Mathematical model of biliary lipid secretion: a quantitative analysis of physiological and biochemical data from man and other species[1]

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Abstract We propose a simple mathematical model to account for the coupling of secretion rates of bile salts, lecithin, and cholesterol into bile. The model assumes that: *1*) molecules of "biliary" lecithin and cholesterol enter a functional compartment located in the endoplasmic reticulum of the hepatocyte from which they are secreted into bile, and in the case of cholesterol, also catabolized to bile salts; *2*) the rates at which lecithin and cholesterol enter the "secretory" compartment are regulated independently by feedback loops that control their synthesis and/or uptake; *3*) lecithin secretion is coupled by an unknown transport mechanism, possibly micellar or vesicular, to the flux of bile salts passing through the compartment; *4*) cholesterol secretion is coupled by a similar mechanism to lecithin secretion and not to bile salt secretion directly; and *5*) bile salt synthesis is proportional to the cholesterol content of the compartment. The model predicts that in the steady state the dependences, lecithin secretion vs bile salt secretion; cholesterol secretion vs lecithin secretion; and cholesterol secretion vs bile salt secretion, will all have the form of rectangular hyperbolae. Four independent parameters related to the postulated mechanisms of biliary lipid synthesis, uptake, and transport determine the quantitative features of these hyperbolae. These four "secretion parameters" also determine how the biliary lipid composition of hepatic and "fasting" gallbladder bile varies with bile salt secretion rate. A quantitative analysis of biochemical and physiological data on biliary lipid secretion in rat, dog, and man confirms the general predictions of the model. Deductions of the secretion parameters are made for each species and are compared with other relevant data on biliary lipid metabolism. From this analysis, we offer new insights into: *i*) the species differences in biliary lipid secretion and bile composition; *ii*) the influence of obesity on biliary lipid secretion in man; and *iii*) the causes of cholesterol supersaturation in fasting gallbladder bile.—**Mazer, N. A., and M. C. Carey.** Mathematical model of biliary lipid secretion: a quantitative analysis of physiological and biochemical data from man and other species. *J. Lipid Res.* 1984. **25:** 932–953.

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For more than a decade, physiological and biochemical studies have been performed in man (1–4) and other species (5–11) in an effort to identify the mechanisms that control the secretion rates of bile salt (BS), lecithin (L), and cholesterol (Ch) into bile. There is agreement that the transhepatic flux of BS "drives" the hepatocytes to secrete L and Ch, and also to generate the bulk of water and electrolyte flow into bile (1, 6, 7, 12). The apparent coupling of BS secretion (BS_{sec}) to the outputs of L and Ch has been regarded as evidence for the role of mixed micelle formation (13) in the secretion of these otherwise insoluble lipids (2, 5, 6). However, in man (1–3), dog (2, 7), and rat (6, 8, 9), both L secretion (L_{sec}) and Ch secretion (Ch_{sec}) vary nonlinearly as functions of BS_{sec} and tend to reach limiting values at high BS outputs. This suggests that the hepatic synthesis and/or availability of L and Ch can also influence biliary lipid secretion (4, 7–10, 14, 15). Moreover, these nonlinear relationships imply that when bile is formed at low BS outputs, it will be relatively enriched in Ch and L compared with bile formed at high BS outputs (1–3, 12, 16). In humans, whose bile generally contains more Ch than other species (12, 17), these relationships result in the formation of greatly supersaturated hepatic bile (3, 12, 13, 18), especially during fasting when BS_{sec} is lowest (19–21).

Abbreviations: BS, bile salt; L, lecithin; Ch, cholesterol; BS_{sec}, L_{sec}, Ch_{sec}, the biliary secretion rates of BS, L, Ch; Q_L, Q_{Ch}, the quantities of L, Ch in the secretion compartment of the hepatocyte; BS_{syn}, L_{syn}, Ch_{syn}, hepatic synthesis rates of BS, L, Ch; X^H_{BS}, X^H_L, X^H_{Ch}, mole fractions of BS, L, Ch in hepatic bile; X^{GB}_{BS}, X^{GB}_L, X^{GB}_{Ch}, mole fractions of BS, L, Ch in gallbladder bile; $\overline{BS}_{sec}$, the time averaged BS secretion rate during the fasting state; X^{max}_{Ch}, maximum mole fraction of Ch that can be solubilized in BS-L-Ch micellar solutions in vitro.

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In the present work we develop a mathematical model of biliary lipid secretion that provides a quantitative biophysical understanding of the relationships between BS_{sec}, L_{sec}, and Ch_{sec} and the pathophysiological consequences of these relationships (e.g., cholesterol saturation of bile and gallstone formation).

The relevant "structural elements" of the model (e.g., the hepatocellular components involved in the control of biliary lipid secretion) have been based, where possible, on experimental data from the literature. In contrast, the mathematical expressions needed to represent these elements are not known experimentally and must be developed on theoretical grounds. In constructing such expressions we have tried to keep their mathematical complexity to a minimum. As a result it has been possible to derive relatively simple algebraic relationships between BS_{sec}, L_{sec}, and Ch_{sec} which use a total of only four independent parameters (e.g., the "secretion parameters" of our model).

To test the assumptions and predictions of the model, we have analyzed secretion data from the literature on the rat (6, 8, 9), dog (2, 7, 22), and man (1–3), including data from non-obese and obese human beings with and without cholesterol gallstones (23–28). For each species (or subpopulation) the "secretion parameters" have been deduced by regression analysis. A comparison of the parameters between groups and with other data on biliary lipid metabolism offers further checks of the model's consistency and reveals certain striking similarities and differences between man and other species.

In the final section the model is used to analyze the biliary lipid composition (and cholesterol supersaturation) of "fasting" gallbladder bile samples obtained from various human populations. From this analysis, deductions of the "fasting bile salt secretion rate" are made and shown to be important determinants of cholesterol supersaturation in man.

DESCRIPTION OF THE MODEL

1. Biliary lipid secretion compartment

Based upon many biochemical observations (5, 10, 29–33) we postulate (**Fig. 1**) the existence of a functional compartment within the smooth endoplasmic reticulum (SER) of the hepatocyte that contains quantities of L and Ch (denoted Q_L and Q_{Ch}) destined for secretion into bile. Bile salts flux through this "microsomal" compartment[5] and, in their passage, transport molecules of L and Ch to the canalicular membrane and from there into bile. The secretion rates: BS_{sec}, L_{sec}, and Ch_{sec} are presumed to depend on the flux of BS and its coupling to the quantities Q_L and Q_{Ch}. The latter are determined by the balance of input and output of L and Ch to/from the secretion compartment. The canalicular membrane is assumed to play no direct role in regulating the relative lipid secretion rates, consistent with a variety of biochemical (29–31) and morphologic (34–36) studies. Experimental data on the relationship between BS_{sec} and canalicular lecithin content (35) further argues against the canaliculus being the secretory compartment as will be discussed later.

2. Input to the secretion compartment

a. Bile salt input (BS_{in}). BS_{in} to the secretion compartment is derived from both BS uptake (BS_{uptake}) (endogenous or infused) and BS synthesis (BS_{syn}) as shown in Fig. 1. When the enterohepatic circulation is intact, the latter contribution is exceedingly small, averaging 1.5% of the total BS_{in} over a 24-hr period in man (37). Nevertheless, BS_{syn} does have an important physiological role (included in our model) as it provides a quantitatively significant pathway for Ch output from the liver (15).

b. Lecithin input (L_{in}). As inferred from biliary secretion, L_{in} is not strongly influenced by hepatocellular uptake of L in man (4) but is derived from a rapidly turning-over pool of L synthesized in the liver (4, 8, 10, 11, 30, 31). In the rat, biliary synthesis of L occurs almost exclusively in the SER of the hepatocyte (30, 31, 38) and is modulated by the enzyme CDP choline:1,2-diacylglycerol choline phosphotransferase (8, 38) and by dietary choline (8). Based on these facts, we have assumed that the input of L_{in} to the secretion compartment is determined by hepatic L synthesis in all species and that it is regulated homeostatically by the amount of L in the compartment (Fig. 1). Although the quantitative dependence of L_{in} on Q_L is not known experimentally, we have chosen to represent it with a linear relationship (see **Fig. 2A**):

$$L_{in} = L_{max} - \beta_1 Q_L \qquad Eq.\ 1)$$

where L_{max} represents the maximum input (synthesis) rate to the compartment and β_1 quantifies the inhibitory effect (i.e., negative feedback) of Q_L on L_{in}. This equation is similar to that assumed by Hardison and Apter (14) in modelling their biliary lipid secretion data.

c. Cholesterol input (Ch_{in}). On the basis of studies in man (39) and rat (9, 40, 41) it has been shown that biliary Ch is derived from two principal sources (depicted in Fig. 1): *i*) Ch that is newly synthesized in the liver (Ch_{syn}), and *ii*) Ch that is taken up by the liver (Ch_{uptake})

[5] Although the term "microsomes" refers to an in vitro preparation of cellular membranes (33), we use it synonymously with the smooth endoplasmic reticulum from which it is mainly derived.

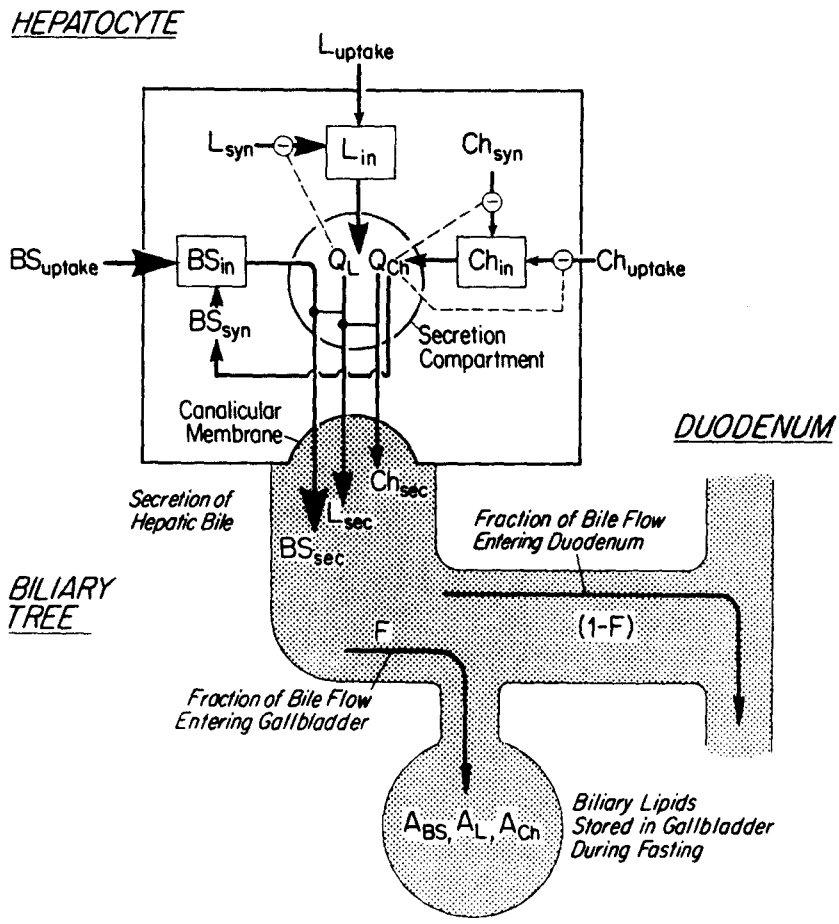

Fig. 1. Schematic model of biliary lipid (bile salt, BS; lecithin, L; cholesterol, Ch) metabolism, secretion, and storage. BS_{uptake}, L_{uptake}, Ch_{uptake} denote the respective uptake rates of the biliary lipids by the hepatocyte. BS_{syn}, L_{syn}, Ch_{syn} denote the intrahepatic synthesis rates. BS_{in}, L_{in}, Ch_{in} denote input rates of the biliary lipids into the postulated biliary secretion compartment (endoplasmic reticulum). Q_L and Q_{Ch} denote the quantities of L and Ch in the compartment. Dashed lines denote feedback mechanisms acting on uptake and synthesis. BS_{sec}, L_{sec}, Ch_{sec} denote the secretion rates of the biliary lipids from the compartment and across the canalicular membrane. (Note that L_{sec} is coupled to BS_{sec}, and Ch_{sec} is coupled to L_{sec}.) A_{BS}, A_L, A_{Ch} denote the amounts of lipid stored in the gallbladder during the fasting state. F and (1-F) indicate the fractions of hepatic bile flow entering the gallbladder and duodenum, respectively (see text for further description).

from various lipoproteins[4] (e.g., chylomicron/VLDL remnants, LDL, and HDL (42–45)). We further assume that the amount of Ch in the secretion compartment, Q_{Ch}, modulates Ch_{in} by negative feedback on both input sources. This view is consistent with a number of observations. Firstly, in the rat hepatic Ch synthesis varies inversely with the amount of influxing chylomicron remnant Ch, an apparent mechanism to homeostatically regulate the Ch content of the liver (46). At the biochemical level, such regulation appears to be provided by the enzyme HMG-CoA reductase, whose activity in the rat has been shown (47, 48) to vary inversely with the concentration of microsomal Ch. Secondly, there is extensive evidence (42–45) documenting a number of feedback mechanisms which influence the hepatic uptake of Ch (mainly esterified) via the regulation of LDL receptors, analogous to those controlling LDL metabolism in extrahepatic tissues (49). Although the biochemical basis of such regulation is not fully understood (42–45), it seems reasonable to suggest that the biliary pool of microsomal Ch (Q_{Ch}) could in part modulate hepatic Ch_{uptake} (via LDL, VLDL, or chylomicron remnant receptors) as illustrated in Fig. 1. As in the case of L_{in}, the functional dependence of Ch_{in} on Q_{Ch} is not known experimentally, and will likewise be approximated using a linear relationship[5] (see Fig. 3A):

[4] This may include Ch that was previously synthesized in the liver.

[5] Although nonlinear "sigmoidal" functions could have been chosen to represent Ch_{in} vs Q_{Ch} and L_{in} vs Q_L, the increased mathematical complexity of these functions would be an unnecessary complication in view of the absence of appropriate experimental data. Similar linear relationships as given in equations *1* and *2* have been used successfully to describe the feedback control of granulopoiesis (see ref. 50).

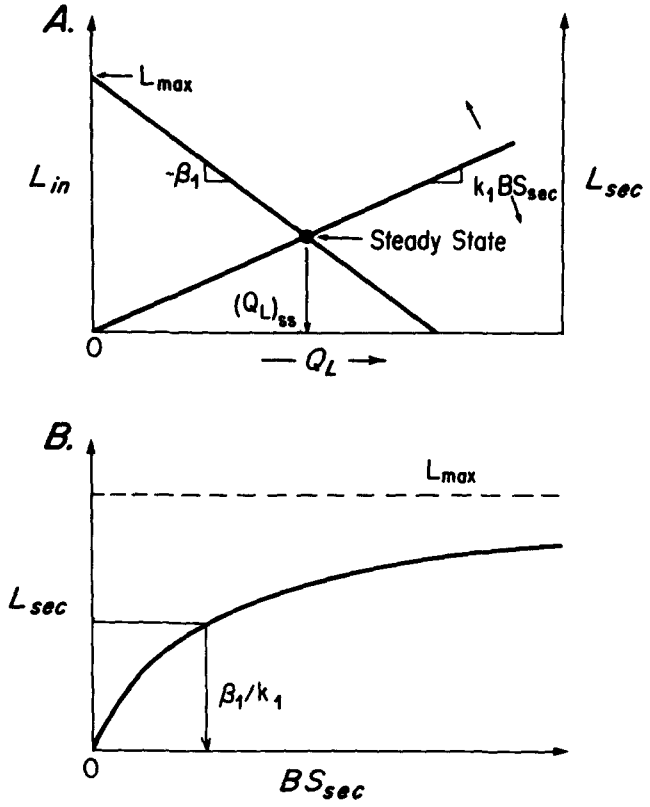

Fig. 2. A, Theoretical dependences of lecithin input (L_{in}) and secretion (L_{sec}) on Q_L, as given by equations *1* and *3*, respectively. Point of intersection of L_{in} and L_{sec} gives the steady state. B, Hyperbolic dependence of L_{sec} vs BS_{sec} given by equation *7*. Maximum secretion approaches L_{max} and β_1/k_1 gives BS_{sec} at which half-maximal secretion occurs.

$$Ch_{in} = Ch_{max} - \beta_2 Q_{Ch} \qquad Eq.\ 2)$$

where Ch_{max} represents the maximum input rate to the secretion compartment from both synthesis and uptake and β_2 reflects the feedback inhibition of Q_{Ch} on both input sources.

3. Output from the secretion compartment

a. Bile salt output (BS_{sec}). As depicted in Fig. 1, bile salts (BS) are presumed to pass through the secretion compartment at the same rate at which they enter. This rate will be identical to BS_{sec} at the canalicular membrane.

b. Lecithin output (L_{sec}). Consistent with in vitro solubilizing capacities of BS for L liquid-crystalline dispersions (13, 51), we assume that the flux of BS passing through the secretion compartment binds ("solubilizes") L and transports it into bile. Quantitatively, we postulate that L_{sec} is proportional to both BS_{sec} and the amount of L in the compartment (Q_L):

$$L_{sec} = k_1 BS_{sec} Q_L \qquad Eq.\ 3)$$

where k_1 is a coupling parameter that describes the capacity of the BS flux to remove L from the compartment. As illustrated in Fig. 2A, L_{sec} increases linearly with Q_L, with a variable slope given by k_1BS_{sec}.

Equation *3* assumes no particular molecular structure for the "solubilized" L and could be consistent with a variety of transport mechanisms. These include: mixed micelles (51), mixed vesicles (52, 53), and even mechanisms that require carrier proteins or microtubular complexes (29).[6] Although k_1 is a kinetic parameter, it is likely to reflect some thermodynamic aspects of BS-L interaction (13, 51–53) and would therefore be expected to depend on the chemical composition of the bile salt pool which differs among species (54) and can be influenced pharmacologically (3, 18, 19).

c. Cholesterol output (Ch_{sec} + BS_{syn}). Ch output into bile occurs by two pathways: *i*) the direct secretion of Ch molecules (Ch_{sec}), and *ii*) the conversion of Ch into BS molecules (BS_{syn}) which are then secreted (15, 46). Although there is some evidence (32, 40, 55) that two subpools may exist for these pathways, we shall assume for simplicity (as did Quarfordt and Greenfield (56)) that a single pool (i.e., Q_{Ch}, Fig. 1) provides Ch for both purposes.

A variety of experimental results (2, 3, 8, 57) suggests that Ch_{sec} is more closely coupled to L_{sec} than to BS_{sec}. The strongest evidence is provided by Robins and Armstrong's study (discussed in detail later) of the influence of dietary choline on Ch_{sec} and L_{sec} in the rat (8). Physical-chemical studies (13, 58) also suggest a more direct coupling between Ch and L outputs, in that Ch is solubilized 3–10-fold greater in BS-L mixed micelles (or mixed vesicles) than in pure BS micelles. For these reasons we assume that Ch_{sec} is linked to L_{sec} by:

$$Ch_{sec} = k_2 L_{sec} Q_{Ch} \qquad Eq.\ 4)$$

where k_2 is a second coupling parameter (see *Eq. 3*)) reflecting the capacity of the L flux to remove Ch from the secretion compartment. Although k_2 defines a physical interaction between L and Ch, different BS species could in principle affect this interaction, similar to their influence on the Ch-solubilizing capacities of BS-L solutions in vitro (13).

The rate-limiting step in the biosynthesis of BS is regulated by the enzyme 7α-hydroxylase (15, 32, 46), and under physiological conditions is believed to be determined by both the amount of active enzyme and the amount of Ch substrate available (32, 59–61). Although newly synthesized Ch may be the preferred substrate for 7α-hydroxylase (40, 55), we will assume

[6] It is not necessary that lecithin be transported intracellularly by micellar aggregates. For example, BS and L form mixed vesicles (52) at BS concentrations that are well below the CMC at which simple or mixed micelles are formed. To be consistent with equation *3*, any mechanism must allow the L/BS ratio of the transported form (i.e., L_{sec}/BS_{sec}) to vary in proportion to Q_L.

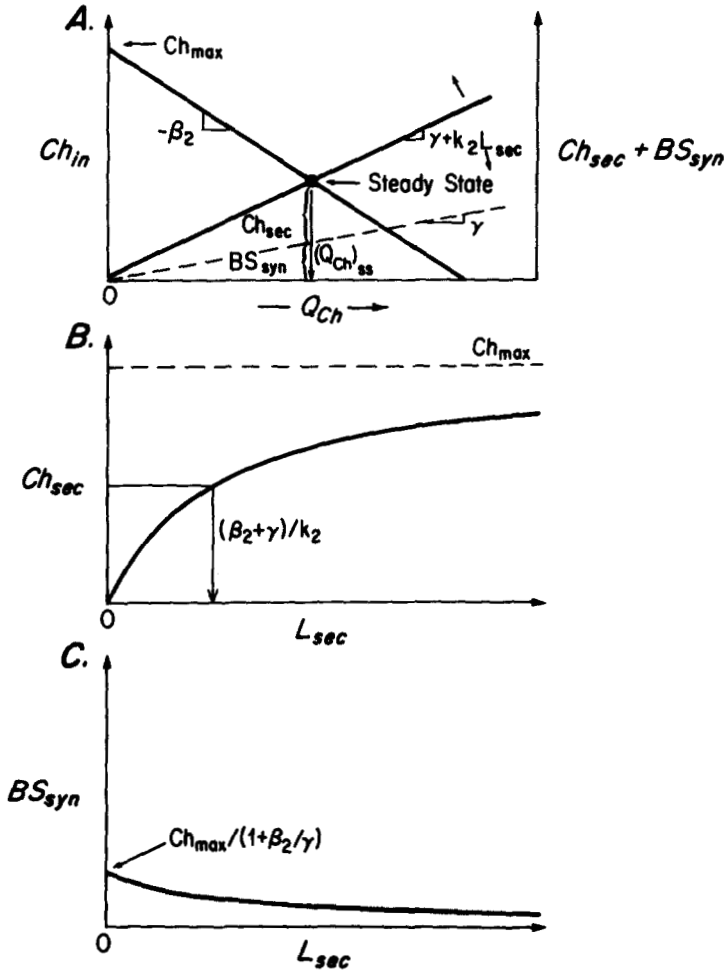

Fig. 3. A, Theoretical dependences of cholesterol input (Ch_{in}), secretion (Ch_{sec}), and conversion to bile salts (BS_{syn}) on Q_{Ch}, as given by equations *2*, *4*, and *5*, respectively. Point of intersection between Ch_{in} and Ch_{sec} + BS_{syn} gives the steady state. B, Hyperbolic dependence of Ch_{sec} vs L_{sec} as given by equation *9*. Maximum secretion approaches Ch_{max}, and $(\beta_2 + \gamma)/k_2$ gives L_{sec} at which half-maximal secretion occurs. C, Theoretical dependence of BS_{syn} on L_{sec} as given by equation *10*.

that the rate of BS_{syn} is proportional to the total content of Ch in the secretion compartment (Q_{Ch}):

$$BS_{syn} = \gamma Q_{Ch} \qquad Eq.\ 5)$$

where the factor γ is a rate constant for the conversion of Ch to BS.[7] While γ may also vary with changes in the concentration of enzyme and effector molecules (including possibly BS and steroid hormones (59, 61)) we assume that such variations play a secondary role in comparison to variations in Q_{Ch} in influencing BS_{syn}.

As shown graphically in **Fig. 3A,** the total Ch output from the secretion compartment (Ch_{sec} + BS_{syn}) increases linearly with Q_{Ch}, with a slope equal to $\gamma + k_2 L_{sec}$.

[7] This equation is similar to that given by Quarfordt and Greenfield (56) in their compartmental model of cholesterol turnover, and would be consistent with the Michaelis-Menten theory of enzyme kinetics (62) under conditions where Q_{Ch} is much less than K_m.

4. Steady state secretory relationships

a. Dependence of L_{sec} on BS_{sec}. In the steady state (ss), the input and output of L to/from the secretion compartment are equal. As shown in Fig. 2A, this corresponds to the point of intersection between the functions L_{in} and L_{sec} and thus determines the L content, $(Q_L)_{ss}$ necessary to maintain the steady state. By setting $L_{in} = L_{sec}$, equations *1* and *3* give the value of $(Q_L)_{ss}$:

$$(Q_L)_{ss} = \frac{L_{max}}{\beta_1 + k_1 BS_{sec}} \qquad Eq.\ 6)$$

and the dependence of L_{sec} on BS_{sec}, which simplifies to:

$$L_{sec} = \left(\frac{L_{max}}{\beta_1/k_1 + BS_{sec}}\right) BS_{sec}. \qquad Eq.\ 7)$$

Equation *7* has the form of a rectangular hyperbola (Fig. 2B) and predicts that with increases in BS_{sec}, L_{sec}

will asymptotically approach L_{max}. The ratio β_1/k_1 corresponds to the value of BS_{sec} at which half-maximal L_{sec} is attained. Although a hyperbolic dependence was theoretically predicted by Wheeler and King (7) to account for L_{sec} in the dog, their model was based on a different set of assumptions and made no connection between L_{syn} and L_{sec}.

b. Dependence of Ch_{sec} on L_{sec}. For Ch, the steady state corresponds to the point of intersection between Ch_{in} and $Ch_{sec} + BS_{syn}$. As illustrated in Fig. 3A, this point is characterized by the Ch content $(Q_{Ch})_{ss}$, the secretion rate, Ch_{sec}, and the BS synthesis rate, BS_{syn}. The mathematical dependences of these variables on L_{sec} can be derived from equations *3–5* and are given by:

$$(Q_{Ch})_{ss} = \frac{Ch_{max}}{\beta_2 + \gamma + k_2 L_{sec}} \qquad Eq.\ 8)$$

$$Ch_{sec} = \left(\frac{Ch_{max}}{(\beta_2 + \gamma)/k_2 + L_{sec}}\right) L_{sec} \qquad Eq.\ 9)$$

$$BS_{syn} = \frac{\gamma}{k_2}\left(\frac{Ch_{max}}{(\beta_2 + \gamma)/k_2 + L_{sec}}\right). \qquad Eq.\ 10)$$

Equation *9* shows that the relationship between Ch_{sec} and L_{sec} is a rectangular hyperbola, with a maximal secretion rate corresponding to Ch_{max}, and half-maximal rate when L_{sec} equals $(\beta_2 + \gamma)/k_2$. In contrast, the dependence of BS_{syn} on L_{sec} (equation *10*) decreases monotonically from a "hypothetical maximum" equal to $Ch_{max}/(1 + \beta_2/\gamma)$. Graphical representations of equations *9* and *10* are shown in Figs. 3B and 3C, respectively.

c. Dependence of Ch_{sec} on BS_{sec}. Although the BS flux is not viewed here as the primary vehicle for Ch secretion into bile, a secondary relationship between Ch_{sec} and BS_{sec} does exist because L_{sec} is "driven" by BS_{sec}. Substituting equation *7* into *9*, we obtain:

$$Ch_{sec} = \left[\frac{Ch_{max}\left(\frac{L_{max}}{(\beta_2 + \gamma)/k_2 + L_{max}}\right)}{\beta_1/k_1\left(\frac{(\beta_2 + \gamma)/k_2}{(\beta_2 + \gamma)/k_2 + L_{max}}\right) + BS_{sec}}\right] BS_{sec}. \qquad Eq.\ 11)$$

This equation also predicts a hyperbolic dependence for Ch_{sec} on BS_{sec}, but implies a maximal secretion rate that is smaller than Ch_{max} by the factor $\frac{L_{max}}{(\beta_2 + \gamma)/k_2 + L_{max}}$. This reflects the fact that L_{sec} can never exceed L_{max}, which in turn limits Ch_{sec} in accordance with equation *9*. Comparison of equation *11* with equation *7* further shows that the value of BS_{sec} required to attain half-maximal Ch_{sec} is smaller than β_1/k_1 (the value for half-maximal L_{sec}) by the factor $\frac{(\beta_2 + \gamma)/k_2}{(\beta_2 + \gamma)/k_2 + L_{max}}$. Thus a plot of Ch_{sec} vs BS_{sec} should flatten off at lower BS_{sec} values than the corresponding plot of L_{sec} vs BS_{sec}. This constitutes one of the major predictions of our model and is ultimately a consequence of the primary coupling of Ch_{sec} to L_{sec} (equation *4*) rather than to BS_{sec}.

The mathematical results derived in Section 4 (a, b, c) show that in the steady state the dependences, L_{sec} vs BS_{sec}; Ch_{sec} vs L_{sec}; and Ch_{sec} vs BS_{sec}, all have the form of rectangular hyperbolae. Quantitatively these relationships are determined by four independent parameters, L_{max}, Ch_{max}, β_1/k_1, and $(\beta_2 + \gamma)/k_2$, which have been defined in terms of the mechanisms of biliary lipid synthesis, uptake, and transport postulated in our model. In the following two sections these "secretion parameters" are further shown to be important determinants of the relative lipid composition of hepatic and gallbladder bile.

5. Dependence of hepatic bile composition on BS_{sec}

The biliary lipid mole fractions X^H_{BS}, X^H_L, and X^H_{Ch} in hepatic bile are related to the respective biliary lipid secretion rates by:

$$X^H_{BS} = \frac{BS_{sec}}{BS_{sec} + L_{sec} + Ch_{sec}} \qquad Eq.\ 12A)$$

$$X^H_L = \frac{L_{sec}}{BS_{sec} + L_{sec} + Ch_{sec}} \qquad Eq.\ 12B)$$

$$X^H_{Ch} = \frac{Ch_{sec}}{BS_{sec} + L_{sec} + Ch_{sec}}. \qquad Eq.\ 12C)$$

By substituting equations *7* and *11* into equations *12A, B, C,* one can obtain the dependences of X^H_{BS}, X^H_L, and X^H_{Ch} on BS_{sec}. As these dependences are somewhat cumbersome to write out explicitly, we shall discuss only their qualitative behavior here and give graphical examples later. By comparing the rate of change of the numerator and denominator in equations *12A, B, C,* it follows that X^H_{BS} will be an increasing function of BS_{sec}, while X^H_L and X^H_{Ch} will generally be decreasing. The magnitude and precise curvature of these functions will depend on the values of the four "secretion parameters."

Quantitatively, it is simpler to express the dependence of the molar ratios X^H_L/X^H_{BS} and X^H_{Ch}/X^H_L on BS_{sec}:

$$\frac{X^H_L}{X^H_{BS}} = \frac{L_{max}}{\beta_1/k_1 + BS_{sec}} \qquad Eq.\ 13A)$$

$$\frac{X^H_{Ch}}{X^H_L} = \frac{Ch_{max}}{(\beta_2 + \gamma)/k_2 + \left(\frac{L_{max}}{\beta_1/k_1 + BS_{sec}}\right) BS_{sec}}. \qquad Eq.\ 13B)$$

In the former case, X_L^H/X_{BS}^H decreases monotonically from an initial value of $L_{max}/(\beta_1/k_1)$, and approaches zero at high BS_{sec}. In the latter case, X_{Ch}^H/X_L^H also decreases (although less rapidly) from an initial value of $Ch_{max}/[(\beta_2 + \gamma)/k_2]$ to a lower limit of $Ch_{max}/[(\beta_2 + \gamma)/k_2 + L_{max}]$. Finally, from equations *13A, B* we can express the values of X_{BS}^H, X_L^H, and X_{Ch}^H in the hypothetical limit of "zero BS_{sec}."

$$X_{BS}^H(0) = \frac{[L_{max}/(\beta_1/k_1)]^{-1}}{[L_{max}/(\beta_1/k_1)]^{-1} + 1 + Ch_{max}/[(\beta_2 + \gamma)/k_2]} \quad \textit{Eq. 14A)}$$

$$X_L^H(0) = \frac{1}{[L_{max}/(\beta_1/k_1)]^{-1} + 1 + Ch_{max}/[(\beta_2 + \gamma)/k_2]} \quad \textit{Eq. 14B)}$$

$$X_{Ch}^H(0) = \frac{Ch_{max}/[(\beta_2 + \gamma)/k_2]}{[L_{max}/(\beta_1/k_1)]^{-1} + 1 + Ch_{max}/[(\beta_2 + \gamma)/k_2]}. \quad \textit{Eq. 14C)}$$

These expressions show that the limiting lipid composition depends on the four secretion parameters, solely in terms of the ratios $L_{max}/(\beta_1/k_1)$ and $Ch_{max}/[(\beta_2 + \gamma)/k_2]$.

6. Composition of fasting gallbladder bile in man

In the fasting state it has been estimated (20, 21) that only a 30–50% fraction (F) of the hepatic bile flow enters the gallbladder, while the remaining fraction of bile flow (1-F) enters the duodenum (see Fig. 1).

The quantities of BS, L, and Ch (A_{BS}, A_L, A_{Ch}) sequestered by the gallbladder over a period of hours (T) will depend on the hepatic secretion rates of the three lipids (which may fluctuate in time) and the fraction F (assumed to be constant) by the following integral relationships:

$$A_{BS} = F\int_0^T BS_{sec}(t)dt \equiv F \cdot \overline{BS}_{sec} \cdot T \quad \textit{Eq. 15A)}$$

$$A_L = F\int_0^T L_{sec}(t)dt \equiv F \cdot \overline{L}_{sec} \cdot T \quad \textit{Eq. 15B)}$$

$$A_{Ch} = F\int_0^T Ch_{sec}(t)dt \equiv F \cdot \overline{Ch}_{sec} \cdot T. \quad \textit{Eq. 15C)}$$

The variables $\overline{BS}_{sec}$, $\overline{L}_{sec}$, $\overline{Ch}_{sec}$ defined here represent the time-averaged hepatic secretion rates over the interval T. If the fluctuations in $BS_{sec}(t)$ are not too large, it can be shown that $\overline{L}_{sec}$ and $\overline{Ch}_{sec}$ will be close to the values of L_{sec} and Ch_{sec} given by equations *7* and *11* with $\overline{BS}_{sec}$ substituted for BS_{sec}.[8] We denote the latter values as $L_{sec}[\overline{BS}_{sec}]$ and $Ch_{sec}[\overline{BS}_{sec}]$.

From equations *15A, B, C* it follows that the mole fractions X_{BS}^{GB}, X_L^{GB}, and X_{Ch}^{GB} corresponding to the lipid composition of gallbladder bile stored over the time interval T will be approximately[9] given by:

$$X_{BS}^{GB} \approx \frac{\overline{BS}_{sec}}{\overline{BS}_{sec} + L_{sec}[\overline{BS}_{sec}] + Ch_{sec}[\overline{BS}_{sec}]} \quad \textit{Eq. 16A)}$$

$$X_L^{GB} \approx \frac{L_{sec}[\overline{BS}_{sec}]}{\overline{BS}_{sec} + L_{sec}[\overline{BS}_{sec}] + Ch_{sec}[\overline{BS}_{sec}]} \quad \textit{Eq. 16B)}$$

$$X_{Ch}^{GB} \approx \frac{Ch_{sec}[\overline{BS}_{sec}]}{\overline{BS}_{sec} + L_{sec}[\overline{BS}_{sec}] + Ch_{sec}[\overline{BS}_{sec}]}. \quad \textit{Eq. 16C)}$$

These equations are identical in form to equations *12A, B, C*. Thus the composition of human gallbladder bile collected after an overnight fast should approximately correspond to the composition of hepatic bile formed at the time-averaged secretion rate, $\overline{BS}_{sec}$, and will depend similarly on the four secretion parameters (which determine the functions $L_{sec}[\overline{BS}_{sec}]$ and $Ch_{sec}[\overline{BS}_{sec}]$). Given knowledge of these parameters, equations *16A, B, C* permit one to deduce the apparent value of $\overline{BS}_{sec}$ corresponding to a particular gallbladder bile composition.

RESULTS AND DISCUSSION

1. Testing predictions of the model

a. Hyperbolic relationships of biliary lipid secretion. To test the quantitative relationships predicted by equations *7, 9,* and *11,* we have compiled biliary lipid secretion data from the literature on rat (6, 8, 9), dog (2, 7, 22), and man (1–3). The latter data were derived from studies of non-obese cholesterol gallstone patients whose bile was obtained post-operatively via T-tube drainage (1–3). In order to control for intraspecies variation in body weight and to provide a basis for interspecies comparison, the experimental secretion rates were normalized per kg total body weight.

The experimental dependences: *a*) L_{sec} vs BS_{sec}, *b*) Ch_{sec} vs L_{sec}, and *c*) Ch_{sec} vs BS_{sec} are plotted for rat, dog, and man in **Figs. 4(A, B, C), 5(A, B, C),** and **6(A, B, C),** respectively. A least squares fit of equations *7* and *9* was performed[10] on the pooled experimental data

[8] Using the data on $BS_{sec}(t)$ given in reference 20 and the secretion parameters deduced for non-obese subjects (Table 3), we calculate $(\overline{L}_{sec} - L_{sec}[\overline{BS}_{sec}])/L_{sec}[\overline{BS}_{sec}]$ and $(\overline{Ch}_{sec} - Ch_{sec}[\overline{BS}_{sec}])/Ch_{sec}[\overline{BS}_{sec}]$ to be −6.2 and −7.9%, respectively.

[9] The approximation is due to the use of $L_{sec}[\overline{BS}_{sec}]$ and $Ch_{sec}[\overline{BS}_{sec}]$ in place of $\overline{L}_{sec}$ and $\overline{Ch}_{sec}$. For the example mentioned in footnote 8, the mole fractions given by equations *16A, B, C* are all within +2 to −7% of the exact values.

[10] The fitting procedure was based on the statistical method given by Johansen and Lumry (63). In analyzing the dependences of L_{sec} vs BS_{sec} and Ch_{sec} vs L_{sec}, we assumed the experimental uncertainties of the ordinates (either L_{sec} or Ch_{sec}) to be proportional to their value.

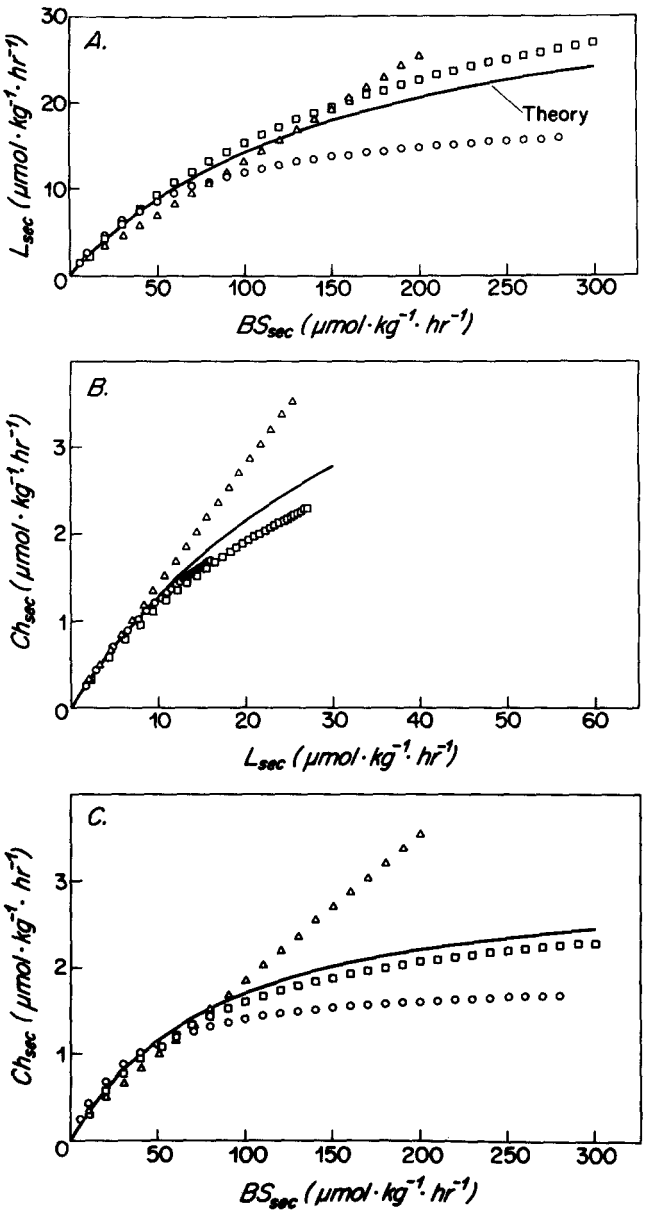

Fig. 4. Biliary lipid secretion data from studies in the rat: O, Hardison and Apter (6); Δ, Turley and Dietschy (9); □, Robins and Armstrong, choline deficient (8). A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; C, Ch_{sec} vs BS_{sec}. Plotted points are derived from regression curves fitted to original data (normalized per kg body weight) and have been spaced in proportion to the number of actual measurements. Solid curves in A and B result from least squares fit of equations *7* and *9* to all three data sets. Solid curve in C is based on equation *11* using parameters derived from panels A and B.

for each species and the resulting hyperbolic functions (including equation *11*) have been plotted as theoretical curves in the respective figures. These curves account well for the non-linearity of the secretion data, which are clearly seen in all species. It is further evident that the dependence of Ch_{sec} on BS_{sec} reaches half-maximal secretion at a smaller BS_{sec} rate than the dependence of L_{sec} on BS_{sec}. This finding confirms an important prediction of the model (see section 4C) and is a necessary consequence of the hyperbolic dependence of Ch_{sec} on L_{sec}. Numerical deductions of the four secretion parameters for each species are given in **Table 1** and will be discussed in detail later.

b. Relationship of L_{sec} to L synthesis. According to equation *7* of our model, the maximum rate of L_{sec} reflects

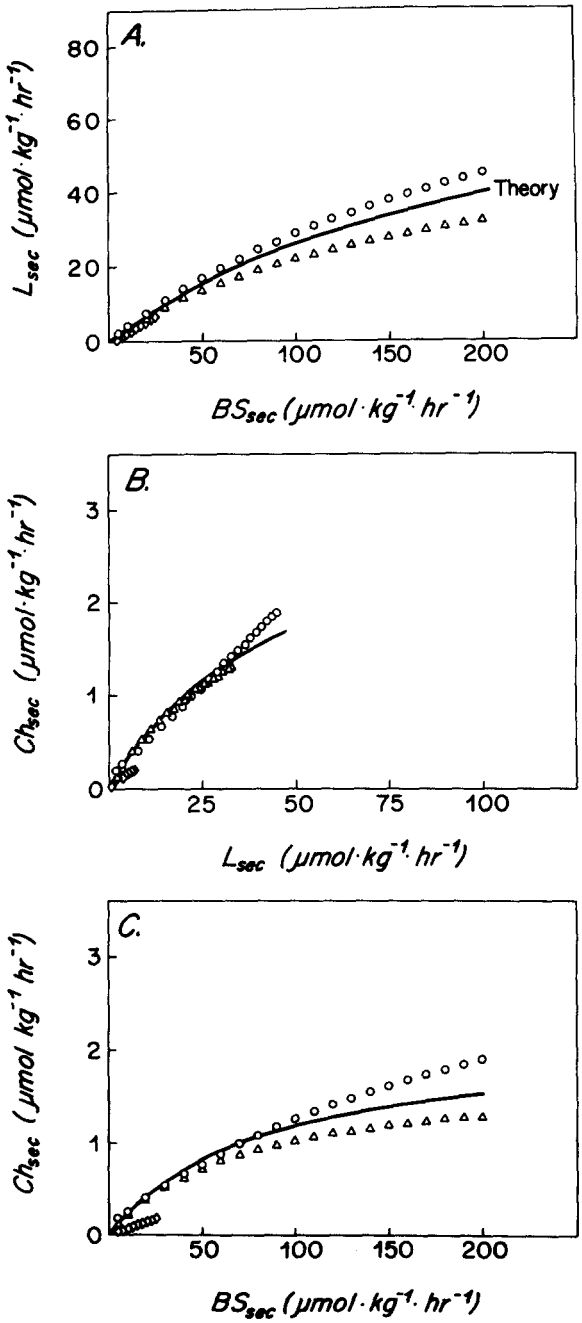

Fig. 5. Biliary lipid secretion data from studies in the dog: O, Wheeler and King (7); Δ, Wagner et al. (2); ◇, Hoffman et al. (22). A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; C, Ch_{sec} vs BS_{sec}. Plotted points and theoretical curves are derived as in Fig. 4.

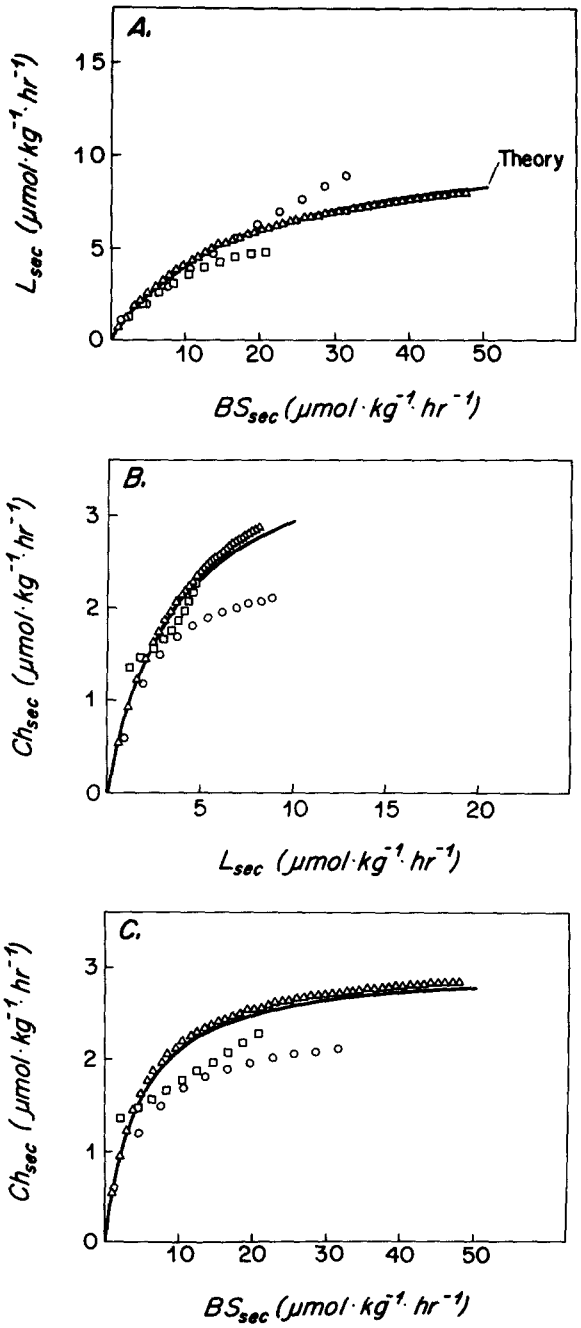

Fig. 6. Biliary lipid secretion data from studies in man, using postoperative T-tube drainage of non-obese cholesterol gallstone patients: O, Lindblad et al. (3); Δ, Wagner et al. (2); □, Scherstén et al. (1). A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; C, Ch_{sec} vs BS_{sec}. Plotted points and theoretical curves are derived as in Fig. 4.

the liver's maximum capacity to synthesize and deliver L to the biliary secretion compartment (L_{max}).[11]

Robins and Armstrong (8) studied biliary lipid secretion in rats that were either deprived of, or supplemented with, dietary choline, an essential precursor of L biosynthesis. Their data for L_{sec} vs BS_{sec} are plotted for the two rat populations in **Fig. 7A.** Fitting these data to equation 7 gives L_{max} values of 81.4 and 46.3 μmol·kg^{-1}·hr^{-1} in the choline-supplemented and -deficient states, respectively; while the parameter β_1/k_1 is approximately the same in both groups (Table 1). This twofold difference quantifies the important influence of L_{syn} on L_{sec}, and is consistent with Robins and Armstrong's observation that the rate of ^{32}P incorporation into biliary L was twofold higher in the choline-supplemented group (8).

Earlier workers have estimated that total hepatic L_{syn} in "normal" rats[12] ranges from ~100 to ~300 μmol·kg^{-1}·hr^{-1} (64). Synthesis rates of this magnitude would be able to provide sufficient L for biliary secretion even under maximum conditions (e.g., $L_{sec} \approx L_{max}$). Recently, Robins and Brunengraber (65) estimated hepatic L synthesis to be only ~10 μmol·kg^{-1}·hr^{-1} in the isolated perfused rat liver and concluded that exogenous (plasma) L is needed to maintain the biliary L pool. This conclusion is at variance with the results of many previous studies (4, 8, 10, 11, 30, 31, 38).

The experimental finding that BS infusion increases the rate of hepatic L synthesis in rat (38), sheep (66), and man (4) provides additional support for the relationship between L_{sec} and L_{syn}, postulated in our model. This phenomenon is readily explained in Fig. 2A (and equation 6) which shows that an increase in BS_{sec} leads to a decrease in $(Q_L)_{ss}$, and thereby reduces the inhibition on L_{syn}. The apparent stimulation of L_{syn} by BS is thus a secondary feedback effect rather than a direct influence on the synthetic apparatus, a conclusion similar to those of Balint et al. (38), Nilsson and Scherstén (4), and Hardison and Apter (14).

Several lines of indirect evidence support the view that the smooth endoplasmic reticulum of the hepatocyte (or some portion of it) corresponds to the biliary lipid secretion compartment of our model. In a quantitative electron micrographic analysis of rat liver sections, Jones et al. (34) found a lobular gradient in the quantity of SER which varied oppositely to the lobular gradient for BS uptake and secretion demonstrated by Gumucio and Katz (67). The SER content of centrilobular hepatocytes was also found to vary inversely with bile flow under conditions of selective biliary obstruction (34). Although such studies showed variation in other organelles (i.e., Golgi-rich area), they nevertheless demonstrate that the SER, taken as a whole, varies inversely with BS_{sec} and

[11] In this regard the biliary lipid secretion model proposed by Hardison and Apter (14) is similar to ours. However, the model of Wheeler and King (7) makes no such prediction.

[12] This refers to rats fed "normal diets," without choline supplementation. It is possible that such rats become choline-deficient during prolonged biliary drainage.

TABLE 1. Deductions of biliary lipid secretion parameters for rat, dog, and man

Species	Weight	L_{max}	β_1/k_1	Ch_{max}	$(\beta_2 + \gamma)/k_2$	γ/k_2 (range)[a]	$\frac{L_{max}}{\beta_1/k_1}$	$\frac{Ch_{max}}{(\beta_2 + \gamma)/k_2}$
	kg			$\mu mol \cdot kg^{-1} \cdot hr^{-1}$				
Rat								
Pooled	0.2 ± 0.1	38.0 ± 2.5[b]	167.6 ± 13.5	6.8 ± 0.8	43.2 ± 5.6	16–30	0.23[c]	0.16[c]
Choline deficient	0.14 ± 0.04	46.3 ± 5.1	206.5 ± 26.2	5.4 ± 0.9	36.1 ± 6.4		0.22	0.15
5% Choline supplement	0.17 ± 0.02	81.4 ± 13.0	206.6 ± 34.8	10.1 ± 4.2	64.6 ± 27.4		0.39	0.16
Dog	15 ± 5	83.2 ± 9.4	221.2 ± 27.4	3.6 ± 0.3	53.0 ± 4.7	38–53	0.38	0.07
Man (non-obese gallstone patients)	65 ± 5	11.2 ± 0.3	18.5 ± 0.7	4.0 ± 0.2	3.6 ± 0.3	0.5–2	0.61	1.11

[a] The range for γ/k_2 is derived from inequalities (Eq. *18*).
[b] Represents estimate of the standard deviation as determined from the residuals of the fit (ref. 63).
[c] These quantities are dimensionless molar ratios.

thus exhibits the functional behavior of the secretion compartment implied by equation *6*. In contrast, Nemchausky, Layden, and Boyer (35) have shown that the amount of *canalicular* L in rats actually *increases* following a taurocholate infusion. This finding argues against the canaliculus being the secretory compartment and presumably results from the increased movement of "microsomal" L across the canalicular membrane.

c. Relationship between Ch_{sec} and L_{sec}. Equation *4* postulates the existence of a single rate-controlling transport mechanism that couples the movement of L and Ch from the secretion compartment into bile. Direct evidence for such a mechanism is provided by Robins and Armstrong's study of choline-deficient and -supplemented rats (8). In contrast to the large difference in L_{sec} vs BS_{sec} for both groups (Fig. 7A), the dependences of Ch_{sec} vs L_{sec} exhibit considerable overlap (Fig. 7B). Although analysis of the two data sets gives different values for Ch_{max} and $(\beta_2 + \gamma)/k_2$ (Table 1), these differences are not statistically significant and the result-

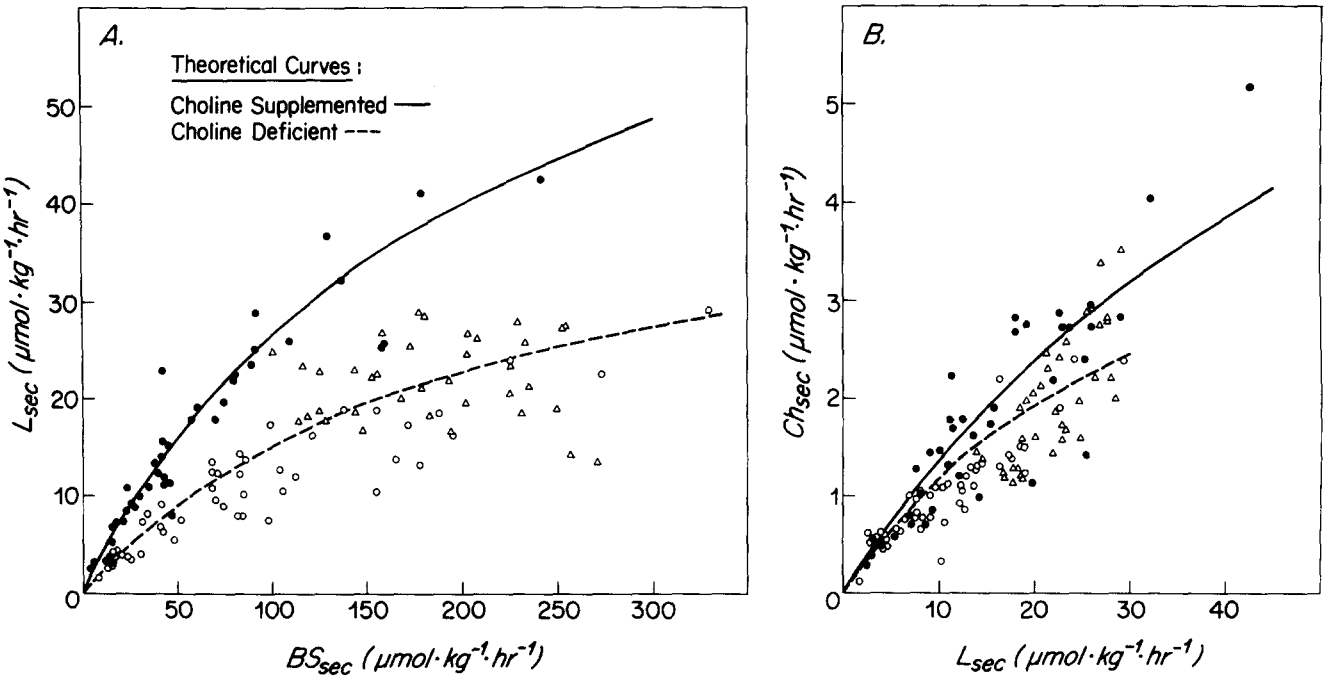

Fig. 7. Biliary lipid secretion data from studies in choline-deficient and choline-supplemented rats after Robins and Armstrong (8). A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; ●, from bile salt depletion experiments in 5% choline-supplemented rats; ○, from bile salt depletion experiments in choline-deficient rats; Δ, from taurocholate infusion studies in choline-deficient rats. Plotted points represent original data normalized per kg body weight. Solid curves in A and B are derived from least squares analysis of the data using equations *7* and *9*, respectively.

ing hyperbolic functions describe quite similar curves. As pointed out by Robins and Armstrong (8), this striking similarity offers strong evidence for the primary coupling between Ch_{sec} and L_{sec}, and could not easily be explained if Ch_{sec} were directly coupled to BS_{sec}.

It is important to point out that the hyperbolic dependence of Ch_{sec} vs L_{sec} predicted in equation *9* accounts well for the experimental data seen in all species (Figs. 4B, 5B, 6B, and 7B). Based on the nonlinearity of these and similar data, previous investigators (7, 14, 16) have postulated multiple Ch transport mechanisms or have suggested altered *coupling* (and uncoupling) between Ch_{sec} and L_{sec} at high and low BS_{sec} rates. Our model shows that the former assumption is not *necessary* to explain the secretion data, per se. It further shows that the ratio Ch_{sec}/L_{sec} need not remain constant, but instead varies in proportion to Q_{Ch} (equation *4*), whose value changes in order to maintain a steady state between cholesterol input, secretion, and catabolism to BS (equation *8*). Although mixed micellar and liquid crystalline phases containing Ch have been observed in model (58) and native (53, 68) bile, it is conceivable that one or both of these phases are formed after the biliary lipids are secreted into the canalicular lumen. As yet the intracellular and transcanalicular mechanisms of biliary lipid transport remain to be elucidated experimentally.

2. Species variation of biliary lipid secretion and hepatic bile composition

a. Comparison of secretion parameters in rat, dog, and man. In deducing and comparing physiological or pharmacokinetic parameters between species it is generally not known a priori whether the "raw data" should be normalized by body weight, a particular organ weight, or some more complex allometric function (69, 70). As the ratio of liver weight-to-body weight varies only slightly among the species (0.035 rat, 0.030 dog, 0.025 man; ref. 71) we believe that the body weight normalization employed in Figs. 4–6 should provide a useful starting point for an interspecies comparison of the biliary secretion parameters.

The L_{max} values (Table 1) show a marked variation, ranging from 11.2 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ in man to 83.2 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$ in the dog. In view of the similarity in L_{sec} for choline-deficient rats (8) and those fed "standard" diets (6, 9) (Fig. 4A), all three studies were combined to assess the secretion parameters for "normal" rats (see footnote 12) which had an L_{max} of 38 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. In contrast, the choline-supplemented rats had an L_{max} of 81.4 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$, which is comparable to that of the dog.

The β_1/k_1 values also differ markedly for the three species, being roughly ten times larger in rat and dog than in man. As a result, L_{sec} in man approaches the plateau value of L_{max} at much lower BS_{sec} rates than in the other species. Within the framework of our model, these variations in L_{max} and β_1/k_1 reflect quantitative differences in hepatic phospholipid metabolism between the species.

A striking finding from our data analysis (Table 1) is that, for all three species, Ch_{max} has a value comparable to that in man, ~ 4 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. In terms of equation *2* this means that the maximum *input rate* of Ch to the biliary secretion compartment is approximately constant when normalized by body weight (or liver weight). Since biliary Ch is derived from both endogenous synthesis and dietary intake, in proportions which may vary markedly between species (15, 39–41) and change physiologically (40, 41, 46), it is remarkable that the net effect of these processes appears to keep Ch_{max} relatively unchanged.

To investigate this result further, we analyzed other parameters of Ch metabolism found in the literature. From Ch balance studies in rat (72, 73), dog (74, 75), and man (76, 77) under conditions of "no Ch intake," the normalized fecal excretion rates of endogenous Ch are: rat, 2.1 ± 1.0 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; dog, 1.47 ± 0.56 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; and man, 1.1 ± 0.2 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. Again one finds a striking similarity between these species, with fecal excretion rates that are roughly 25–50% of the Ch_{max} values deduced previously. This relative constancy supports our previous finding and is consistent with the view that *unabsorbed* biliary Ch is a major source of neutral fecal sterol (46, 73). In fact, if biliary Ch secretion is maintained at or near the plateau region of the Ch_{sec} vs BS_{sec} curves (Figs. 4C, 5C, 6C), then a fractional intestinal absorption of $\sim 50\%$ would still leave enough Ch to account for all of the fecal excretion. This fraction is consistent with experimental estimates in man (15, 76, 78).

In contrast to Ch_{max}, the parameter $(\beta_2 + \gamma)/k_2$ is tenfold smaller in man than in rat and dog (Table 1). It is more likely that this species variation results from differences in the Ch feedback coefficient β_2 or BS synthesis coefficient γ, rather than from k_2, which describes a physical interaction between L and Ch. If β_2 or γ are in fact the cause, one would predict from Fig. 3A (or equation *8*) that the steady state Ch content in the secretion compartment, $(Q_{Ch})_{ss}$, would reach higher values in man than in the other species if measured at low L_{sec} rates. Present estimates of the total microsomal Ch in human (18, 79–81) and rat liver (47, 48) are both on the order of 60 $\mu mol/g$ of microsomal protein and thus do not bear out this prediction. Nevertheless, we shall present evidence below which suggests that the value of γ/k_2 is, in fact, markedly smaller in man than in rat or dog.

b. Deductions of γ/k_2: species variation in BS synthesis. As implied by equation *10* and Fig. 3C, the instantaneous rate of BS_{syn} will depend inversely on the magnitude of L_{sec}. Since L_{sec} can theoretically range between 0 and L_{max}, one can place the following theoretical limits on BS_{syn}:

$$\frac{\gamma}{k_2}\left(\frac{Ch_{max}}{(\beta_2+\gamma)/k_2 + L_{max}}\right) < BS_{syn} < \left(\frac{\gamma}{k_2}\frac{Ch_{max}}{(\beta_2+\gamma)/k_2}\right). \quad Eq.\ 17)$$

These inequalities in turn imply that γ/k_2 must lie between:

$$\left(\frac{BS_{syn}}{Ch_{max}}(\beta_2+\gamma)/k_2\right) < \frac{\gamma}{k_2} < \left(\frac{BS_{syn}}{Ch_{max}}[(\beta_2+\gamma)/k_2 + L_{max}]\right) \quad Eq.\ 18A)$$

or

$$< (\beta_2+\gamma)/k_2), \quad \text{if smaller than the above expression.} \quad Eq.\ 18B)$$

Experimental studies in rat (73, 82–84), dog (74), and man (37, 76, 77), have determined the magnitude of BS_{syn} under normal conditions (i.e., intact enterohepatic circulation, low Ch diet) for the three species: rat, 2.5 ± 0.5 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; dog, 2.6 ± 0.5 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; and man (non-obese), 0.45 ± 0.1 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. Using these values in conjunction with the inequalities (equation *18*), we have calculated the ranges within which γ/k_2 must lie for the three species (see Table 1). Taking the midpoint of each range as an arbitrary estimate of γ/k_2 yields the following values: rat, 27 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; dog, 45.5 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$; and man, 1.25 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$. These results indicate that γ/k_2 is markedly smaller in man than in rat or dog and that this constitutes the major source of the variation of $(\beta_2 + \gamma)/k_2$ (see Table 1). Assuming that the differences in γ/k_2 reflect changes in γ (and not k_2) we must conclude that the rat and dog divert a greater fraction of Ch to BS synthesis than does man. This view is consistent with other metabolic studies (15, 46, 73, 85) but its connection to the "shape" of the Ch_{sec} vs L_{sec} dependence has not been demonstrated previously. As yet the biochemical basis for the species variation of γ has not been determined. The available data do not demonstrate any marked differences in the activity of 7α-hydroxylase in rat (59–61) or man (79, 80).

c. Dependence of hepatic bile composition on BS_{sec}. Using equations *7*, *11*, and *12A, B, C* together with the secretion parameters given in Table 1, we depict in **Fig. 8A, B, C** the mole fractions of X^H_{BS}, X^H_L, and X^H_{Ch} as functions of BS_{sec} for rat, dog, and man, respectively. The qualitative behavior of X^H_{BS}, X^H_L, and X^H_{Ch} (discussed in section 5) is similar for all three species; X^H_L and X^H_{Ch} decrease monotonically with increasing BS_{sec}, while

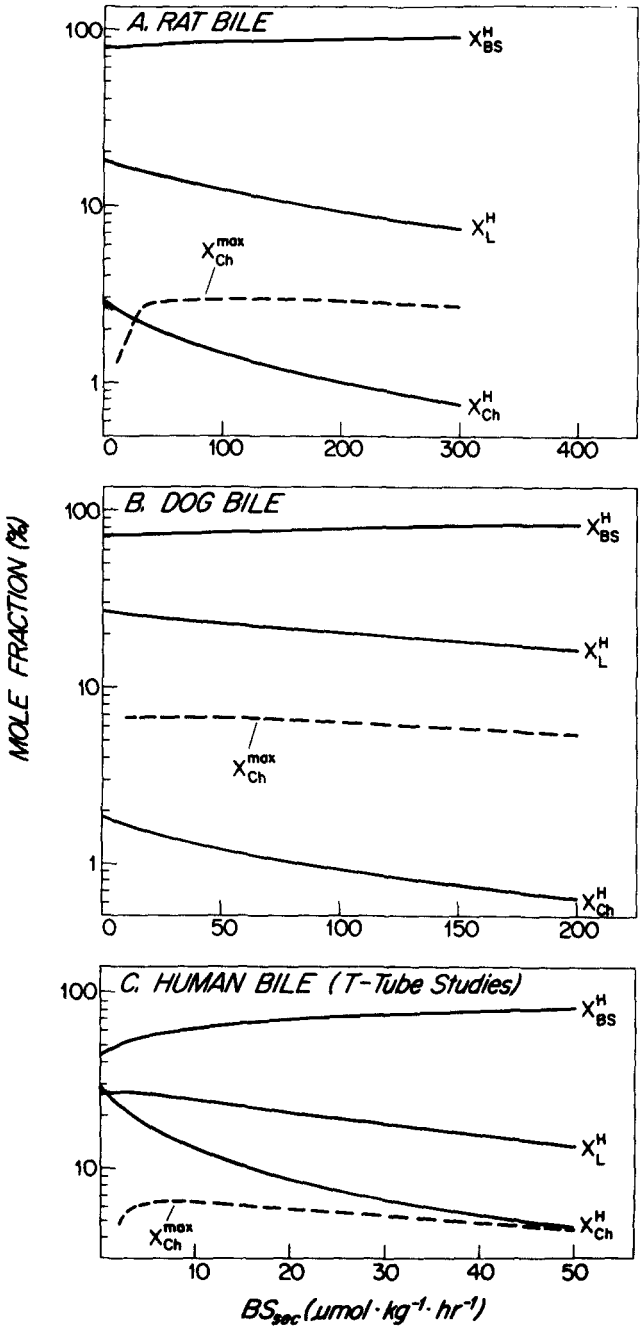

Fig. 8. Mole fractions of bile salt (X^H_{BS}), lecithin (X^H_L), and cholesterol (X^H_{Ch}) in hepatic bile as functions of BS_{sec}: A, rat; B, dog; and C, man. Curves are derived from equations *7*, *11*, and *12A, B, C* using parameters for each species given in Table 1. Dashed curves represent the maximum mole fraction of cholesterol (X^{max}_{Ch}) that can be solubilized by a micellar solution of taurocholate and lecithin with total lipid concentration comparable to hepatic bile (see Appendix).

X^H_{BS} shows a small increase. The ratio X^H_L/X^H_{BS} is seen[13] to decrease similarly with BS_{sec} in all species; whereas, the ratio X^H_{Ch}/X^H_L exhibits a significant species variation. In the dog and rat, X^H_{Ch}/X^H_L has the smallest values (0.03–0.1) and the least variation with BS_{sec}, whereas in man X^H_{Ch}/X^H_L is largest (approaching ~1) and decreases markedly with BS_{sec} to a limiting value of ~0.3.

These features, which have been noted previously by others (2, 16, 41), can be understood quantitatively from equations *13A, B.* In particular, the magnitude of X_{Ch}/X_L (at low BS_{sec}) depends largely on the ratio of parameters $Ch_{max}/[(\beta_2 + \gamma)/k_2]$, whereas its degree of variation depends on the relative sizes of $(\beta_2 + \gamma)/k_2$ and L_{max}. In this regard, the major difference between man and the other species can be attributed to his markedly smaller value of $(\beta_2 + \gamma)/k_2$.

d. Ch saturation of hepatic bile. The dependence of X^H_{Ch} on BS_{sec} has important pathophysiological significance when related to the maximum mole fraction of Ch (X^{max}_{Ch}) that can be solubilized in biliary lipid micellar systems. Based on the calculations described in the Appendix, the dependence of X^{max}_{Ch} on BS_{sec} is shown as the dashed curves in Fig. 8A, B, C. In the case of the dog (Fig. 8B), the entire X^H_{Ch} curve falls below X^{max}_{Ch}. In rats (Fig. 8A), $X^H_{Ch}(0)$ exceeds X^{max}_{Ch}; however, for BS_{sec} rates in excess of 25 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$, X^H_{Ch} lies below the solubilization limit. In man (Fig. 8C), X^H_{Ch} exceeds X^{max}_{Ch} for nearly all BS_{sec} values.

Although the X^{max}_{Ch} curves show a small species variation, the major reason for the greater supersaturation of human hepatic bile (especially at low BS_{sec} rates) is the upward shift of the X^H_{Ch} curve. In terms of our model, the production of lithogenic bile in man is thus a further result of the decreased value of $(\beta_2 + \gamma)/k_2$, which appears to be caused by a decreased ability to convert Ch to BS (i.e., a smaller γ value).

3. Biliary lipid secretion in obese and non-obese man: influence of Ch synthesis

a. Stimulated secretion studies. A considerable body of literature on biliary lipid secretion in obese and non-obese man has been obtained using the methodological approach developed (86) and validated by Grundy and co-workers (23–26). In this method the rates of BS, L, and Ch secreted into the duodenum are measured under stimulated steady state conditions induced by continuous infusion of a liquid formula diet.

In order to analyze the functional dependences, L_{sec} vs BS_{sec}; Ch_{sec} vs L_{sec}; and Ch_{sec} vs BS_{sec}, previous authors (23, 27, 28) combined stimulated secretion data from a large group of subjects, assuming that the variations in lipid outputs for the group as a whole would be comparable to the variations seen in individuals studied by the T-tube drainage and infusion method. As yet this assumption has never been rigorously tested.

In order to quantify the lipid secretory relationships of non-obese and obese subjects within the framework of our model, we have also combined individual data taken from a number of published investigations (23–28). The subject characteristics of our two groups are given in **Table 2.** As in the case of interspecies comparison, it is necessary to normalize the lipid secretion rates in order to eliminate the effects of body (or liver) size, per se. Following the rationale of Bennion and Grundy (25) we have normalized the data by the subjects *ideal* body weight and present the results for non-obese and obese populations in **Figs. 9(A, B, C)** and **10(A, B, C),** respectively. Although there is considerable scatter in the data, it is clear that the hyperbolic functions of our model (equations *7, 9,* and *11*) provide a good representation for the curvature of the relationships and that the lipid secretion parameters (Table 3) are well estimated. Furthermore, it is evident that these relationships differ quantitatively between the two groups; the most significant differences being dependences of Ch_{sec} on L_{sec} and Ch_{sec} on BS_{sec} which are shifted upward in the obese state relative to the non-obese state.

b. Parameter estimation and interpretation. In deducing the secretion parameters for obese and non-obese groups, we have found it useful to analyze unnormalized secretion data, as well as data normalized by actual and ideal body weight. In **Table 3** our deductions of L_{max}, β_1/k_1, Ch_{max}, and $(\beta_2 + \gamma)/k_2$ are listed for both populations, based on all three approaches. Comparison of the parameters deduced from the un-normalized data shows that all four parameters are significantly *larger* for the obese population than the non-obese population. When the data are normalized by actual body weight, L_{max}, β_1/k_1, and $(\beta_2 + \gamma)/k_2$ are found to be *smaller* in the obese group while Ch_{max} has nearly the *same* value in both groups. Lastly, when ideal body weight is used to normalize the data (as in Figs. 9 and 10), L_{max}, β_1/k_1, and Ch_{max} are all *larger* in the obese state, while $(\beta_2 + \gamma)/k_2$ is nearly the *same* in both groups. When the data from subjects with and without Ch gallstones were analyzed separately within each population (results not presented), differences in the four lipid secretion parameters were not found to be statistically significant. Thus, in agreement with Shaffer and Small (27), we find that the functional relationships between BS_{sec}, L_{sec}, and Ch_{sec}, obtained by Grundy's method, do not differ significantly between control and gallstone groups provided that their degree of obesity is comparable.

It is noteworthy that Ch_{max}, when normalized by actual body weight, is roughly the same for both populations, i.e., 4 $\mu mol \cdot kg^{-1} \cdot hr^{-1}$, and furthermore is

[13] The ratios X^H_L/X^H_{BS} (and X^H_{Ch}/X^H_L) are related "inversely" to the spacing between X^H_{BS} and X^H_L (X^H_{Ch} and X^H_L), respectively, on the semilogarithmic plots in Fig. 8(A, B, C).

TABLE 2. Non-obese and obese patient populations

Condition	Study	Year	Patients (number/sex)	Weight (kg)	Percent Ideal Weight	Characteristics
Non-obese						
Without gallstones	Grundy et al.	1972	5/M 3/F	69.4 ± 12.5	108.9 ± 7.6	American Indian
	Grundy et al.	1974	14/F	53.2 ± 4.5	99.4 ± 8.4	Caucasian
	Bennion and Grundy	1975	6/M 5/F	64.2 ± 6.9	103.6 ± 6.8	Five Caucasian subjects following weight loss
	Shaffer and Small	1977	8/M 5/F	69.1 ± 12.2	103.0 ± 11	Caucasian
	Valdivieso et al.	1979	14/F	51.6 ± 5.2	90.1 ± 7.8	Chilean
Total			19/M 41/F	60.5 ± 11	100 ± 10	
With cholesterol stones	Grundy et al.	1972	4/F	51.2 ± 3.3	104.3 ± 9.8	American Indian, post-operative
	Grundy et al.	1974	3/F	54.7 ± 4	112.3 ± 11.7	Caucasian, post-operative
	Shaffer and Small	1977	14/M 7/F	72.5 ± 10.9	103.1 ± 7.2	13 Caucasian subjects, post-operative
	Valdivieso et al.	1979	9/F	53 ± 7.6	95.2 ± 11.7	Chilean
Total			14/M 23/F	64 ± 13	103 ± 10	
Pooled non-obese			55/M 42/F	62 ± 12	101 ± 10	
Obese						
Without gallstones	Grundy et al.	1972	2/M 3/F	88 ± 24.6	148.2 ± 20.6	American Indian
	Benion and Grundy	1975	36/M 13/F	115 ± 26.8	168.1 ± 38.9	American Indian and Caucasian, different feeding states
	Shaffer and Small	1977	3/M 4/F	133 ± 31.0	189.3 ± 35.3	Caucasian
	Mok et al.	1979	4/M	131.9 ± 26	183 ± 31	Race not specified
Total			45/M 20/F	115 ± 29	170 ± 37	
With cholesterol stones	Grundy et al.	1972	13/F	69.5 ± 11.8	139.5 ± 16.5	American Indian, post-operative
	Grundy et al.	1974	5/F	91.6 ± 11.3	167.6 ± 24.2	Four Caucasian, one Black, post-operative
	Shaffer and Small	1977	1/M 3F	109.5 ± 11.1	180.5 ± 22	Caucasian, post-operative
Total			1/M 21/F	82 ± 20	153 ± 25	
Pooled obese			46/M 41/F	107 ± 27	166 ± 34	

comparable to the normalized values given in Table 1 for rat, dog, and man (by the T-tube method). It would thus appear that the maximum rate at which Ch can be made available for biliary secretion is proportional to *total body mass,* independent of the degree of obesity. This point is consistent with the Ch balance studies of Miettinen (77), who found neutral sterol excretion (with no Ch intake) to be roughly the same in obese and non-obese controls when normalized by total body weight ($\sim 1.2\ \mu mol \cdot kg^{-1} \cdot hr^{-1}$). Thus, the correlation between Ch_{max} and fecal Ch excretion noted previously for rat, dog, and non-obese man can be extended to obese human populations.

If, on the other hand, Ch_{max} is normalized by ideal body weight (IW), a significantly larger value of 5.9 $\mu mol \cdot kg\ IW^{-1} \cdot hr^{-1}$ is obtained for the obese group. This increase is nearly the same as the % ideal body weight in these subjects, and suggests a simple relationship between Ch_{max} and the degree of obesity:

$$Ch_{max} \simeq 4.0\left(\frac{\%\ \text{ideal body weight}}{100}\right)\mu mol \times kg\ IW^{-1} \cdot hr^{-1}. \quad \textit{Eq. 19)}$$

The metabolic basis for equation *19* is presumably the increased synthesis of Ch in the obese state (25, 77, 87, 88), which apparently provides a greater input of Ch to the biliary secretion compartment, than in non-obese patients.

In contrast to Ch_{max}, the parameter $(\beta_2 + \gamma)/k_2$ shows little change when normalized by ideal body weight, suggesting that the coefficients β_2 and γ both vary in proportion to ideal weight (or possibly liver mass) rather than actual weight. Using the earlier inequalities (equation *18*) together with Miettinen's measurement of acidic sterol excretion (i.e., BS_{syn}) in obese patients (77), we have deduced ranges for γ/k_2, and find no significant difference between non-obese and obese groups (Table 3). Thus, in terms of our model (equations *9* and *11*), the upward shift of the Ch_{sec} vs L_{sec} and Ch_{sec} vs BS_{sec} curves in obese man is due solely to the increased value of Ch_{max}. This analysis offers a clear theoretical explanation for the empirical correlation between Ch_{sec} and obesity noted previously by others (24, 25, 27). It further illustrates that, in man, an uncompensated increase in Ch_{syn} can lead to increased Ch_{sec}, analogous to the relationship between L_{syn} and

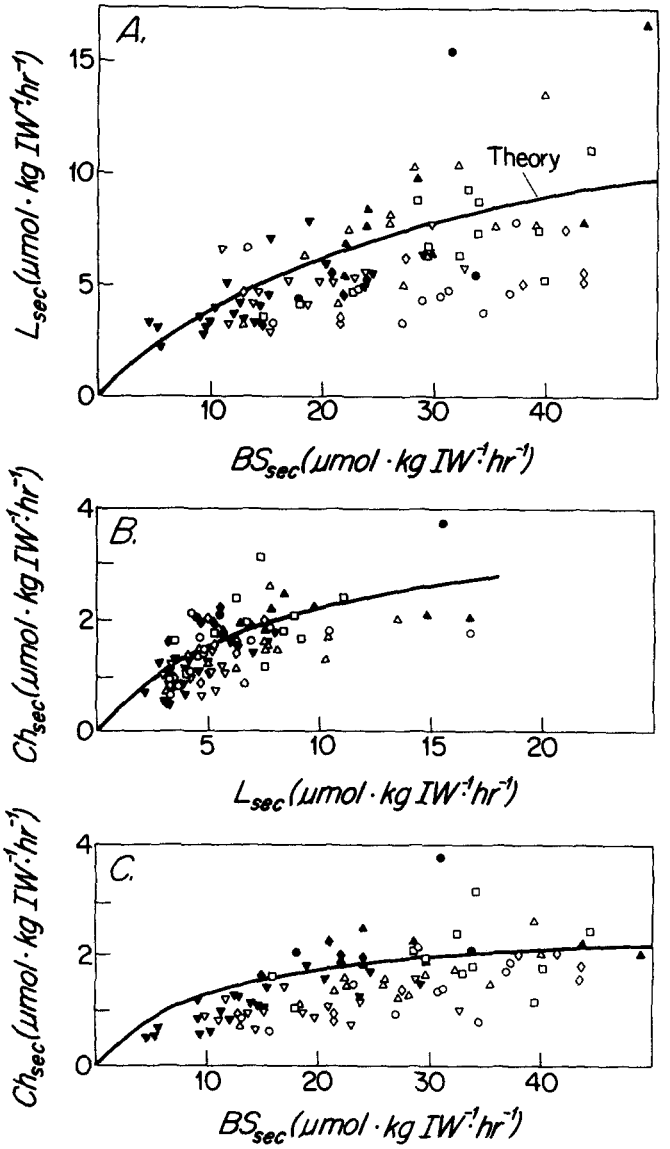

Fig. 9. Biliary lipid secretion data from studies of non-obese humans with and without cholesterol gallstones. A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; C, Ch_{sec} vs BS_{sec}. Subjects with gallstones (closed symbols) and without gallstones (open symbols) were taken from the following studies: ♦/◇, Grundy et al., 1972 (23); ●/○, Grundy et al., 1974 (24); none/□, Bennion and Grundy, 1975 (25); ▼/▽, Shaffer and Small, 1977 (27); ▲/△, Valdivieso et al., 1979 (28). Plotted points represent original data normalized per kg ideal weight. Solid curves are derived from fitting theory to experimental data as described in Fig. 4.

L_{sec} in the rat (8). Excessive Ch synthesis in the hamster has also been shown to increase Ch_{sec} (41).

It is important to note that the secretion parameters derived from non-obese gallstone patients (Table 1) and from pooled non-obese populations (with or without gallstones) (Table 3) are not in complete agreement. This discrepancy may be due in part to the different methodologies employed to obtain data on these two groups. It is now appreciated in the context of pharmacokinetic analysis (89) that the deduction of parameters from individual subjects (i.e., as in the T-tube studies) or from large populations (i.e., Grundy's method) need not give the same results. Moreover in the present

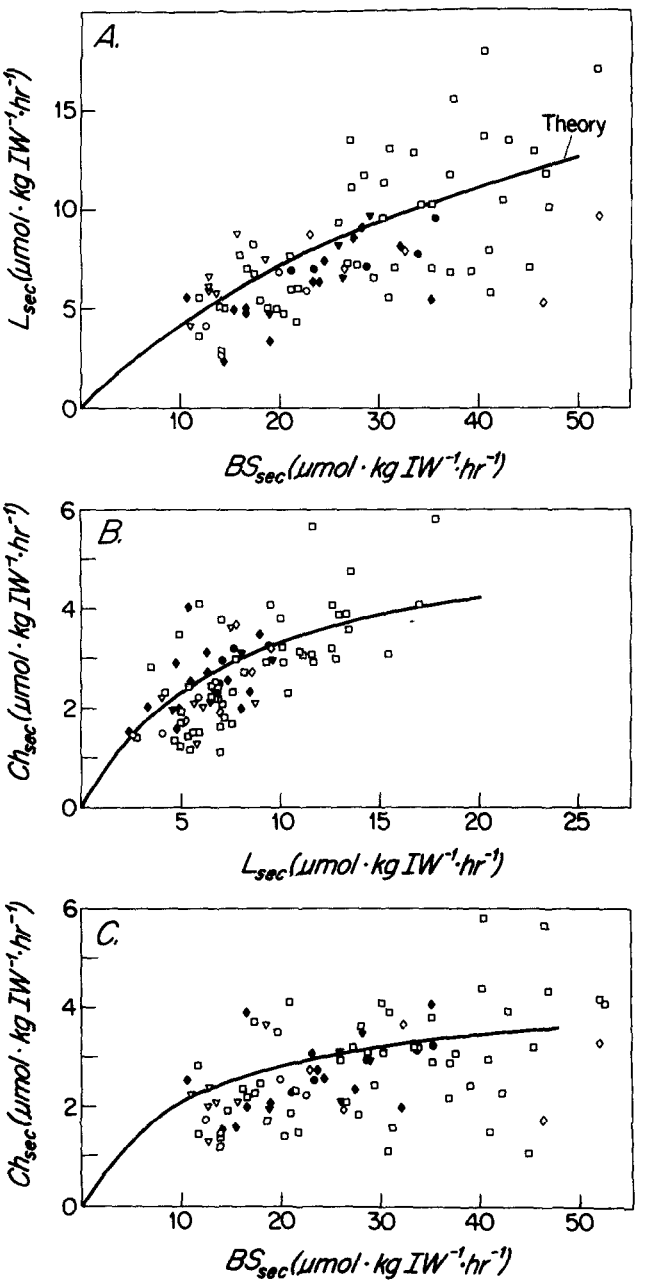

Fig. 10. Biliary lipid secretion from studies of obese humans with and without cholesterol gallstones. Data are normalized for ideal weight. A, L_{sec} vs BS_{sec}; B, Ch_{sec} vs L_{sec}; C, Ch_{sec} vs BS_{sec}. Subjects with gallstones (closed symbols) and without gallstones (open symbols) were taken from the following studies: ♦/◇, Grundy et al., 1972 (23); ●/none, Grundy et al., 1974 (24); none/□, Bennion and Grundy, 1975 (25); ▼/▽, Shaffer and Small, 1977 (27); none/○, Mok et al., 1979 (26). Plotted points represent original data normalized per kg ideal weight. Solid curves are derived from fitting theory to data as described in Fig. 4.

TABLE 3. Biliary lipid secretion parameters in non-obese and obese human populations

Population	L_{max}	β_1/k_1	Ch_{max}	$(\beta_2+\gamma)/k_2$	γ/k_2 (range)[a]	$\frac{L_{max}}{\beta_1/k_1}$	$\frac{Ch_{max}}{(\beta_2+\gamma)/k_2}$
	$\mu mol \cdot kg\ IW^{-1} \cdot hr^{-1}$						
Pooled non-obese							
Un-normalized data ($\mu mol \cdot hr^{-1}$)	816 ± 111[b]	1433 ± 270	181 ± 28	300 ± 69		0.57[c]	0.60[c]
Normalized by actual weight ($\mu mol \cdot kg^{-1} \cdot hr^{-1}$)	17.2 ± 2.6	35.5 ± 6.6	4.0 ± 0.7	8.3 ± 1.8		0.48	0.48
Normalized by ideal weight ($\mu mol \cdot kg\ IW^{-1} \cdot hr^{-1}$)	16.2 ± 2.4	32.4 ± 5.9	4.2 ± 0.9	8.9 ± 2.2	0.8–2.4	0.50	0.47
Pooled obese							
Un-normalized data ($\mu mol \cdot hr^{-1}$)	2531 ± 896	5962 ± 2200	389 ± 51	530 ± 92		0.42	0.73
Normalized by actual weight ($\mu mol \cdot kg^{-1} \cdot hr^{-1}$)	13.3 ± 2.5	24.9 ± 5.6	3.6 ± 0.6	4.6 ± 1.1		0.53	0.78
Normalized by ideal weight ($\mu mol \cdot kg\ IW^{-1} \cdot hr^{-1}$)	25.5 ± 5.8	52.0 ± 13.3	5.9 ± 0.9	7.8 ± 1.6	1.0–4.0	0.49	0.76

[a] The range for γ/k_2 is derived from inequalities (Eq. *18*).
[b] Represents estimate of standard deviation derived from the residuals of the fit (ref. 63).
[c] These quantities are dimensionless molar ratios.

case, the "stimulated" biliary secretion rates obtained by Grundy's method could differ from secretion data obtained by T-tube drainage even in the same individual. For both of these reasons one cannot conclude that the secretion relationships given in Figs. 6 and 9 (and the associated parameters) are necessarily different for the two subject populations. We believe that further biliary secretion studies using identical experimental techniques are needed to resolve whether the secretion parameters for non-obese Ch gallstone formers are significantly different from controls.[14]

c. Hepatic bile composition and Ch saturation. Using the four secretion parameters deduced for the non-obese and obese populations in Table 3, we have computed the dependence of X^H_{BS}, X^H_L, and X^H_{Ch} on BS_{sec} (normalized for ideal body weight) (**Fig. 11A, B**). While the dependences of X^H_{BS} and X^H_L on BS_{sec} rate are similar, the dependence of X^H_{Ch} on BS_{sec} differs significantly between the two populations and results in different degrees of supersaturation when compared with the solubilization limits X^{max}_{Ch}. For the non-obese population, X^H_{Ch} never exceeds 14 moles percent, and is unsaturated with respect to X^{max}_{Ch} at BS_{sec} rates greater than ~20 $\mu mol \cdot kg\ IW^{-1} \cdot hr^{-1}$. In contrast, X^H_{Ch} for the obese population approaches 20 moles percent and exceeds X^{max}_{Ch} over a much wider range of BS_{sec}. According to our model this difference largely reflects the increased Ch_{max} (per ideal weight) in the obese group (equation *19*), which produces a greater "driving force" to secrete biliary Ch at all L_{sec} and BS_{sec} rates.

4. Analysis of gallbladder bile composition in man

a. Deduction of $\overline{BS}_{sec}$ in the fasting state. In **Table 4** we list experimental values for X^{GB}_{BS}, X^{GB}_L, and X^{GB}_{Ch} taken from well controlled studies of non-obese (13, 18, 25, 91) and obese patients (13, 25, 26) with and without Ch gallstones. Data from patients with nonfunctioning gallbladders (91) have also been analyzed and will be discussed later.

As mentioned earlier, equations *16A, B, C* predict that the relative composition of "fasting" gallbladder bile should correspond hypothetically to the relative composition of hepatic bile produced at the time-average secretion rate $\overline{BS}_{sec}$. To test this prediction, we have used the dependences of X^H_{BS}, X^H_L, and X^H_{Ch} on BS_{sec} derived for non-obese and obese populations (Fig. 11A, B) in an attempt to fit the experimental data of Table 4. The fitting procedure involved choosing the values of $\overline{BS}_{sec}$ (identified as BS_{sec} in Fig. 11A, B) that allowed the closest agreement between the hepatic bile curves and the gallbladder bile compositions. Although the value of X^{GB}_{Ch} had the greatest influence on the choice of $\overline{BS}_{sec}$, it was possible in all cases to obtain a good fit to all three biliary lipid mole fractions (see theoretical deductions, Table 4). This success offers important support for the assumptions underlying equations *16A, B, C*. For non-obese patients, $\overline{BS}_{sec}$ is found to be 17

[14] Using the kinetic analysis model of Quarfordt and Greenfield (56), Hepner and Quarfordt (90) have found that the fractional conversion of Ch to BS is reduced in non-obese gallstone patients relative to controls. In the context of our model, this would imply a smaller γ value in these patients.

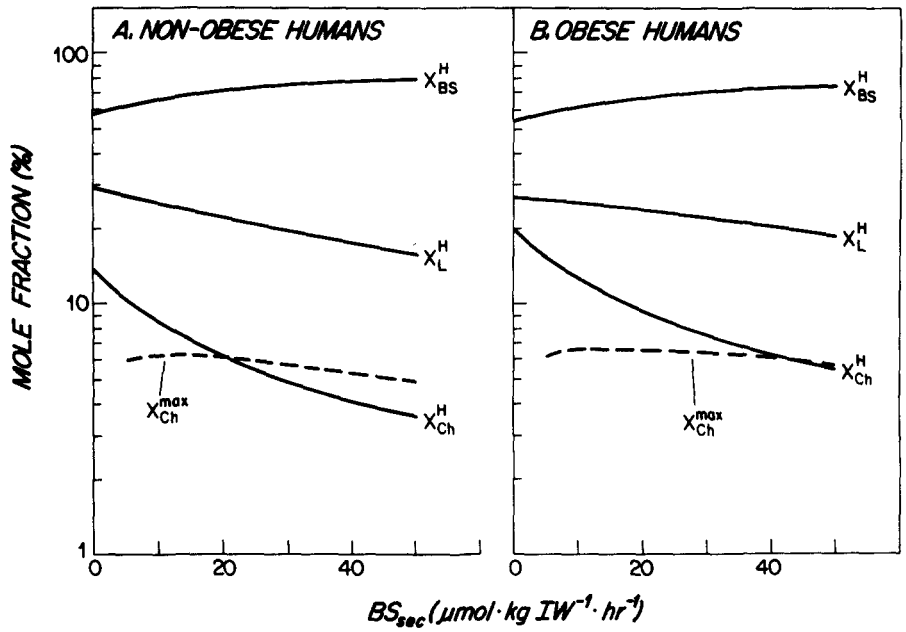

Fig. 11. Mole fractions of bile salt (X^H_{BS}), lecithin (X^H_L), and cholesterol (X^H_{Ch}) as functions of BS_{sec} for hepatic bile in A, non-obese humans; and B, obese humans. Curves are derived from equations *7*, *11*, and *12A*, *B*, *C* using parameters for both groups given in Table 3. Dashed curves represent the maximum mole fraction of cholesterol (X^{max}_{Ch}) that can be solubilized in micellar solution (see Appendix).

μmol·kg IW^{-1}·hr^{-1} in those without gallstones and 7 μmol·kg IW^{-1}·hr^{-1} in those with gallstones. For obese patients, the corresponding values are 14 and 4 μmol·kg IW^{-1}·hr^{-1}, respectively. Thus it would appear that, during the *fasting state,* gallstone patients secrete bile salts at a significantly lower rate than controls. For non-obese gallstone patients, the lower $\overline{BS}_{sec}$ rate itself is sufficient to account for the elevated value of X^{GB}_{Ch}; whereas for obese gallstone patients the markedly elevated X^{GB}_{Ch} values are consequences of the decreased $\overline{BS}_{sec}$ rate and the hypersecretion of Ch associated with obesity.

It is possible to compare our indirect deductions of $\overline{BS}_{sec}$ with recent experimental estimates of fasting hepatic BS_{sec} in man (20, 21). Van Berge Henegouwen and Hofmann (20) have quantified the diurnal variation in hepatic BS output in non-obese patients without stones and in non-obese patients whose stones had been dissolved previously by treatment with chenodeoxycholic acid. We have computed $\overline{BS}_{sec}$ from their data for the time interval beginning 90 min after the evening meal and ending prior to the morning meal. In the control group, $\overline{BS}_{sec}$ was 12.9 μmol·kg^{-1}·hr^{-1}, whereas in the "former gallstone" group, $\overline{BS}_{sec}$ was 9.7 μmol·kg^{-1}·hr^{-1}. Although not computed in their original paper (20), these average fasting secretion rates are remarkably close to our indirect deductions and show a similar trend between controls and stone formers. In a second study, Mok, von Bergmann, and Grundy (21) estimated mean hepatic BS_{sec} during the fasting state to be 11.8 μmol·kg^{-1}·hr^{-1} for non-obese controls, while in five Ch gallstone patients $\overline{BS}_{sec}$ was 9.0 μmol·kg^{-1}·hr^{-1}. These results are also consistent with our deductions. As yet, similar studies have not been conducted in obese subjects.

b. Effect of gallbladder function on $\overline{BS}_{sec}$. Redinger (91) found a marked change in the lipid composition of fasting gallbladder bile following the development of gallbladder dysfunction (i.e., failure to concentrate bile) in non-obese Ch gallstone patients. The experimental results (Table 4) indicate a significant decrease in X^{GB}_{Ch} and significant increase in X^{GB}_{BS} relative to gallstone patients with functioning gallbladders. In the context of our model, these changes could be explained by an increase in the fasting $\overline{BS}_{sec}$ rate from 7 to 29.0 μmol·kg IW^{-1}·hr^{-1} after the concentrating defect developed. Presumably the increased $\overline{BS}_{sec}$ rate would result from a decrease in the fraction of bile entering the gallbladder during fasting (↓F, Fig. 1) and a consequent increase in the enterohepatic circulation of bile salts. Such an argument has been suggested by others (91, 92) and is supported by the observation that the bile of these patients contained a larger fraction of secondary BS (deoxycholate) after developing the concentrating defect.

c. Relationship between Ch saturation and $\overline{BS}_{sec}$. The relationship between fasting gallbladder bile composition and $\overline{BS}_{sec}$ is illustrated graphically in **Fig. 12,** where the biliary lipid compositions of the various patient groups

TABLE 4. Biliary lipid compositions of fasting gallbladder biles: deductions of $\overline{BS}_{sec}$

Population	Study	Year	X_{BS}^{GB} [a]	X_{L}^{GB} [a]	X_{Ch}^{GB} [a]
Non-obese					
Without stones	Bennion and Grundy	1975	70.4	22.9	6.7
	Carey and Small	1978	73.3	20.0	6.7
	Ahlberg et al.	1981	69.4	23.9	6.8
		Avg:	71.0 ± 2.0	22.3 ± 2	6.7 ± 0.1
Theoretical deductions: $\overline{BS}_{sec}$ = 17 μmol·kg $IW^{-1}\cdot hr^{-1}$			70.3	23.0	6.7 [b]
Cholesterol stones	Redinger	1976	65.6	24.8	9.7
	Carey and Small	1978	69.8	21.1	9.2
	Ahlberg et al.	1981	64.5	25.4	10.2
		Avg:	66.6 ± 2.8	23.8 ± 2.3	9.7 ± 0.5
Theoretical deductions: $\overline{BS}_{sec}$ = 7 μmol·kg $IW^{-1}\cdot hr^{-1}$			64.2	26.4	9.4 [b]
Cholesterol stones/nonfunctioning gallbladders	Redinger	1976	76.3 ± 3.4	18.1 ± 3.1	5.6 ± 0.7
Theoretical deductions: $\overline{BS}_{sec}$ = 29.0 μmol·kg $IW^{-1}\cdot hr^{-1}$			75.0	20.0	5.0 [b]
Obese					
Without stones	Bennion and Grundy	1975	62.6	26.3	11.1
			67.2	23.1	9.7
	Mok et al.	1979	65.7	24.5	9.8
			66.1	22.2	11.6
			65.1	22.2	12.7
		Avg:	65.3 ± 1.7	23.7 ± 1.8	11.0 ± 1.2
Theoretical deductions: $\overline{BS}_{sec}$ = 14 μmol·kg $IW^{-1}\cdot hr^{-1}$			64.1	24.8	11.1 [b]
Cholesterol stones	Carey and Small	1978	58.8 ± 3.2	26.1 ± 3.2	15.2 ± 1.4
Theoretical deductions: $\overline{BS}_{sec}$ = 4 μmol·kg $IW^{-1}\cdot hr^{-1}$			58.4	26.2	15.4 [b]

[a] Indicates mole fractions of bile salt (BS), lecithin (L), and cholesterol (Ch) in gallbladder bile.
[b] Theoretical deductions correspond to the mean BS secretion rate during the fasting state ($\overline{BS}_{sec}$) and the associated biliary lipid mole fractions, as derived from Fig. 11. See text for explanation. IW, ideal weight.

(Table 4) have been plotted (ovals) on a triangular micellar phase diagram, corresponding to 10 g/dl total biliary lipid concentrations (13). The two solid curves represent theoretical biliary lipid compositions of non-obese and obese populations derived from the biliary compositional dependences on $\overline{BS}_{sec}$ given in Fig. 11A, B. At the points of intersection between the theoretical curves and experimental results, the $\overline{BS}_{sec}$ values associated with those theoretical compositions are given. Where the theoretical curves cross the X_{Ch}^{max} boundary, the "threshold" $\overline{BS}_{sec}$ rates (at which supersaturation occurs) can be deduced. For the obese state, the threshold value is ~33 μmol·kg $IW^{-1}\cdot hr^{-1}$, and for the non-obese state the threshold is ~11 μmol·kg $IW^{-1}\cdot hr^{-1}$. From the curves in Fig. 12 it is clear that the fasting $\overline{BS}_{sec}$ rate is a critical determinant of the Ch mole fraction of gallbladder bile, and that the degree of supersaturation increases markedly as $\overline{BS}_{sec}$ falls below the threshold values.

It is beyond the scope of the present work to examine the mechanisms that determine $\overline{BS}_{sec}$. As discussed in a recent review (93), bile salt pool size (37, 94), intestinal motility (95, 96, 97), gallbladder function (91, 92, 96–98), and the specific effects of different bile salt species (93, 99) all appear to influence $\overline{BS}_{sec}$. A quantitative understanding of how some of these mechanisms determine the dynamics of the enterohepatic circulation is beginning to emerge (100).

CONCLUSIONS

We have presented a theoretical model which describes the quantitative relationships between BS, L, and Ch secretion into bile, and have applied this model to analyze a wide range of physiological and biochemical data from man and other species. Four parameters are shown to control the dependences: L_{sec} vs BS_{sec}, Ch_{sec} vs L_{sec}, and Ch_{sec} vs BS_{sec}, all of which have the form of rectangular hyperbolae. These parameters reflect a combination of physical and biochemical interactions between biliary lipids, and their numerical values have been deduced and compared with other parameters of

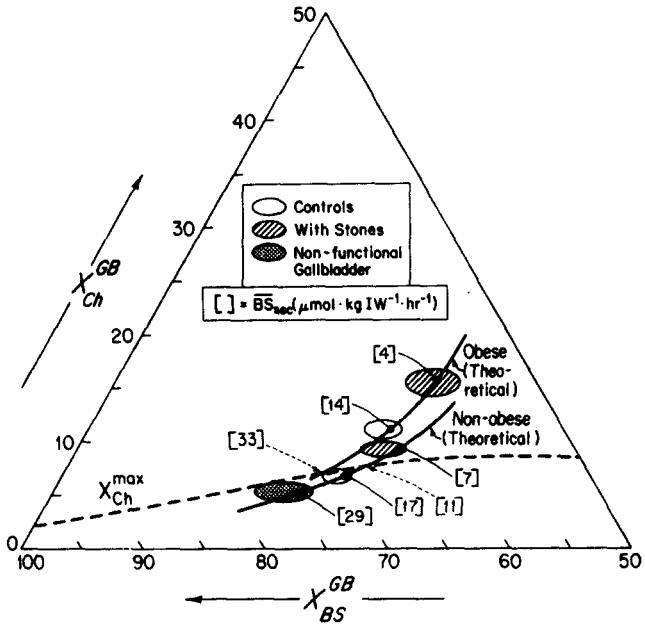

Fig. 12. Biliary lipid compositions of fasting gallbladder biles plotted on triangular coordinates. Data (ovals) are from non-obese and obese populations with and without gallstones as presented in Table 4. Data from gallstone patients with non-concentrating gallbladders are also shown. Dashed curve, X_{Ch}^{max}, denotes micellar solubilization limits at 10 g/dl total lipid composition as derived by Carey and Small (13). Solid curves are theoretical calculations giving bile compositions at various $\overline{BS}_{sec}$ rates for non-obese and obese populations. The numbers in square brackets give $\overline{BS}_{sec}$ rates in μmol·kg IW^{-1}·hr^{-1} appropriate to the biliary lipid composition of each patient group, and also identify the threshold secretion at which $X_{Ch} = X_{Ch}^{max}$.

biliary lipid metabolism in rat, dog, and man. We have also shown how these secretion parameters quantitatively determine the relative lipid composition and Ch saturation of *hepatic* bile as a function of BS_{sec}, and have extended this formalism to relate the composition and Ch saturation of fasting *gallbladder* bile in man to the time-averaged BS secretion rate, $\overline{BS}_{sec}$, during the fasting state.

The quantitative analyses presented in this paper illustrate a number of important concepts about the mechanisms controlling biliary lipid secretions. *1*) The rate of biliary L secretion is determined by *both* the transhepatic flux of BS and the capacity of the liver to synthesize L (4, 8, 14, 10). *2*) Biliary Ch secretion is coupled directly to L secretion rather than to BS secretion (2, 3, 8, 57). *3*) The markedly smaller percentage of Ch found in rat and dog bile (as compared to man), is related to the greater capacity of these species to convert Ch into BS (15, 46, 73). *4*) The increased secretion of Ch in obesity is the result of an uncompensated increase in Ch synthesis. *5*) A decreased "fasting" BS secretion rate appears to be an important pathogenic factor in the formation of supersaturated bile in non-obese gallstone patients (16, 101).

We hope that this model will provide a set of quantitatively stated "working hypotheses" of how biliary lipid secretion is regulated in the liver. Further experimental and theorertical work is underway to test and refine these hypotheses, and to extend the model to other aspects of biliary physiology and Ch metabolism.

APPENDIX

Calculation of the Ch solubilization limits in hepatic bile

As noted previously, X_{Ch}^{max} varies as a function of BS_{sec} due to its dependence on the L/BS molar ratio (X_L/X_{BS}) and the total lipid concentration (C_T) of hepatic bile. The L/BS ratio varies with BS_{sec} in accordance with equation *13A*. The dependence of C_T on BS_{sec} is related to the secretion rates of all three lipids and on the rate of bile flow, BF, by the following expression:

$$C_T = \frac{M_{BS}BS_{sec} + M_L L_{sec} + M_{Ch}Ch_{sec}}{BF} \qquad A\text{-}1)$$

where M_{BS}, M_L, and M_{Ch} are the molecular weights of the three biliary lipids. Based on studies in rat (102, 103), dog (104), and man (1, 105), we have determined the following numerical expressions for BF as a function of BS_{sec}:

$$\text{Rat: BF (ml·kg}^{-1}\text{·hr}^{-1}) = 3.05 + 0.014BS_{sec} \qquad A\text{-}2A)$$

$$\text{Dog: BF (ml·kg}^{-1}\text{·hr}^{-1}) = 0.13 + 0.008BS_{sec} \qquad A\text{-}2B)$$

$$\text{Man: BF (ml·kg}^{-1}\text{·hr}^{-1}) = 0.21 + 0.011BS_{sec} \qquad A\text{-}2C)$$

where BS_{sec} has units of μmol·kg^{-1}·hr^{-1}. Using equations *A-1*, *A-2*, *7*, and *11*, we have computed C_T as a function of BS_{sec} for all three species.

Finally, to estimate X_{Ch}^{max} we employed Carey and Small's (13) computer plots of X_{Ch}^{max} as functions of $X_L/(X_{BS} + X_L)$ and total lipid concentration, which were derived from phase equilibria studies of taurocholate-L-Ch systems at 37°C.

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REFERENCES

1. Scherstén, T., S. Nilsson, E. Cahlin, M. Filipson, and G. Brodin-Persson. 1971. Relationship between the biliary

excretion of bile acids and the excretion of water, lecithin, and cholesterol in man. *Eur. J. Clin. Invest.* **1:** 242–247.
2. Wagner, C. I., B. W. Trotman, and R. D. Soloway. 1976. Kinetic analysis of biliary lipid excretion in man and dog. *J. Clin. Invest.* **57:** 473–477.
3. Lindblad, L., K. Lundholm, and T. Scherstén. 1977. Influence of cholic and chenodeoxycholic acid on biliary cholesterol secretion in man. *Eur. J. Clin. Invest.* **7:** 383–388.
4. Nilsson, S., and T. Scherstén. 1970. Influence of bile acids on the synthesis of biliary phospholipids in man. *Eur. J. Clin. Invest.* **1:** 109–111.
5. Swell, L., C. C. Bell, Jr., and C. Entenman. 1968. Bile acids and lipid metabolism. III. Influence of bile acids on phospholipids in liver and bile of the isolated perfused dog liver. *Biochim. Biophys. Acta.* **164:** 278–284.
6. Hardison, W. G. M., and J. T. Apter. 1972. Micellar theory of biliary cholesterol excretion. *Am. J. Physiol.* **222:** 61–67.
7. Wheeler, H. O., and K. K. King. 1972. Biliary excretion of lecithin and cholesterol in the dog. *J. Clin. Invest.* **51:** 1337–1350.
8. Robins, S. J., and M. J. Armstrong. 1976. Biliary lecithin secretion. II. Effects of dietary choline and biliary lecithin synthesis. *Gastroenterology.* **70:** 397–402.
9. Turley, S. D., and J. M. Dietschy. 1979. Regulation of biliary cholesterol output in the rat: dissociation from the rate of hepatic cholesterol synthesis, the size of the hepatic cholesteryl ester pool, and the hepatic uptake of chylomicron cholesterol. *J. Lipid Res.* **20:** 923–934.
10. Balint, J. A., D. A. Beeler, D. H. Treble, and H. L. Spitzer. 1967. Studies in the biosynthesis of hepatic and biliary lecithins. *J. Lipid Res.* **8:** 486–493.
11. Zilversmit, D. B., and E. Van Handel. 1958. The origin of bile lecithin and the use of bile to determine plasma lecithin turnover rates. *Arch. Biochem. Biophys.* **73:** 224–232.
12. Redinger, R. M., and D. M. Small. 1972. Bile composition, bile salt metabolism and gallstones. *Arch. Intern. Med.* **130:** 618–630.
13. Carey, M. C., and D. M. Small. 1978. The physical chemistry of cholesterol solubility in bile. Relationship to gallstone formation and dissolution in man. *J. Clin. Invest.* **61:** 998–1026.
14. Hardison, W. G. M., and J. T. Apter. 1974. Use of hybrid computers to analyze behavior of detailed models of biological systems. II. System parameters used in bile salt stimulated biliary cholesterol excretion. *Comput. Biol. Med.* **4:** 3–17.
15. Grundy, S. M. 1978. Cholesterol metabolism in man. *West. J. Med.* **128:** 13–25.
16. Mok, H. Y. I., K. von Bergmann, and S. M. Grundy. 1978. Factors affecting bile saturation at low outputs of biliary bile acids in man. Falk Symposium 26—Biological Effects of Bile Acids. G. Paumgartner, A. Stiehl, and W. Gerok, editors. M.T.P. Press Ltd., Lancaster, England. 39–51.
17. Nakayama, F. 1969. Composition of gallstone and bile: species difference. *J. Lab. Clin. Med.* **73:** 623–630.
18. Ahlberg, J., B. Angelin, and K. Einarsson. 1981. Hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and biliary lipid composition in man: relation to cholesterol gallstone disease and effects of cholic acid and chenodeoxycholic acid treatment. *J. Lipid Res.* **22:** 410–422.
19. Hofmann, A. F. 1977. The enterohepatic circulation of bile acids in man. *Clin. Gastroenterol.* **6:** 3–24.
20. Van Berge Henegouwen, G. P., and A. F. Hofmann. 1978. Nocturnal gallbladder storage and emptying in gallstone patients and healthy subjects. *Gastroenterology.* **75:** 879–885.
21. Mok, H. Y. I., K. von Bergmann, and S. M. Grundy. 1980. Kinetics of the enterohepatic circulation during fasting: biliary lipid secretion and gallbladder storage. *Gastroenterology.* **78:** 1023–1033.
22. Hoffman, N. E., D. E. Donald, and A. F. Hofmann. 1975. Effect of primary bile acids on bile lipid secretion from perfused dog liver. *Am. J. Physiol.* **229:** 714–720.
23. Grundy, S. M., A. L. Metzger, and R. D. Adler. 1972. Mechanisms of lithogenic bile formation in American Indians and women with cholesterol gallstones. *J. Clin. Invest.* **51:** 3026–3043.
24. Grundy, S. M., W. C. Duane, R. D. Adler, J. M. Aron, and A. L. Metzger. 1974. Biliary lipid outputs in young women with cholesterol gallstones. *Metabolism.* **23:** 67–73.
25. Bennion, L. J., and S. M. Grundy. 1975. Effects of obesity and caloric intake on biliary lipid metabolism in man. *J. Clin. Invest.* **56:** 996–1011.
26. Mok, H. Y. I., K. Von Bergmann, J. R. Crouse, and S. M. Grundy. 1979. Biliary lipid metabolism in obesity. Effects of bile acid feeding before and during weight reduction. *Gastroenterology.* **76:** 556–567.
27. Shaffer, E. A., and D. M. Small. 1977. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. *J. Clin. Invest.* **59:** 828–840.
28. Valdivieso, V., R. Palma, F. Nervi, C. Covarrubias, C. Severin, and C. Antezana. 1979. Secretion of biliary lipids in young Chilean women with cholesterol gallstones. *Gut.* **20:** 997–1000.
29. Gregory, D. H., Z. R. Vlahcevic, M. F. Prugh, and L. Swell. 1978. Mechanism of secretion of biliary lipids: role of a microtubular system in hepatocellular transport of biliary lipids in the rat. *Gastroenterology.* **74:** 93–100.
30. Kawamoto, T., G. Okano, and T. Akino. 1980. Biosynthesis and turnover of individual molecular species of phosphatidylcholine in liver and bile. *Biochim. Biophys. Acta.* **619:** 20–34.
31. Kawamoto, T., T. Akino, M. Nakamura, and M. Mori. 1980. Metabolism of individual molecular species of phosphatidylcholines in the liver, subcellular membranes and bile. *Biochim. Biophys. Acta.* **619:** 35–47.
32. Balasubramaniam, S., K. A. Mitropoulos, and N. B. Myant. 1973. Evidence for the compartmentation of cholesterol in rat liver microsomes. *Eur. J. Biochem.* **34:** 77–83.
33. Palade, G. E., and P. Siekevitz. 1956. Liver microsomes—an integrated morphological and biochemical study. *J. Biophys. Biochem. Cytol.* **2:** 171–212.
34. Jones, A. L., D. L. Schmucker, J. S. Mooney, R. D. Adler, and R. K. Ockner. 1978. A quantitative analysis of hepatic ultrastructure in rats during enhanced bile secretion. *Anat. Rec.* **192:** 277–287.
35. Nemchausky, B. A., T. J. Layden, and J. L. Boyer. 1977. Effects of chronic choleretic infusion of bile acids on the membrane of the bile canaliculus—a biochemical and morphological study. *Lab. Invest.* **36:** 259–267.
36. Layden, T. J., and J. L. Boyer. 1978. Bile acids influence bile canalicular membrane morphology and the lobular gradient in canalicular size. *Lab. Invest.* **39:** 110–119.

37. Mok, H. Y. I., K. von Bergmann, and S. M. Grundy. 1977. Regulation of pool size of bile acids in man. *Gastroenterology.* **73:** 684–690.
38. Balint, J. A., D. A. Beeler, E. C. Kyriakides, and D. H. Treeble. 1971. The effect of bile salts upon lecithin synthesis. *J. Lab. Clin. Med.* **77:** 122–131.
39. Schwartz, C. C., M. Berman, Z. R. Vlahcevic, L. G. Halloran, D. H. Gregory, and L. Swell. 1978. Multicompartmental analysis of cholesterol metabolism in man. *J. Clin. Invest.* **61:** 408–423
40. Turley, S. D., and J. M. Dietschy. 1981. The contribution of newly synthesized cholesterol to biliary cholesterol in the rat. *J. Biol. Chem.* **256:** 2438–2446.
41. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1983. Alteration of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed and newly synthesized cholesterol. *Gastroenterology.* **84:** 253–264.
42. Attie, A. D., R. C. Pittman, and D. Steinberg. 1982. Hepatic catabolism of low density lipoprotein: mechanisms and metabolic consequences. *Hepatology.* **2:** 269–281.
43. Koelz, H. R., B. C. Sherrill, S. D. Turley, and J. M. Dietschy. 1982. Correlation of low and high density lipoprotein binding in vivo with rates of lipoprotein degradation in the rat. *J. Biol. Chem.* **257:** 8061–8072.
44. Packard, C. J., and J. Shepherd. 1982. The hepatobiliary axis and lipoprotein metabolism: effects of bile acid sequestrants and ileal bypass surgery. *J. Lipid Res.* **23:** 1081–1098.
45. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein receptors in the liver. Control signals for plasma cholesterol traffic. *J. Clin. Invest.* **72:** 743–747.
46. Dietschy, J. M., and J. D. Wilson. 1970. Regulation of cholesterol metabolism. *N. Engl. J. Med.* **282:** 1128–1138, 1179–1183, 1241–1249.
47. Jakoi, L., and S. H. Quarfordt. 1974. The induction of hepatic cholesterol synthesis in the rat by lecithin mesophase infusions. *J. Biol. Chem.* **249:** 5840–5844.
48. Jakoi, L., and S. H. Quarfordt. 1977. Alteration of rat hepatic cholesterogenesis by heterologous lipoproteins. *J. Biol. Chem.* **252:** 6856–6860.
49. Brown, M. S., P. T. Kovanen, and J. T. Goldstein. 1981. Regulation of plasma cholesterol by lipoprotein receptors. *Science.* **212:** 628–635.
50. Von Schulthess, G. K., and N. A. Mazer. 1982. Cyclic neutropenia (CN): a clue to the control of granulopoiesis. *Blood.* **59:** 27–37.
51. Mazer, N. A., G. B. Benedek, and M. C. Carey. 1980. Quasi-elastic light scattering studies of aqueous biliary lipid systems. Mixed micelle formation in bile salt-lecithin solutions. *Biochemistry.* **19:** 601–615.
52. Schurtenberger, P., N. A. Mazer, W. Känzig, and R. Preisig. 1984. Quasielastic light scattering studies of the micelle to vesicle transition in aqueous solutions of bile salt and lecithin. *In* Proceedings of the International Symposium on Surfactants in Solution, Lund, Sweden; 1982. K. L. Mittal, editor. Plenum Press. **2:** 841–855.
53. Mazer, N. A., P. Schurtenberger, M. C. Carey, R. Preisig, K. Weigand, and W. Känzig. 1984. Quasielastic light scattering studies of native bile from the dog. Comparison with aggregative behavior of biliary lipid systems. *Biochemistry.* **23:** 1994–2005.
54. Haslewood, G. A. D. 1964. Bile Salts. R. Peters and F. G. Young, editors. Methuen Co. Ltd., London.
55. Einarsson, K., J. Ahlberg, B. Angelin, and B. Holmström. 1979. Evidence for the presence of different hepatic cholesterol precursor pools in man. *In* The Liver: Quantitative Aspects of Structure and Function. R. Preisig and J. Bircher, editors. Editio Cantor, Aulendorf, Germany. 233–238.
56. Quarfordt, S. H., and M. F. Greenfield. 1973. Estimation of cholesterol and bile acid turnover in man by kinetic analysis. *J. Clin. Invest.* **52:** 1937–1945.
57. Mok, H. Y. I., K. von Bergmann, and S. M. Grundy. 1978. Effects of interruption of enterohepatic circulation on biliary lipid secretion in man. *Dig. Dis. Sci.* **23:** 1067–1075.
58. Mazer, N. A., and M. C. Carey. 1983. Quasielastic light scattering studies of aqueous biliary lipid systems. Cholesterol solubilization and precipitation in model bile systems. *Biochemistry.* **22:** 426–442.
59. Shefer, S., S. Hauser, V. Lapar, and E. H. Mosbach. 1973. Regulatory effects of sterols and bile acids on hepatic 3-hydroxy-3-methylglutaryl CoA reductase and cholesterol 7α-hydroxylase in the rat. *J. Lipid Res.* **14:** 573–580.
60. Mitropoulos, K. A., S. Balasubramaniam, and N. B. Myant. 1973. The effect of interruption of the enterohepatic circulation of bile acids and of cholesterol feeding on cholesterol 7α-hydroxylase in relation to the diurnal rhythm in its activity. *Biochim. Biophys. Acta.* **326:** 428–438.
61. Van Cantfort, J., and J. Gielen. 1975. Cholesterol 7α-hydroxylase. 2. Biochemical properties and participation of endogenous cholesterol in the assay in vitro. *Eur. J. Biochem.* **55:** 33–40.
62. Michaelis, L., and M. L. Menten. 1913. Die Kinetik der Invertinwirkung. *Biochem. Z.* **49:** 333–369.
63. Johansen, G., and R. Lumry. 1961. Statistical analysis of enzymic steady-state rate data. *C. R. Trav. Lab. Carlsberg.* **32:** 185–214.
64. Sundler, R., and B. Åkesson. 1975. Biosynthesis of phosphatidylethanolamines and phosphatidylcholines from ethanolamine and choline in rat liver. *Biochem. J.* **146:** 309–315.
65. Robins, S. J., and H. Brunengraber. 1982. Origin of biliary cholesterol and lecithin in the rat: contribution of new synthesis and preformed hepatic stores. *J. Lipid Res.* **23:** 604–608.
66. Heath, T., I. W. Caple, and P. M. Redding. 1970. Effect of the enterohepatic circulation of bile salts on the flow of bile and its content of bile salts and lipids in sheep. *Q. J. Exp. Physiol.* **55:** 93–103.
67. Gumucio, J. J., and M. E. Katz. 1979. The acinar organization for bile salt transport. *In* The Liver: Quantitative Aspects of Structure and Function. R. Preisig and J. Bircher, editors. Editio Cantor, Aulendorf Germany. 179–184.
68. Olszewski, M. F., R. T. Holzbach, A. Saupe, and G. H. Brown. 1973. Liquid crystals in human bile. *Nature.* **242:** 336–337.
69. Dedrick, R. L., K. B. Bischoff, and D. Z. Zaharko. 1970. Interspecies correlation of plasma concentration history of methotrexate (NSC-740). *Cancer Chemother. Rep. Part I.* **54:** 95–101.
70. Boxenbaum, H. 1982. Interspecies scaling, allometry, physiological time and the ground plan of pharmacokinetics. *J. Pharmacokinet. Biopharm.* **10:** 201–227.
71. Prothero, J. W. 1982. Organ scaling in mammals: the liver. *Comp. Biochem. Physiol.* **71A:** 567–577.

72. Ivy, A. C., H. M. Janecek, and R. Wojciech. 1961. Relation of body weight to excretion of endogenous sterol in rat. *Am. J. Physiol.* **201:** 194–196.
73. Wilson, J. D. 1964. The quantification of cholesterol excretion and degradation in the isotopic steady state in the rat: the influence of dietary cholesterol. *J. Lipid Res.* **5:** 409–417.
74. Abell, L. L., E. H. Mosbach, and F. E. Kendall. 1956. Cholesterol metabolism in the dog. *J. Biol. Chem.* **220:** 527–536.
75. Wojciech, R., H. M. Janecek, and A. C. Ivy. 1961. Endogenous excretion and intestinal capacity for absorption of cholesterol in the dog. *Am. J. Physiol.* **201:** 190–193.
76. Grundy, S. M., and E. H. Ahrens. 1969. Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
77. Miettinen, T. A. 1971. Cholesterol production in obesity. *Circulation.* **44:** 842–850.
78. Grundy, S. M., and H. Y. I. Mok. 1977. Determination of cholesterol absorption in man by intestinal perfusion. *J. Lipid Res.* **18:** 263–271.
79. Salen, G., G. Nicolau, S. Shefer, and E. H. Mosbach. 1975. Hepatic cholesterol metabolism in patients with gallstones. *Gastroenterology.* **69:** 676–684.
80. Carulli, N., M. Ponz De Leon, F. Zironi, A. Pinetti, A. Smerieri, R. Iori, and P. Loria. 1980. Hepatic cholesterol and bile acid metabolism in subjects with gallstones: comparative effects of short term feeding of chenodeoxycholic acid and ursodeoxycholic acid. *J. Lipid Res.* **21:** 35–43.
81. Carulli, N., M. Ponz De Leon, F. Zironi, R. Iori, and P. Loria. 1980. Bile acid feeding and hepatic sterol metabolism: effect of deoxycholic acid. *Gastroenterology.* **79:** 637–641.
82. Eriksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. Bile acids and steroids. *Proc. Soc. Exp. Biol. Med.* **94:** 578–582.
83. Lindstedt, S., and A. Norman. 1956. The turnover of bile acids in the rat. *Acta Physiol. Scand.* **38:** 121–128.
84. Myant, N. B., and H. A. Eder. 1961. The effect of biliary drainage upon the synthesis of cholesterol in the liver. *J. Lipid Res.* **2:** 363–368.
85. Grundy, S. M., E. H. Ahrens, and J. Davignon. 1969. The interaction of cholesterol absorption and cholesterol synthesis in man. *J. Lipid Res.* **10:** 304–315.
86. Grundy, S. M., and A. L. Metzger. 1972. A physiological method for estimation of hepatic secretion of biliary lipids in man. *Gastroenterology.* **62:** 1200–1217.
87. Nestel, P. J., H. M. White, and D. S. Goodman. 1969. Distribution and turnover of cholesterol in humans. *J. Clin. Invest.* **48:** 982–991.
88. Miettinen, T. A. 1970. Enhanced cholesterol metabolism in obesity. *Scand. J. Clin. Lab. Invest.* **25 (Suppl. 113):** 28 (Abstract).
89. Sheiner, L. B., and S. L. Beal. 1980. Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-Menten model: routine clinical pharmacokinetic data. *J. Pharmacokinet. Biopharm.* **8:** 553–571.
90. Hepner, G. W., and S. H. Quarfordt. 1975. Kinetics of cholesterol and bile acids in patients with cholesterol cholelithiasis. *Gastroenterology.* **69:** 318–325.
91. Redinger, R. N. 1976. The effect of loss of gallbladder function on biliary lipid composition in subjects with cholesterol gallstones. *Gastroenterology.* **71:** 470–474.
92. Soloway, R. D. 1976. Effects of gallbladder function on bile composition. *Gastroenterology.* **71:** 880–881 (Selected Summaries).
93. Carey, M. C. 1982. The enterohepatic circulation. *In* The Liver: Biology and Pathobiology. I. M. Arias, H. Popper, D. Schacter, and D. A. Shafritz, editors. Raven Press, New York. 429–465.
94. Hoffman, N. E., and A. F. Hofmann. 1977. Metabolism of steroid and amino acid moieties of conjugated bile acids in man. V. Equations for the perturbed enterohepatic circulation and their application. *Gastroenterology.* **72:** 141–148.
95. Hardison, W. G. M., N. Tomaszewski, and S. M. Grundy. 1979. Effect of acute alteration in small bowel transit time upon the biliary excretion rate of bile acids. *Gastroenterology.* **76:** 568–574.
96. Duane, W. C., and K. C. Hanson. 1978. Role of gallbladder emptying and small bowel transit in regulation of bile acid pool size in man. *J. Lab. Clin. Med.* **92:** 859–872.
97. Everson, G. T., M. J. Lawson, C. McKinley, R. Showalt, and F. Kern, Jr. 1983. Gallbladder and small intestinal regulation of biliary lipid secretion during intraduodenal infusion of standard stimuli. *J. Clin. Invest.* **71:** 596–603.
98. Shaffer, E. A. 1979. The role of the gallbladder in gallstone formation. *In* Gallstones. M. M. Fisher, C. A. Goresky, E. A. Shaffer, and S. M. Strasberg, editors. Plenum Press, New York. 223–249.
99. Sama, C., N. F. LaRusso, V. Lopez del Pino, and J. L. Thistle. 1982. Effects of acute bile acid administration on biliary lipid secretion in healthy volunteers. *Gastroenterology.* **82:** 515–525.
100. Hofmann, A. F., G. Molino, M. Milanese, and G. Belforte. 1983. Description and simulation of a physiological pharmacokinetic model for the metabolism and enterohepatic circulation of bile acids in man. Cholic acid in healthy man. *J. Clin. Invest.* **71:** 1003–1022.
101. Metzger, A. L., R. Adler, S. Heymsfield, and S. M. Grundy. 1973. Diurnal variation in biliary lipid composition—possible role in cholesterol gallstone formation. *N. Engl. J. Med.* **288:** 333–336.
102. Balabaud, C., K. A. Kron, and J. J. Gumucio. 1977. The assessment of the bile salt-nondependent fraction of canalicular bile water in the rat. *J. Lab. Clin. Med.* **89:** 393–399.
103. Boyer, J. L., and G. Klatskin. 1970. Canalicular bile flow and bile secretory pressure. Evidence for a non-bile salt dependent fraction in the isolated perfused rat liver. *Gastroenterology.* **59:** 853–859.
104. Preisig, R., H. L. Cooper, and H. O. Wheeler. 1962. The relationship between taurocholate secretion rate and bile production in the unanesthetized dog during cholinergic blockade and during secretin administration. *J. Clin. Invest.* **41:** 1152–1162.
105. Linblad, L., and T. Scherstén. 1976. Influence of cholic and chenodeoxycholic acid on canalicular bile flow in man. *Gastroenterology.* **70:** 1121–1124.