Is Intracellular pH and/or Intracellular Bicarbonate a Determinant of Bile Salt Independent Canaliculular Bile Formation? The Subject Revisited

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Canalicular bile formation is a complex process that involves basolateral and apical cell membrane transport, paracellular transport and vesicular transport, all of which may be subject to regulation by pH. We review the concept that apical cell membrane bicarbonate secretion promotes bile salt independent canalicular bile formation. We show that the presence of paracellular electrolyte transport imposes a severe restriction in interpreting data from ion substitution experiments aimed at demonstrating pH or bicarbonate dependent bile formation. Furthermore, we report on experiments that all show stimulation of bile flow under three disparate experimental conditions: i) intracellular alkalinization in the absence of $\text{[HCO}_3^{-}]$, or associated with a decrease of $\text{[HCO}_3^{-}]$, ii) intracellular alkalinization with an increase of $\text{[HCO}_3^{-}]$, and iii) intracellular acidification with increase of $\text{[HCO}_3^{-}]$. It is suggested that both, intracellular pH and intracellular bicarbonate may modulate canalicular bile salt independent bile formation, but it remains conjectural which mechanism is the prevailing one under a given experimental setting.

INTRODUCTION

This article gives a short overview of the concept of bicarbonate dependent canalicular bile formation, and it discusses some recent studies.

The model of separating bile flow into a canalicular (hepatocellular) and ductular fraction is based on clearance studies and on the paradigm that the sugar alcohols erythritol and mannitol freely permeate the biliary tree exclusively at the level of its fine canalicular branches but not at the level of the larger bile ductules and bile ducts [1].

Species that exhibit a large fraction of ductular bile formation (e.g., guinea pigs, humans) respond to secretin or parasympathomimetic stimuli with an increase of bile flow attributed to ductular secretion of bicarbonate. In rats, erythritol clearance is nearly equal to total bile flow suggesting that ductular bile secretion is low or absent. Only after inducing bile duct hyperplasia is secretin-stimulated bicarbonate-rich bile flow seen in this species [2, 3], and stimulation of bile flow by either hyperkapien or metabolic alkalosis was only observed in the special case of hypercholeresis induced by ursodeoxycholate [4].

It was, therefore, a challenging observation that Hardison and Wood [5] reported that up to 50 percent of bile salt independent bile formation depends on bicarbonate secretion in the isolated perfused rat liver. This conclusion was mainly based on the observation that bile flow was reduced when perfusate bicarbonate was replaced with the buffer tricine. However, these data did not discriminate whether the reduction of bile flow was due to the

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$^b$ Abbreviations: DIDS, 4,4'-Diisothiocyanatostilbene-2,2'-disulfonic acid.
reduce the bicarbonate in the perfusate or to the addition of tricine, a buffer that presumably exhibits a poor perfusate to bile permeability (see below).

Furthermore, it remained questionable whether a bile to perfusate pH (or bicarbonate) gradient can be maintained at the canalicular level. Studies in the isolated perfused liver [6] and in isolated hepatocyte couplets [7] showed a high ion permeability of the paracellular pathway suggesting that tight junctions may not be tight enough to prevent bicarbonate diffusion. Indeed, no pH gradient between “bile” and bathing fluid could be demonstrated in liver cell couplets [8, 9]. The concept that canalicular bile flow may be driven by bicarbonate secretion received support from studying the polar distribution of pH regulatory systems in hepatocytes, the alkali loading systems (Na+/H+ exchange, Na+-HCO3- cotransport) being localized to the basolateral cell membrane [e.g., 10, 11], whereas the alkali extruding Cl-/HCO3- exchange resides in the apical cell membrane [12, 13]. Combined, these systems would allow for vectorial transport of bicarbonate into bile while simultaneously maintaining intracellular pH at a constant level [14, 15, 16] (Figure 1).

It has been tested, therefore, whether maneuvers that alter intracellular pH and bicarbonate concentration do affect bile flow in the perfused rat liver [17, 18]. One study measured bile flow and cell pH simultaneously [17]; the other reports on correlated changes of bile flow, bile pH or biliary bicarbonate concentration [18]. In both studies, intracellular...
pH was varied by two sets of experiments: either by shifting perfusion between media with high NaHCO₃/CO₂ (50 mM/10 v/v percent) and low NaHCO₃/CO₂ (25 mM/5 v/v percent) at constant pH or by application or withdrawal of NH₄Cl (20 mM) in the continuous presence of NaHCO₃/CO₂ (25 mM/5 v/v percent). During both maneuvers, isotonicity was maintained by appropriate variation of NaCl concentration.

It appears worthwhile to consider the passive consequences of these maneuvers first: abrupt changes of cell pH (pHᵢ) result from the rapid diffusion across the cell membrane of either CO₂ or NH₃, the non-ionic, gaseous components of the two buffer systems: H⁺ + HCO₃⁻ ↔ H₂CO₃ ↔ H₂O + CO₂ (equilibration being accelerated through carbonic anhydrase), and NH₄⁺ ↔ H⁺ + NH₃, respectively. Cell pH at any given point of time results from the changes of the intracellular ratio of [HCO₃⁻]/[CO₂] or [NH₄⁺]/[NH₃], respectively. The resulting changes of cell [HCO₃⁻] are, therefore, different in the two sets of experiments: application of high NaHCO₃/CO₂ results in acidification with simultaneous generation of HCO₃⁻ in the presence of H⁺ accepting buffers (B⁻) by: B⁻ + H₂CO₃ ↔ BH + HCO₃⁻ (Figure 2). The amplitude of the change of pHᵢ therefore, depends on the buffer capacity. In analogy, after equilibration at high NaHCO₃/CO₂, reducing perfusate NaHCO₃/CO₂ results in alkalinization with a buffer dependent reduction of [HCO₃⁻]. In contrast, intracellular alkalinization by application of NH₄Cl results in production of HCO₃⁻, by uptake of NH₃, which draws H⁺ from BH and H₂CO₃ generating HCO₃⁻ by: H₂CO₃ + NH₃ ↔ NH₄⁺ + HCO₃⁻ (Figure 2). In addition, these experimental maneuvers will alter canalicul bile pH and HCO₃⁻ concentration not only as a function of apical cell membrane HCO₃⁻ transport but also directly, as a result of diffusion of CO₂ and NH₃ into bile, paracellular permeation of HCO₃⁻ and NH₄⁺ and the presence of other buffers in bile.

**Figure 2. Mechanisms of modulation of intracellular pH and [HCO₃⁻] by changing the extracellular buffer composition.** The presentation of the hepatocyte is as in Figure 1. Two maneuvers are shown that result in an increase of intracellular [HCO₃⁻] which may stimulate apical Cl⁻/HCO₃⁻ exchange. Upper part: elevation (or addition) of extracellular HCO₃⁻ and CO₂ (at constant extracellular pH) results in rapid diffusion of CO₂ into the cell. Intracellular H₂CO₃ lowers cell pH and serves to generate HCO₃⁻ by buffer anions (B⁻) accepting H⁺ and being converted to the undisassociated acid (BH). Opposite intracellular effects result from reduction of extracellular HCO₃⁻ and CO₂.
With respect to the activity of the apical Cl⁻/HCO₃⁻ exchanger, two components have to be considered: i) for thermodynamic reasons, the exchanger will only operate if out of equilibrium, i.e., if [HCO₃⁻]_{bile}/[HCO₃⁻]_{i} is different from [Cl⁻]_{bile}/[Cl⁻]_{i}. Approximate values for these ratios, for control conditions, may be close to 2 and 5 for the concentration ratios of HCO₃⁻ and Cl⁻, respectively [6], allowing for operation in the direction of a HCO₃⁻ extruder; ii) the activity of the exchanger appears to be subject to pH dependent allosteric modulation, i.e., its turnover rate appears to increase by intracellular alkalinization [13] (Figure 1).

Combined, both studies mentioned above [17, 18] showed that both extracellular reduction of NaHCO₃/CO₂ or application of NH₄Cl transiently increased cell pH, bile flow and biliary bicarbonate output (only after application of NH₄Cl) of the isolated perfused rat liver. It may be recalled that the two maneuvers affect cell [HCO₃⁻] differently in that reduction of NaHCO₃/CO₂ decreases [HCO₃⁻] whereas application of NH₄Cl increases [HCO₃⁻] (see Figure 2). Furthermore, the study by Bruck et al. [18] demonstrated that the anion transport inhibitor DIDS inhibited the effects produced by reduction of NaHCO₃/CO₂. In addition, application of colchicine inhibited the effects of both maneuvers. These data suggest that the increase of bile flow was due to stimulation of apical Cl⁻/HCO₃⁻ exchange and to an increase of microtubule-dependent insertion of this transporter into the apical cell membrane. Furthermore, Ba²⁺ was applied in order to prevent cell membrane potential hyperpolarization that results from alkalinization induced increase of K⁺ conductance [19, 20]. Ba²⁺ did not inhibit the increase of bile flow following reduction of NaHCO₃/CO₂ [18]. From these studies, it appears that the alkalinization induced effects on bile flow are neither due to potential dependent lowering of [Cl⁻]_{i} nor to an increase of [HCO₃⁻]_{i}, the two primary driving forces of Cl⁻/HCO₃⁻ exchange, but rather due to alkalinization-induced activation of this transporter. Moreover, the lack of effects of Ba²⁺ argues against the possibility that alkalinization induced cell membrane hyperpolarization may affect electric transporters that could support bile salt independent bile formation.

We present here additional experiments that test i) the direct effect on bile flow and cell pH of adding tricine to the perfusion medium at constant perfusate [HCO₃⁻], ii) the effect on bile flow of cell alkalinization in the absence of HCO₃⁻ and iii) the effect on bile flow when cell pH and [HCO₃⁻]_{i} are changed in the opposite direction (acidification with simultaneous increase of [HCO₃⁻]_{i}). The data show i) that tricine inhibits bile flow without affecting cell pH and [HCO₃⁻]_{i}, ii) that cell alkalinization in the absence of HCO₃⁻ increases bile salt independent bile flow, and iii) that an increase of [HCO₃⁻]_{i} stimulates bile flow despite cell acidification.

**MATERIAL AND METHODS**

Male Louvain rats weighing 200 to 250 g were used as liver donors. Livers were perfused in a non-recirculating system at a rate of approximately 3.5 ml/g/min. The standard perfusion solution contained (mM): NaCl 118.4, KCl 4.75, CaCl₂ 2.57, KH₂PO₄ 1.19, MgSO₄ 1.19, NaHCO₃ 25; pH was adjusted to 7.4 after equilibration with 5 v/v percent CO₂, 95 v/v percent O₂. In solutions that contained tricine, 25 mM NaCl was iso-osmotically replaced by tricine titrated to pH 7.4. Hepes buffered solutions were bicarbonate-free, were gas-equilibrated with 100 percent O₂ and contained 10 mM Hepes titrated to pH 7.4 with NaOH, and NaCl was increased to 133.4 mM in order to maintain isoosmolarity. Additions of NH₄Cl were at expense of NaCl. Experimental maneuvers were carried out after 30 min had elapsed after the beginning of liver perfusion in order to allow for clearance of endogenous bile acids.
Bile flow was measured automatically by the time intervals and by the weight of bile drops falling from the bile duct cannula.

Intracellular pH was continuously monitored as previously described [17]. In brief, 1,3-dihydroxy-pyrene-6,8-disulfonic acid was used as an intracellular sensor, which was loaded into the cells of the perfused liver in the form of its diacetoxy ester. Surface fluorescence of the organ was measured at 512 nm during alternating excitation at 464 and 420 nm, the latter being the isosbestic wavelength of the pH sensitive dye.

RESULTS

In order to test whether application of tricine buffer in the perfused liver may affect bile flow independently of changes of intracellular pH or [HCO₃⁻], we replaced NaCl (instead of NaHCO₃, compare [5]) by tricine buffer but kept HCO₃⁻/CO₂ in the perfusate constant. Figure 3 shows that this maneuver resulted in an abrupt reduction of bile flow, which returned to near control values after perfusion was shifted back to tricine-free

Figure 3. Effects of replacing 25 mM NaCl in the perfusion medium by tricine buffer on intracellular pH (surface fluorescence ratio) (upper panel; A) and bile flow (lower panel; B) of the isolated perfused liver. The experiment was carried out in the continuous presence of 25 mM NaHCO₃/5 v/v percent CO₂. Note that application tricine exhibits only an insignificant effect on pHᵢ but causes a substantial reduction of bile flow. Independent calibration studies reveal a control pHᵢ of approx. 7.45, the increase by tricine amounting to approx. 0.05 pH units.
Table 1. Effects of intracellular alkalization on bile flow in the presence and absence of bicarbonate in the perfused liver

| Perfusion Buffer | Bile Flow (mg/g liver/min) |
|------------------|---------------------------|
|                  | Control                  | 20 mM NH₄Cl a |
| 25 mM HCO₃⁻/5% CO₂ b | 0.80 ± 0.05              | 1.14 ± 0.07   |
| 10 mM HEPES     | 0.58 ± 0.03 c            | 0.87 ± 0.04 c |

a: peak values after addition of NH₄Cl.
b: values taken from Ref. [17].
c: mean values ± SEM, N = 4.

Figure 4. Effects of addition of NaHCO₃/CO₂ (25 mM/5 v/v percent) (bar length) in expense of NaCl on intracellular pH (upper panel, A) and bile flow (lower panel, B) of the isolated perfused liver. 25 mM tricine buffer was present throughout the experiment. Note that application of NaHCO₃/CO₂ results in transient acidification (by approx. 0.5 pH units) whereas removal of NaHCO₃/CO₂ results in transient alkalinization by about the same amplitude. Bile flow increases following application of NaHCO₃/CO₂, presumably by rapid generation of intracellular HCO₃⁻ and decreases after removal of NaHCO₃/CO₂. The data show that choleresis is already elicited during cell acidification and that alkalinization fails to produce choleresis.
solution. In addition, replacement of NaCl by tricine buffer was not associated with a significant change of intracellular pH (Figure 3) indicating that addition of tricine buffer inhibits bile flow independently of modulation of pH.

As shown in Table 1, application of 20 mM NH₄Cl in the nominal absence of HCO₃⁻/CO₂ resulted in an increase of bile flow (see Figure 2 with respect to the experimental maneuver). The amplitude of the increase of bile flow was indistinguishable from the effect of NH₄Cl application seen in the presence of 25 mM HCO₃⁻ / 5 v/v percent CO₂ [17] indicating that stimulation of bile flow by cell alkalization does not require the presence of HCO₃⁻.

The typical experimental protocol to activate Cl⁻/HCO₃⁻ exchange involves reduction of extracellular NaHCO₃/CO₂ concentration at constant extracellular pH [13]. The preceding equilibration at high NaHCO₃/CO₂ leads to a high level of intracellular [HCO₃⁻] and the following reduction of extracellular NaHCO₃/CO₂ results in alkalization by rapid diffusion of CO₂ out of the cell (compare Figure 2). Both, high [HCO₃⁻] and the increase of cell pH, activate Cl⁻/HCO₃⁻ exchange, which results in HCO₃⁻ extrusion and subsequent decrease of pH (compare Figure 1). This maneuver results in stimulation of bile flow of the isolated liver, whereas an increase of perfusate NaHCO₃/CO₂ concentration leads to inhibition [17, 18]. These effects do not clearly discriminate whether bile flow is stimulated by the increase of pH alone (independent of activation of Cl⁻/HCO₃⁻ exchange; see above and Table 1) or whether this stimulation is due to activation of apical Cl⁻/HCO₃⁻ exchange by both high [HCO₃⁻] and cell alkalization. The following experiments aim at discriminating between these possibilities in that an increase of [HCO₃⁻] was induced while cell pH was simultaneously lowered.

As shown in Figure 4, NaHCO₃/CO₂ -free perfusion (with tricine as the perfusate buffer) results in a low rate of bile flow (compare Figure 3). Addition of NaHCO₃/CO₂ led to intracellular acidification followed by recovery of pH, and withdrawal of NaHCO₃/CO₂ led to alkalization and subsequent return of pH to control level. Addition of NaHCO₃/CO₂ was associated with an increase of bile flow, and withdrawal of NaHCO₃/CO₂ led to a gradual decrease of bile flow towards control level. The data show that generation of intracellular HCO₃⁻ by extracellular addition of NaHCO₃/CO₂ (compare Figure 2) is accompanied by an increase of bile flow despite transient intracellular acidification.

**DISCUSSION**

In reviewing the development of the concept that bile salt-independent canaliculal bile formation is supported by Cl⁻/HCO₃⁻ exchange at the apical liver cell membrane, we are faced with an abundance of experimental data, but many unknown components of a complex system hamper their interpretation. In the following, we discuss experiments and arguments that are in favor of, or against, the concept that canaliculal HCO₃⁻ secretion promotes bile salt-independent bile formation.

With making the compromise of reducing the perfused rat liver to a three-compartment system (perfusate-cell interior-bile) we remain uncertain about whether changes in bile flow and bile composition in response to changes of perfusate composition reflect properties of paracellular or transcellular transport. Thus, we show that replacement of NaCl in the perfusate by tricine buffer has no substantial effect on pH (Figure 3) but produces a greater than 50 percent reduction of bile flow similar to that seen when NaHCO₃ was replaced by the buffer, thus invalidating this latter approach to suggest that a large fraction of canaliculal bile formation depends on HCO₃⁻ secretion [5]. The observed pattern of bile-flow changes following ion substitution is rather similar to that seen when Na⁺
or Cl\(^-\) are replaced by less permeable ions such as choline or acetildiglycinate, respectively [6] and indicates a poor permeability of tricine.

We observe that cell alkalization by application of NH\(_4\)Cl (at the expense of NaCl) in the absence of HCO\(_3^-\)/CO\(_2\) in the perfusate results in the same choleric effect as cell alkalization produced by NH\(_4\)Cl in the presence of HCO\(_3^-\)/CO\(_2\) (Table 1). This may indicate that cell alkalization, rather than an increase of HCO\(_3^-\) secretion into bile, promotes choleresis. It is possible, though, that endogenous CO\(_2\) production is sufficient for the generation of HCO\(_3^-\) upon entry of NH\(_3\) into the cell. Alternatively, entry of NH\(_3\) and alkalization may generate other buffer anions from their undisassociated acids, whose canalicular secretion may promote bile formation. In this context, it may be recalled that the anionic components of the biliary cation-anion-gap have been only partially identified, but their secretion (increase of the cation-anion-gap) was shown to increase biliary osmolarity [6], and a large fraction of bile salt-independent canalicular bile formation could be attributed to secretion of glutathione [21].

DIDS inhibited choleresis and the increase of biliary HCO\(_3^-\) output following application of NH\(_4\)Cl in the presence of HCO\(_3^-\)/CO\(_2\) [18]. Although this effect is consistent with inhibition of apical Cl\(^-\)/HCO\(_3^-\) exchange [13], the interaction of DIDS with biliary HCO\(_3^-\) secretion appears to be more complex: DIDS is efficiently secreted into bile and secretion is associated with choleresis and a reduction of biliary HCO\(_3^-\) output [22]. A major mechanism appears to be that DIDS replaces HCO\(_3^-\) in bile as it was shown i) that excretion of DIDS was inhibited by reduction of perfuse HCO\(_3^-\), and that ii) reduction of HCO\(_3^-\) excretion by DIDS was independent of the presence of Cl\(^-\) in the perfusion medium, the latter observation making participation of Cl\(^-\)/HCO\(_3^-\) exchange appear unlikely. These data rather suggest that reduction of biliary HCO\(_3^-\) by DIDS results from secretion of DIDS via exchange at the apical cell membrane by the HCO\(_3^-\)/SO\(_4^2^-\) antiporter which appears to accept DIDS as a substrate [22, 23].

It appears possible that the choleric effect of replacing NaCl isosmotically by NH\(_4\)Cl is due to the higher paracellular permeability of the latter. Colchicine was shown to reduce the choleric effect of NH\(_4\)Cl application and of the parallel increase of biliary bicarbonate output in the presence of HCO\(_3^-\)/CO\(_2\) [18]. This observation is consistent with reduced apical bicarbonate secretion in that the number of apical Cl\(^-\)/HCO\(_3^-\) exchangers is reduced by inhibition of membrane insertion from submembraneous vesicles whose membranes contain this transporter [18]. On the other hand, colchicine appears to increase paracellular permeability [24], which may also modulate the effect of NH\(_4\)Cl on bile flow.

Intracellular alkalization may also result in cell swelling, which may increase bile flow [25] by mechanisms that are independent of activation of Cl\(^-\)/HCO\(_3^-\) exchange.

The most direct experimental maneuver to demonstrate cellular Cl\(^-\)/HCO\(_3^-\) exchange involves reduction of extracellular NaHCO\(_3^-\)/CO\(_2\) concentration at constant extracellular pH [13]. This maneuver leads to a sudden alkalization of cell pH whose amplitude depends on the intracellular buffering power. Alkalization stimulates the exchanger and results in HCO\(_3^-\) extrusion and recovery of cell pH to control level, effects that are inhibited by DIDS or by removal of extracellular Cl\(^-\). In the perfused liver, reduction of extracellular NaHCO\(_3^-\)/CO\(_2\) concentration from 25 mM/ 5 v/v percent to 0 mM/ 0 v/v percent leads also to an increase of cell pH, which is associated with stimulation of bile formation [17, 18]. This association suggests that activation of apical Cl\(^-\)/HCO\(_3^-\) exchange promotes bile flow but, as noted above, cell alkalization stimulates bile flow also in the absence of HCO\(_3^-\).

Lastly, the experiment shown in Figure 4 employs an experimental condition where generation of intracellular HCO\(_3^-\) is associated with cell acidification. In this experiment,
control [HCO$_3^-$]$_i$ is expected to be close to zero during NaHCO$_3$/CO$_2$-free perfusion (with tricine as the only extracellular buffer). As depicted in Figure 2, addition of extracellular NaHCO$_3$/CO$_2$ at constant extracellular pH results in diffusion of CO$_2$ into the cell, which leads to acidification and to generation of HCO$_3^-$. Generation of HCO$_3^-$ will be facilitated by carbonic anhydrase, and it depends on the presence of intracellular buffers that accept H$^+$ from the dissociation of carbonic acid. Figure 4 shows that bile flow is increased during the expected rise of [HCO$_3^-$]$_i$ (despite transient intracellular acidification) and, following removal of extracellular NaHCO$_3$/CO$_2$, bile flow falls as cells are expected to become depleted of HCO$_3^-$. Choleretic is thus associated with the rise of [HCO$_3^-$]$_i$, which may be considered to promote bile flow through activation of Cl$^-$/HCO$_3^-$ exchange. This experiment differs from those reported in the preceding paragraph in two aspects: i) intracellular [HCO$_3^-$] is expected to vary here between approximately 0 and 20 mM following addition of NaHCO$_3$/CO$_2$, which may be sufficient to activate Cl$^-$/HCO$_3^-$ exchange despite the transient fall of pH to approx. 7.0. In contrast, activation of Cl$^-$/HCO$_3^-$ exchange in the experiments reported above [17, 18] was achieved by cell alkalization at an initial [HCO$_3^-$]$_i$ expected to be greater than 30 mM, and ii) the experiments shown in Figure 4 were performed in the continuous presence of tricine as perfusate buffer. It may be noted that tricine could serve as a buffer anion to generate HCO$_3^-$ by accepting H$^+$ by the reaction: B$^- +$ CO$_2 +$ H$_2$O $\leftrightarrow$ BH + HCO$_3^-$ ($pK_a$ of tricine = 8.1). Provided that tricine enters the cells, it may add to the intrinsic intracellular buffering power, which, in the absence of HCO$_3^-$/CO$_2$, is low at pH$_i$ values above 7.3 [13]. Thus, the presence of tricine may assist to generate intracellular HCO$_3^-$ upon addition of NaHCO$_3$/CO$_2$. In addition, this buffering reaction generates osmolytes by increasing the sum of ([B$^- + BH +$ HCO$_3^-$]). With tricine being secreted the reaction may also take place in canalicular bile and generation of osmolytes by diffusion of CO$_2$ into bile could be responsible for the rapid increase of bile flow seen upon addition of NaHCO$_3$/CO$_2$ (Figure 4). Thus, the experiments shown in Figure 4 suggest that generation of intracellular HCO$_3^-$ promotes bile formation by activation of Cl$^-$/HCO$_3^-$ exchange but, as with other experimental approaches, the data allow for alternative interpretations.

SUMMARY

In this article, we have attempted to critically review several experimental approaches that appear(ed) to support the concept that active secretion of HCO$_3^-$ by hepatocellular Cl$^-$/HCO$_3^-$ exchange supports canalicular bile formation. We show that alternative interpretations are more adequate for some experimental settings. One principal restriction in interpretation of experimental data is that the paracellular permeability of HCO$_3^-$ remains undetermined. Furthermore, experimental maneuvers that increase intracellular pH and/or intracellular [HCO$_3^-$] cannot distinguish which of the two parameters is the main determinant in modulating bile flow. Thus, choleretic can be induced by i) intracellular alkalization in the nominal absence of [HCO$_3^-$]$_i$ (application of NH$_4$Cl in the absence of extracellular NaHCO$_3$/CO$_2$), ii) alkalization at either high [HCO$_3^-$]$_i$ (reduction of extracellular NaHCO$_3$/CO$_2$ concentration) or alkalization associated with an increase of [HCO$_3^-$]$_i$ (application of NH$_4$Cl in the presence of NaHCO$_3$/CO$_2$), and iii) increase of [HCO$_3^-$]$_i$ with simultaneous reduction of cell pH (addition of NaHCO$_3$/CO$_2$ following NaHCO$_3$/CO$_2$-free perfusion with tricine buffer). We therefore conclude that some selected data support the concept that hepatocellular secretion of HCO$_3^-$ via apical Cl$^-$/HCO$_3^-$ exchange promotes canalicular bile formation (without providing a definite proof) but changes of pH$_i$ appear to alter other components of bile salt independent bile formation also (e.g., secretion of buffer anions other than HCO$_3^-$) that may be generated.
by intracellular alkalization). The quantitative role of the former component in contributing to bile salt independent canalicular bile formation and the mechanism(s) and significance of the latter remain to be elucidated.

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