review

Mechanisms for the intestinal absorption of bile acids

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ABSTRACT In this review experimental data are summarized which indicate that at least four different transport mechanisms account for net movement of bile acids across the gastrointestinal tract. These are active transport and the passive mechanisms of ionic, nonionic, and micellar diffusion.

Of these four, active transport and passive nonionic diffusion are quantitatively of the greatest importance. Active transport is confined to the ileum and probably plays a dominant role in the absorption of conjugated bile acids. Passive nonionic diffusion may occur at any level of the gastrointestinal tract and probably is the major mechanism for the absorption of unconjugated bile acids.

KEY WORDS bile acids · conjugated bile acids · small bowel · large bowel · bile acid transport · passive · active · facilitated diffusion · exchange diffusion · micelles

BILE ACIDS ARE THE END PRODUCT of the catabolism of cholesterol by the liver, and, indeed, they represent one of the major means for the ultimate excretion of sterols from the body (1–6). In addition, however, they serve a number of other important physiologic functions. For example, within the intestinal lumen bile acids play a crucial role in the absorption of fat by bringing into micellar solution the fatty acids and monoglycerides that are the end products of the action of pancreatic lipase upon dietary triglyceride (7, 8). Also, they are required for the effective absorption of cholesterol and steroid hormones from the small intestine (9–11).

Abbreviations: CMC, critical micellar concentration; CMT, critical micellar temperature.

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In addition to promoting the movement of fat-soluble substances across the intestinal mucosa, bile acids may also act intracellularly to exert a rate-controlling effect upon several important metabolic pathways (12). It has been suggested, for example, that in the liver bile acids play a feedback-regulatory role in the synthesis both of cholesterol from acetate and of bile acid itself from cholesterol (13, 14). Bile acids have also been shown to alter the rate of cholesterogenesis in the small intestine (15–17). Finally, some evidence (18–22) implicates both conjugated and unconjugated bile acids as possible regulatory agents in such diverse metabolic processes as oxidative phosphorylation, ATPase activity, and various synthetic and transport systems.

In the intact animal, bile acids are absorbed from the gastrointestinal tract and transported via the portal blood to the liver where they are excreted into the bile and so again gain access to the intestinal lumen. Because this enterohepatic circulation is efficient, the bile acid pool is cycled several times daily through the intestine and liver, and only a relatively small amount of bile acid is synthesized de novo each day to replace that which escapes absorption and so is excreted in the stool as acidic steroids. Interruption of this cycle leads to a marked reduction in the intraluminal and tissue concentrations of bile acid, and this in turn leads to changes in the rate of fat absorption as well as the rates of several intracellular metabolic processes (14, 16, 17, 23, 24).

One of the crucial factors for maintenance of the enterohepatic circulation is the ability of the gastrointestinal tract to absorb bile acids; the mechanisms of this absorptive process are the subject of the present review. The discussion includes three sections in which particular emphasis is given to (*a*) the intraluminal phase, (*b*) the

mucosal phase, and (*c*) the delivery phase of this absorptive process.

INTRALUMINAL PHASE OF THE ABSORPTIVE PROCESS

The physicochemical characteristics of bile acids as they exist in simple solutions as well as in the intestinal contents have been the subject of much recent investigation. In the present review only those features of micellar solutions that effect the rate of intestinal absorption of bile acids will be considered. For a more detailed discussion of this topic, the reader is referred to the recent excellent reviews by Hofmann (25) and by Hofmann and Small (26).

The bile acid molecule bears a resemblance to cholesterol but differs from this parent compound in several important respects: an isopropyl group has been cleaved from the cholesterol side chain and the terminal carbon atom (C-24) forms part of a carboxyl group; the steroid nucleus has been saturated in such a way that the A ring is in a *cis* configuration with respect to the B ring, i.e., bile acids are 5β-H sterols; and finally, hydroxyl groups have been substituted for hydrogen atoms at various sites on the steroid nucleus. Such substitutions are commonly, although not exclusively, in the α-configuration. Bile acids may be monohydroxy, e.g. lithocholic acid (3α-hydroxycholanoic acid); dihydroxy, e.g. deoxycholic and chenodeoxycholic acid (3α,12α- and 3α,7α-dihydroxycholanoic acid, respectively); or trihydroxy, e.g. cholic acid (3α,7α,12α-trihydroxycholanoic acid).

In addition, bile acids may be conjugated through a peptide linkage at C-24 to either of the amino acids glycine or taurine. Such conjugation profoundly alters the pK_a of the bile acids and so, as will be apparent in later sections, has a marked effect upon their diffusion through the intestinal membrane. Thus, the pK_a for unconjugated bile acids is approximately 6.0, while those for the glycine and taurine conjugates are approximately 4 and 2, respectively (26).

As illustrated in Fig. 1, the prototype bile acid, in this case taurocholic acid, may be considered as a planar molecule with three important features. First, the lower surface (as oriented in Fig. 1) containing the angular methyl groups of the steroid nucleus is hydrophobic. Second, the upper surface containing one or more hydroxyl groups is hydrophilic. Third, at sufficiently high pH the end of the molecule containing the carboxyl group or, in the case of conjugated bile acids, the glycine or taurine moiety, possesses a negative charge and, therefore, is highly polar.

As a consequence of this structure in which the molecule contains both hydrophobic and hydrophilic regions, i.e., is amphipathic, bile acids possess many of the properties of surface active agents or detergents. One of these properties that is important in the present discussion is the ability of such molecules to form macromolecular aggregates or micelles. At low concentrations bile acids exist in aqueous solution in monomeric form as shown diagrammatically in Fig. 2A. As the concentration of such a solution is increased, a point is reached, the critical micellar concentration or CMC, at which spontaneous association of monomers to form dimers, tetramers, or even larger structures occurs (Fig. 2B–D). Increasing the concentration of bile acid above this level results in an increased concentration of micelles while the concentration of monomer increases at a diminished rate or actually remains almost fixed at a value approximately equal to the CMC. A concentrated solution of bile acid, therefore, consists of two molecular species—individual molecules of bile acid and aggregates of molecules in micelles—in equilibrium.

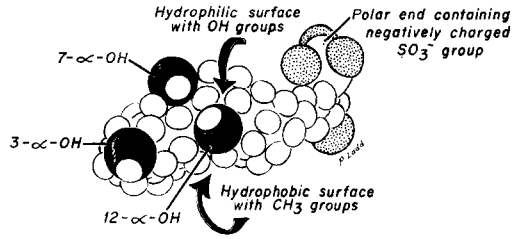

FIG. 1. Diagrammatic representation of a typical bile acid molecule, that of taurocholic acid (3α,7α,12α-trihydroxycholanoic acid). The molecule is inverted from the usual manner of presentation in order to show the hydrophilic surface containing the three α-hydroxyl groups. The hydrophobic surface containing the methyl groups is not seen in this view of the molecular model.

Small has recently published data (27, 28) which suggest that the smaller micelle structures, i.e., the dimers, tetramers, etc., are held together by hydrophobic bonding between the hydrophobic surfaces of the bile acid molecules while the hydrophilic sides and polar end groups are thrust outward into the aqueous phase as shown in Fig. 2B. In the case of some bile acids larger aggregates may be formed by the coalescence of the smaller micelles; such a structure presumably would be maintained by hydrogen bonding between the outwardly directed hydrophilic surfaces of the bile acid molecules composing the smaller micelles (Fig. 2D).

The exact concentration at which aggregation of monomers begins, as well as the number of molecules present in the micelle, i.e., the aggregation number, is different for different bile acids; furthermore, for any given bile acid in solution the CMC and aggregation number are not fixed but are dependent upon such factors as the temperature, the pH, and the presence of counter-ions or a second amphipath. For example, micelle formation will not begin until the temperature of the solution has reached a certain crucial value known as

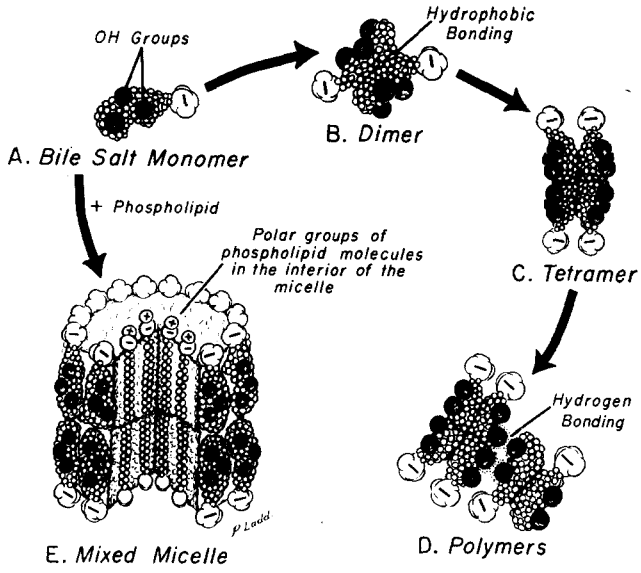

FIG. 2. Diagrammatic representation of recently proposed micelle structures for bile acids. In solution, bile acids may exist as monomers (A) or as various macromolecular complexes. Small complexes, e.g., dimers and tetramers (B and C), consist of groups of monomers held together by hydrophobic bonding between the hydrophobic surfaces of adjacent bile acid molecules, while the hydrophilic surfaces and polar groups are oriented outward into the aqueous phase. Such small complexes have been termed primary micelles and are thought to represent the structure of the aggregates formed by the trihydroxy bile acids, which have relatively low aggregation numbers of 2 to 10. Larger secondary micelles are formed by aggregation of primary particles as shown in D; it has been proposed that hydrogen bonding is responsible for maintaining the structure of these large complexes. The dihydroxy bile acids, which have larger aggregation numbers (>10), probably form such large micelles. The addition of a second amphipath to a solution of bile acids results in a completely different structure, as shown in E for phospholipid. In this mixed micelle the hydrocarbon chains of phospholipid are in the interior of the complex, while the polar end groups point outward on the surface of the cylindrical structure. The hydrophobic core is coated with bile acid molecules oriented with their hydrophilic surface pointed outward into the aqueous phase. Solutions of such mixed micelles usually have a lower CMC and a higher aggregation number than solutions of the bile acid alone. (This diagram is based upon experimental data and concepts presented by Small in references 27 and 28).

the critical micelle temperature (CMT); elevation of the temperature above this value, in general, will raise the value of the CMC for a given bile acid while its aggregation number will remain unchanged or decline slightly (26, 27). The presence of counter-ions commonly results in a decrease in the CMC but an increase in the aggregation number, while a downward shift in the pH causes a striking increase in the aggregation number, particularly for the dihydroxy bile acids. For detailed information on the interactions, the reader is referred to references 7, 26, 27, 29.

Despite these complex permutations, a number of generalizations are possible which apply to solutions of bile acids under the conditions of pH, ionic strength, and temperature commonly utilized in the study of bile acid transport; some of these are given in Table 1. First, the CMT is well above 37°C for unsubstituted cholanoic acid and for lithocholic acid, but well below body temperature for the common di- and trihydroxy bile acids. Conjugation of the free acids does not appear to alter these values significantly. Thus, at physiologic temperatures substances such as cholic, deoxycholic, and chenodeoxycholic acid will form micelles while lithocholic acid will not. Second, in physiologic solutions micelle formation begins at lower concentrations in the case of dihydroxy bile acids (2–4 mM) than with the trihydroxy bile acids (3–8 mM). Again, these values for the critical micelle concentration are not markedly different for either the taurine or glycine conjugates. Third, the aggregation numbers for the free or conjugated dihydroxy bile acids are substantially greater (falling into the range 10–23) than those of the trihydroxy bile acids (5–6). Fourth, as noted earlier, the value of the pK_a is essentially independent of the number of hydroxyl substitutions on the steroid nucleus but, primarily, is a function of the group conjugated at C-24; thus, these substances become increasingly acidic as one goes from unconjugated bile acids ($pK_a \sim 6.0$) to glycine conjugates ($pK_a \sim 4.0$) to taurine conjugates ($pK_a \sim 2.0$). Finally, when a second amphipath such as phospholipid is dissolved in a micellar solution of bile acid (shown diagrammatically in Fig. 2E), the general effect is to greatly expand the size of the micelle while, at the same time, reducing the value of the CMC (28, 30–32, and personal communication from D. M. Small).

TABLE 1 THREE PHYSICAL CONSTANTS OF SELECTED BILE SALTS IN SOLUTION

Type of Bile Salt (All Sodium)	Critical Micellar Temperature*	Critical Micellar Concentration†	Aggregation Number‡
	°C	mM	
No hydroxyl groups			
Cholanoate	60	—	—
Monohydroxy			
Lithocholate	50	—	—
Dihydroxy			
Chenodeoxycholate	< 0	2–4	10–21
Deoxycholate	< 0	2–4	16–23
Trihydroxy			
Cholate	< 0	3–8	5–6

* CMT values determined in bile acid solutions of 10 mM as reported by Hofmann and Small (26).

† CMC values determined in solutions also containing 150 mM NaCl (26).

‡ Approximate aggregation numbers of bile acids determined by light scattering at 37°C in solutions containing 150 mM NaCl at pH 8–9. Extrapolated from the data of Small (27).

MUCOSAL PHASE OF THE ABSORPTIVE PROCESS

Schiff, in 1870, first established that bile participates in an enterohepatic circulation by demonstrating (33) the dependence of biliary secretion upon the absorption of some component of bile from the gastrointestinal tract. It was later appreciated, however, that the bile acid constituent of whole bile, and not the bile pigments, was absorbed from the gastrointestinal tract. 8 yr later, in 1878, Tappeiner provided the first evidence (34) that regional differences exist in the ability of various areas of the intestine to bring about such reabsorption when he found more rapid uptake of bile acids from the ileum than from the proximal small bowel. This finding was confirmed by Fröhlicher in 1936 (35). On the other hand, results apparently contradictory to these were published in 1928 by Greene, Aldrich, and Rowntree (36) and in 1955 by Sjövall and Åkesson (37). Greene et al., for example, administered a solution of bile salts intragastrically to dogs with external biliary fistulas and found that the maximum rate of hepatic secretion of these salts was achieved by the end of the second 30 min collection period. Furthermore, when the bile salt solution was instilled directly into the duodenum, a rise in the bile acid content of portal blood was detected within 15 min (36). Similarly, Sjövall and Åkesson (37) found that after the oral administration of taurocholic acid-^{14}C to rats with external biliary fistulas, the labeled bile acid appeared in the biliary drainage material within minutes and reached a maximum rate of excretion (and, therefore, intestinal absorption) 20–30 min after instillation of the isotope. It seems unlikely, as stressed by Sjövall (38), that in these two experiments the bile acid could have traversed the whole length of the jejunum and so reached the ileum, the postulated site of bile acid absorption, in so short a time; therefore, these data suggest, by inference at least, that bile acid absorption may also occur in the proximal small intestine.

More recent investigations undertaken in vivo have tended to support the concept that bile acid absorption occurs in both the proximal and distal small intestine although the latter location appears quantitatively more important. Baker and Searle, for example, showed (39) that absorption of crude ox bile took place from all areas of the small bowel of the rat, but the rate of uptake varied from approximately 1.5 μmoles/cm per hr in the proximal small bowel to 4.0 μmoles/cm per hr in the distal ileum. Using polyethylene glycol as a nonabsorbable marker, Webling (40) detected uptake of taurocholic acid equal to 21 ± 9%, 57 ± 5%, and 84 ± 11% of the dose placed in tied-off segments of duodenum, jejunum, and ileum, respectively, in the chick. Tidball (41) similarly demonstrated absorption of cholic acid from both the jejunum and ileum of the rat (18% and 45% of the administered dose in 1 hr, respectively). In contrast to these studies, however, it should be emphasized that Searle and Baker (42), Weiner and Lack (43), and Sullivan (44), working with the dog, immature guinea pig, and rat, respectively, detected no significant bile acid absorption from the proximal small intestine.

These various investigations, which were all undertaken in vivo, provide no information concerning the mechanism(s) of transport responsible for the intestinal absorption of bile acids. In every case the chemical gradient for bile acid was undoubtedly in the direction mucosa-to-serosa. Thus, any one of several passive mechanisms, as well as active transport, could be evoked as a possible explanation for the observed absorption. In addition, a variety of bile acid preparations were utilized; these ranged from crude ox bile, which contains several different conjugated and unconjugated bile acids, to purified preparations of radiolabeled cholic and taurocholic acid. It is necessary, therefore, to turn to experiments performed under in vitro conditions in which factors such as the chemical, electrical, and pH gradients can be precisely manipulated and measured in order to delineate the mechanisms responsible for the transmucosal movement of bile acids.

MECHANISMS OF TRANSPORT ACROSS THE SMALL INTESTINE OF ANIMALS

Active Transport

The first studies of this type were reported by Lack and Weiner in 1961 (45). These authors utilized the everted gut sac preparation originally described by Wilson and Wiseman (46) to study systematically the transport of taurocholic and glycocholic acid at all levels of the small intestine of the rat and guinea pig. Beginning with equal concentrations of bile acids in both the serosal and mucosal fluids, they found that after a 90 min incubation only intestinal sacs prepared from the terminal quarter of the small bowel were capable of net transport of bile acids across the bowel wall, so that the ratio of the concentration of bile acid in the serosal fluid to that in the mucosal fluid achieved a value significantly greater than 1. Similar results were subsequently reported by Playoust and Isselbacher (47) and by Holt (48). An example of this type of experiment performed in the author's laboratory is shown in Fig. 3; in this case the entire small intestine of the rat was divided into 10 segments and everted sacs were prepared and incubated with taurocholic acid-^{14}C. As is apparent, at the end of the incubation the serosal/mucosal ratios of the concentrations of bile acid were about 1 throughout the proximal half of the bowel, while distally the ratios increased progressively, reaching maximum values of approximately 9–10 in the terminal

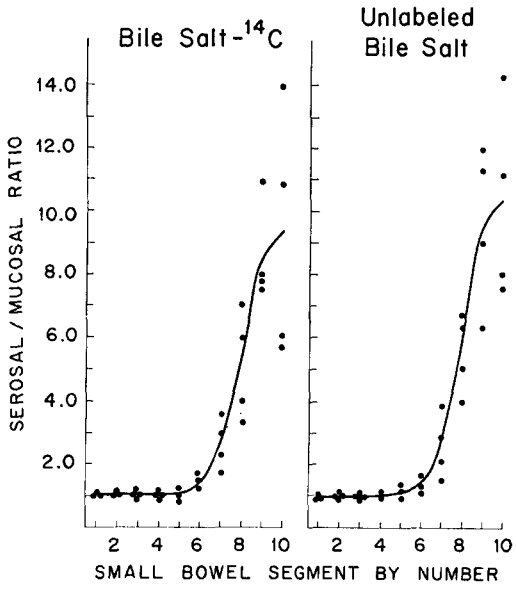

FIG. 3. Taurocholic acid transport in the everted gut sac. The entire small bowel of the rat was divided into ten segments of equal length, numbered from 1 to 10, proximal to distal, and everted gut sacs were prepared. These were incubated for 90 min at 37°C in Krebs bicarbonate buffer containing taurocholic acid-^{14}C at an initial concentration of 0.2 mM. The results are expressed as the ratio of the final concentration of bile acid in the serosal fluid to that in the mucosal fluid at the end of the incubation. The left panel shows the values of the ratios determined by radioassay; ratios in the right panel were obtained by chemical analysis of the bile acid concentrations (unpublished data from the author's laboratory).

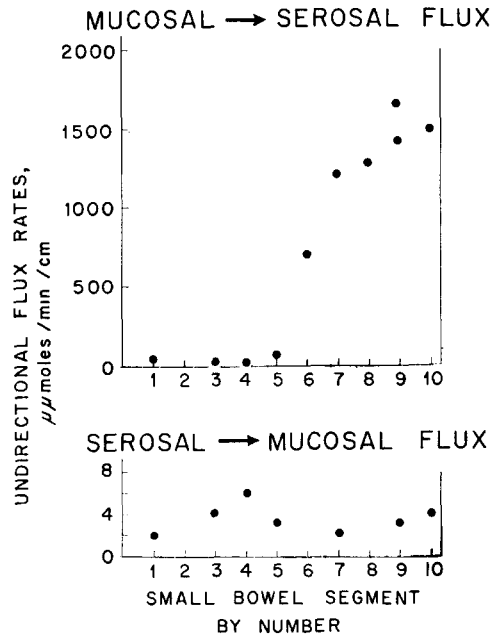

FIG. 4. Unidirectional flux rates of taurocholic acid along the length of the small intestine. The upper and lower panels show the mucosal-to-serosal and serosal-to-mucosal flux rates, respectively, for taurocholic acid at every level of the small intestine of the rat. These flux rates were measured in an in vitro perfusion apparatus in which both the activity and electrical gradients were fixed at zero and the solvent drag effects could be corrected for (50).

two ileal segments. Thus, as originally emphasized by Lack and Weiner (45), only the ileum appears capable of moving bile acid against a concentration gradient. In addition, it should be emphasized that such data constitute strong evidence for the presence of an active transport mechanism in these segments, for if such high serosal/mucosal ratios were simply the passive consequence of a favorable electrical gradient, then a transmural potential difference of approximately 60 mv, serosa-positive, would be required. In point of fact, the potential difference across the wall of these sacs equals only a few millivolts at most (49); thus, in these experiments bile acid clearly is moving against an electrochemical gradient.

Additional evidence in support of active bile acid transport in the rat ileum is provided by the flux ratio data published from this laboratory (50). Fig. 4 illustrates the values of the unidirectional flux rates for taurocholic acid measured in vitro at 10 levels of the small intestine when both the chemical and electrical gradients across the bowel wall were fixed at zero. Under these experimental conditions the Ussing flux ratio equation (51) would predict a value of 1 for the ratio of the mucosal-to-serosal flux rate to the serosal-to-mucosal flux rate if movement of bile acid across the intestinal wall were dictated solely by the existing electrochemical gradient, i.e., if such movement were passive. The value of the flux ratio was indeed found to approximately equal 1 in the proximal small intestinal segments; in contrast, however, values much greater than 1 were found in all segments taken from the ileum—a finding which strongly supports the concept of Lack and Weiner that an active transport process for bile acid exists in this area of the intestine.

In their original observations Lack and Weiner also demonstrated that the ability of ileal sacs to transport bile acids against a concentration gradient is inhibited by anoxia or by several metabolic inhibitors (45). These findings have been confirmed and extended in several other laboratories, so that transport has been shown to be inhibited by incubation of the everted sacs with an atmosphere of nitrogen, 2,4-dinitrophenol, sodium azide, potassium cyanide, 4,6-dinitro-*o*-cresol, sodium iodoacetate, sodium fluoride, benzmalecene, or ouabain (45, 47, 48, 50, 52). The effect of ouabain appears to be species-specific since inhibition occurs in the intestine of guinea pig (53, 54) but not in that of hamster (47). Finally, the equimolar substitution of K^+ for Na^+ in the incubation media also inhibits bile acid transport (47). It is of interest, however, that this process is not dependent upon the presence of glucose, the rates of taurocholic acid transport being the same, for example, whether glucose is present in the incubation medium at a level of 200 mg/100 ml or is totally absent (47, 50). Nor does phlori-

dizin at concentrations sufficient to block glucose transport interfere with the uptake of bile acid (47).

Fragmentary observations on the kinetics of this active transport process have appeared from four different laboratories during the past 6 yr. In their initial paper, Lack and Weiner demonstrated that the amount of taurocholic acid transported by guinea pig ileum appeared to reach a limiting velocity as the concentration of bile acid in the incubation medium was progressively increased (45). Playoust and Isselbacher further showed (47) that the amount of taurocholic acid-^{35}S transported by hamster ileal sacs is related linearly to the concentration of bile acid in the perfusing media when each of these parameters is plotted as the reciprocal value, i.e., in a Lineweaver–Burk plot (55). These authors further calculated that the apparent values of V_{max} and K_m for taurocholic acid in this species are 10.9 μmoles for a 10 cm sac incubated for 45 min (approximately 24,000 $\mu\mu$moles/min per cm) and 1.34 μmoles/ml, respectively. Similar kinetics have been reported by Holt (48) in experiments in which tissue accumulation in slices of ileum rather than net bile acid transport was measured. Finally, Dietschy, Salomon, and Siperstein (50), using an in vitro perfusion apparatus in which the mucosal concentration of bile acid could be kept constant during the measurement of the active flux—an experimental circumstance not possible with the everted gut sac—determined V_{max} for the transport of taurocholic acid by the terminal ileum of the rat. The value obtained was 1,380 $\mu\mu$moles/min per cm.

It is of interest to point out that the flux rates shown in the upper panel of Fig. 4 are close to the values of V_{max} at each level of the ileum. This is true because the mucosal-to-serosal flux rate measured in each segment can be equated with the net active flux rate since the passive serosal-to-mucosal flux in this study was insignificant. Furthermore, the concentration of taurocholic acid in the bathing medium equaled 500 mμmoles/ml—a concentration 5 times the value of K_m observed in this study—so that the measured active flux rate approached the maximal transport velocity. Thus, the value of V_{max} for the active transport process progressively increases down the length of the distal small bowel and accounts for the apparent gradient of absorptive activity. Such a finding is consistent with the concept that there is a gradual increase in the density of transport sites per unit length of bowel from the midintestine to the terminal ileum rather than a relatively uniform number of sites with various affinities for the bile salt molecule.

The stereospecificity of the ileal transport system is of considerable importance since several different bile acids are commonly present in the mammalian intestine. Early observations suggested that differences exist in the rate of transport of bile acids of different structures (45, 47, 48). In a more recent publication Lack and Weiner (53) have reported an extensive investigation of the ability of everted intestinal sacs prepared from guinea pig ileum to transport a variety of bile acids. Their data are partially summarized in Table 2. The highest serosal/mucosal ratios were found for tauro- or glycocholic acids. Intermediate values for the serosal/mucosal ratios (and, therefore, presumably less rapid transport) were found with conjugated dihydroxy bile acids and the triketo derivative, but movement against a concentration gradient did, nevertheless, occur. Cholic acid conjugated to other negatively charged groups besides taurine or glycine also was transported. However, conjugation of this trihydroxy bile acid to compounds having either two negative charges (Nos. 5, 6, and 7, Table 2) or a positively charged group (No. 8) resulted in bile acids which were either poorly transported or were not transported at all. These authors concluded that the one crucial structural feature which appeared necessary for transport is the presence of a negative charge on the bile acid side chain.

That these various bile acids are all transported by a common carrier rather than by several related carriers is suggested by data demonstrating competition among the various conjugated and unconjugated bile acids for transport sites. Thus, Playoust and Isselbacher have reported

TABLE 2 EFFECT OF MOLECULAR STRUCTURE ON THE ACTIVE TRANSPORT OF BILE ACIDS ACROSS THE EVERTED GUT SAC

Bile Acid Derivative	Serosal/Mucosal Ratio
Trihydroxycholanoic acid derivatives	
1. Cholic acid	3.7
2. Taurocholic acid	13.0
3. Glycocholic acid	11.2
4. *N*-Cholyl-*n*-δ-aminovaleric acid	5.2
5. *N*-Cholylaspartic acid	1.2
6. *N*-Cholyl-*O*-phosphoryl ethanolamine	0.85
7. *N*-Cholylaminoethylphosphoric acid	2.0
8. *N*-Cholyl-trimethylethylenediamine	0.71
Dihydroxycholic acid derivatives	
9. Taurodeoxycholic acid	5.5
10. Glycodeoxycholic acid	1.8
11. Taurochenodeoxycholic acid	6.5
12. Glycochenodeoxycholic acid	2.1
13. Taurohydrodeoxycholic acid	5.7
14. Glycohyodeoxycholic acid	6.9
15. 7,12-Dihydroxycholanoyltaurine	4.5
16. *N*-3-Chloro-7,12-dihydroxycholanoylglycine	2.0
Triketocholanoic acid derivative	
17. Glycodehydrocholic acid	3.4

This table is the result of adapting data presented by Lack and Weiner (53) and shows the mean serosal/mucosal ratios developed after incubation of everted gut sacs prepared from the terminal quarter of the small intestine of the guinea pig with the bile acids and derivatives listed.

(47) that both cholic and glycocholic acids compete with taurocholic acid for transport across the hamster ileum. Holt (48) has further demonstrated competitive inhibition by endogenous bile acids, i.e., those bile acids present in the mucosa at the time of tissue preparation, of the uptake of taurocholic acid into slices of ileum. Lack and Weiner (53) reported similar competitive phenomena and further observed that dihydroxy bile acids inhibit transport of a second bile acid more effectively than the trihydroxy bile acid, and that this last-named acid was, in turn, more potent than the triketo derivative.

Unfortunately, most of these data on the comparative transport of bile acids have been obtained in experiments that measured serosal/mucosal ratios or uptake in the everted gut sac. Such measurements represent only an indirect measure of active transport; furthermore, since the ratios have, for the most part, been measured at only one concentration of bile acid in the mucosal solution, essentially no data on the K_m values for the different bile acids are available. Thus, a difference in the ratios achieved by two bile acids might be due to a difference in the values of V_{max} or K_m for the active transport of these acids or to a different rate of back-diffusion from the serosal to mucosal compartments. Conclusions, therefore, concerning the relative rates of transport of different bile acids based upon a comparison of the serosal/mucosal ratios developed in the everted gut sac must be interpreted with caution.

The only published data on the comparative kinetics of the transport of different bile acids are those of Playoust and Isselbacher (47) in which, in the everted hamster gut sac, the K_m values for taurocholic, glycocholic, and cholic acids were found to be 1.34, 0.90, and 1.95 μmoles/ml, respectively. In studies from this laboratory (E. Schiff, unpublished) the values of V_{max} for the trihydroxy bile acids have been found to be about 2–4 times greater than the values of V_{max} for the dihydroxy bile acids; furthermore, the concentration of bile acid in the mucosal solution necessary to achieve half maximal transport velocity (K_m) of the unconjugated bile acids is 4–6 times greater than the values of K_m for the taurine and glycine conjugated acids. Such results probably account for the lower serosal/mucosal ratios found in the everted gut sac with the conjugated dihydroxy bile acids and unconjugated bile acids reported by several investigators (Table 2 and references 47, 50, 53).

One final interesting feature of the ileal transport system is that net movement of conjugated bile acid is accomplished without hydrolysis of the C-24 peptide linkage. This point was clearly established by Playoust and Isselbacher (47), who demonstrated that the specific activity of taurocholic acid-^{35}S did not decrease when this bile acid was transported across the ileum in the presence of a large amount of unlabeled taurine.[1]

In summary, there exists a transport mechanism in the small intestine that can bring about the net movement of bile acid against an electrochemical gradient, is inhibited by a variety of metabolic inhibitors, is characterized by saturation kinetics, and manifests substrate structural specificity and competition for transport between related bile acids. By currently accepted criteria this transport mechanism can therefore be considered active. Sites for active bile acid transport are not present in the jejunum but first appear in the proximal ileum; present data suggest that the density of these sites per unit length of intestine progressively increases down the length of the ileum, reaching maximum values in the terminal segments of the small bowel. Finally, it should be emphasized that this active transport mechanism has been identified in various species (56) and is probably present in man (57).

Passive Transport

Net movement of a given substance across a membrane by a passive mechanism can take place only in the presence of a favorable electrochemical gradient. In the case of bile acids, the chemical gradient at every level of the bowel is in the direction intestinal lumen → portal blood; in addition, the luminal surface is negatively charged with respect to the serosal side of the mucosal membrane (49). Thus, it is apparent that the electrochemical gradient for bile acids is oriented strongly in the direction of lumen → portal blood. If the mucosal membrane of the intestine is permeable to bile acids, significant net absorption of bile acids could occur by passive means. Three passive mechanisms have been studied to date; these include passive ionic diffusion, passive nonionic diffusion, and facilitated diffusion.

Passive Ionic Diffusion. Conjugated bile acids have relatively low pK_a values and therefore exist in the luminal contents almost entirely as negatively charged ions. Since biologic membranes are, in general, relatively impermeable to charged molecules, one would expect this species to diffuse only slowly across the intestinal wall. This indeed appears to be so. When the flux of bile acid across the jejunum was studied (50) for solutions of bile acids at concentrations below the CMC, it was found (*a*) that the rate of flux is low but is proportional to the electrochemical gradient, (*b*) that no saturation phenomenon could be detected even at the highest concentrations tested, i.e., that the kinetics were first-order throughout, and (*c*) that the diffusion rate was not significantly altered by a decrease in the temperature of the perfusing media or by metabolic inhibitors (50). These studies,

[1] It is of historical interest that this study confirms the observation of Weiss in 1884 that glycocholic acid appeared to be absorbed intact across the bowel of the dog (*cited by* H. Sobotka. 1937. *In* Physiological Chemistry of the Bile. The Williams & Wilkins Company, Baltimore. 30).

then, clearly establish that passive ionic diffusion of bile acid does take place across the small intestine.

The absolute rate of diffusion is dependent upon the structure of the particular bile acid and is inversely related to the number of hydroxyl groups on the molecule and to the size of the moiety conjugated at the C-24 position (N. Small, unpublished observations made in this laboratory). Thus, of the common bile acids, taurocholic acid has the lowest diffusion constant and unconjugated lithocholic acid, the highest. In addition, for any one bile acid there appears to be important species differences in the passive permeability characteristics of the bowel wall. Glasser, Weiner, and Lack demonstrated, for example (56), that the amount of taurocholic acid absorbed passively during a 90 min period varied from 0.9 to 13.2% of a given load of bile acid placed in the jejunum of the rabbit, rat, guinea pig, dog, or spider monkey.

This passive process now appears to be one step more complicated. Heretofore, passive diffusion has been carefully quantified only from solutions of bile acids at concentrations lower than the CMC. The rate of passive diffusion should be proportional to the chemical activity of the bile acid in the test solution; this activity has, in turn, been assumed to be closely related to the concentration of bile acid monomer. Since the concentration of monomer becomes almost constant as the total concentration of bile acid in solution is raised to and beyond the CMC, the rate of passive diffusion similarly should reach a limiting value at this same point if bile acid monomer were the only species diffusing passively across the bowel wall. However, in a test of this hypothesis (Fig. 5A), the rate of passive transport actually increased as the concentration of bile acid in the luminal test solution was raised above the CMC. From this and a variety of other experiments, it now appears that passive diffusion from solutions of bile acids below their CMC must be considered separate from passive diffusion from solutions above their CMC. For the purpose of this review these two transport mechanisms will be referred to as passive monomer and passive micellar diffusion, respectively. As shown in the study in Fig. 5A, for example, monomeric taurocholic acid diffuses through the membrane at a rate of only 42 $\mu\mu$moles/min per cm per mmole, while micellar taurocholic acid moves across at the faster rate of 84 $\mu\mu$moles/min per cm per mmole. In solutions containing only bile acids, the rate of micellar diffusion appears to be directly proportional to the micellar aggregation number. If, on the other hand, the size of the micelle is greatly expanded by the addition of a second amphipath such as phospholipid—an experimental circumstance in which the small compact taurocholic acid micelle (Fig. 2C) presumably is converted to a bulky mixed micelle (Fig. 2E)—then, as shown in Fig. 5B, the rate of micellar diffusion of taurocholate is greatly retarded. This experimental result is similar to and probably explains the earlier observation of Annegers (58) that oleic acid inhibits the absorption of cholate from Thiry-Vella loops.

Passive Nonionic Diffusion. Since the value of the pK_a (about 6.0) for unconjugated bile acids is much higher than for conjugated bile acids, significant amounts of these acids may exist in the unionized form at the pH levels commonly encountered in the intestinal contents. In general, the unionized form of a weak acid or base will penetrate the intestinal wall much faster than the corresponding ionized acid or base (59). In studies reported

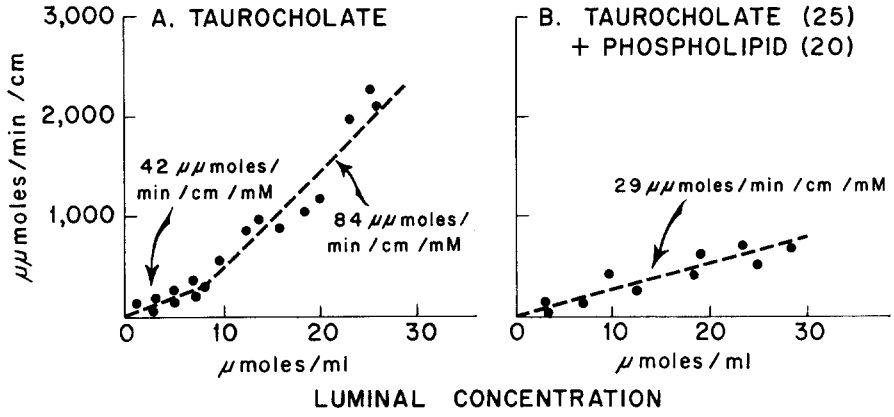

FIG. 5. Passive mucosal-to-serosal flux rates of taurocholic acid across the jejunum of the rat. An in vivo preparation of isolated jejunal loops similar to that described in reference 50 was used, and passive flux rates were measured as a function of the mean intraluminal concentration of bile acid in the test segment. Data in the left panel illustrate results obtained with luminal solutions containing only taurocholic acid in 150 mM sodium chloride solution, while the right panel shows results obtained with luminal solutions containing taurocholic acid and phospholipid at a constant molar ratio of 25:20. The dashed lines were fitted by the method of least squares, and the slope of these lines is indicated in each panel (unpublished data by N. Small from this laboratory).

from this laboratory, this was found also to be true for bile acids; at a given luminal concentration, unionized cholic acid is passively absorbed at a rate that is 5–6 times greater than the rate of absorption of cholate ion (50).

Since nearly all of the bile acid present in the proximal small bowel is conjugated, significant nonionic diffusion probably does not occur across the jejunum under normal conditions. This process may, however, be important physiologically in two other situations. (*a*) Deconjugation of bile acid does occur in the distal ileum and in the colon of the intact animal (12, 60). Nonionic diffusion may play a significant or even predominant role in the absorption of such unconjugated bile acid in these areas of the intestinal tract. (*b*) In pathologic conditions such as the blind loop syndrome, massive bacterial contamination of the upper small bowel leads to deconjugation of bile acids in the jejunum (61). Rapid absorption of these unconjugated bile acids across the jejunum by nonionic diffusion undoubtedly occurs and, in a sense, leads to "short-circuiting" of the normal enterohepatic circulation, which in turn causes a decrease in the intraluminal concentration of bile acid. It is also conceivable that similar excessive jejunal absorption of bile acid might occur in the absence of deconjugation, provided that the intraluminal pH was at sufficiently low levels. The pK_a for bile acids conjugated to glycine is approximately 4.0; thus in pathologic states in which massive gastric hypersecretion of H^+ leads to acidification of the jejunal contents, marked loss of glycine-conjugated bile acids from the intestinal lumen would be expected to occur via nonionic diffusion.

Facilitated Diffusion

In an abstract published in 1967 De Laey has presented preliminary evidence[2] that uptake of bile acid in both the jejunum and ileum may be treated as facilitated diffusion. Judgment of this hypothesis must await publication of the definitive experimental data, but hitherto available data do not support this concept. According to current theory, the observation that bile acid in the ileum is transported against an electrochemical gradient rules out facilitated diffusion as the mechanism responsible for ileal transport of bile acid (62). Furthermore, both the passive ionic and nonionic transport mechanisms described above demonstrate no saturation phenomena over a wide range of bile acid concentrations (from 0.2 to 28 μmoles/ml), have low temperature coefficients, are not sensitive to metabolic inhibition, and manifest no competitive inhibition between related bile acids. All these observations argue strongly against facilitated diffusion as a transport process for bile acid in the small bowel.

[2] De Laey, P. 11th International Conference on the Biochemistry of Lipids. Jerusalem, Israel. 6–11 August 1967.

Solvent Drag

Since the isosmotic absorption of water and electrolytes occurs from all levels of the small intestine, it is important to quantify the effects of bulk water flow on the net movement of bile acids, i.e., the effects of "solvent drag." This has been studied (50) by measurement of the changes in passive flux of taurocholic and cholic acid across the jejunum brought about by changes in the magnitude of bulk water flow. The reflection coefficient of bile acid (σ_{BA}) proved to be about 0.96.

The significance of this value becomes apparent when one considers that the net movement of a given substance across the bowel wall brought about by solvent drag can be calculated as the product of the net water flux times the concentration of that substance in the mucosal solution, times the quantity $(1-\sigma)$. For bile acid the value of the term $(1-\sigma_{BA})$ is so small that solvent drag brings about little net movement of this substance. As will become apparent later, this finding is of considerable importance in explaining the variations in concentration of bile acids down the length of the small intestine.

Exchange Diffusion

If present, exchange diffusion could account for bidirectional flux of bile acid across the bowel wall but not for net transport. Nevertheless, exchange diffusion of bile acid has been specifically looked for in rat intestine and has not been found (50).

In summary, four separate transport phenomena have been identified which bring about net movement of bile acid across the small bowel. They are active transport, passive ionic diffusion, passive nonionic diffusion, and, finally, a process tentatively identified as passive micellar diffusion. Solvent drag plays no significant role, while facilitated diffusion and exchange diffusion of bile acid do not seem to occur.

Transport Across the Colon

Few data are available concerning the movement of bile acids across the colon. Everted sac preparations of rat colon do not transport bile acids against a concentration gradient (48). On the other hand, both conjugated and unconjugated bile acids are absorbed from the large bowel in vivo (44, 63, and unpublished observations in this laboratory). It therefore appears reasonable to assume that passive ionic and nonionic diffusion but not active transport of bile acids take place across the colonic mucosa. No quantitative data are at present available.

Transport in the Human

Throughout this review only those investigations that allow precise definition of transport phenomena have been stressed. Such precision is not possible in studies performed in the human, but several reported investigations

are available that allow speculation as to the operation of one or more of these transport mechanisms in the intestine of man. Three investigations, for example, have shown that little net absorption of bile acid occurs from the proximal small intestine (57, 64, 65). In contrast, other studies have demonstrated a marked increase in the turnover rate of bile acids in patients with ileectomy (24, 66, 67), an observation which provides strong inferential evidence that the most significant site for absorption of bile acids in man, as in other mammals, is the ileum. A detailed analysis of movement of bile acids across the jejunum of man has been reported in abstract form by Hislop, Hofmann, and Schoenfield (68). In this study the rate of uptake during a 30 min test period for unconjugated, glycine-conjugated, and taurine-conjugated bile acids equaled 60 ± 5.1%, 24.5 ± 5.6%, and 3.3 ± 0.8% of the load placed in the small bowel, respectively. In addition, these authors found that the rate of disappearance from the intestinal lumen was influenced by the number and position of hydroxyl groups on the bile acid molecule; thus, cholic, deoxycholic, and chenodeoxycholic acid were absorbed by the jejunum in amounts of 32.8 ± 3.0%, 66.2 ± 5.0%, and 85.7 ± 3.2% of the administered dose, respectively. From these various studies it seems likely that the two quantitatively significant transport phenomena identified in rodents, i.e., active transport and nonionic diffusion, also occur in man.

DELIVERY PHASE OF THE ABSORPTIVE PROCESS

After absorption across the intestinal mucosa, bile acids presumably diffuse equally well into radicals of both the portal venous system and the intestinal lymphatics. Yet, despite this circumstance, Josephson and Rydin presented evidence in 1936 which indicated that bile acids are absorbed predominantly by the portal route (69). This finding was confirmed in 1955 by Sjövall and Åkesson, who demonstrated that only trace amounts of bile acid appeared in the intestinal lymph following the oral administration of taurocholic acid-^{14}C to the rat (37).

The mechanism of this nearly complete shunting of bile acid into the portal circulation has recently been clarified by Reinke and Wilson (70). These authors demonstrated that, during the steady-state absorption of taurocholic acid-^{14}C, the concentration of bile acid in portal blood serum is nearly twice that found in intestinal lymph. This difference, in turn, was shown to correlate well with the relative concentrations of albumin (or total protein) in each of these fluids, a finding which is consistent with the observation of Rudman and Kendall that bile acid binds to albumin and other plasma proteins (71). Reinke and Wilson concluded that even though absorbed bile acids gain access equally well to the portal and lymphatic systems, the facts that (*a*) twice as many binding sites are available in portal blood as in lymph and that (*b*) the flow of portal blood exceeds that of intestinal lymph by a factor of 500 readily accounts for the observation that approximately 99.9% of bile acid absorption takes place via the portal circulation.

TABLE 3 CONCENTRATION AND COMPOSITION OF BILE ACIDS IN THE PORTAL SERUM OF RATS FED A COMMERCIAL CHOW DIET

Bile Acid	% of Total	% Unconjugated
Cholic acid	67 ± 1.6	31 ± 9
β-Muricholic acid	11 ± 2.0	25 ± 9
Hyodeoxycholic acid	11 ± 1.3	45 ± 9
Chenodeoxycholic acid	5 ± 0.4	40 ± 9
Deoxycholic acid	5 ± 1.0	42 ± 10

The total bile acid concentration was 56.4 ± 13.1 μM (36 ± 9% unconjugated).

Data represent mean values ± SEM (n = 8) and were determined by gas-liquid chromatography (60).

The relative concentrations of the various bile acids in portal blood is of considerable interest since these values not only reflect the concentration of different bile acids in the intestinal contents, but are also a function of the ability of the intestinal mucosa to absorb each bile acid. For technical reasons such data have been available in only fragmentary form in the past (72, 73); however, in a recent publication, Cronholm and Sjövall, who used modern analytic procedures, have presented in considerable detail the pattern of bile acids present in the portal blood of the rat (60). These studies are partially summarized in Table 3. For the purposes of this discussion two points warrant emphasis: all the quantitatively important di- and trihydroxy bile acids present in the intestinal contents of the rat are absorbed; and of the total bile acid present in the portal blood, as much as 36% is unconjugated.

CONCLUSIONS

Throughout this review the different mechanisms of transport have been considered as isolated events; this is necessary if the transport phenomena operative in bile acid absorption are to be precisely defined. It is obvious however, that many of these mechanisms may act in concert to bring about the net absorption of bile acid in the intact animal. By way of summary, data will be reviewed with particular reference to the situation in the intact animal.

The concentrations of conjugated bile acids down the length of the small bowel of the fed rat are shown in Panel A, Fig. 6 (17). Hepatic bile has a bile acid concentration of about 36 μmoles/ml. Dilution with digestive

secretions occurs so that in the duodenum the concentration falls to about 10 μmoles/ml. In the jejunum, however, the concentration of conjugated bile acids progressively increases so that maximal values are found in the distal jejunum and proximal ileum. In the distal two-thirds of the ileum, the concentration then progressively falls, reaching values of only 2–3 μmoles/ml. The reason for these changes in concentrations will become evident later in this discussion.

A similar concentration profile down the length of the small bowel is seen when pure taurocholic acid is administered to the rat in vivo, as shown in panel B (Fig. 6). In this experiment, however, the bile acid was administered along with a nonabsorbable marker, inulin-^{3}H, so that it was possible to deduce not only changes in concentration of taurocholic acid but also net movement of this bile acid out of the intestinal lumen. As shown in panel C, there was a definite but slow absorption of conjugated bile acid along the length of the jejunum. The rate of absorption increases markedly in the proximal ileum, and absorption is nearly but not quite complete by the time the terminal small bowel segments are reached.

These results may be correlated with the transport phenomena reviewed in this discussion. First, four observations readily explain the events that occur in the proximal small bowel. (*a*) This area of the bowel is devoid of active transport sites; therefore, only passive transport mechanisms come into play. (*b*) Since only conjugated bile acids are present in the jejunum, passive ionic, but not the more rapid nonionic, diffusion takes place. (*c*) In the presence of phospholipid, fatty acids, and monoglycerides the bile acid micelle is expanded, which in turn lowers the rate of the micellar diffusion component of the passive diffusion process. Together, these three factors explain the low net rate of absorption of bile acids seen in the jejunum (panel C, Fig. 6). (*d*) Finally, the high reflection coefficient accounts for the increase in *concentration* of bile acid down the length of the proximal small bowel; thus, even though net bile acid absorption occurs in the jejunum, the disproportionately higher rate

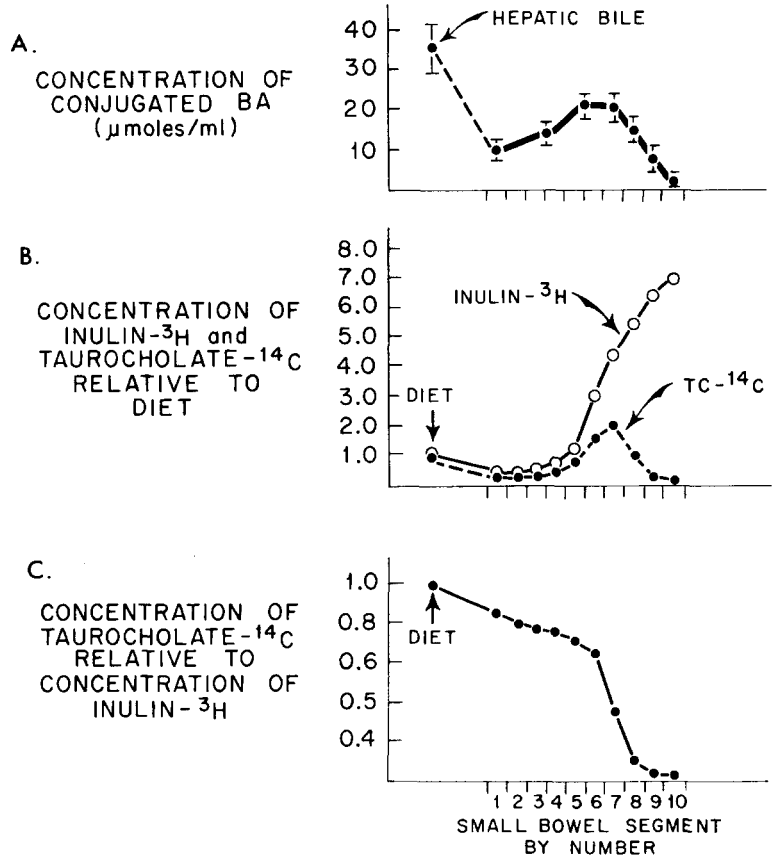

FIG. 6. Bile acid absorption in the intact animal. Panel A illustrates the concentration of conjugated bile acid down the length of the small intestine of rats allowed to eat ad lib. and killed at midnight when their stomachs and intestines were filled with food (17). In another experiment rats were prepared surgically by ligation of the common hepatic duct; after recovery (24 hr) these animals were fed intragastrically a liquid diet containing dextrose, casein hydrolysate, fat, taurocholic acid-^{14}C (10 μmoles/ml), and inulin-^{3}H. 6 hr later the animals were killed, and the concentrations of bile acid and inulin were determined at each level of the small intestine. The results of this experiment are shown in panels B and C (unpublished data from the author's laboratory). BA, bile acid; TC, taurocholic acid.

of isosmotic water and electrolyte absorption results in an increase in bile acid concentration. As a result of all these events, therefore, the intraluminal concentration of bile acids in the proximal small bowel is maintained at a high level—a circumstance which, in view of the role of bile acids in bringing about micellar solubilization of lipids, is teleologically highly favorable to the animal.

In the distal small intestine four phenomena also account for rapid bile acid absorption. (*a*) Sites for the active transport of bile acids are present in this area of the small bowel. (*b*) Passive ionic diffusion of conjugated bile acids presumably occurs in the ileum as in the jejunum. (*c*) Selective absorption proximally of the lipid components of the mixed micelle leads to a higher rate of passive micellar diffusion in the ileum. (*d*) Significant deconjugation of bile acids occurs in the ileum, and this provides the opportunity for rapid passive absorption via nonionic diffusion.

From a consideration of rate constants it seems that of these various transport mechanisms, active transport is of paramount importance for the absorption of conjugated bile acids. This may not be true for the unconjugated bile acids, however. As reported by Playoust and Isselbacher (47) and confirmed in this laboratory (E. Schiff, unpublished data), the K_m values of unconjugated bile acids are 2–6 times higher than those of the corresponding conjugated bile acids. In addition, at any level of the ileum, the concentration of conjugated bile acids is usually much higher than that of unconjugated bile acids. Thus, if all bile acids in the ileum are competing for a common transport site, the presence of high concentrations of conjugated bile acids (with low K_m values) would virtually block the active transport of unconjugated bile acids present at low concentrations (with high K_m values). Despite this circumstance, as much as 36% of the bile acid in rat portal blood is unconjugated (60). These data, therefore, provide strong circumstantial evidence that one of the other transport mechanisms besides active transport is also of considerable quantitative importance. Presumably this mechanism is nonionic diffusion. Whether such diffusion occurs predominantly across the ileum or across the colon cannot be evaluated on the basis of data currently available.

In concluding this review it seems appropriate to emphasize the unusual complexity of studying the transport of substances such as bile acids, for care is required in defining not only the usual physical phenomena across the membrane, i.e., activity and electrical gradients, solvent drag, etc., but also the different phases present in solutions of these amphipaths. Solutions of bile acids are not homogeneous but may contain a variety of molecular species, including ionized bile acid monomers, unionized bile acid monomers, pure micelles, and mixed micelles. As is apparent from this review, these species may be transported at grossly different rates across biologic membranes. Failure to appreciate this fact has led to many of the apparently contradictory results reported in the literature. Thus, the observation in some studies but not in others that rapid absorption of bile acid occurs from the proximal small bowel can be explained by the difference in the relative rates of ionic and nonionic diffusion since some investigators have used free bile acids or crude ox bile (which contains unconjugated bile acids), while others have utilized conjugated compounds. Similarly, the fact that conjugated bile acids in simple saline solution disappear relatively rapidly from the small intestine, whereas these same bile acids placed in whole bile or mixed with other amphipaths are absorbed very slowly reflects the important differences in the diffusion rate of monomers and small micelles and that of large mixed micelles. Thus, meaningful data on the transport of bile acids can be obtained only when strict attention is paid both to the physicochemical conditions on both sides of the membrane *and* to the phase phenomena in the bulk solutions of bile acids.

The author gratefully acknowledges that Doctors T. Cronholm, D. M. Small, J. Sjövall, and J. D. Wilson made available manuscripts that were not yet published at the time this review was being prepared.

This work was supported by Research Grant HE-09610 and Training Grant AM-06506 from the National Institutes of Health and by the John and Mary Markle Foundation.

Manuscript received 23 October 1967.

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