Active and Passive Bile Acid Absorption in Man

PERFUSION STUDIES OF THE ILEUM AND JEJUNUM

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ABSTRACT Absorption of the major human bile acids was studied in 12 healthy volunteers by steady state perfusion of the ileum in 112 experiments and of the jejunum in 48 experiments. Use of a randomized order of four perfusions on 1 day of study and use of up to 4 consecutive days of study in a subject allowed important comparisons of data from the same individuals. That there is active ileal absorption of chenodeoxycholic, glycochenodeoxycholic, and taurocholic acids in man was supported by the finding of saturation kinetics and of competition for absorption among conjugated bile acids. Values for apparent kinetic constants (apparent maximal transport velocity [$*V_{max}$] and apparent Michaelis constant) in man are similar to those in other species. The ileum absorbed chenodeoxycholic acid more rapidly than its glycine conjugate, due mainly to a ninefold greater permeability for the free acid. Taurocholate had the highest $*V_{max}$ and was absorbed more rapidly than glycochenodeoxycholate. Passive permeability of the jejunum to bile acids was twice that of the ileum, and the permeabilities to free and glycine-conjugated chenodeoxycholate were in the same ratio as in the ileum (9:1). Jejunal permeability to chenodeoxycholic acid was three times that to cholic acid. Variation of intraluminal pH by up to 1.4 units did not influence jejunal uptake of free bile acids. These results, which are comparable with those from animal experiments, provide a basis for estimation of intestinal reabsorption of bile acids in intact man.

INTRODUCTION

Current concepts of the enterohepatic circulation of bile acids in man (1, 2) include a small total pool (2.5–4 g) (1–5) cycling 6–12 times daily (3, 6, 7) and thereby constituting a large effective pool. To maintain a steady state, hepatic synthesis of bile acids must equal the fecal loss. This loss amounts to only 0.2–0.8 g daily (6, 8), implying that intestinal conservation of bile acids is efficient. When the degree of impaired intestinal reabsorption exceeds the capacity of hepatic synthesis to compensate, jejunal concentrations of bile acids can decrease to levels inadequate for normal assimilation of fat (9). Studies in animals (10–12) and in man (7, 13) have localized the major site of bile acid reabsorption to the ileum.

Ileal absorption of bile acids is thought to be active because: (*a*) absorption occurs against concentration gradients (14–16), and bile acids are absorbed when their electrochemical gradient is zero (17); (*b*) absorption is blocked by metabolic inhibitors and anoxia (14–18); (*c*) individual bile acids inhibit the absorption of other bile acids, suggesting competition for the same transport mechanism (16, 19, 20); (*d*) the kinetics of absorption display saturation phenomena (14, 16–18, 20, 21); and (*e*) absorption is dependent on the presence of sodium ions in the lumen (16, 18).

Absorption also may be passive, and two mechanisms have been proposed: ionic diffusion and nonionic diffusion (17, 18, 20). The relative contribution of each is determined by the principles governing transport of weak acids (22) and depends on intraluminal pH, pK_a of the bile acid, and the permeability and partition coefficients of the ionic and nonionic species. Ionized forms should traverse lipid plasma membranes less readily than the nonionized species. Nonionic diffusion is implied by the pH dependency of absorption (17, 20) and by studies in which partition coefficients and absorption have been related (23, 24).

In the jejunum, absorption is passive because: (*a*) flux is proportional to electrochemical gradients; (*b*) metabolic inhibitors do not influence absorption; and (*c*) no evidence exists for competition among bile acids, saturation phenomena, or dependency on sodium ions (17, 18, 20).

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Most of our information on bile acid absorption comes from experiments in animals, and few studies have been performed in man. The present experiments describe absorption of the major human bile acids in the ileum and jejunum as measured by a technique of intraluminal perfusion in healthy man.

METHODS

Perfusion procedure. Experiments were performed on 12 healthy volunteers (9 men, ages 21–45 yr, and 3 postmenopausal women). All gave written informed consent and had no signs or symptoms of gastrointestinal disease. Perfusions of 25-cm intestinal segments within the distal 35 cm of ileum or in the proximal jejunum were performed as described previously (25, 26). Experiments were conducted on up to 4 successive days during which the tube was kept in place. Variations in the location of the tube between successive days and after each day of study were less than 10 cm (27).

On the day of study, after the volunteer had fasted overnight, the balloon was inflated and perfusates (at 37°C) were pumped in at a rate of 10 ml/min. Samples were collected 25 cm distally, by intermittent suction in the ileum and siphonage in the jejunum, and separated into 10-min aliquots. Occlusion of the intestinal lumen by the balloon was monitored by the color of aspirates. Occasionally the clear aspirates became contaminated with bile from above the balloon. Reinflation and a longer period of perfusion was then necessary; bile-stained samples were not analyzed. Reflux of perfusates, monitored by counting for [^{14}C]polyethylene glycol (PEG) [1] in aspirates from above the balloon, was negligible.

For each perfusing solution, an equilibration period of 15–30 min was followed by the collection of four to six sequential 10-min samples; these samples comprised an experimental period. Steady-state conditions were verified by stable concentrations of PEG during these periods.

Composition of perfusates. The solutions contained: NaCl, 90 mmol/liter; KCl, 5 mmol/liter; $NaHCO_3$, 45 mmol/liter; D-xylose, 10 mmol/liter (in 144 perfusions); PEG, 5 g/liter; and [^{14}C]PEG (New England Nuclear, Boston, Mass.), 5 μCi/liter; they were 280 mosmol/kg. The pH was 8, except for one series of studies performed at pH 6 (see below), without buffer. Bile acids were added as indicated below.

Sources of unconjugated bile acids, their purification, and the preparation of glycine- and taurine-conjugated bile acids have been described elsewhere (26, 28). When two bile acids were perfused simultaneously, [^{14}C]chenodeoxycholic acid (CDC) or [^{14}C]glycochenodeoxycholic acid (GCDC) (New England Nuclear) was included, and [^{14}C]PEG was not used.

Experimental design. During each day of study, the subject received four different perfusions successively, i.e., four different bile acid solutions. A total of 40 study days yielded 160 separate perfusion experiments in the 12 subjects.

The test solutions perfused each day in each group of subjects are listed in Table I. The sequence of perfusions on each day ($N = 4$) was randomized within each group of subjects ($N = 4$) to fulfill a 4×4 Latin square design (26). The block of perfusions used each day was also randomized, except when jejunal perfusion (group E) followed 2 days of ileal perfusion (group B).

Analytical methods. Xylose was measured by the *o*-toluidine method (29), and bile acids, by the method of Iwata and Yamasaki (30). Radioactivity was measured with a toluene-based "cocktail" and liquid scintillation spectrometry; quench correction was made by external standardization (31). When a ^{14}C-labeled bile acid was present in a solution, nonradioactive PEG was used alone and estimated turbidimetrically (31).

Mathematical treatment. Water, bile acid, and xylose absorptions were calculated by standard formulas (31). Statistical analyses were by paired *t* test and linear regression (method of least squares). For kinetic analysis, absorptions of CDC, GCDC, and taurocholic acid (TC) (per 25 cm of ileum) were expressed by (20):

$$J = \left({}^{*}V_{\max} \cdot \frac{C_m}{{}^{*}K_m + C_m} \right) + (({}^{*}P) \cdot (C_m))$$

in which J = absorption in $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$; ${}^{*}V_{max}$ = apparent maximal transport velocity in $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$; C_m = logarithmic mean intraluminal bile acid concentration in mmol/liter; ${}^{*}K_m$ = apparent Michaelis constant in mmol/liter; ${}^{*}P$ = apparent permeability coefficient of the bile acid in $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$ per mmol/liter at pH 8. The first term describes a rectangular hyperbola, taken to represent active absorption; the second term is a straight line and represents passive absorption (20). It is apparent that, with increasing C_m, the first term approximates a constant, and J approaches a linear function of C_m. The experimental results yielded such a relationship; from this, ${}^{*}P$ was calculated, and the passive component was substracted from the measured total absorption. The active component so calculated was used to establish values for ${}^{*}V_{max}$ and ${}^{*}K_m$, based on the Lineweaver-Burk plot.

RESULTS

Studies in ileum

Absorption Kinetics of Free and Glycine-Conjugated CDC

Fig. 1 shows the relationships between intraluminal concentration and absorption of CDC and GCDC in groups A and C. Inclusion of results from the latter group is justified because we could not demonstrate inhibition of individual bile acid absorptions when CDC and GCDC were perfused together (see below). In group C experiments, CDC was absorbed significantly faster than GCDC ($P < 0.001$).

In Fig. 2 the relationships between intraluminal concentrations and absorptions of CDC and GCDC are separated into their two components, active and passive, as described above. At most concentrations, a passive component of CDC absorption was quantitatively important, whereas for GCDC, the active component always pre-

[1] *Abbreviations used in this paper:* C, cholic acid; CDC, chenodeoxycholic acid; C_m, logarithmic mean intraluminal bile acid concentration; DC, deoxycholic acid; GC, glycocholic acid; GCDC, glycochenodeoxycholic acid; ${}^{*}K_m$, apparent Michaelis constant; ${}^{*}P$, apparent permeability coefficient of the bile acid; PEG, polyethylene glycol (mol wt, 4,000); TC, taurocholic acid; ${}^{*}V_{max}$, apparent maximal transport velocity.

TABLE I

Experimental Design: Subjects and Bile Acids Perfused

Study group* and subjects	Bile acids perfused			
	Day 1	Day 2	Day 3	Day 4
			mmol/liter	
Studies in ileum				
Group A	CDC 0.25	GCDC 0.25	CDC 0.50	CDC 0.50
(subjects 1–4)	CDC 0.50	GCDC 0.50	DC 0.50	CDC 0.50 + GCDC 1.00
	CDC 0.75	GCDC 0.75	C 0.50	GCDC 0.50
	CDC 1.00	GCDC 1.00	GC 0.50	CDC 1.00 + GCDC 0.50
Group B	TC 0.25	TC 0.50		
(subjects 5–8)	TC 0.75	TC 0.50 + GCDC 1.00		
	TC 1.00	GCDC 0.50		
	TC 2.00	TC 1.00 + GCDC 0.50		
Group C	No bile acid			
(subjects 9–12)	CDC 0.62 + GCDC 0.62			
	CDC 1.25 + GCDC 1.25			
	CDC 2.50 + GCDC 2.50			
Studies in jejunum				
Group D	CDC 0.25	GCDC 0.25		
(subjects 1–4)	CDC 0.50	GCDC 0.50		
	CDC 0.75	GCDC 0.75		
	CDC 1.00	GCDC 1.00		
Group E			C (pH 8.0) 0.5	
(subjects 5–8)			C (pH 6.0) 0.5	
			CDC (pH 8.0) 0.5 and 1.0‡	
			CDC (pH 6.0) 0.5 and 1.0‡	

* Group D was same as group A, but studies were performed during a second intubation, 3 mo after group A studies. Group E was same as group B and studies were performed during same intubation as group B; the tube was withdrawn from the ileum and positioned, under fluoroscopic control, in the jejunum.

‡ Two subjects perfused with 0.5 mmol/liter and two with 1.0 mmol/liter.

dominated. Values for $*V_{max}$, $*K_m$, and $*P$ of CDC and GCDC in the ileum are listed in Table II.

Absorption Kinetics of Conjugated Trihydroxy Bile Acid

Fig. 3 shows the relationship between mean ileal concentration and absorption of TC. Passive ileal absorption was ignored, because passive absorption of TC in the human jejunum is negligible (23), and the hyperbola $J = *V_{max} \cdot (C_m/[*K_m + C_m])$ was fitted to the data. The kinetic constants for TC in the ileum are listed in Table II.

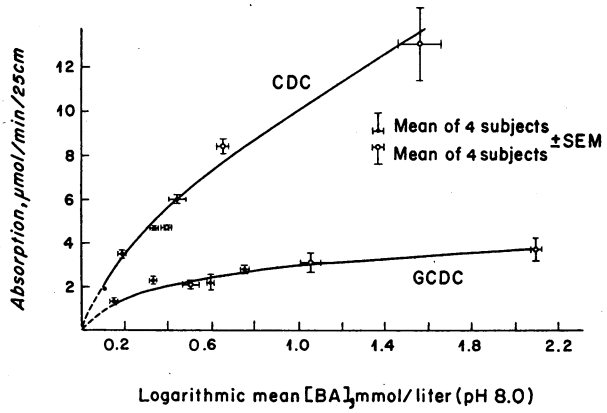

FIGURE 1 Relationship between intraluminal bile acid concentration ([BA]) and bile acid absorption for CDC and GCDC in human ileum. Open symbols are means from four subjects perfused with equimolar mixtures of CDC and GCDC (group C, Table I); closed symbols are means from four different subjects perfused with either CDC or GCDC alone (group A, Table I).

Inhibition of Absorption of One Bile Acid by Another

Two conjugated bile acids (TC and GCDC). When TC was perfused at 0.5 mmol/liter with and without GCDC at 1.0 mmol/liter, absorption of TC was signifi-

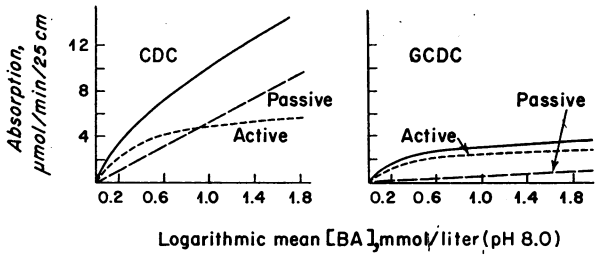

FIGURE 2 Relationship between intraluminal concentration and absorption of CDC and GCDC in human ileum. Total absorption (solid line) has been separated into active and passive components by assuming that linear kinetics contribute to total absorption at all concentrations, by computing an apparent passive permeability, and by fitting a hyperbolic function to the remaining data (20). [BA], bile acid concentration.

TABLE II

Kinetic Constants of Bile Acid Absorption in Man*

Bile acid	*V_{max}	*K_m	*P Ileum	*P Jejunum
	μmol/min per 25 cm	*mmol/liter*	*μmol/min per 25 cm per mmol/liter*	
CDC	6.4±0.8	0.4±0.1	5.3±0.4	12.8±0.6
GCDC	3.1±0.2	0.3±0.1	0.6±0.1	1.4±0.1
TC	13.8±1.4	0.6±0.1	‡	‡
C	—	—	—	4.4±1.3

* Each value is mean (±SE) of studies in four subjects at pH 8.0.
‡ Assumed to be negligible.

cantly ($P < 0.001$) decreased (38%) in the presence of GCDC (Table III). Similarly, addition of TC at 1.0 mmol/liter resulted in a significant ($P < 0.01$) decrease (11%) in absorption of GCDC.

A free (CDC) and a conjugated (GCDC) bile acid. When examined in a similar way, addition of CDC caused an insignificant decrease (3%) of GCDC absorption, and addition of GCDC caused an insignificant decrease (7%) of CDC absorption.

COMPARISON OF ABSORPTION RATES AMONG DIHYDROXY AND TRIHYDROXY BILE ACIDS

When perfused at the same concentrations (0.5 mmol/liter), deoxycholic acid (DC), CDC, cholic acid (C), and glycocholic acid (GC) showed the same rates of absorption (Table IV).

INTRALUMINAL pH

All perfusates were pH 8.0–8.1 when introduced; effluent pH was 7.8–7.9.

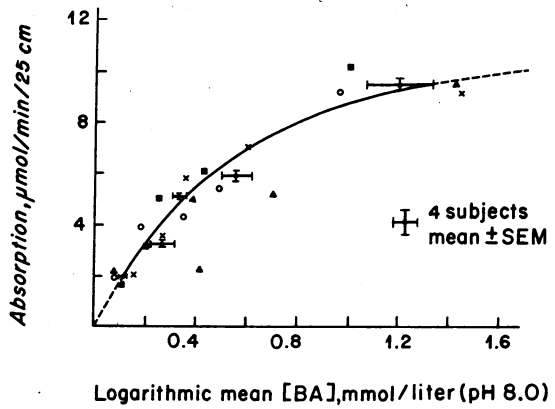

FIGURE 3 Relationship between concentration and absorption of TC in human ileum. Individual results in different symbols and group means of four subjects are shown. The line shows the hyperbolic function of best fit, assuming that passive permeability of the ileum to taurocholate is negligible (see text). [BA], bile acid concentration.

TABLE III

Influence of One Bile Acid on Absorption of a Second Bile Acid in Human Ileum

Test solution		Bile acid absorption*		
Bile acid	Concn	Total	TC or CDC	GCDC
	mmol/liter	*μmol/min per 25 cm*		
Both conjugated				
TC‡	0.5	3.23±0.14	3.23±0.14	—
TC and GCDC	0.5 and 1.0	5.64±0.36	1.99±0.09§	3.65±0.27
GCDC‡	0.5	2.87±0.20	—	2.87±0.20
GCDC and TC	0.5 and 1.0	7.03±0.52	4.49±0.38	2.54±0.20‖
One conjugated				
CDC	0.5	3.05±0.25	3.05±0.25	—
CDC and GCDC	0.5 and 1.0	6.46±0.50	2.83±0.21	3.63±0.37
GCDC	0.5	2.61±0.18	—	2.61±0.18
GCDC and CDC	0.5 and 1.0	8.94±0.33	6.41±0.28	2.53±0.21

* Each value is mean (±SE) of studies in four subjects.
‡ Predicted absorption, using kinetic parameters (Table II), TC 0.5 mmol/liter is 3.76 $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$; GCDC 0.5 mmol/liter is 1.91 $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$.
§‖ For effect of second bile acid: § $P < 0.001$; ‖ $P < 0.01$.

TABLE IV
Absorption of Dihydroxy and Trihydroxy Bile Acids in Human Ileum

Bile acid	Absorption*
	μmol/min per 25 cm
CDC	3.17±0.14
DC	3.06±0.17
C	2.76±0.34
GC	3.00±0.26

* Each value represents mean (±SE) of studies in same four subjects at pH 8.0. Perfusates contained bile acid 0.5 mmol/liter; perfusion rate was 5 μmol/min.

Studies in jejunum

Absorption of Free and Conjugated CDC

Fig. 4 compares the relationships between concentration and absorption of CDC and GCDC. In these studies, perfusates were introduced at pH 8.1, and the mean effluent pH was 7.7. The intercepts of linear regressions on the X and Y axes do not differ significantly from zero. The slopes of the lines, expressing *P, are significantly different ($P < 0.001$), with that of CDC being approximately nine times that of GCDC.

Influence of pH on Passive Absorption of CDC and C

Under the conditions of our studies, changes of intraluminal pH did not influence the absorption of CDC or C (Table V). When CDC was used initially at 0.50 mmol/liter, it was almost completely absorbed; later studies used CDC at 1.0 mmol/liter. These experiments also provided an assessment of the *P of C in the jejunum (Table II).

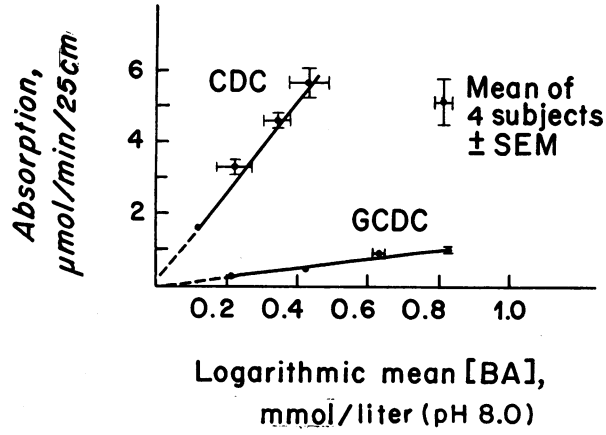

FIGURE 4 Absorption of CDC and GCDC in jejunum of four subjects perfused with four concentrations of each. Where not shown, the dispersion (SE) of results was too small for illustration. The slopes of linear regression lines reflect the passive permeability of the jejunum to the bile acid, that for CDC being approximately nine times that for GCDC. [BA], bile acid concentration.

Comparisons between jejunum and ileum

In the ileum the ratio of *P of CDC to *P of GCDC was 9:1, as it was in the jejunum. The apparent permeability of the jejunum to both GCDC and CDC was twice that of the ileum.

Xylose was also absorbed twice as fast in the jejunum (mean±SE, 5.1±0.3 mg/min per 25 cm) as in the ileum (2.5±0.1 mg/min per 25 cm).

Net water movement

Net water absorption (as assessed by PEG concentration) was observed in 45 studies in the jejunum and in 70 studies in the ileum. Fluid secretion, which usually was small in amount, occurred only when the highest concentration of dihydroxy bile acids was perfused.

DISCUSSION

Experimental technique and design. Our perfusion technique has been validated (25) and used previously to study segments of human jejunum (26) and ileum (27). The ligament of Treitz is a reproducible landmark for perfusion of the jejunum. By using the ileocecal junction as our guide, it was possible to perfuse reproducibly a segment within the distal 35 cm of ileum (27), which permitted multiple studies in the same subject. When combined with a randomization scheme that eliminated the influence of perfusion sequence, our design allowed important comparisons to be made on the same individuals.

Our assumption that disappearance of bile acids from the lumen is equivalent to absorption is supported by findings in the rat in which disappearance from the

TABLE V
*Influence of pH on Passive Absorption of C and CDC in Human Jejunum**

Bile acid‡	pH		Absorption (% of amount perfused)
	Perfusate	Aspirate	
C	8.1±0.0	7.7±0.0	43.5±9.9
C	6.3±0.1	7.2±0.1	52.0±11.5
CDC	8.1±0.0	7.7±0.0	91.0±2.2
CDC	6.4±0.1	7.3±0.0	90.8±4.6

* Each value represents mean (±SE) of studies in same four subjects.

‡ Cholic (C) acid was perfused at 0.50 mmol/liter; chenodeoxycholic (CDC) acid was perfused at 0.50 mmol/liter in two subjects and 1.0 mmol/liter in two (see text).

lumen coincided with appearance in biliary drainage (20). However, our methods demonstrate net effects only, and the kinetic constants we calculated should be considered as estimates which test current concepts of bile acid transport in intact man. In earlier studies of ileal bile acid absorption in man (27), we used high concentrations of bile acids. These concentrations were above the critical micellar concentrations and also evoked intestinal secretion of fluid, circumstances that we consider inappropriate for quantification of bile acid absorption. In the present studies, lesser concentrations of bile acids were used, and fluid absorption was the rule.

Active absorption of bile acids. The concept of active absorption of bile acids in the human ileum is supported by the following observations. First, the presence of one conjugated bile acid inhibited the absorption of a second conjugated bile acid. These findings agree with animal studies by Heaton and Lack (19) and Lack and Weiner (32); they demonstrated initially the presence of inhibitory phenomena in vitro (32) but could not exclude nonspecific effects. Later, in vivo, they demonstrated mutual inhibitory phenomena between various conjugated bile acids. Using ratios of inhibitor to substrate of 3:1 and 4:1, rather than 2:1 as we did, they found that GCDC inhibited TC absorption by 67% and that TC inhibited GCDC absorption by 39% (19). They found no evidence of nonspecific effects and attributed the phenomena to competition for a common transport process (19).

Our failure to demonstrate similar competition between CDC and GCDC may be explained by the data shown in Fig. 2. An effect of GCDC on absorption of CDC may not be recognizable in our system, because passive absorption comprised approximately 50% of the total absorption of the free bile acid; in the reciprocal experiment, rapid absorption of CDC decreased its intraluminal concentration sharply, thereby decreasing its influence on GCDC absorption.

A second line of evidence is the relationship between ileal concentrations of CDC and GCDC and their rates of absorption; these relationships are consistent with a saturable phenomenon. For these calculations we assumed, like Schiff, Small, and Dietschy (20), that ileal absorption involves active and passive mechanisms. With this premise, a hyperbolic function could be constructed to provide a good fit for the component of active absorption. For TC, which was perfused at low concentrations only, we have insufficient data for adequate assessment of the passive component. However, passive absorption of TC should be less than that of GCDC (23) and probably negligible.

The kinetic parameters listed in Table I show that $*V_{max}$ values (in $\mu mol \cdot min^{-1} \cdot 25\ cm^{-1}$) for GCDC, CDC, and TC have the relationship 1:2:4, which approximates that calculated from the data (pmol/min per cm) of Dietschy's group (17, 18, 20), 1:3:9. Our values for $*K_m$ (mmol/liter)—GCDC, 0.3; CDC, 0.4; TC, 0.6—showed no significant differences between individual bile acids; Dietschy's group's values—0.21, 0.38, and 0.23 mmol/liter, respectively—include significantly lower $*K_m$ for the two conjugated bile acids (20), although earlier results (17) had indicated similar values for $*K_m$ of C and TC. Thus, data from man and rat are in general agreement, but we cannot separate the $*K_m$ values with respect to conjugation. Quantitatively, $*V_{max}$ per unit length of intestine was greater in man by a factor of 10^2–10^3. This difference is more than that predicted from considerations of differences in surface area, which should be in the range of 10–10^2.[3] Differences in methods, particularly the use of an in vitro system for the rat, might contribute to this disparity.

Passive absorption of bile acids. Animal studies, which have been reviewed extensively (33) and reevaluated recently (18, 20), have consistently failed to produce any evidence of active absorption of bile acids in the jejunum or the colon. At these intestinal loci, bile acid absorption is explicable by passive diffusion. The linear relationships between concentration and absorption of CDC and GCDC in the human jejunum suggest the presence of passive absorption alone. Our experiments cannot exclude a mechanism saturable at higher intraluminal concentrations, but no evidence for such has been found in other species (18). Earlier perfusion studies in man (23, 24) also are consistent with passive jejunal absorption; furthermore, they suggest that nonionic diffusion is the major mechanism of uptake. Thus, free bile acids were absorbed faster than conjugates; of the major conjugates, glycine dihydroxy acids were absorbed fastest (23, 24).

The observations on passive jejunal absorption also support our concepts of ileal absorption. The relative passive permeabilities we derived for CDC and GCDC in the ileum showed $*P$ of CDC to be nine times that of GCDC. Passive jejunal absorption, in the same individuals, was in the same ratio, CDC: GCDC = 9:1.

When $*P$ in the jejunum and ileum were compared, the ratio (jejunum: ileum) for CDC and GCDC was 2:1. Dietschy's group (17, 20) found a ratio of 5:4 in the rat and attributed the difference to different mucosal surface areas per unit length of jejunum and ileum. As a reference compound, we used xylose, which is thought to be absorbed predominantly by passive means (34, 35) and to have only slight affinity for active carrier mechanisms. It has been proposed that xylose absorption is determined mainly by exposure of the perfusate to the

[3] Based on the assumption that the diameter of the ileum in the rat is no less than 1/30 that in man (3–4 cm).

absorptive surface (34). Relative rates of xylose absorption in jejunum and ileum were 2:1, as for the bile acids (our ratio of xylose absorption was somewhat less than reported previously [34], 3:1 and 5:1).

In contrast to Dietschy's group's (20) results with perfusion of rat jejunum in vivo, we found that absorption of C and CDC in the jejunum was not influenced by changes of pH. The reasons for this difference are uncertain. We can quantify the change of pH only as being between 0.4 and 1.8 units, the minimal and maximal differences of pH between perfused and recovered solutions. Also, the relationship between the pH of the intraluminal contents and the pH at the mucosal surface is uncertain. Hogben, Tocco, Brodie, and Schanker (22) proposed that these are not necessarily identical and that the juxtamucosal ("virtual") pH was between 5 and 6. Dietschy's group (17, 18, 20) has suggested the existence of both ionized and nonionized passive diffusion and used observations at two levels of pH to establish mucosal permeabilities of the ionized and nonionized species. Since we could detect no significant effect of pH, we cannot calculate these constants and prefer to consider our permeability constants as "apparent net" values.

Indeed, mucosal uptake of ionized bile acids by passive diffusion can be questioned. The molecular dimensions of both the conjugated and unconjugated compounds are larger than those generally considered to preclude passage through aqueous "pores"; in the absence of appreciable lipid solubility, compounds of molecular weight greater than 100–150 are generally absorbed poorly (36). Furthermore, a ninefold difference in uptake between free and conjugated CDC at a luminal pH favoring ionization is not readily explicable in terms of ionic diffusion but is consistent with differences between partition coefficients of the free and conjugated acids (24).

However, our experiments do not elucidate the mechanisms of passive absorption of bile acids further. Extensive experimental observations and relevant theoretical considerations have been reported by Dietschy's group (17, 18, 20), including evaluation of the role played by the juxtamucosal "unstirred water layer" as a potential rate-limiting step for less polar compounds. Additional models have been discussed by Ho and Higuchi (37) and Ochsenfahrt and Winne (38), who considered the influence of bulk fluid movement (net absorption or secretion of water). The presence of an unstirred water layer also distorts calculations of active transport kinetics (39, 40), leading to falsely high estimates of $*K_m$, and dictates that any attempt to translate our values to the biochemical mechanism of transport would be unwise.

Physiologic implications. Our findings are consistent with the existence, in the human ileum, of an active transport mechanism available to CDC, GCDC, and TC; in other species, trihydroxy and dihydroxy bile acids, in the conjugated and unconjugated forms, utilize the active carrier (33, 41). Thus, it seems likely that all natural bile acids of man participate in active absorption in the ileum. The values of $*K_m$ in man were less than 1 mmol/liter, implying that active ileal absorption should effectively clear conjugated bile acids. Postprandial concentrations of bile acids are above this concentration (42); moreover, free bile acids are also present in the healthy ileum (43) and are increased in amount in disease (44). Passive diffusion could provide an effective alternative route of absorption for unconjugated acids.

Our results permit some approximations of ileal absorptive capacity in man. If (see Appendix) the intraluminal concentration of GCDC is 2 mmol/liter, active absorption would be 7.2 g/24 h, and the passive component would be 3.2 g/24 h. If GCDC were deconjugated in part and the respective intraluminal concentrations of GCDC and CDC were 1.5 and 0.5 mmol/liter, total bile acid absorption would be 22 g/day, rather than 10 g. The pool of CDC is approximately 1 g, and it circulates 6–12 time/day. However, the time available for absorption is probably less than 24 h, being represented mainly by the postprandial periods. Thus, maximal ileal reabsorption may not exceed the physiologic requirements very greatly. When the pool of CDC is enlarged by exogenous bile acids, diarrhea may occur (45), possibly because the ileal absorptive capacity is exceeded and bile acid enters the colon where it inhibits water reabsorption (46). Ileal absorption of TC should be predominantly active; active transport would yield an absorption of 31 g/day at a mean concentration of 2 mmol/liter. The higher $*V_{max}$ of the trihydroxy acid and its predicted lower passive permeability contributed presumably to our failure to demonstrate a difference between ileal absorption rates for C and GC.

Jejunal absorption could contribute significantly to maintenance of the enterohepatic cycle, particularly for glycine dihydroxy acids. At a mean concentration of 2 mmol/liter, 100 cm of jejunum could absorb 7 g of GCDC/24 h. This would increase markedly with deconjugation. However, the presence in the proximal small bowel of mixed micelles containing bile acids and biliary and dietary lipids could modify passive uptake. Further documentation of bile acid absorption in vivo will require sequential analysis of postprandial intestinal contents at different levels of the bowel.

APPENDIX

Calculation of bile acid absorption assumes the ileum and jejunum each to be 100 cm long and to be exposed constantly to the stated concentrations of bile acids. Values for $*V_{max}$, $*K_m$, and $*P$ were used in conjunction

with the assumed bile acid concentration. Thus, for GCDC, at 2 mmol/liter, the active component was equal to $3.1 \times (2.0/[0.3 + 2.0]) \times 60$ (min) $\times 24$ (h) $\times 4$ (100 cm) $\times$ (450/1,000) (to convert to grams) $= 7.2$ g/24 h. Similarly, the passive component ($^{*}P \cdot C_m$) was equal to 3.2 g/24 h. Passive transport of trihydroxy bile acids was assumed to be negligible, and active transport of all bile acids in the jejunum was assumed to be negligible.

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