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Research Article

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Canalicular Bile Secretion in Man

STUDIES UTILIZING THE BILIARY CLEARANCE OF [^{14}C]MANNITOL

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ABSTRACT [^{14}C]Mannitol was administered i.v. as a bolus injection to five postcholecystectomy patients with indwelling T-tubes and re-established enterohepatic circulations to evaluate the biliary clearance of [^{14}C]-mannitol as a means of estimating canalicular bile flow in man. [^{14}C]Mannitol appeared in collections of bile 9–22.5 min after intravenous injection, rose to a peak, and thereafter paralleled the plasma [^{14}C]mannitol disappearance curve. Bile-plasma [^{14}C]mannitol ratios and [^{14}C]mannitol clearances were determined during control and choleretic periods after correction of the bile [^{14}C]mannitol points for the transit time of a given sample. After i.v. injection of sodium dehydrocholate in five studies, bile flow and mannitol clearance increased proportionately. However, when ductular secretion was stimulated with an i.v. bolus of secretin in three other studies, [^{14}C]mannitol clearance remained essentially unchanged, indicating that [^{14}C]mannitol entered bile at the level of the hepatocyte and could be utilized as a marker of canalicular flow in man.

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INTRODUCTION

Experimental observations in animals indicate that bile is secreted from two sites: the canaliculus of the hepatocyte, which is the major source of bile secretion in most species, and the biliary ductules (1, 2). Bile salts and perhaps sodium are believed to be actively transported across the canalicular membrane, forming osmotic gradients that then result in the passive diffusion of water and other electrolytes into the canalicular lumen (1–3). In contrast, distal secretion in the bile ducts is stimulated by the hormones secretin and gastrin, producing a watery bicarbonate-rich fluid (1, 2, 4). Reabsorption of water, electrolytes, and glucose may also occur along the biliary epithelium (4, 5).

Considerable evidence has accumulated that indicates that as much as 60% of canalicular secretion in some species may be formed by processes independent of the osmotic effects of bile salt transport (6–9). Although the mechanism of bile salt-independent canalicular secretion is as yet uncertain, active transport of sodium has been suggested, since bile salt-independent canalicular flow may be inhibited or stimulated in vivo by a variety of drugs and compounds that also inhibit or stimulate Na^+,K^+-ATPase in vitro (2, 7, 9).

Whether such mechanisms are applicable to the formation of bile secretion in man is not known, primarily because studies of biliary secretion are more difficult than in animals and because markers of canalicular secretion, which have advanced our understanding of biliary secretion in animals, have not yet been applied analogously to humans. In the present study we evaluate the use of [^{14}C]mannitol as a marker of canalicular secretion during spontaneous flow of bile and after secretin and dehydrocholate (triketocholanoic acid) choleresis.

METHODS

Patient selection and experimental protocol. After informed consent was obtained, five patients with indwelling T-tubes (three women and two men, ranging from 28 to 71 yr of age) were studied on a metabolic ward 2–5 wk after cholecystectomy for gallstone disease. Liver function tests were normal. Each patient consumed a regular diet and was studied after an overnight fast, at least 72 h after the enterohepatic circulation was re-established by clamping the T-tube. On the morning of study the T-tube was vented and drained by gravity. In three patients the T-tubes contained a small balloon on the distal arm of the tube (Baldwin T-tubes), which was inflated to assure complete diversion of biliary contents. Bile volumes were similar in magnitude to those obtained in the two patients whose tubes were not occluded distally, suggesting that gravity drainage removed the majority of the secreted bile, an observation previously made by others (10). An initial 1–2 ml of bile was discarded to remove bile contained in the stagnant arm of the T-tube, and serial timed samples were then collected over 2–5-min periods for the duration of the 2–3 h study. After establishing effective drainage, 50 μCi of sterile [^{14}C]mannitol, prepared by New England Nuclear, Boston, Mass., and more than 99% radiochemically pure, was administered intravenously in 250 mg of unlabeled mannitol as a single bolus through tubing from a saline infusion. Blood samples were removed at 10–15-min intervals from a vein in the opposite arm. After 60–70 min (control period) either 5 cm^3 of 20% sodium dehydrocholate or 75–90 U of secretin-Boots (Warren-Teed Pharmaceuticals, Inc., Columbus, Ohio) were administered intravenously as a single bolus and the study was continued for another 90 min (choleretic period).

Bile samples were analyzed at 5–20-min intervals throughout the study. Samples of bile or plasma (100–200 μl) were added to 10 ml dioxane, placed overnight in the dark at 4°C, and counted in a Nuclear Chicago liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). Corrections for quenching were obtained by the use of internal standards. Total bile acids were measured in separate samples of bile with the purified preparations of hydroxysteroid dehydrogenase (Worthington Biochemical Corp., Freehold, N. J.) used previously in this laboratory (11).

Urine and stool were collected over a 24-h period. Excretion of 68–77% of the administered dose occurred in the urine within 4 h, and essentially all of the activity was eliminated within 24 h. [^{14}C]Mannitol activity in bile and rates of flow and bile acid excretion were referred graphically to the mid-point of a given 2–5-min bile collection period before corrections for differences in transit time.

Determination of mannitol bile transit time (MBTT) [1] *and bile-plasma (B/P) [^{14}C]mannitol ratios.* Because biliary dead space imposes a significant delay between the injection of [^{14}C]mannitol and its detection in collected bile, each point on the bile [^{14}C]mannitol curve of a given study was shifted to the left with respect to the plasma curve by the magnitude of the MBTT for the respective sample. MBTT represents the average time for mannitol to traverse the biliary dead space (the volume in the biliary tree and cannula). Measurements of MBTT during the control period were obtained by two different techniques at the beginning of the study, when bile flow rates were relatively constant. These consisted of: (*a*) a previously described method for determination of biliary transit time in the rat (12), and (*b*) a method used to estimate the mean transit time of material in a linear flow system (13). Biliary dead space volume was then calculated for each study (biliary volume = MBTT × mean flow rate). Although this estimate of biliary transit time was valid during control periods, when bile flow remained relatively constant, it was not correct after a bolus of dehydrocholate, when the bile flow rate changed rapidly. Hacki and Paumgartner (14) have shown in the rat that the biliary transit time for each sample may be calculated under these circumstances if it is assumed that the volume of the biliary tree remains relatively constant (MBTT for individual sample = volume of biliary tree/mean flow rate of sample). Therefore, the times of the bile [^{14}C]mannitol points were shifted to the left by the magnitude of the biliary transit time calculated for the individual sample. B/P ^{14}C ratios were then obtained during control and post-choleretic periods during the period when the plasma and bile [^{14}C]-mannitol curves were parallel. These values must be regarded as estimates, however, since we must assume that biliary volume remains constant.

Biliary clearance of [^{14}C]mannitol. The biliary mannitol clearance for each study was calculated as the product of *MBF* and B/P ratio, where *MBF* = the mean flow rate for the control or choleretic period, and B/P = corrected bile-plasma [^{14}C]mannitol ratios, averaged for that collection period. B/P ratios from individual collections did not vary more than 5% during a control or choleretic study period once [^{14}C]mannitol had equilibrated in bile.

Bile salt-independent flow. The magnitude of the flow independent of bile salt was calculated by extrapolation of linear regression lines as previously described (6–9) by the least square analysis (15). However, as discussed by Hacki and Paumgartner (14), since bile salt excretion and flow rise and fall out of synchrony after a bolus injection of bile salts, because of the time lag in the biliary dead space, both bile salt excretion and flow rates must be calculated after each sample is corrected for the appropriate flow rate generated at the time the bile acids were being secreted at the level of the canaliculus. This corrected flow rate (*CF*) was obtained by extrapolation after first calculating the MBTT for the sample. $CF = F(t_o - \text{MBTT})$, where t_o = midtime of sample collection and F = flow at time sample was formed at the canaliculus (t_o − MBTT).

Bile salt-independent *canalicular* flow was assessed by correlating corrected [^{14}C]mannitol clearances and bile acid excretion rates. A linear regression analysis was obtained from (*a*) pooled data from either the control or dehydrocholate studies, and (*b*) from individual studies during

[1] *Abbreviations used in this paper:* B/P, bile-to-plasma; MBTT, mean bile transit time.

dehydrocholate infusions. Bile salt-independent canalicular flow could not be assessed in individual control experiments because mannitol clearance and bile salt secretion rates did not vary sufficiently to permit extrapolation of a significant linear regression line.

RESULTS

As illustrated in a representative study in Fig. 1, plasma [^{14}C]mannitol activity fell rapidly after i.v. injection, so that 16–25% of the isotope remained in the plasma by 10 min. This initial disappearance of activity from plasma was mainly due to rapid equilibration with extracellular water, as has been shown in the rat (16). Thereafter, the plasma disappearance rate of [^{14}C]mannitol was much slower (0.5–1.5%/min of the plasma pool of [^{14}C]mannitol lost), reflecting the rate of renal excretion after the isotope had equilibrated. As noted in Fig. 1 and Table I, there was a significant delay from the time of injection until the isotope was first detected in the collected bile. This initial appearance time varied between patients from 9 to 22.5 min, presumably as the result of variable lengths in biliary catheters and differences in bile flow rates. Fairly close agreement in transit time was observed, however, when the study was repeated in the same patient (Table I). Thereafter the activity rose to a peak (equilibration time) and then decreased in parallel with the plasma [^{14}C]mannitol disappearance curve.

As pointed out by Barber-Riley, who made similar observations in the rat with bromosulphthalein (12), the lag in the biliary appearance of intravenously administered solutes is most likely due to the time required for the solute to traverse the biliary tree and catheter dead space. The sigmoid shape of the appearance of [^{14}C]mannitol in bile presumably reflects the different path lengths that mannitol must traverse in passing through the biliary canaliculi and ducts. Barber-Riley derived a MBTT by taking half of the time from appearance of the biliary solute to its peak concentration in bile and adding this to the initial appearance

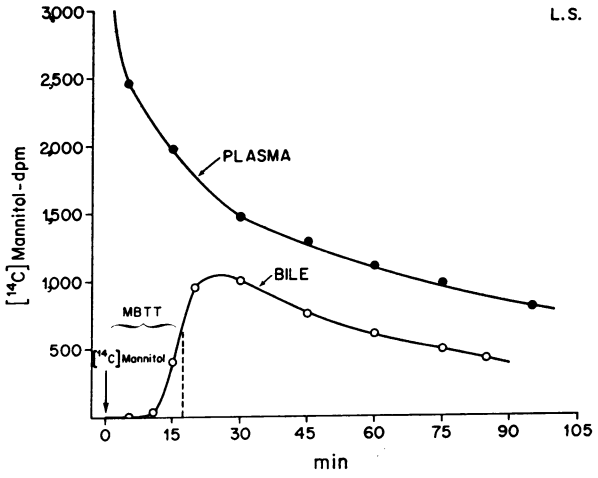

FIGURE 1 A representative [^{14}C]mannitol plasma disappearance curve demonstrating the lag in appearance of i.v. mannitol in bile. MBTT represents the average time for mannitol to pass from hepatocytes to the distal end of the biliary cannula. After the peak activity is reached in bile, biliary [^{14}C]mannitol declines in parallel with plasma activity.

TABLE I
Control Studies

Patient	Initial [^{14}C]mannitol appearance time	B/P equilibration time	Estimated MBTT	B/P [^{14}C]mannitol	Bile flow	[^{14}C]mannitol clearance
	min	*min*	*min*		*ml/min*	*ml/min*
L. S.—1	10.0	25.5	17.8	0.44	0.41	0.18
L. S.—2	10.0	22.5	16.3	0.50	0.42	0.21
W. G.—1	22.5	42.0	32.0	0.45	0.43	0.19
W. G.—2	21.0	43.0	32.0	0.60	0.31	0.19
L. W.	15.0	34.0	24.5	0.68	0.35	0.24
A. G.—1	13.0	30.0	21.5	0.79	0.34	0.26
A. G.—2	10.0	27.0	18.5	0.55	0.49	0.27
V. W.	9.0	21.0	15.0	0.95	0.68	0.65
Mean	13.8	30.6	22.2	0.62	0.43	0.27
±SD	±5.3	±8.4	±6.7	±0.18	±0.12	±0.16
Range	9–22.5	21–43	15.0–32.3	0.44–0.95	0.31–0.686	0.18–0.65

Use of [^{14}C]mannitol in determining mannitol clearance in bile. Mannitol appearance time = time from i.v. [^{14}C]mannitol injection until initial appearance in bile. Equilibration time = time from injection until equilibration with plasma disappearance curve. MBTT ≡ average estimated time for mannitol to pass from the liver to the end of the biliary cannula.

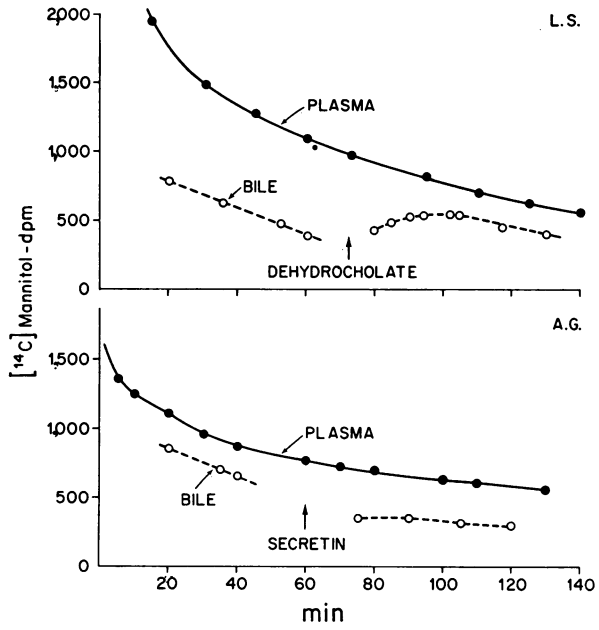

FIGURE 2 B/P [^{14}C]mannitol curves in two experiments after adjustment of each bile sample for its respective MBTT. Dehydrocholate was administered at 70 min in the top study while secretin was administered at 60 min in the other. B/P [^{14}C] ratios were calculated for the respective control and post-choleretic periods when the bile and plasma curves were parallel. Comparable patterns were observed for each of the five dehydrocholate and three secretin studies.

time. While this calculation applies strictly to the situation with a linear rather than sigmoid increase in the concentration of a biliary solute, Barber-Riley's method for calculating MBTT in the present study was in close agreement (96±4.0%) with calculations of transit time based on measurement of the area circumscribed by the bile appearance curve up to the point of equilibration with plasma (13).

When the bile and plasma [^{14}C]mannitol determinations were corrected for the MBTT of each bile collection (Fig. 2), B/P [^{14}C]mannitol ratios averaged 0.62±0.18 ($\bar{x}$±SD) in the eight control studies, and mannitol clearances averaged 0.27±0.16 ml/min (Table I). 5–10 min after sodium dehydrocholate infusions, bile flow increased to 3–4 times control values, followed by a lag in the rise of bile salt excretion (Fig. 3). [^{14}C]Mannitol ratios are increased, generally resulting in an increment in mannitol clearance that paralleled the change in bile flow (Fig. 2, 4 and Table II).

In contrast, although bile flow increased when secretin was infused, (Fig. 4, Table II), B/P [^{14}C]mannitol ratios fell (Fig. 2). Mannitol clearance remained unchanged from control values, demonstrating that

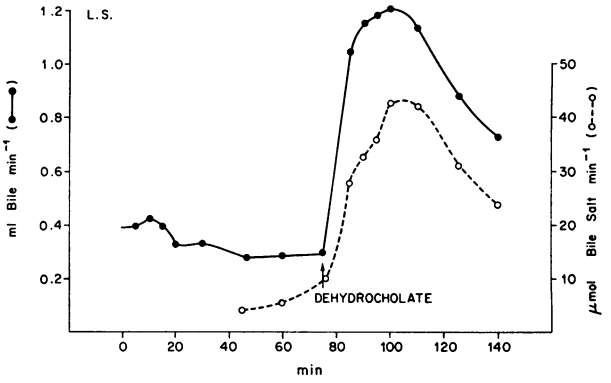

FIGURE 3 Bile flow (solid line) and bile acid excretion rates (dotted line) in a representative study, illustrating the slow change in flow during the control period, the rapid increase in flow after an i.v. bolus of sodium dehydrocholate, and the lag in the appearance of bile acids as a result of the biliary dead space. Similar curves were observed in other experiments and were utilized to determine the appropriate flow rate after correction for the MBTT (see text). Small corrections were necessary during control studies while significant corrections were necessary during dehydrocholate choleresis (also see Fig. 6).

stimulation of ductular secretion did not result in significant increments of mannitol in bile.

When bile flow was correlated with fasting levels of bile salt secretion during control studies, a linear relationship was observed, and a significant amount of flow (0.298 ml/min) was found to be bile salt-independent when the regression line was extrapolated to zero bile acid secretion (Fig. 5A). When the same determinations were made after infusions of sodium dehydrocholate, a positive intercept was again observed (0.402 ml/min), which was not statistically different from control studies. Calculation of the slope of the regres-

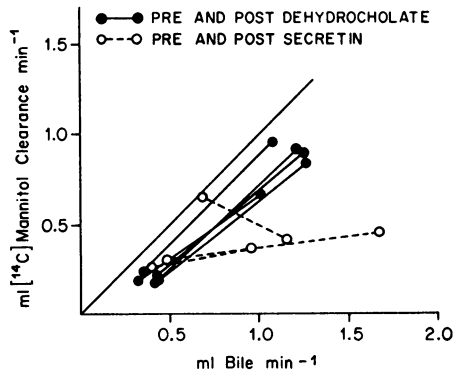

FIGURE 4 The effect of dehydrocholate (solid dot) and secretin (open circle) on bile flow (ordinate) and mannitol clearance (abscissa). The solid line represents the unit slope. Control and postcholeretic values are connected by lines.

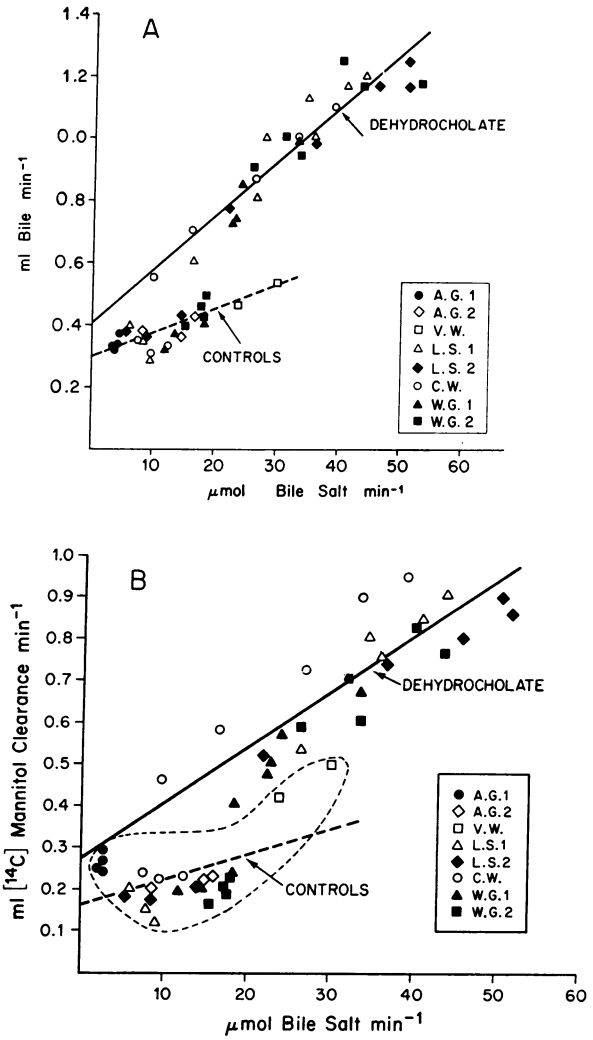

FIGURE 5 A: The relationship between bile salt excretion and bile flow after correction for delay in transit in the biliary tree. Control values from eight studies and post-dehydrocholate values from five studies are represented. Individual experiments are identified by separate symbols. The regression lines were calculated by the method of least squares and were significant to the $P < 0.001$ level. 6.7 μl of bile was obligated by each μmol of bile acid during control studies, while 17.3 μl of bile was excreted with each μmol of bile acid during dehydrocholate experiments. The positive y intercept defines the bile salt-independent component which consists of both canalicular and ductular sources. The control and post-dehydrocholate y intercepts were not significantly different. Control: $y = 0.298 + 0.0067\,x$, $r = 0.74$, $P < 0.001$. Dehydrocholate: $y = 0.403 + 0.0173\,x$, $r = 0.93$, $P < 0.001$. B: The relationship between bile salt excretion and [^{14}C]mannitol clearances in control and post-dehydrocholate studies represented in Fig. 5A, demonstrating a bile salt-independent canalicular component to bile flow in man. The dotted line encircles points from control data. Although the correlation coefficients for the regression lines in this figure were significant in both studies, B/P [^{14}C]mannitol ratios varied considerably between patients (Table I) and resulted in considerable scatter in values for [^{14}C]mannitol clearance for a given bile salt excretion rate. Control: $y = 0.155 + 0.0062\,x$, $r = 0.52$, $P < 0.01$; Dehydrocholate: $y = 0.275 + 0.0129\,x$, $r = 0.85$, $P < 0.001$). Values for the two estimates of bile salt-independent canalicular flow (y intercepts) did not differ significantly.

sion line in control studies indicated that approximately 7 μl of bile was formed for each μmol of bile acid excreted/min while the slope of the regression line was substantially elevated during dehydrocholate infusion so that 17 μl of bile was now formed per μmol excreted bile acid (Fig. 5A).

During dehydrocholate infusion, when flow rates were changing rapidly (Fig. 3), careful attention to the MBTT for each sample was necessary to adjust the collected sample of bile salts to its appropriate flow rate (14). Failure to make this correction resulted in distortion of the relationship between bile acid secretion and bile flow, as illustrated in Fig. 6.

When bile salt-independent canalicular flow was estimated by comparing [^{14}C]mannitol clearance with bile salt excretion, values of 0.155 ml/min and 0.275 ml/min were obtained during control and dehydrocholate infusions, respectively (Fig. 5B). These estimates of bile salt-independent canalicular secretion did not differ significantly and should be viewed as approximations, since B/P [^{14}C]mannitol ratios varied considerably between patients (0.44–0.95, Table I), resulting in a substantial scatter in points when the data is pooled (Fig. 5B). Nevertheless, when [^{14}C]mannitol clearance and bile acid excretion were correlated in individual experiments during dehydrocholate infusions (Fig. 7), extrapolation of a highly significant regression line resulted in a positive y intercept that also defined a portion of canalicular flow that was independent of the osmotic effects of bile salt excretion. As seen in Fig. 7, the values of the y intercepts for these studies were quite similar to values obtained from the pooled observations (Fig. 5B).

DISCUSSION

The fraction of total bile flow derived from the canaliculus can be estimated from the B/P ratio of a solute that selectively enters bile at the level of the canaliculus in the same concentration as in plasma (6–9). [^{14}C]Mannitol was utilized for this purpose in the present study, and B/P ^{14}C ratios were obtained after adjustment for the delay in the appearance of [^{14}C]-mannitol in collected bile. By shifting the bile [^{14}C]-mannitol points to the left by the magnitude of the MBTT, B/P mannitol ratios could be obtained for any given bile collection (Fig. 2), so that mannitol clearance could be estimated. Implicit in this method is the assumption that mannitol equilibrates rapidly with liver

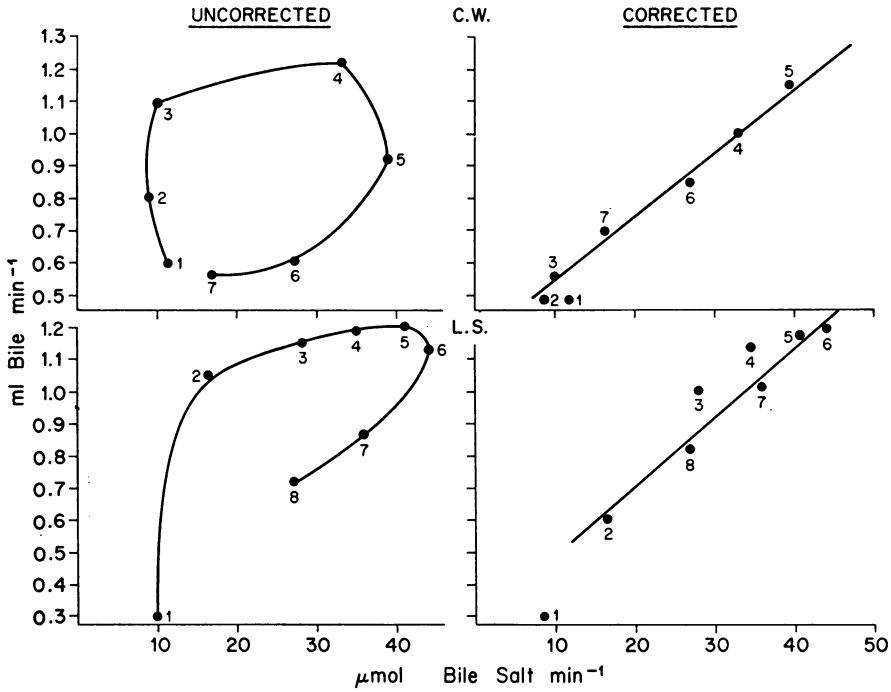

FIGURE 6 The effect of biliary dead space on the relationship between bile acid excretion and bile flow during dehydrocholate infusion in two experiments. Failure to account for the rapidly changing transit times in the biliary tree results in the uncorrected "loop" on the left rather than the expected linear regression which obtains when the rate of bile flow is corrected by calculating the MBTT (see Methods). The numbers show the sequence in which samples were obtained.

cell water. This occurs within several minutes in animals (17), but must be assumed for humans.

The validity of the measurement of canalicular flow also depends on assumptions tested previously in rats, rabbits, dogs, and guinea pigs (6–9, 18–20), which indicate that mannitol and other low molecular weight solutes, such as erythritol, enter bile passively at the level of the canaliculus in response to the osmotic force produced by the active transport of solutes such as bile salts. Mannitol presumably entered bile at the level of the hepatocytes in these human studies as well, since mannitol clearance increased linearly when canalicular flow was enhanced with dehydrocholate but not when ductular secretion was stimulated with secretin (Fig. 4).

Since mannitol clearance paralleled changes in dehydrocholate-induced choleresis, it is unlikely that mannitol was significantly restricted in crossing the canalicular membrane in these studies (the increase in mannitol clearance averaged 0.65 ml/min while increases in total bile flow averaged 0.79 ml/min), or that significant amounts of mannitol-free ductular secretion were stimulated by dehydrocholate or dehydrocholate metabolites (21). Although small increments in mannitol clearance were also observed in two studies after

TABLE II

Effect of Dehydrocholate and Secretin on Bile Flow, [^{14}C]Mannitol B/P Ratios, and Mannitol Clearance

	Dehydrocholate (5)			Secretin (3)		
	Pre	Post dehydrocholate	*P*	Pre	Post	*P*
Bile flow, *ml/min*	0.38±0.05	1.17±0.1	<0.001	0.50±0.18	1.26±0.37	<0.001
B/P ^{14}C ratio	0.54±0.10	0.74±0.07	<0.001	0.76±0.20	0.36±0.06	<0.001
Mannitol clearance, *ml/min*	0.20±0.02	0.85±0.11	<0.001	0.39±0.22	0.42±0.03	NS

Number of experiments is given in parenthesis, while values represent the mean ±SD.

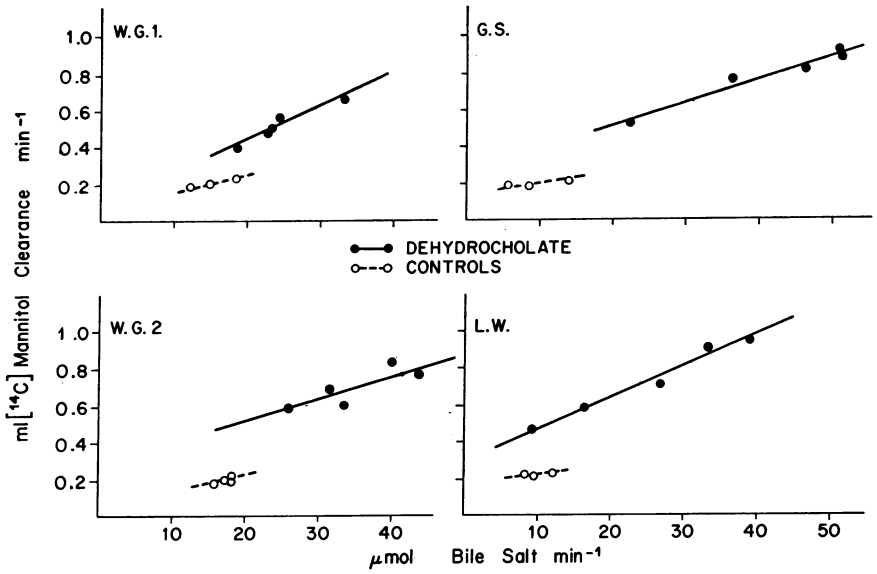

FIGURE 7 Relationship between bile acid secretion rates and [^{14}C]mannitol clearance in four individual experiments (after correction for the lag in biliary dead space). Each solid line is calculated by the method of least squares and gives highly significant correlation coefficients during changes in flow and bile acid secretion produced by the infusion of dehydrocholate. Extrapolation of the regression lines define the y intercept, which represents bile salt-independent canalicular flow in these individual studies. Minimal changes in flow rates during control observations prevented determinations of the linear regression line in the individual patient, although in each case the apparent regression line extrapolated to a positive y intercept. W. G. 1: $y = 0.18 + 0.018\ x$, $r = 0.99$, and $P < 0.002$. W. G. 2: $y = 0.274 + 0.012\ x$, $r = 0.81$, and $P < 0.05$. G. S.: $y = 0.263 + 0.012\ x$, $r = 0.99$, and $P < 0.002$. L. W.: $y = 0.288 + 0.017\ x$, $r = 0.96$, and $P < 0.01$.

secretin administration (Fig. 4), the differences, when compared with control measurements, were not significant. It therefore seems unlikely that more than a small fraction of canalicular secretion could be stimulated by secretin, as has been previously suggested in studies in dogs (22), or that mannitol crossed ductular epithelium in appreciable amounts during secretin choleresis.

Rapid loss of mannitol from plasma to urine might also affect these determinations if plasma concentrations declined more rapidly than in bile. Although approximately 75% of the injected label was excreted in urine within 4 h in these studies, the slopes of the plasma and bile disappearance curves were comparable, indicating that mannitol was equilibrated in the plasma and bile pools at the time of the measurements. Despite these potential limitations, the biliary clearance of mannitol correlated well with changes in canalicular secretion, as in previous studies in animals (Fig. 4), findings which suggest that [^{14}C]mannitol clearance may provide a useful approximation of canalicular flow in man.

Bile salt-independent canalicular secretion. During acute changes of flow induced by a bolus of bile salt, flow rates rise and fall out of synchrony with bile salt excretion rates (Fig. 3) as a direct consequence of the time lag in the biliary dead space. This phenomenon, first observed in the rat by Hacki and Paumgartner (14), distorts the expected linear regression between bile salt excretion and bile flow, and may lead to error in both the slope and intercept of the linear regression and thus the magnitude of bile salt-independent flow. Studies with bolus injections of bile salts in humans are particularly prone to this error, as demonstrated in the present study (Fig. 6), because of the relatively large time delay between events that occur at the canaliculus and subsequent recovery of that sample of bile from the T-tube. However, when the bile flow rate of a given sample was adjusted for the time lag in the biliary dead space, as discussed previously in the Methods, the expected correlation between bile salt secretion and flow was then observed (Fig. 6).

These studies suggest that bile salt-independent secretion represents a significant portion of canalicular flow in man, as has been previously demonstrated for a variety of species, including the rat (8, 9, 23–25), rabbit (7), hamster (26), and dog (6, 27). The magnitude of this portion of canalicular flow varies in given species, but represents the major source of bile in animals such as the rat and rabbit. In the present study,

estimates of bile salt-independent canalicular flow during control periods averaged 0.155 ml/min or 37% of the average basal flow rate (Table I). In a preliminary report by Prandi, Erlinger, Glasinovic, and Dumont, nearly identical values (0.16 ml/min) were obtained with [^{14}C]erythritol in patients with normal liver function who had been operated on for gallstone disease (28). Many other quantitative studies of bile secretion in man have been performed in T-tube patients, either after surgery for common duct obstruction when biliary secretion was large (10, 29), or after interruption of the enterohepatic circulation where the bile salt pool was probably depleted. However, in studies where the enterohepatic circulation was artificially maintained in cholecystectomized patients with T-tube drainage, the estimated total 24-h volume averaged 500–600 ml (10, 30–31), values similar to the 24-h volume we would estimate from our data in the present study.

Correlations of bile salt secretion and bile flow have also been made previously in cholecystectomized patients by Preisig, Bucher, Stirnemann, and Tauber (29), and by Shersten, Nilsson, Cahlin, Filipson, and Brodina-Persson (32). Shersten et al. observed a bile salt-independent fraction of 288 ml/24 h, while Preisig et al. estimated 216 ml/24 h, although neither study used markers to estimate canalicular flow.

According to the present studies, the bile salt-dependent component of biliary secretion obligated 6.7 μl bile/μmol bile acid secreted (Fig. 5A), a value somewhat less than reported by Shersten et al. in man (14 μl/μmol bile salt) but similar to the value obtained by Wheeler and Ramos in the dog (8 μl/μmol bile salt) (4). In addition, the increase in flow during dehydrocholate infusion (17 μl/μmol bile salt) was nearly identical to that obtained previously with dehydrocholate infusions in the isolated perfused rat liver (18 μl/μmol bile salt) (8).

On the basis of our control observations and the assumption that the bile acid pool averaged 3 g (33) and circulated six times daily, approximately 33 mmol bile acid would be anticipated to be excreted by the liver, providing for approximately 200 ml of bile salt-dependent flow each day. Bile salt-dependent (200 ml) plus bile salt-independent canalicular flow (223 ml) would average 423 ml daily. If ductular secretion were 30% of total flow, as suggested by the present study, the total 24-h bile volume would be 604 ml, close to the 500–600 ml 24-h volumes obtained from T-tube drainage during artificial maintenance of the enterohepatic circulation (10, 30–31).

According to these estimates, approximately 70% of daily bile production is canalicular in origin and at least one-third is bile salt-independent. Thus this latter secretory process would appear to provide a substantial contribution to the net production of bile in man, whatever its mechanism. It will be necessary to determine whether gallstone disease or biliary tract surgery influenced these values.

Dehydrocholate was selected to test the relationship between mannitol clearance and canalicular bile flow in the present study because of its large choleretic effect. Although the mechanism whereby this synthetic bile salt stimulates canalicular secretion is also poorly understood, the larger increment in bile secretion after bile salt excretion (Fig. 5) may be related to diminished aggregation of dehydrocholate metabolites in micelles, so that more of the bile acid is in monomeric form in bile and therefore osmotically active (21).

Further studies of bile secretion in man will be necessary to examine these questions and also to determine if bile salt-independent canalicular secretion plays a role in the pathogenesis of cholestasis.

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