salts associated with a vesicle or organelle, which was suggested originally from proliferation of the Golgi region and of pericanalicular vesicles during active bile salt transport (Boyer *et al.*, 1979; Jones *et al.*, 1979; Goldsmith *et al.*, 1983), and from autoradiographic studies (Jones *et al.*, 1980a; Suchy *et al.*, 1983) (but with rather imprecise localization), is far less likely since transcytotic events appear to be much slower (e.g. IgA takes 20–25 min; Lowe *et al.*, 1985) and are affected by colchicine at concentrations which have much less effect on bile salt transport (Barnwell *et al.*, 1984; Lowe *et al.*, 1985). The explanation of the proliferation of vesicles in the pericanalicular and Golgi regions during bile salt transport may be more related to the provision of biliary lipid (see below).

Intracellular bile salt concentrations, under physiological conditions, although difficult to measure, are of the order of 0.2 mM (Okishio & Nair, 1966; Oh & Du Pont, 1975), but may in reality be much lower in free solution due to the presence of the cytosolic binding proteins. Bile salt concentration in hepatic bile is of the order of 20 mM; bile salts are thus secreted into the canaliculus against a very unfavourable electrochemical gradient. Sealed o:o canalicular membrane vesicles have been used to study this transporter; they can pump bile salts in the physiological direction and have shown saturation kinetics, specificity (conjugates > unconjugated) and competition; the transport is sodium-independent and they appear to derive the energy for this pumping from the membrane potential (Inoue *et al.*, 1982, 1984; Meier *et al.*, 1984a; Boyer, 1986; Arias, 1986).

The potential across the canalicular membrane, measured in isolated cell couplets, is of the order of 40 mV (cytosol is negative) (Graf & Peterson, 1978; Graf *et al.*, 1984, 1985), but this is insufficient to account for the magnitude of the bile salt concentration gradient (Blitzer & Boyer, 1982; Meier *et al.*, 1984a; Boyer, 1986; Arias, 1986) (approx. 100-fold, whereas a 10-fold difference of concentration requires +58 mV). Since bile salts form micelles above their critical micellar concentration, a 'micellar sink' has often been suggested to lower the intraluminal concentration of bile salts effectively to around 2 mM, but recent work with a non-micellar bile salt analogue, which can be secreted into the lumen to yield concentrations of 25–70 mM,

Fig. 1. Possible mechanisms for the secretion of lipid into bile

Bile salt (BS) is secreted via a transport protein. Vesicles, composed of biliary-type lipid, are moved to and fuse with the bile-canalicular membrane by a process that involves the cytoskeleton. The microdomain of lipid thus produced persists until the action of the bile salts, already present in the lumen of the canaliculus, causes the release of the membrane components. Processes A, B and C are discussed in the text.

suggests that other systems than the 'micellar sink' may be operating (O'Maille & Hofmann, 1986).

Biliary lipids

Biliary phospholipid is predominantly (80–95%) phosphatidylcholine with a unique fatty acid pattern, i.e. $C_{16:0}$, $C_{18:2}$ and $C_{16:0}$, $C_{18:1}$ (Balint *et al.*, 1965; Yousef & Fisher, 1975; Kawamoto *et al.*, 1980); this is distinct from the phospholipid and fatty acid profiles seen in biological membranes (Yousef & Fisher, 1975; Kremmer *et al.*, 1976; Evans *et al.*, 1976). Biliary cholesterol is almost entirely unesterified.

In a general sense, biliary lipid secretion is dependent upon bile salt secretion and, at physiological rates of bile salt secretion, the relationship is approximately linear (Hoffman *et al.*, 1975b). This does not hold, however, at very low or very high bile salt outputs (Wheeler & King, 1972; Hardison & Apter, 1972), indicating that other factors are involved.

The effectiveness of the bile salts varies in relation to their overall hydrophobicity; chenodeoxycholate and deoxycholate provoke more lipid secretion per molecule, at lower biliary bile salt concentrations, than does cholate. The maximum rates of phospholipid output are, however, approximately the same in all cases (Barnwell *et al.*, 1986a,b). Very hydrophilic, i.e. non-micelle-forming, bile salt analogues, e.g. taurodehydrocholate, produce very little lipid output (Hardison & Apter, 1972; Barnwell *et al.*, 1984).

Sequence of bile salt and lipid secretions. In bile, the biliary lipids and bile salts are often found associated as mixed micelles and it has been suggested that these mixed micelles might originate inside hepatocytes (Forker, 1977; Reuben & Allen, 1986). This is unlikely however, since intracellular bile salt concentrations (< 0.2 mM) are well below the micellar range (> 2 mM) and bile salt secretion can be separated in time from lipid secretion. By using single-pass perfusion techniques and short

sampling times, it has been shown that bile salt output can precede biliary lipid output by several minutes (Lowe *et al.*, 1984).

This dissection of lipid secretion from bile salt secretion is supported by a number of other observations: (i) the canalicular bile salt transporter is effective at $10\text{--}50\text{ }\mu\text{M}$ (Meier *et al.*, 1984b), which is far below micellar concentration; (ii) colchicine reduces biliary lipid secretion at concentrations having little effect on bile salt secretion (Barnwell *et al.*, 1984); (iii) the non-micellar analogue taurodehydrocholate can be secreted without lipid secretion (Barnwell *et al.*, 1984); (iv) several molecules, e.g. ampicillin (Apstein & Robins, 1982) and iodipamide (Apstein & Russo, 1985), reduce biliary lipid secretion but have little effect on bile salt secretion.

The sequence of events, therefore, seems to be the primary secretion of bile salts into the canaliculus, followed by the biliary lipids and then the secondary association of the bile salts and lipids to form mixed micelles (Lowe *et al.*, 1984; Coleman *et al.*, 1986). In a continuously flowing system, as *in vivo*, the formation of mixed micelles would appear to be continuous but the lipid would not necessarily be forming micelles with the bile salts initially invoking their secretion.

Canalicular processing and lipid resupply. The only sources of lipids available to bile salts in the canalicular lumen are those already present in the canalicular membrane, or shed into the lumen. Several experiments presenting bile salts to the extracytoplasmic surface of intact cells, both erythrocytes and hepatocytes, have shown that these detergents can remove limited amounts of membrane components without causing rupture of the plasma membrane (Billington & Coleman, 1978; Billington *et al.*, 1980). Their effectiveness was dependent upon the composition and location (Coleman *et al.*, 1980) and structural order (Lowe & Coleman, 1981) of the membranes.

The lipid composition of the bile canaliculus membrane is more complicated than that of the biliary lipid (Evans *et al.*, 1976) and, moreover, its overall fluidity is low (Lowe & Coleman, 1982; Storch *et al.*, 1983; Whetton *et al.*, 1983). It has been suggested that biliary lipid may therefore be removed preferentially from more fluid microdomains within the membrane and that this lipid is continuously resupplied, *in vivo*, from within the cell in order to avoid extensive damage (Coleman *et al.*, 1977; Barnwell *et al.*, 1984; Lowe *et al.*, 1984). Some specificity of lipid pattern has been observed on treating bile-canaliculus-rich membrane preparations with bile salts (Yousef & Fisher, 1975), but the absence of the resupply factor limits the resolution of such studies, probably due to the amount of 'biliary lipid' available.

Resupply of lipid to the bile canaliculus might take one of several forms: (i) via specific lipid transport proteins; (ii) as components of the contents of a specific vesicle; (iii) as components of the membrane of a specific vesicle. Colchicine, a microtubule-depolymerizing drug which interferes with vesicle movement, markedly reduces biliary lipid output (Barnwell *et al.*, 1984; Coleman *et al.*, 1986), supporting (ii) and (iii) above, rather than (i). Lipid vesicles supplying plasma membrane components have been reported for other cells (De Silva & Siu, 1981; De Grella & Simoni, 1982; Lange & Matthias, 1984), and an increased number of vesicles have been observed in the vicinity of the bile canaliculus during extensive bile salt secretion (Jones *et al.*, 1979; Boyer *et al.*, 1979; Goldsmith *et al.*, 1983). Most strikingly, this occurred during taurodehydrocholate secretion in which, although bile salt (analogue) is secreted in abundance, little lipid secretion is invoked by this non-membrane-seeking analogue (Barnwell *et al.*, 1984). All of these phenomena point to increased vesicle movement consequent upon bile salt secretion and the observations with dehydrocholate may indicate that continuous exocytosis of vesicle contents is unlikely.

The chain of events during lipid secretion may therefore involve creation of specific microdomains in the canaliculus membrane resulting from the fusion of 'biliary lipid' precursor vesicles, but without a random mixing with the bulk membrane (as is the case in several other cells; see Lowe *et al.*, 1984). These microdomains would be more fluid than the bulk membrane and therefore more susceptible to bile salts; they would then vesiculate outwards, become solubilized directly, leaving behind the bulk, less fluid, membrane more able to withstand the further effects of the bile salts and therefore protecting the cell from damage during normal bile formation (Lowe *et al.*, 1984; Coleman *et al.*, 1986).

Important in these considerations will be the isolation of the putative intracellular vesicles rich in biliary-type lipids, but in view of the large numbers of intracellular vesicles involved in other processes in hepatocytes (Evans & Flint, 1985; Evans & Hardison, 1985) the search does not promise to be an easy one.

Alteration of the rate of bile salt secretion within the physiological range brings about a parallel adjustment in biliary lipid secretion and implies that transport of bile salts is somehow sensed by the cell and affects the control of some part(s) of lipid provision, vesicle assembly, vesicle transport and membrane processing (Rahman *et al.*, 1986). Kinetic experiments interrupting the bile salt supply have shown that control over the signalling can vary and also that a compound not itself invoking

substantial lipid secretion (taurodehydrocholate) can increase the processes of lipid provision or delivery to the bile canaliculus (Rahman & Coleman, 1987). Whether the bile salts are signalling molecules directly, or whether another molecule, e.g. a bile salt binding protein, is involved, and what the target processes are, remain to be established. In this context, the earlier radioautographic findings of the localization of bile salt analogues over the Golgi apparatus and other intracellular sites (Suchy *et al.*, 1983) may provide further clues.

Although different amounts of lipid are secreted per molecule for each bile salt species, the maximum phospholipid secretion rate is similar whatever the bile salt. Beyond this point (the SR_m for each bile salt), secretory parameters decline and the liver becomes cholestatic. Increases in amounts of non 'biliary-type' phospholipids have been detected in bile just beyond the SR_m and it has been suggested that this coincides with the maximum rate of lipid provision and delivery. Plasma membrane preparations from such cholestatic livers show a reduction in phospholipid:cholesterol ratio, suggesting an impaired membrane repair following the excessive bile-salt-induced lipid loss. The efficiency of membrane functions, e.g. transport proteins, in the cholesterol-rich, less fluid membrane which remains may thus decline and be responsible for the reduction in secretory parameters (Barnwell, 1987; Barnwell *et al.*, 1986a,b, 1987; Yousef *et al.*, 1987). Intracellular bile salt concentrations will then rise and may promote the onset of a bile salt toxicity.

Physical form of lipids in bile. Phospholipid and cholesterol are found in bile both as vesicles and as mixed micelles with bile salts (Sömjen & Gilat, 1983, 1985; Sömjen *et al.*, 1986; Carey, 1985); the balance between these two forms is not constant and represents a continuously shifting equilibrium with the less stable vesicles becoming the more stable micelles, depending upon such factors as bile salt concentration and type, location in the biliary tract, storage time (in the gall bladder), temperature, and other less well-defined variables (Sömjen & Gilat, 1985, 1986, 1987; Carey, 1985; Sömjen *et al.*, 1986; Carey & Cohen, 1987).

Lipid vesicles appear to be able to maintain a higher cholesterol:phospholipid ratio than micelles and the conversion of vesicles to micelles thus brings about a more saturated or supersaturated (with cholesterol) bile (Sömjen, 1986). In some cases, due to the presence of nucleating factors or absence of anti-nucleating factors, cholesterol may then precipitate out from equilibrium and form cholesterol crystals and, subsequently, cholesterol gallstones (Bouchier, 1984; Holzbach *et al.*, 1984; Kibe *et al.*, 1985; Sömjen & Gilat, 1986, 1987; Sömjen *et al.*, 1986; Carey & Cohen, 1987; Holzbach, 1987; Harvey *et al.*, 1987).

A second source of biliary cholesterol? In some species, the molecular ratio of cholesterol:phospholipid in bile is not always constant, notably at low bile salt secretion rates (Hardison & Apter, 1972; Wheeler *et al.*, 1973); this may complicate the tendency to develop cholesterol gallstones. One possibility for this variable stoichiometry is a second, non-bile-salt-dependent cholesterol secretory pathway whose influence will be most seen at lower bile salt secretion rates. It is interesting that, in the rat, at low bile salt secretion rates, biliary cholesterol secretion is

more susceptible than phospholipid secretion to phalloidin inhibition and may yield further clues as to the origin of the second source of biliary cholesterol (Rahman & Coleman, 1986).

Organic anions

In addition to the bile salts, a number of other organic anions are cholephilic and their concentrations in bile are sometimes 10–1000 times that of their plasma precursors (Klaasen & Watkins, 1984). Some of these anions are of endogenous origin, e.g. bilirubin diglucuronide, and the glucuronides and sulphates of oestrogens. Other anions are xenobiotics, secreted: (i) directly, e.g. Indocyanin Green, Rose Bengal, tartarazine; (ii) after conjugation, e.g. glucuronides of valproate and phenolphthalein; (iii) after metabolic conversion (Phase I biotransformation), e.g. phenobarbital $\rightarrow$ 5-ethyl-4-hydroxy-phenylbarbituric acid; (iv) after initial metabolism and subsequent conjugation (Phase I and II biotransformations), e.g. biphenyl $\rightarrow$ 4,4'-dihydroxybiphenyl glucuronide (see Smith, 1973).

Phase I metabolism causes the molecule to become more polar; subsequent conjugation with a more strongly ionizable group introduces (or increases) a negative charge on the molecule such that the organic anion generated can then become handled by the anion transporter(s) in the canalicular membrane. In many cases the biotransformed molecules are water-soluble and less toxic than their parent compounds but, in some cases, may be more toxic, either directly, or after subsequent bacterial metabolism (Chipman, 1982). Some of these may cause damage to the biliary system itself (e.g. chlorpromazine, erythromycin, oestrogen glucuronides) (see Klaasen & Watkins, 1984) or act as carcinogens in the biliary tree, especially in the lower part of the biliary and pancreatic ducts (see Chipman, 1982).

Small organic anions, i.e. < 3000 Da, are excreted exclusively by the kidney. When the molecular mass of the anion exceeds a threshold of 325 (rat), 400 (guinea pig), 475 (rabbit) or 500 (man) Da the ions are preferentially excreted into bile. Conjugation with glucuronic acid (+ 176 Da), glutathione (+ 306 Da), etc. serves to increase the molecular mass of the parent compound and may therefore be a necessary prerequisite for its biliary excretion (Smith, 1973; Levine, 1978).

The high biliary:plasma concentration ratio of these organic anions suggest carrier-mediated active transport. This is further supported, in individual cases, by observations of transport maxima, specificity, competition, binding proteins and an energy requirement (Berk & Stremmel, 1986; Klaasen & Watkins, 1984).

Carriage of organic anions, or their precursors, in blood is largely by high-affinity binding to albumin; free concentrations are low. Overall kinetics of uptake by liver cells appear to correlate better with total (albumin-bound + free) concentrations than with the free concentrations and, since the amount of material extracted in a single pass through the liver is often greater than that in free solution, albumin receptors have been postulated to account for anion uptake (see Berk & Stremmel, 1986). The existence of these receptors is, however, still controversial and many of the phenomena can be accounted for by rapid dissociation.

Hepatic uptake of many cholephilic anions does not appear to be via the Na^+ -linked bile salt receptor in the sinusoidal plasma membrane and also itself does not

appear to be sodium-dependent. A mutual competition for hepatocellular uptake of sulphobromophthalein, bilirubin and Indocyanin Green has been observed and, using plasma membrane preparations (mainly sinusoidal), high-affinity binding sites have been characterized. The binding to these of sulphobromophthalein could be inhibited by bilirubin and Indocyanin Green, suggesting that several anions can share a common site. From these membrane preparations a binding protein (subunit mass 55 kDa, but distinct immunologically from the 54 kDa Na^+ -linked bile salt transporter) has been isolated; it has a higher affinity for sulphobromophthalein than does albumin, and may therefore displace the anions from the plasma carrier (see Berk & Stremmel, 1986).

Since intracellular concentrations of the cholephilic anions appear to be higher in bile than in plasma, and their binding affinities to ligandin and other minor intracellular binding proteins are only of the same order as those to albumin, it thus appears that the uptake of anions is unlikely to be by facilitated diffusion but will require some form of energy input. The nature of the energy linkage to the plasma membrane transporter (binding protein) is, however, unclear since: (i) the entry of these anions does not appear to be linked to the sodium gradient; (ii) a direct involvement of ATP appears unlikely and (iii) the membrane potential is unfavourable. The most likely candidate so far appears to be linkage to a Cl^- antiport (Berk & Stremmel, 1986).

After their intracellular movement, possibly associated with ligandin, and any biotransformation which may have occurred (usually on the membranes of the endoplasmic reticulum), the organic anions are subsequently secreted into the bile canalicular lumen. The anion transporter(s) involved have been little characterized, due to their anatomical inaccessibility. What information is available is largely based on inference from comparisons of the characteristics of overall transcellular transport, hepatocellular uptake, and rates of biotransformations (see Klaasen & Watkins, 1984) but the recent availability of sealed vesicles from the canalicular membrane has shown that energy linkage may involve the membrane potential but, as for bile salt transport, this is of insufficient magnitude to account, on its own, for the gradients generated *in vivo* (Boyer, 1986).

The high concentrations in bile of organic anions and their counterions may, in some cases (e.g. valproate glucuronides) be responsible for an osmotic cholerisis; this coincides with the peak output of the anion. Where the anion is radio-opaque, e.g. the conjugates of iodinated compounds such as iopodate, ioglycamide and iodipamide, the concentrative secretion in bile is used to provide X-ray contrast in the biliary tract for clinical diagnoses (Smith, 1973).

The nature and origin of the biliary secretion threshold for anions is intriguing. One possibility for the discrimination is a molecular size specificity shown by the canalicular anion transporter(s); such specificity, however, remains to be demonstrated. Another possibility lies in the molecular sieving properties of the tight junction, i.e. all anions may be secreted but the smallest ones may leak back into plasma across the junction; the different thresholds for each animal species may thus be due to the characteristics of the junctions in each species (Elias *et al.*, 1983). Experiments in which different degrees of damage to junctions have been imposed have

shown that increasing damage results in increasing permeability to molecules of higher M_r , presumably as the discrimination of the molecular sieving is progressively lost (Elias *et al.*, 1984; Kan & Coleman, 1986).

Biliary proteins

The protein content of hepatic bile in many species is in the range 1–5 mg/ml (see La Russo, 1984); in the gall bladder this bile may then be subsequently concentrated or have proteins added to, or removed from, it (see Godfrey *et al.*, 1981; Bouchier, 1983; Reuben, 1984; La Russo, 1984). If there is contamination with protein-rich pancreatic juice (see Billington *et al.*, 1986), protein content may be artificially high.

Proteins in hepatic bile are derived either from: (i) plasma or (ii) from the cells of the biliary system, i.e. hepatocytes and bile duct cells. Plasma proteins appear to make up the major part of the protein complement of bile in most instances (see Mullock *et al.*, 1978, 1985; Reuben, 1984; La Russo, 1984) and, due to the greater relative availability of appropriate antibodies, a larger number of individual 'biliary' plasma proteins have been identified for man than for other species.

General features of entry from plasma. Since most plasma proteins are initially made in the liver their presence in bile could reflect either a direct secretion into bile or an entry from the plasma compartment. Results from isolated perfused rat liver studies essentially eliminate a direct secretion, since perfusion with a medium devoid of rat plasma proteins results both in a substantial fall in total protein concentration (Barnwell *et al.*, 1983a) and a progressive elimination of rat plasma proteins from the bile (Barnwell *et al.*, 1983a; Kloppel

et al., 1986); this indicates that the secretory polarity of the hepatocyte is normally absolute and that the newly synthesized albumin, transferrin, etc. are secreted only via the sinusoidal pole of the hepatocyte (Barnwell & Coleman, 1983). Moreover, the labelling kinetics of several proteins appearing in bile show that these proteins, prior to their appearance in bile, are first delivered to the sinusoidal pole of the cell, i.e. its membrane or its extracellular fluid (Mullock *et al.*, 1985; Kloppel *et al.*, 1986). Only when the secretory polarity is abolished and its microtubular guiding system destroyed, e.g. with colchicine or vinblastine (Barnwell & Coleman, 1983), is there a direct secretion into the bile canaliculus from misdirected secretory vesicles ('blundersomes') (Barnwell & Coleman, 1983; Coleman *et al.*, 1984, 1986).

The normal mode of entry into bile for plasma proteins is, therefore, from the plasma, i.e. they are plasma-derived proteins. This derivation has been demonstrated with: (i) endogenous (radiolabelled) proteins presented in the circulation of animals (Dive *et al.*, 1974) or of isolated livers (Lowe *et al.*, 1985; Kloppel *et al.*, 1986); (ii) exogenous proteins, e.g. horseradish peroxidase and bovine albumin, presented in plasma (Renston *et al.*, 1980; Jones *et al.*, 1982; Schiff *et al.*, 1984) or perfusion fluid (Barnwell *et al.*, 1983a; Lowe *et al.*, 1985; Kan & Coleman, 1986) to isolated livers. Such exogenous proteins are identified in bile by their biological activity, e.g. as enzymes or by antibody reaction, suggesting that they have been derived from plasma as intact molecules. Comparison of the biliary concentrations of these proteins with their concentrations in plasma or perfusion fluid reveals that they fall into two main groups: (i) those with low bile:plasma ratios (< 0.03), and (ii) those with high (or higher) bile:plasma ratios, i.e. $0.03 - > 1$ (see La Russo, 1984; Mullock *et al.*, 1985). Proteins showing the higher ratios are those for which receptor-linked pathways are superimposed upon the common entry pathways exemplified by the low ratio group.

Addition of an exogenous protein to the recycling circulation of an isolated rat liver results in the build-up of its concentration in the bile until a plateau is reached about 20 min after initial presentation (Barnwell *et al.*, 1983a; Kloppel *et al.*, 1986). Presentation as a single short pulse in a single-pass perfusion shows the biliary output to have two components, an initial peak at ~ 5 min and a second peak at ~ 20 min (Lowe *et al.*, 1985). In recycling perfusion the combination of these would clearly result in a plateau after 20 min. The same two kinetic components appear to operate for endogenous proteins, e.g. replacement of plasma with fluid devoid of rat plasma proteins causes disappearance of albumin etc. from the bile of an isolated liver as two kinetic components, one with $t_{\frac{1}{2}} \sim 5$ min, the second with $t_{\frac{1}{2}} \sim 25$ min (Kloppel *et al.*, 1986). Recycling perfusion of isolated livers with protein solutions of different concentration results in parallel increases in biliary protein concentration and therefore a constant bile:perfusate ratio, suggesting that neither of the two kinetic components exhibit saturation kinetics (Thomas *et al.*, 1982; Barnwell *et al.*, 1983a).

Paracellular permeability. One of the mechanisms involved in this non-specific transfer from blood to bile is probably paracellular movement by sieving across the tight junctions between adjacent hepatocytes or bile duct

Fig. 2. Time sequence of appearance in bile of a protein added as a bolus into a single-pass perfusion of an isolated rat liver

The protein (e.g. horseradish peroxidase, 40 kDa) was added to the perfusion fluid at 0 min (arrow) and the output of protein in bile was followed with time. The characteristics of the two transport components are indicated above their respective peaks of output. Derived from Lowe *et al.* (1985). Abbreviation: ANIT, α -naphthylisothiocyanate.

Fig. 3. Schematic representation of possible mechanisms of secretion of various biliary components

The details of the processes can be found in the appropriate part of the text. Abbreviations: BA⁻, bile acid anion; X⁻, organic anion; Y, xenobiotic molecule being transformed to Y-OH and conjugated to form the anionic compound YA⁻; Alb, albumin; -S, receptor for polymeric IgA; S, secretory component; *, polymeric IgA.

cells. This is supported by an inverse relationship to the molecular size, i.e. low- M_r proteins show higher bile:plasma ratios than do large ones, resulting in a different balance between the various plasma proteins in bile and in plasma in relation to their molecular sizes (Dive *et al.*, 1974; Elias *et al.*, 1978; Mullock *et al.*, 1985). This paracellular permeability is probably represented by the initial (~ 5 min) peak in single-pass perfusion (Lowe *et al.*, 1985) which is: (i) coincident with a peak for low(er)- M_r molecules normally considered to enter bile predominantly by the paracellular route (see Lowe *et al.*, 1985); (ii) increased by pre-treatment of the liver with α -naphthylisothiocyanate (Lowe *et al.*, 1985), a drug which increases paracellular permeability in the liver (Krell *et al.*, 1982; Jaescke *et al.*, 1983); (iii) not reduced by inhibitors of vesicle transport, e.g. colchicine (Lowe *et al.*, 1985). Progressive treatment with α -naphthylisothiocyanate brings about a progressive reduction in the molecular sieving effect, resulting in increasing permeability to molecules of higher M_r (Kan & Coleman, 1986). Colchicine, in a way as yet unknown, also appears to increase junctional permeability somewhat (Thomas *et al.*, 1985; Lowe *et al.*, 1985), but much less dramatically than does α -naphthylisothiocyanate (Lowe *et al.*, 1985).

Pinocytosis/transcytosis. The second non-specific pathway from plasma to bile is the non-specific endocytosis (pinocytosis) of plasma components (fluid phase markers) into vesicles at the sinusoidal plasma membrane, vesicular transcytosis and subsequent exocytosis of vesicle contents into bile (Renston *et al.*, 1980; Jones *et al.*, 1982; Reuben, 1984; La Russo, 1984; Coleman *et al.*, 1986). The overall pathway is probably responsible for the second kinetic peak (~ 20 min) of biologically active protein in single-pass perfusions (Lowe *et al.*, 1985). This peak is unaffected by pretreatment of the liver with α -naphthylisothiocyanate but is greatly reduced by colchicine (Lowe *et al.*, 1985). In recycling perfusion a plateau of output is thus reached at about 20–25 min (Barnwell *et al.*, 1983a; Kloppel *et al.*, 1986) but in intact animals the 30 min peak and plateau are more complicated due to mixing kinetics in the plasma compartment (see Lowe *et al.*, 1985).

Vesicles carrying biologically intact (or radiolabelled) proteins have been identified in the pericanalicular cytoplasm within 10 min of initial presentation of a protein pulse (Renston *et al.*, 1980; Jones *et al.*, 1982) and probably represent the protein en route to the bile canaliculus. This transhepatocyte transport, which may have routed via the endosomal compartment, constitutes the 'direct pathway' to the canaliculus (Renston *et al.*, 1980; Jones *et al.*, 1982). It is necessary, however, to stress the importance of the identification of biologically intact proteins in the vesicles, or subsequently in bile, since radiolabelled materials could also be due to degraded protein fragments passing through the lysosome system (see below).

Relative contributions of the two pathways. Pinocytosis (and subsequent transcytosis) is completely indiscriminating in relation to molecular size whereas paracellular sieving discriminates in favour of lower- M_r proteins. The biliary protein profile of many animal species therefore may reflect the relative contributions of the two pathways. Man especially (together with pig, chicken

and sheep) shows a greater influence of the paracellular pathway whereas in the rat (and in dogs, cats and rabbits), the paracellular pathway makes a relatively smaller contribution (see Mullock *et al.*, 1985). It is of interest, therefore, that man shows the greatest threshold for anion excretion whereas the rat shows the lowest threshold and this too may be due to differences in paracellular permeability.

The locations in the liver in which these two pathways are operating are little documented. In the rat, electron microscopy has demonstrated active horseradish peroxidase in the pericanalicular cytoplasm of hepatocytes (Renston *et al.*, 1980; Jones *et al.*, 1982); the role of the duct cells in both pathways is much less clear.

Receptor-mediated endocytosis/transcytosis. For those biliary proteins whose concentrations are significantly higher than would be expected from a combination of paracellular sieving and pinocytosis/transcytosis, receptor-mediated vesicle transports are suspected, or have been identified. Polymeric, mainly dimeric, IgA, i.e. two monomeric IgA joined by a J chain, is a quantitatively important protein in many species, occurring in bile as secretory IgA (sIgA). In rats, for example, secretory IgA is a major biliary protein; it is also present in substantial amounts in some, but not all, humans and in rabbits but is largely absent from sheep and guinea pig bile (see Mullock *et al.*, 1985). Secretory IgA also occurs in bile as a complex with antigen (Socken *et al.*, 1982; Harmatz *et al.*, 1982).

Secretory IgA (~ 385 kDa; Annen *et al.*, 1985) contains polymeric IgA linked to an ~ 80 kDa dalton polypeptide (Kloppel *et al.*, 1983; Sztul *et al.*, 1983), secretory component, which is the proteolysed fragment of a polymeric IgA receptor found on the sinusoidal surface of rat hepatocytes and which protects the polymeric IgA itself from proteolytic digestion. This receptor is a transmembrane glycoprotein of 120 kDa (Sztul *et al.*, 1985) which binds polymeric IgA, polymeric IgA-antigen complexes and, to a lesser extent, polymeric IgM, possibly via the J chain; after endocytosis, receptor and ligand are transported to the canalicular membrane in specific vesicles (Takahashi *et al.*, 1982). This vesicular movement, which probably involves endosomal, but not lysosomal, fusion (Mullock & Hinton, 1981; Mullock *et al.*, 1983), takes about 25 min (Lowe *et al.*, 1985) and can be inhibited by colchicine (Mullock *et al.*, 1980b; Barnwell & Coleman, 1983; Goldman *et al.*, 1983; Lowe *et al.*, 1985). The polymeric IgA, or polymeric IgA-antigen, complex with secretory component is shed into the canaliculus following proteolysis which releases the exposed polypeptide portion of the receptor as secretory component; proteolysis probably takes place at the plasma membrane rather than when the vesicle is in transit. Each polymeric IgA transfer therefore requires a new receptor and therefore the term 'sacrificial receptor' was used to describe the system (Kuhn & Krachenbuhl, 1982); continuous loss of the receptor protein represents considerable metabolic expenditure by the cell (Mullock & Hinton, 1981). This loss of secretory component even occurs when no polymeric IgA is available for transport and thus the bile of a perfused rat liver contains secretory component, without polymeric IgA attached (Mullock *et al.*, 1980c; Kloppel *et al.*, 1983) as a major protein contributor (Kloppel *et al.*, 1986). The role of this continuous movement through the cell may be to

provide both a cleaning of the plasma from polymeric IgA-antigen complexes and to deliver active polymeric IgA for the immunological protection of the biliary and intestinal tracts (Kleinman *et al.*, 1982; Annen *et al.*, 1985). In the rat the polymeric IgA transport system is located in hepatocytes, but in man this system, which is of much lower intensity, appears to reside in bile duct cells (Delacroix *et al.*, 1983, and see Reuben, 1984; Mullock *et al.*, 1985).

Receptor-mediated endocytosis and subsequent transcytosis bypassing lysosomes, i.e. a 'direct pathway', seem to be involved in the biliary secretion of a number of other plasma-derived molecules, e.g. haemoglobin-haptoglobin complex, IgM, β_2 -glycoprotein, caeroplasmin, insulin and asialoglycoproteins (Renston *et al.*, 1980; Mullock & Hinton, 1981; Jones *et al.*, 1982; Schiff *et al.*, 1984; and see Reuben, 1984; Mullock *et al.*, 1985). In most cases the details of the receptors and processes involved are much less well-characterized than for polymeric IgA; for asialoglycoproteins it represents only a small part of the liver uptake (Jones *et al.*, 1982; Schiff *et al.*, 1984) and has been attributed to mis-sorting, probably at the endosome (Schiff *et al.*, 1984).

Lysosomal breakdown products and lysosomal enzymes. Although some of the proteins above, e.g. secretory component, insulin, etc. may have been clipped proteolytically, they are nevertheless delivered to bile still as biologically active molecules and the clipping has taken place at a non-lysosomal site, probably at the canalicular membrane, rather than showing the more vigorous degradations characterizing lysosomal proteolysis (Renston *et al.*, 1980; Jones *et al.*, 1982; Schiff *et al.*, 1984; and see La Russo, 1984; Reuben, 1984). The major part of the cellular uptake of many proteins entering liver cells, however, both by receptor mechanisms (e.g. asialoglycoproteins, low-density lipoprotein, epidermal growth factor) or by pinocytosis (e.g. horseradish peroxidase), is normally routed to lysosomes where it is degraded to small, biologically inert fragments (Renston *et al.*, 1980; Jones *et al.*, 1982; and see La Russo, 1984; Reuben, 1984). Most of these fragments are taken up into cell metabolism but some may enter bile through lysosomal discharge (see La Russo, 1984). If such fragments bear a recognizable label, e.g. ^{125}I , or other recognizable component, e.g. cholesterol from lipoprotein catabolism, then, when this material is discharged into bile, it may be attributed initially to the original protein; molecular sieving chromatography, however, identifies the presence of the small fragments indicating the lysosomal route (Renston *et al.*, 1980; Jones *et al.*, 1982; Schiff *et al.*, 1984). The lysosomal (indirect) route to bile takes at least 30 min before appreciable lysosomal products are released into the canaliculus (Jones *et al.*, 1982; Schiff *et al.*, 1984) in contrast with the non-lysosomal (direct) route for intact proteins which takes about 20 min (see above).

Other lysosomal contents are found in bile, e.g. the lysosomal enzymes acid phosphatase, β -glucuronidase, etc. (see La Russo, 1984); these enzymes may be present in partially inhibited form due to the presence of low- M_r inhibitors (Godfrey *et al.*, 1981). These enzymes, which may amount to 3–5% of the total lysosomal enzyme content per day (La Russo & Fowler, 1979; Godfrey *et al.*, 1981), nevertheless make up only a small part of the biliary protein. Whether they have a function in bile is

not clear, but the intrabiliary hydrolysis of some of the glucuronides could have important implications for enterohepatic cycling of xenobiotic materials and for gallstone formation.

Plasma membrane-derived enzymes. The lysosomal enzymes and secretory component are examples of proteins originated from liver itself, rather than from plasma. Other liver-derived proteins occurring in bile are mucus glycoproteins secreted, particularly, from gallbladder cells (Pearson *et al.*, 1982; Bouchier, 1983; Reuben, 1984) and enzymes derived from the plasma membrane of cells lining the biliary tract. These enzymes, e.g. 5'-nucleotidase, alkaline phosphodiesterase I and leucine aminopeptidase, etc. appear to be released into bile in response to bile salt secretion. Their output is low if the bile salt pool is depleted but rises if bile salt output is increased (Godfrey *et al.*, 1981; Hatoff & Hardison, 1982; Barnwell *et al.*, 1983a,b) and are probably derived mainly from the membrane of the bile canaliculus rather than from duct cells. Bile salts differ in their ability to promote the release of these enzymes, with the effectiveness for the conjugated bile salts in the order deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate > dehydrocholate (Billington *et al.*, 1980; Barnwell *et al.*, 1983a,b). At low bile salt concentrations a proportion of the enzymes can be sedimented (Godfrey *et al.*, 1981; Hatoff & Hardison, 1982); this, coupled with model experiments with the membranes of isolated cells, suggests that the mechanism for their presence in bile may involve an initial microvesiculation of a region of the canaliculus membrane followed by its solubilization as the bile salt concentration in the canaliculus lumen increases (Coleman *et al.*, 1980; Barnwell *et al.*, 1983a). The enzymes appear to have no function in bile, but have been used most effectively for many years as serum indicators of biliary reflux in liver disease affecting the biliary tract.

Cholestasis—defective biliary secretion

It can be seen from what has been written above that bile is a complex fluid and that its formation involves the intermeshing of the secretion of many components. Reduction in bile secretion or of some of its components—cholestasis—is a feature of many liver malfunctions and is often accompanied by elevated levels of biliary components or their immediate precursors in the blood, e.g. bilirubin and its glucuronides. The reduction in bile secretion may occur due to some blockage or constriction within the biliary tract (extrahepatic cholestasis) or due to some defect in liver cells (intrahepatic cholestasis). The latter could be at one or more of many locations: canalicular membrane, tight junction, cytoskeleton, endoplasmic reticulum, individual pumps or enzymes. It is too large a subject to be attempted in the remaining few lines of the Review, but the author hopes that he has given some background to enable the interested reader to approach recent reviews in this area (see Elias, 1981; Tuchweber *et al.*, 1986; Duffy & Boyer, 1986; Phillips *et al.*, 1986).

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