Quantitative estimation of transcellular and paracellular pathways of biliary sucrose in isolated perfused rat liver

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A method was developed to estimate the relative contributions of paracellular and transcellular pathways to the total biliary clearance of sucrose in isolated perfused rat liver. When livers were perfused with a sucrose-containing medium (1 mM), biliary sucrose concentration reached an equilibrium of $165 \pm 27 \mu\text{M}$ within 10 min, without further significant change up to 40 min. After removal of sucrose from the perfusate, the decrease of the sucrose concentration in bile was found to obey biphasic first-order kinetics, showing a rapid initial decrease (half-life 3.3 ± 0.5 min) and then a slower decrease (half-life 29.4 ± 5.7 min). Both phases of decrease were further characterized. (1) Pretreating rats with the cholestatic agents α -naphthyl isothiocyanate (ANIT), oestradiol valerate (OV) and colchicine increased the biliary equilibrium concentration and decreased the half-life of the fast phase of the biliary sucrose elimination. The slow phase was unaffected in the livers of ANIT- and OV-treated rats. (2) The slow phase of biliary sucrose efflux was sensitive to colchicine treatment. (3) A close correlation was observed between the slow-phase fraction of the biliary sucrose and the corresponding sucrose content of the liver. By quantitative analysis of the efflux kinetics the relative contribution of the paracellular pathway to the biliary clearance of sucrose was estimated to be $83 \pm 2\%$ in control livers, which increased to about 90% in livers of pretreated animals. These results are important in view of the use of sucrose in evaluating the paracellular-pathway permeability in intra- and extra-hepatic cholestasis.

INTRODUCTION

Bile formation is known to be an iso-osmotic secretion process (Boyer, 1980; Strange, 1984). All epithelia that show iso-osmotic secretion are characterized by high ion conductivity across the junctional complexes (Schultz, 1977; Diamond, 1982). In the liver, bile acids and other osmotically active compounds should therefore induce a paracellular flow of water and electrolytes (Graf & Peterlik, 1976). Inert solutes like sucrose (Forker, 1969, 1970; Elias *et al.*, 1980), inulin (Forker, 1970; Elias *et al.*, 1980) and poly(ethylene glycol) (Javitt *et al.*, 1979) were also found to cross the junctional complex. These compounds were extensively used to investigate the mechanism of drug-induced intrahepatic cholestasis (Forker, 1969; Harkavy & Javitt, 1969; Layden *et al.*, 1978; Elias *et al.*, 1980; Krell *et al.*, 1982; Jaeschke & Krell, 1983; Jaeschke *et al.*, 1983), elevated bile-to-plasma ratios and clearance rates of inert solutes being indicative of increased permeability of the paracellular pathway. However, the validity of these methods was questioned, since sucrose (Graf, 1983) and inulin (Lorenzini *et al.*, 1982) were identified within intracellular vesicles, suggesting a transcellular transport, rather than a paracellular movement, of these substances. In recent reports the parallel existence of different pathways of entry had been qualitatively described for various compounds (Jaeschke, 1983; Lake *et al.*, 1985; Lowe *et al.*, 1985). It can be concluded that sucrose shares the paracellular pathway with erythritol and a transcellular route with markers of higher M_r (Lake *et al.*, 1985). Lowe *et al.* (1985) showed that horseradish peroxidase may enter bile via two routes: a faster paracellular

pathway and a slower transcellular transport within vesicles. However, the quantitative contribution of each pathway to the total biliary clearance of these solutes remained unclear. In the present study a method is described to estimate the relative contributions of the two main routes to the biliary clearance of sucrose. Evidence is provided that the increased biliary clearance of sucrose observed during treatment with cholestatic agents like α -naphthyl isothiocyanate (ANIT) and oestradiol valerate (OV) is caused solely by stimulation of the paracellular pathway, whereas the transcellular route was unaffected. Colchicine, on the other hand, inhibited the transcellular transport and increased the paracellular influx of sucrose.

MATERIALS AND METHODS

Materials

[^{14}C]Sucrose and [^3H]water were obtained from New England Nuclear (Dreieich, Germany), ANIT was purchased from Eastman-Kodak (Rochester, NY, U.S.A.), OV (Progynon) from Schering A.G. (Berlin, Germany) and colchicine from Serva G.m.b.H. (Heidelberg, Germany). All other chemicals were products of Merck A.G. (Darmstadt, Germany).

Animals

Male Wistar rats (200–250 g) fed a standard laboratory diet (Altromin, Lage, Germany) were used in all experiments. Some of the animals were pretreated as follows: (a) OV was dissolved in olive oil (1 mg/ml) and injected subcutaneously for 12 weeks in weekly doses of

Abbreviations used: ANIT, α -naphthyl isothiocyanate; OV, oestradiol 17 β -valerate.

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1 mg of OV/kg body wt. (b) ANIT was suspended in olive oil (200 mg/ml) and administered orally (250 mg/kg body wt.) 9 h before the liver perfusion. (c) Colchicine was dissolved in saline (10 mg/ml) and injected intraperitoneally at a dose of 10 mg/kg body wt. 2 h before the experiment.

Isolated perfused rat liver

Livers were perfused as described previously (Sies, 1978; Krell *et al.*, 1984) with haemoglobin- and albumin-free Krebs-Henseleit bicarbonate buffer containing 18 mM- NaHCO_3 , 122 mM- NaCl , 5.9 mM- KCl , 1.18 mM- MgCl_2 , 1.24 mM- NaH_2PO_4 , 1.2 mM- Na_2SO_4 and 1.2 mM- CaCl_2 . All experiments started with a pre-perfusion period of 40 min, during which the effluent medium was discarded (open perfusion). In order to evaluate the distribution of sucrose in liver, 200 ml of perfusate, containing 1 mM-sucrose labelled with 5 μCi of [^{14}C]sucrose, was recirculated for various time intervals (5–60 min). Subsequently the liver was perfused for 3 min with sucrose-free perfusate to remove the radioactivity from the extracellular spaces. The concentration of sucrose in the effluent medium decreased to $< 2 \mu\text{M}$ within this time. The organ was homogenized with 5% HClO_4 , protein was removed by centrifugation and the [^{14}C]sucrose concentration in the supernatant was determined by liquid-scintillation counting. Only 1–3% of the total radioactivity of the liver could be detected in the sediment. The total amount of sucrose in the supernatant was assumed to be intracellular and referred to liver wet wt. The extracellular sucrose ($< 0.2 \text{ nmol/g}$ liver wt. in the sinusoidal space and about 0.2–0.3 nmol/g of liver wt. in the biliary space) represented less than 0.5–2% of the total sucrose content of the liver (20–80 nmol/g of liver wt.). To calculate the intracellular sucrose concentration, a water content of 72% of liver wet wt. was assumed (Schanker & Hogben, 1961; Forker, 1970; Javitt *et al.*, 1979), 15% of this water being extracellular and 85% intracellular (Schanker & Hogben, 1961).

In a second series of experiments the livers from controls, ANIT-, OV- or colchicine-pretreated animals were isolated and perfused for 40 min as described above. After a recirculation period of 10 or 40 min with 200 ml of sucrose-containing perfusate, the system was changed to a single-pass perfusion with sucrose-free medium. During another 55 min a decrease in the concentration of sucrose in bile and perfusate was observed. At the end of the experiment the sucrose remaining in the liver was determined as described above.

The duration of the longest experiment was 140 min. The viability of the perfused livers was demonstrated by the following criteria (Jaeschke *et al.*, 1983; Krell *et al.*, 1984): (1) slow linear decrease of the bile flow ($1.26 \pm 0.08 \mu\text{l/min}$ per g of liver wt. at 30 min; 0.86 ± 0.10 at 140 min ($n = 10$); (2) stable lactate-to-pyruvate ratios of 5–6; (3) slight increase of the lactate dehydrogenase efflux ($< 0.5 \text{ unit/h}$ per g of liver wt. at 30 min; $< 2 \text{ units/h}$ per g of liver wt. at 140 min); (4) unchanged hepatic oxygen consumption of $2.5 \mu\text{mol/min}$ per g of liver wt. These viability criteria did not change in cholestatic livers (except the bile flow). The bile flow of ANIT-treated rats slowly decreased from $0.77 \pm 0.10 \mu\text{l/min}$ per g of liver wt. at 30 min to 0.52 ± 0.06 at 140 min ($n = 4$), in OV-treated animals from 0.93 ± 0.12 at 30 min to 0.72 ± 0.07 at 140 min

($n = 4$), and in colchicine-treated rats from 1.08 ± 0.12 at 30 min to 0.31 ± 0.06 at 140 min.

Statistics

All results are expressed as mean values $\pm$ S.D. The data were analysed by Dunnett's (1964) test for multiple comparisons with a control. $P < 0.05$ was considered to be significant.

RESULTS

Transcellular and paracellular pathways of sucrose into bile

In order to compare the time course of the appearance of sucrose in bile and in liver cells, livers were perfused with [^{14}C]sucrose in a recirculating system. The liver sucrose content was determined after various time intervals (5–60 min). As shown in Fig. 1, sucrose increased to a maximum of 80 nmol/g of liver wt. within the first 20 min. In contrast with the results obtained *in vivo* (Forker, 1970), no further augmentation of the liver sucrose was observed up to 60 min. To exclude effects of different perfusion times in these experiments, all livers were perfused over a total period of 100 min, including the time of pre-perfusion without sucrose. Thus livers which were recirculated for 5 min had a pre-perfusion phase of 95 min, livers recirculated for 60 min had been pre-perfused for 40 min.

In contrast with its slow accumulation in liver cells, sucrose reached an equilibrium concentration of $177 \mu\text{M}$ in bile within 10 min (Fig. 2a). Compared with [^3H]-water, only a slight delay of the larger molecule, sucrose, was observed. The terminal bile flow was $1.01 \mu\text{l/min}$ per g of liver wt., corresponding to a biliary sucrose clearance (product of bile-to-perfusate ratio of sucrose and bile flow) of $0.18 \mu\text{l/min}$ per g of liver wt. Sucrose already reached its equilibrium concentration in bile when the intracellular content was about 50% of the

Fig. 1. Time-dependence of the hepatic [^{14}C]sucrose content

After a pre-perfusion period of 40 min, livers were perfused with Krebs-Henseleit buffer containing 1 mM-sucrose. After various periods of perfusion (5–60 min) with labelled sucrose, the extracellular sucrose was removed by flushing the liver with sucrose-free perfusate for 2 min. Afterwards the organ was homogenized and the intracellular sucrose content was determined as described in the Materials and methods section. Values shown are means $\pm$ S.D. for four animals.

Fig. 2. Time-dependence of the biliary [^{14}C]sucrose (○) and $^3\text{H}_2\text{O}$ (★) concentration

(a) After a pre-perfusion phase of 40 min, the liver was perfused with sucrose- and $^3\text{H}_2\text{O}$ -containing perfusate for another 40 min. Subsequently the perfusion was continued without radioactivity for 55 min. The biliary equilibrium concentrations of sucrose ($177\ \mu\text{M}$) and $^3\text{H}_2\text{O}$ (100 c.p.m./ μl) were taken as 100%. (b) The biphasic decrease of the biliary sucrose concentration of the control perfusion (a) is plotted semilogarithmically. Calculated regression lines are given for the rapid- (I) and slow- (II) phase kinetics. Iterative corrections of the one kinetics for the contribution of the other increased the slope of the first kinetics and reduced the slope of the second. The corrected value of the extrapolated slow-phase kinetics is 18.8% of the total biliary clearance of sucrose. An individual perfusion is shown. The mean values for repeated experiments are shown in Tables 1–3.

maximal value. This provides further evidence for the existence of a rapid pathway of sucrose entry into bile. On the other hand, if such a fast route were the only way for sucrose to enter bile, one would expect a rapid and total disappearance of sucrose from bile after changing to a perfusate without label. The results shown in Fig. 2(a), however, indicate that the kinetics of sucrose entry into bile were of a biphasic nature. It can be described as the sum of two exponential functions, as demonstrated by the semilogarithmic plot of the biliary sucrose concentration against time (Fig. 2b). If each kinetic phase was iteratively corrected for the contribution of the other, the half-life of the fast and the slow phases were calculated as 3.5 and 35.4 min respectively. The extra-

polation of the slow-phase curve back to the time of the change of the perfusate revealed a concentration of $33.2\ \mu\text{M}$ (18.8% of the total sucrose concentration).

The following experiments were performed in order to investigate the relationship between the fast and the slow decrease of sucrose in bile to postulated trans- and para-cellular pathways.

Modulation of the rapid-phase kinetics of sucrose in bile

In order to modulate the paracellular route of sucrose, rats were pretreated with ANIT or OV, which are known to increase the permeability of the biliary tract (Krell *et al.*, 1982; Jaeschke *et al.*, 1983; Jaeschke & Krell, 1983; Lowe *et al.*, 1985). In livers of treated animals the concentration of sucrose in bile and the clearance of sucrose were found to be markedly increased (Table 1). The intracellular content of sucrose, however, did not differ significantly from the content of control livers either during sucrose perfusion (Table 1) or after a sucrose-free period of 55 min (Table 2). At the end of the experiments the concentration of sucrose in bile had decreased to $12.9 \pm 3.8\ \mu\text{M}$ in untreated animals. No differences (except in bile flow) between controls and ANIT- and OV-treated rats were seen (Table 2). The analysis of the sucrose-efflux kinetics in cholestatic livers revealed an acceleration of the first phase with significantly decreased half-lives compared with controls (Table 3). The extrapolated biliary sucrose concentrations were slightly higher only in livers of OV-treated rats. The relative contribution of the transcellular pathway to the total clearance was therefore calculated to be 4% in ANIT-treated and 11% in OV-treated rats compared with 17% in controls.

Modulation of the slow-phase kinetics of sucrose in bile

In order to vary the transcellular portion of sucrose in bile, sucrose was omitted from the perfusate either after 10 min or after 40 min of recirculation. After 10 min the intracellular content was about 50% of the amount obtained after 40 min (Fig. 1). The concentration of sucrose in bile, however, remained essentially unchanged (Table 1). Extrapolation of the curve of the slow-phase decrease in biliary sucrose back to the time when the perfusate was changed yielded significantly decreased values of biliary sucrose after 10 min compared with 40 min of recirculation (Table 3). Thus a 50% decrease of the hepatic sucrose content caused a corresponding decrease in the extrapolated biliary sucrose concentration in the slow phase of the efflux, whereas the half-life was unaffected. The mean values of various parameters measured and calculated from several perfusions of control livers are summarized in Tables 1–3. The data show that bile flow, equilibrium concentration of sucrose in bile, bile-to-perfusate ratio and the biliary sucrose clearance were the same after 10 or 40 min of recirculating perfusion with sucrose-containing medium, whereas the hepatic sucrose content differed significantly (Table 1). After 55 min of sucrose-free perfusion, the biliary sucrose concentration as well as the hepatic sucrose content were significantly different between livers perfused for only 10 min and those perfused for 40 min with the sucrose-containing perfusate (Table 2). The half-lives of both phases did not differ, but the slow-phase fraction contributed only about 10% to the total biliary clearance in the short-time sucrose perfusion (10 min),

Table 1. Sucrose clearance in control and cholestatic livers

Livers were taken from controls, OV-, ANIT- and colchicine-treated animals. All parameters were obtained at the end of a recirculation period of 10 min (C₁₀) or 40 min (C₄₀, OV₄₀, ANIT₄₀, colchicine₄₀) with sucrose-containing perfusate. Values are means $\pm$ S.D. for four animals. Significance: ***P* < 0.01 (C₄₀ versus OV₄₀, ANIT₄₀ or colchicine₄₀; C₄₀ versus C₁₀). Abbreviation used: n.d., not determined.

	C ₁₀	C ₄₀	OV ₄₀	ANIT ₄₀	Colchicine ₄₀
Bile flow (μ l/min per g liver wt.)	1.06 $\pm$ 0.07	1.03 $\pm$ 0.13	0.78 $\pm$ 0.10**	0.72 $\pm$ 0.10**	1.06 $\pm$ 0.07
[Sucrose] (bile) (μ M)	165 $\pm$ 27	189 $\pm$ 29	409 $\pm$ 66**	703 $\pm$ 118**	467 $\pm$ 81**
Sucrose (bile-to-perfusate ratio)	0.18 $\pm$ 0.03	0.20 $\pm$ 0.03	0.43 $\pm$ 0.06**	0.74 $\pm$ 0.12**	0.46 $\pm$ 0.07**
Sucrose clearance (μ l/min per g liver wt.)	0.19 $\pm$ 0.03	0.20 $\pm$ 0.02	0.37 $\pm$ 0.07**	0.53 $\pm$ 0.11**	0.49 $\pm$ 0.07**
[Sucrose] (liver) (nmol/g of liver wt.)	41.6 $\pm$ 6.3**	78.1 $\pm$ 10.0	88.7 $\pm$ 12.8	86.2 $\pm$ 15.2	n.d.

Table 2. Sucrose content in bile and liver cells from control and cholestatic livers after a period of sucrose-free perfusion

Livers were taken from controls, OV-, ANIT- and colchicine-treated animals. After perfusion of the livers with sucrose-containing perfusate for 10 min (C₁₀) or 40 min (C₄₀, OV₄₀, ANIT₄₀, colchicine₄₀), the medium was changed and the livers were perfused for another 55 min with sucrose-free buffer. All parameters were measured at the end of this period. Values are means $\pm$ S.D. for four animals. Significance: ***P* < 0.01 (C₄₀ versus OV₄₀, ANIT₄₀ or colchicine₄₀; C₄₀ versus C₁₀).

	C ₁₀	C ₄₀	OV ₄₀	ANIT ₄₀	Colchicine ₄₀
Bile flow (μ l/min per g of liver wt.)	0.86 $\pm$ 0.08	0.91 $\pm$ 0.06	0.74 $\pm$ 0.11**	0.53 $\pm$ 0.03**	0.31 $\pm$ 0.06**
[Sucrose] (bile) (μ M)	5.2 $\pm$ 1.9**	12.9 $\pm$ 3.8	18.9 $\pm$ 5.4	14.9 $\pm$ 8.9	4.5 $\pm$ 0.9**
[Sucrose] (perfusate) (μ M)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
[Sucrose] (liver) (nmol/g of liver wt.)	12.5 $\pm$ 2.0**	41.8 $\pm$ 10.6	44.3 $\pm$ 10.5	37.6 $\pm$ 3.0	35.7 $\pm$ 2.6

Table 3. Calculated parameters of sucrose-efflux kinetics in bile

Livers from controls and pretreated (OV, ANIT, colchicine) animals were perfused for 10 min (C₁₀) or 40 min (C₄₀, EV₄₀, ANIT₄₀, colchicine₄₀) with sucrose-containing medium, followed by a 55 min period of perfusion without sucrose. The biliary-sucrose-efflux kinetics obtained from every experiment were plotted semilogarithmically. The slow-phase regression lines were calculated by using only values after 70 min (i.e. 30 min of sucrose-free perfusion). At that time, the superposition of the low-phase kinetics by the rapid-phase kinetics is negligible. Extrapolation back to the time when sucrose was omitted from the perfusate revealed the contribution of the slow-phase kinetics (suggested as transcellular) to the total biliary sucrose clearance. The regression lines of the rapid-phase kinetics were corrected by the contribution of the slow-phase kinetics. Values are means $\pm$ S.D. for four animals. Significance: **P* < 0.05 ***P* < 0.01 (C₄₀ versus OV₄₀, ANIT₄₀ or colchicine₄₀).

	C ₁₀	C ₄₀	OV ₄₀	ANIT ₄₀	Colchicine ₄₀
Half-life (min)					
Rapid phase	3.6 $\pm$ 0.3	3.3 $\pm$ 0.5	2.5 $\pm$ 0.4*	1.5 $\pm$ 0.2**	2.4 $\pm$ 0.2*
Slow phase	33.9 $\pm$ 12.9	29.3 $\pm$ 5.7	32.6 $\pm$ 11.7	33.1 $\pm$ 16.9	76.2 $\pm$ 22.5**
[Sucrose] (bile) (μ M)					
Transcellular	16.8 $\pm$ 3.6**	31.3 $\pm$ 3.8	43.8 $\pm$ 7.9*	27.0 $\pm$ 10.6	4.5 $\pm$ 0.9**
Total concn.	165 $\pm$ 27	189 $\pm$ 29	409 $\pm$ 66**	703 $\pm$ 118**	467 $\pm$ 81**

compared with approx. 17% of the 40 min recirculation experiments (Table 3).

The relationship between the slow phase of [¹⁴C]sucrose disappearance from bile and a transcellular transport process was confirmed by correlating the sucrose

concentration in bile with the liver content after a sucrose-free period of 55 min (Fig. 3). These values, which were obtained at the end of different control perfusions, yielded a correlation coefficient of 0.989 by the method of least squares.

Fig. 3. Correlation between the biliary sucrose concentration and the hepatic sucrose content

Values from individual perfusions of controls are shown (●). After the recirculating perfusion, all livers had been perfused with sucrose-free perfusate for 55 min. Additionally, mean values of the hepatic sucrose content after 10 or 40 min of perfusion with sucrose-containing medium (Table 1) were correlated with mean values of the calculated transcellular sucrose (Table 3) (○). A linear regression line was calculated from all values.

Inhibition of transcellular transport by colchicine

The transcellular pathway of larger molecules such as horseradish peroxidase is assumed to occur via a vesicular transport route (Jones *et al.*, 1980) that can be inhibited by colchicine (Mullock *et al.*, 1980; Bajwa & Fujimoto, 1983; Kacich *et al.*, 1983; Lowe *et al.*, 1985). In order to clarify whether such a vesicular transport was involved in the transcellular movement of sucrose, rats were pretreated with colchicine (10 mg/kg body wt.) 2 h before the liver-perfusion experiments. Here, the equilibrium concentration of sucrose in bile became significantly higher than in controls, as found with OV-treated rats (Table 1). After omission of sucrose from the medium, the concentration of sucrose in bile decreased more rapidly than in control livers. The half-life of the rapid-phase process was similar to that in OV-treated livers (Table 3). In contrast, the half-life of the slow phase process was prolonged and the biliary sucrose concentration at the end of the experiment was significantly decreased compared with controls (Table 3). Extrapolation to the time of perfusate change revealed a concentration of $7.7 \pm 0.6 \mu\text{M}$, corresponding to 1.6% of the biliary sucrose. Since the cellular sucrose content was not significantly altered, the inhibition of the transcellular transport cannot be caused by an enhanced reflux from the hepatocytes back into the perfusate. Thus at least part of the transcellular fraction of biliary sucrose can be attributed to a colchicine-sensitive transport mechanism.

DISCUSSION

The present study was designed to evaluate the contribution of both transcellular and paracellular routes to the biliary clearance of sucrose. Analysing the time-dependence of the decrease of the biliary sucrose concentration after omission of sucrose from the perfusate, biphasic first-order kinetics of sucrose elimination was found with half-lives of 3.3 min and 29.4 min in the fast and the slow phase respectively. Considering

the sequence of equilibration of sucrose in the cellular and the biliary space respectively, it seems obvious to attribute the rapid phase to a paracellular route and the slow phase to a transcellular route. These conclusions are supported by results of experiments designed to alter the individual pathways of sucrose elimination.

(1) Removing sucrose from the perfusate at the time when the liver content was half-maximal, it was found that the contribution of the slow phase of biliary elimination was also decreased, whereas its half-life remained unaffected.

(2) An excellent correlation was observed between the slow-phase fraction of the biliary elimination of sucrose and the corresponding sucrose content of the liver.

(3) Restraint of the transcellular vesicle transport by colchicine inhibited the slow-phase fraction of the biliary sucrose elimination and prolonged its half-life.

(4) Pretreating animals with agents known to increase the permeability of the paracellular pathway, such as ANIT (Krell *et al.*, 1982; Jaeschke *et al.*, 1983; Lowe *et al.*, 1985), OV (Jaeschke & Krell, 1983) or colchicine (Bajwa & Fujimoto, 1983), increased the equilibrium concentration of sucrose in bile and the biliary clearance. In these experiments the half-life of the rapid phase was found to be decreased, indicating a reflux of sucrose into the perfusate via the paracellular pathway.

The biphasic first-order sucrose-elimination kinetics in bile is consistent with a two-compartment model of independent routes of entry. The rapid phase can be attributed to wash-out kinetics of the biliary space when the fast influx of sucrose into bile across the junctional complex is stopped. With this hypothesis, integration of the area under the curve of these kinetics should give the total biliary space, including the intercellular space. A half-life of 3.3 min in controls revealed a volume of both spaces of about $4.8 \mu\text{l/g}$ of liver wt., which corresponds to the previous estimates of the biliary-tree volume of $2.2\text{--}3.2 \mu\text{l/g}$ of liver wt. (Forker, 1970; Haecki & Paumgartner, 1973).

The slow phase of sucrose elimination can be attributed to a vesicular transport system described previously (Jones *et al.*, 1980; Mullock & Hinton, 1981). There is no evidence that sucrose is able to penetrate the intact plasma membrane of liver cells by simple diffusion. Rather, sucrose was found in the cytosol of hepatocytes only after permeabilization of the plasma membrane by various treatments (e.g. electric discharges, collagenase treatment) (Gordon & Seglen, 1982; Gordon *et al.*, 1985). Even this freely distributed sucrose was rapidly incorporated into lysosomal vesicles and mitochondria by autophagocytosis (Tolleshaug *et al.*, 1984). Recent reports also identified sucrose within intracellular vesicles of the isolated perfused rat liver (Graf, 1983; Lake *et al.*, 1985). Consistent with these results is our observation that the slow-phase biliary sucrose efflux is sensitive to colchicine, which is known to be an inhibitor of the transcellular vesicle transport (Mullock *et al.*, 1980; Kacich *et al.*, 1983; Bajwa & Fujimoto, 1983; Lowe *et al.*, 1985).

The method here described can be used to investigate the impairment of the junctional complex and transcytotic pathways by cholestatic agents. The present study showed that ANIT and OV altered selectively the permeability of the biliary tree without affecting the transcellular transport of sucrose. This is in accord with the results of Lowe *et al.* (1985), who found enhanced

paracellular movement of horseradish peroxidase in livers of ANIT-treated animals but no effect on the biliary secretion of IgA. In contrast, colchicine affects both pathways of sucrose. Lake *et al.* (1985) have questioned the steady-state analysis of sucrose distribution commonly used to demonstrate increased biliary permeability (Wheeler *et al.*, 1968; Forker, 1969), since agents affecting sucrose distribution may differently influence trans- and para-cellular pathways. If, however, a quantitative estimate can be made, sucrose may well serve as a paracellular marker. We were able to demonstrate that, in cholestatic states induced by ANIT and OV, the transcellular portion of the biliary sucrose transport was not affected. Under such conditions of a constant and rather low contribution of the transcellular route, the use of sucrose should be valid to indicate permeability changes of the paracellular pathway. We therefore suggest that analysis of biliary permeabilities using steady-state sucrose distributions should be accomplished by an estimation of the contribution of the transcellular route.

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