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# Isolation of small polarized bile duct units

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**ABSTRACT** **Fragments of small interlobular bile ducts averaging 20 μm in diameter can be isolated from rat liver. These isolated bile duct units form luminal spaces that are impermeant to dextran-40 and expand in size when cultured in 10 μM forskolin for 24–48 hr. Secretion is $Cl^-$ and $HCO_3^-$ dependent and is stimulated by forskolin > dibutyryl cAMP > secretin but not by dideoxyforskolin, as assessed by video imaging techniques. Secretin stimulates $Cl^-/HCO_3^-$ exchange activity, and intraluminal pH increases after forskolin administration. These studies establish that small polarized physiologically intact interlobular bile ducts can be isolated from rat liver. These isolated bile duct units should be useful preparations for assessing the transport properties of small bile duct segments, which are the primary site of injury in cholestatic liver disorders, known as "vanishing bile duct syndromes."**

Bile duct epithelial cells represent only 3–5% of liver cells and are difficult to isolate. Recently, purified populations of isolated bile duct epithelial cells have been obtained from normal (1, 2) and bile duct-obstructed rats (3, 4) and from human liver (5), using techniques such as cell elutriation or immunomagnetic separation (3, 5). Although specific transport systems have been characterized in these dissociated cells, particularly those related to intracellular pH regulation and bicarbonate secretion (2, 4), all of these preparations have lost structural polarity. Furthermore, secretion cannot be assessed because the biliary lumen has been disrupted. An intact polarized bile duct unit has recently been isolated by enzymatic digestion and microdissection (6) following techniques described (7) for isolated pancreatic ducts. However, the mean duct diameters of these preparations average ≈100 μm and range up to 450 μm; these ducts are presumably derived from the larger interlobular and segmental duct segments. Smaller bile duct units (25–75 μm) form most of the interlobular duct system and are the primary sites of injury in disorders resulting in the cholestatic liver diseases known as "vanishing bile duct syndromes" (8). Because of heterogeneity in the biliary epithelium and the need to develop polarized models in smaller bile duct units, we initiated the present studies. This report provides direct evidence that secretin activates a $Cl^-/HCO_3^-$ exchanger in association with a net increase in an alkaline secretion from small interlobular bile ducts of rat liver.

## MATERIALS AND METHODS

**Materials.** Bovine serum albumin (essentially fatty acid free), penicillin/streptomycin, EDTA, heparin, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), D(+)-glucose, insulin, soybean trypsin inhibitor (type I-s), amiloride, dimethyl sulfoxide, hyaluronidase deoxyribonuclease (DN-25), nigericin, 4,4-diisothiocyano-2,2′-disulfonic acid stilbene (DIDS), 3-isobutyl-1-methylxanthine (IBMX), sodium gluconate, potassium gluconate, hemicalcium gluconate, fast blue BB salt, forskolin, dideoxyforskolin, and $N^6$,2′-*O*-dibutyryladenosine 3′:5′-cyclic monophosphate (dibutyryl cAMP; $Bt_2$cAMP) were purchased from Sigma. 2,7-Bis(carboxymethyl)-5-(and-6)-carboxyfluorescein, acetomethyl ester (BCECF-AM), and $H_2$DIDS were obtained from Molecular Probes. Matrigel was from Collaborative Research, collagenase D was from Boehringer Mannheim Biochemicals, and Pronase was from Calbiochem. Liebowitz-15 (L-15), minimum essential medium (MEM), α-MEM, L-glutamine, gentamicin, and fetal calf serum were from GIBCO. *N*-(γ-1-Glutamyl)-4-methoxy-2-naphthylamide was obtained from Polyscience. Monoclonal anti-cytokeratin 7 and 19 antibodies (RPN 1162 and RPN 1165, respectively) were from Amersham. Secretin (3000 units/mg) was from Bachem.

**Isolation of Bile Duct Units.** Male Sprague–Dawley rats (Camm Laboratory Animals, Wayne, NJ) weighing 200–250 g were housed and anesthetized as described (2). The portal vein was cannulated with a 16-gauge cannula, and the liver was perfused *in situ* for 10 min with $Ca^{2+}$, $Mg^{2+}$-free Hanks' buffer/0.019% EDTA and then for 10–15 min with $Ca^{2+}$, $Mg^{2+}$-containing Hanks' buffer/collagenase D (63.8 units/liter). The portal tissue residue was mechanically separated from parenchymal tissue by first shaking in cold L-15 medium and then using pressure from medium forced through a syringe to dissociate the remaining hepatocytes. The tissue was then finely minced with a scissors in solution A, which contained MEM supplemented with 0.066% collagenase, 0.033% Pronase, 0.006% DNase, 3% fetal calf serum, 0.1% bovine serum albumin, and penicillin/streptomycin at 100,000 units–100 mg/liter). The minced tissue was then poured into a culture flask containing solution A and shaken at 37°C for 30 min, minced again, and sequentially filtered through 100- and 30-μm-mesh Nitex Swiss nylon monofilament screens (Tetko, Lancaster, NY). Fragments remaining on the filters were digested for an additional 30 min in solution A, then digested (30 min) in solution B, in which 0.036% hyaluronidase was substituted for Pronase. Fragments were then again filtered through 10-μm and 30-μm mesh, and those remaining on the 30-μm filter were collected in 4–10 ml of L-15 or α-MEM supplemented with 0.1 μM insulin, 3% fetal calf serum, 2 mM L-glutamine, gentamicin (50 μg/ml), and penicillin/streptomycin (100,000 units–100 mg/liter). Fragments were then plated on small coverslips (4 × 2 mm), layered in 12-mm-diameter tissue culture plastic wells (Corning), covered with a thin layer of Matrigel (Collaborative Research), and incubated at 37°C in an air-equilibrated incubator for L-15 or 95% $O_2$/5% $CO_2$ for α-MEM. The medium was changed after 24 hr, and the experiments were done between 20 and 48 hr after plating. Viability (trypan blue exclusion) was evaluated in plated isolated bile duct units (IBDU) at the beginning and end of the functional studies.

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Abbreviations: IBDU, isolated bile duct units; $Bt_2$cAMP, dibutyryl cAMP; KRB, Krebs–Ringer/bicarbonate; $pH_i$, intracellular pH; DIDS, 4,4-diisothiocyano-2,2′-disulfonic acid stilbene; γGT, γ-glutamyl transpeptidase; IBMX, 3-isobutyl-1-methylxanthine; BCECF, 2,7-bis(carboxymethyl)-5-(and-6)-carboxyfluorescein acetomethyl ester.

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**Characterization of IBDU.** γ-Glutamyl transpeptidase (γGT) cytochemistry (9) and cytokeratin immunocytochemistry (cytokeratins 19 and 7) (4) were done in plated fragments 24 and 48 hr after plating. Transmission electron microscopy was done on IBDU preparations after 48 hr (4).

**Intracellular and Luminal pH Determinations.** Intracellular ($pH_i$) and luminal pH of IBDU was measured by using a microfluorometric single-cell method with a SPEX-AR-CM microsystem (Spex Industries, Edison, NJ). IBDU on glass coverslips were loaded with 12 μM BCECF-AM for 40 min, washed for 10 min with BCECF-AM-free medium, and then transferred into a 37°C thermostated perfusion chamber on the stage of an inverted microscope (Zeiss IM 35). $pH_i$ was measured as described (2, 4). Fluorescence intensity was found to exceed background autofluorescence by at least 40-fold. The 490/440 fluorescence intensity ratio data were converted to $pH_i$ values by using the 12 μM nigericin calibration curve technique, as described (10, 11). Experiments were done 18–26 hr after plating. Over $pH_i$ 6.4–7.6, fluorescence ratio varied in a linear fashion with $pH_0$. Intrinsic ($\beta_i$) and total ($\beta_{tot}$) buffering power was measured ($n$ = 9) as described (2, 5, 12–14) and found to be 69 mM per pH unit at pH 6.5, 25 mM per pH unit at pH 7.0, and 9.2 mM per pH unit at pH 7.7, values similar to described buffering capacity in isolated bile duct epithelial cells (2).

For $pH_i$ measurements, IBDU cultured for 48 hr were perfused with Krebs–Ringer barcarbonate (KRB) on the stage of an inverted microscope as described above. Micropipettes containing 0.5–1.0 mM membrane-impermeant pH-sensitive dye, dextran–BCECF (40,000 $M_r$), in 140 mM NaCl/5 mM $Na_2HPO_4$ (pH 7.2) was injected into the lumens of the IBDU. Once pH was measured the perfusate was switched to KRB containing 10 μM forskolin or the 0.01% dimethyl sulfoxide carrier and perfused for another 15 min. The pH after this 15-min stimulation was compared with baseline in both control and forskolin-stimulated IBDU.

**Quantitative Morphology and Secretory Response.** To assess the size of the IBDU as well as their secretory properties, their cross-sectional diameters and the maximum length and width of their luminal spaces were measured by using Nomarski optics and a Zeiss IM 35 inverted microscope.

Measurements were obtained immediately after isolation on fragments fixed and stained for γGT and then in unfixed specimens at 24 and 48 hr, with and without 10 μM forskolin.

Finally, 48-hr IBDU were perfused on a microscope stage equipped with a video camera (Dage–MTI, Michigan City, IN) connected to analysis software. Coverslips were scanned to find an IBDU that was relatively spherical in shape and did not appear to have connections to other surrounding duct units. Video images were obtained every 5 min in the plane in which luminal size was the largest. After a 15-min control period of buffer alone, the ducts were stimulated for 30 min with either 10 μM forskolin or dideoxyforskolin, 100 μM $Bt_2cAMP$/50 μM IBMX, or 200 nM secretin. All changes in luminal area were expressed as a percentage of baseline (time 0). IBDU were either cultured in $HCO_3^-$-containing medium for 48 hr and perfused with KRB during the experiment or cultured for 24 hr in α-MEM and then switched to $HCO_3^-$-free L-15 medium for the remainder of the culture time. During the experimental period the latter IBDU were perfused in Hepes buffer.

Response to forskolin was also assessed in IBDU preincubated and perfused for 30–40 min in $Cl^-$-free KRB (ion substitutions with gluconate).

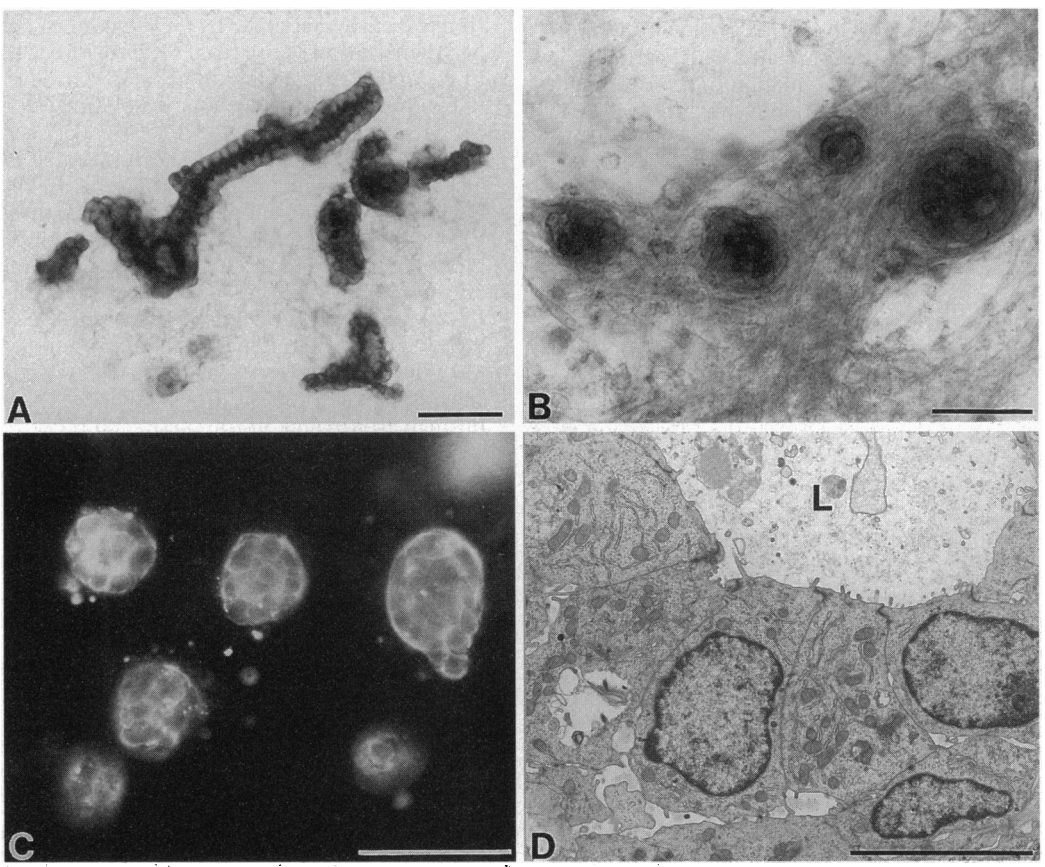


FIG. 1. (*A*) Freshly isolated bile duct fragments collected between 30- and 100-μm filters and stained for γGT. (*B*) Forty-eight-hour-cultured IBDU stained for γGT. (*C*) Forty-eight-hour IBDU stained for α-cytokeratins 19 and 7. (*D*) Electron micrograph of 48-hr-cultured IBDU showing well developed microvilli on the luminal surface and tight junction complexes between single layers of epithelial cells. [Bars = 50 μm (*A–C*) and 5 μm (*D*).]

Table 1. Change in sizes of IBDU with time and forskolin

| | IBDU, μm length × μm width | |
|---|---|---|
| | Duct | Lumen |
| Freshly isolated duct (36) | 21 ± 4* | |
| 24-hr isolated duct (64) | 41 ± 13 × 32 ± 9 | 22 ± 11 × 14 ± 7 |
| + forskolin (92) | 54 ± 22 × 38 ± 12 | 37 ± 21 × 22 ± 12 |
| 48-hr isolated duct (41) | 53 ± 24 × 38 ± 15 | 39 ± 22 × 24 ± 13 |
| + forskolin (54) | 61 ± 23 × 46 ± 15 | 46 ± 22 × 30 ± 14 |

Numbers in parentheses = *n*.
*Width only.

**Solutions.** The composition of solutions has been detailed (2, 4). Secretin was made in perfusion buffer with 1% (wt/vol) bovine serum albumin and infused at a 1:60 (vol/vol) dilution. Forskolin/dimethyl sulfoxide was diluted to 10 μM. Final concentration of dimethyl sulfoxide in the medium was 0.01%. $Bt_2$cAMP/IBMX was diluted to 100 μM/50 μM.

Data are presented as the arithmetic means ± SDs unless otherwise indicated. Statistical analysis used the paired or unpaired Student's *t* test as appropriate or the one-way ANOVA when three groups were compared.

## RESULTS

Immediately after their isolation, duct fragments appeared as tubule-like structures containing segments of intact γGT-positive ducts that averaged 20.8 ± 4.1 μm in width (Fig. 1*A*). Twenty-four to forty-eight hours after the bile duct fragments were cultured, spherical clusters of cells, which contained a defined lumen, could be easily detected using Nomarski optics. These preparations were identified as bile duct epithelial cells after histochemical assays for γGT and immunocytochemical studies stained for cytokeratins 7 and 19 revealed positive staining (Fig. 1 *B* and *C*).

After 24 or 48 hr in culture, 99% of IBDU excluded trypan blue, indicating good viability. At 24 hr, most of their luminal spaces were impermeant to Texas Red–dextran-40 (66 of 75 IBDU from five preparations) and averaged 22.0 × 13.8 μm in maximal diameters by video microscopy ($n = 64$). When incubated in the presence of 10 μM forskolin, which stimulates the catalytic subunit of adenylyl cyclase, the lumens increased in size, averaging 36.9 × 22.2 μm ($n = 92$, $P < 0.003$) (Table 1).

After 24–48 hr the IBDU became progressively leaky, presumably because secretory pressure increased and disrupted the tight junctions between the epithelial cells. At 48 hr, when most of the secretory studies were done, 77% of the IBDU excluded Texas Red–dextran-40 (133 of 173 IBDU from five preparations).

Electron microscopic preparations obtained after 48 hr in culture (Fig. 1*D*) revealed typical ultrastructural characteristics of bile duct epithelial cells, including (*i*) a centrally located multilobulated nucleus, with a small cytoplasmic/nuclear ratio, (*ii*) a paucity of mitochondria compared with hepatic parenchymal cells, and (*iii*) many vesicular structures particularly prominent in the subapical region of the cell. Tight junctions were well developed between cells, and the luminal membrane was amplified by numerous microvilli.

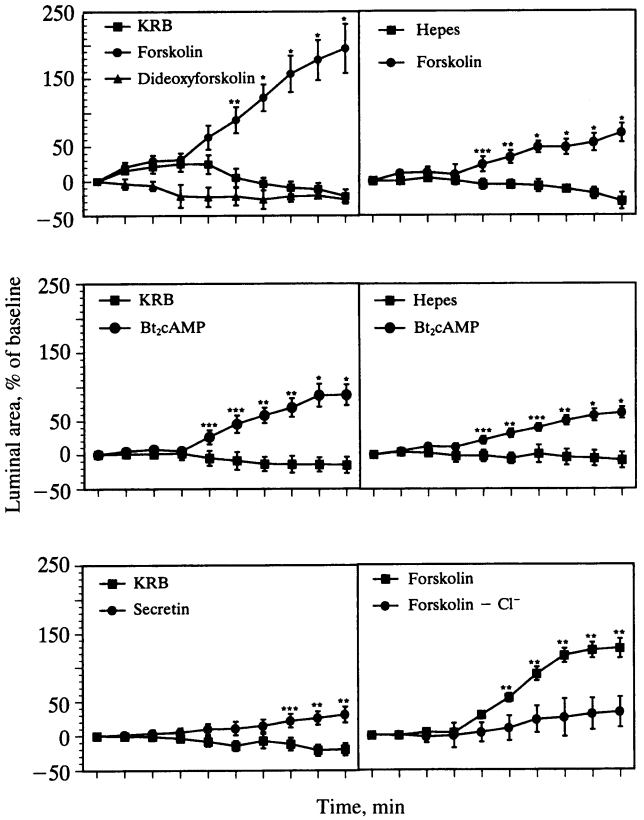


FIG. 2. Effects of 10 μM forskolin or 10 μM dideoxyforskolin, 100 μM $Bt_2$cAMP/50 μM IBMX, or 200 nM secretin on the area of the luminal space measured with video images at 5-min intervals after a 15-min control period in the presence and absence of $HCO_3^-$-containing medium in 48-hr IBDU and after $Cl^-$ removal. Data are expressed as percentages of initial control period ± SEMs. *, $P < 0.001$; **, $P < 0.01$; ***, $P < 0.05$.

The secretory properties of these IBDU preparations were studied further in units cultured for 48 hr. After a 15-min control period, the preparation was perfused with either 10 μM forskolin, 100 μM $Bt_2$cAMP/50 μM IBMX, or 200 nM secretin for an additional 30 min (Fig. 2). During the 15-min control period, luminal diameter did not increase significantly. In contrast, in all preparations, IBDU responded to the secretogues with an increase in luminal area during the 30-min perfusion (Fig. 3), which was greater when the IBDU were perfused with $HCO_3^-$-containing medium compared with Hepes ($P < 0.05$). Larger choleretic responses were observed with forskolin compared with

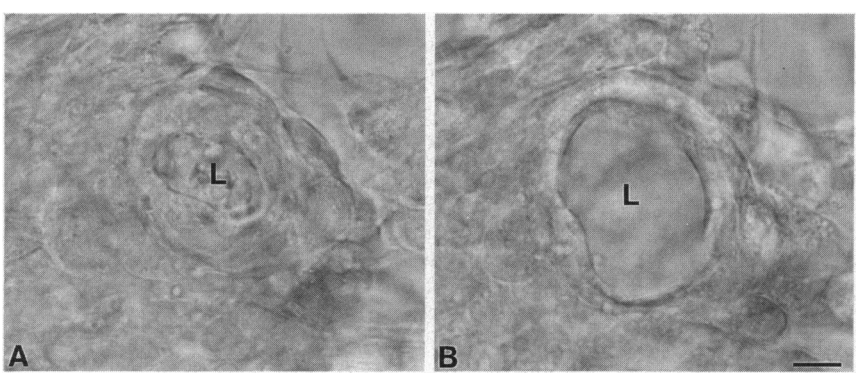


FIG. 3. (*A*) Nomarski image of 48-hr IBDU perfused in KRB and used for quantitative measurements of secretion. (*B*) The same IBDU as in *A*, after 30-min exposure to KRB/10 μM forskolin. Maximal luminal area increased from 287 to 916 $\mu m^2$. L, lumen. (Bar = 10 μm.)

Table 2. Recovery from a 4- to 6-min $NH_4Cl$ acid pulse in Hepes or KRB

| Condition | Basal $pH_i$ | Lowest $pH_i$ | pH recovery rate, units/min | $H^+$ flux, mM/min |
|---|---|---|---|---|
| Hepes (13) | 6.99 ± 0.05 | 6.59 ± 0.08 | 0.20 ± 0.06 | 12.83 ± 3.98 |
| Hepes/amiloride (6) | 7.01 ± 0.04 | 6.46 ± 0.04 | 0.023 ± 0.004 | 1.56 ± 0.36 |
| KRB (8) | 7.21 ± 0.08 | 6.75 ± 0.04 | 0.22 ± 0.045 | 12.24 ± 2.47 |
| KRB/amiloride (8) | 7.22 ± 0.07 | 6.66 ± 0.05 | 0.174* ± 0.045 | 11.28 ± 3.41 |
| KRB/amiloride/DIDS (4) | 7.04 ± 0.04 | 6.65 ± 0.05 | 0.03 ± 0.01 | 1.72 ± 0.34 |
| KRB $Cl^-$ depleted/amiloride (5) | 7.37 ± 0.08 | 6.92 ± 0.08 | 0.226 ± 0.026 | 11.32 ± 1.25 |

Data are means ± SDs.
*$P < 0.05$ vs. paired controls where amiloride was omitted.

$Bt_2cAMP$ ($P < 0.05$). The response to forskolin was diminished when $Cl^-$ was omitted ($P < 0.005$); dideoxyforskolin produced no effect. Secretin (200 nM) also stimulated secretion, but the choleretic responses were smaller than with either forskolin ($P < 0.0001$) or $Bt_2cAMP$ ($P < 0.01$). All of these responses were consistent with a cAMP-stimulated $Cl^-/HCO_3^-$ exchange-mediated secretion.

Basal $pH_i$ of IBDUs measured 7.0 ± 0.06 ($n = 23$) in $HCO_3^-$-free medium and was significantly lower ($P < 0.01$) than in the presence of $HCO_3^-$ (7.20 ± 0.09; $n = 24$). $Na^+/H^+$ exchange was the major acid-extruding system in Hepes, as demonstrated because 1 μM amiloride decreased basal $pH_i$ by 0.15 ± 0.04 pH unit ($n = 4$) and inhibited $pH_i$ recovery from a 20 mM $NH_4Cl$ acute acid load by ≈90% (Table 2). In the presence of bicarbonate (KRB), amiloride ($n = 5$) had no effect on basal $pH_i$, indicating that this exchanger is not involved in $pH_i$ maintenance but is activated only after an acute acid load (Table 2, see KRB/amiloride vs. KRB alone). A $Na^+$, $HCO_3^-$ symport was demonstrated by the $Na^+$-dependent, $Cl^-$-independent, DIDS-inhibitable recovery from a 20 mM $NH_4Cl$ acute acid load. Indeed, the amiloride-insensitive component of pH recovery was abolished by $Na^+$ omission, was not inhibited by $Cl^-$ depletion (Table 2, $Cl^-$ depleted/amiloride vs. KRB/amiloride), and was almost completely inhibited by DIDS pretreatment (Table 2; see KRB/amiloride/DIDS vs. KRB/amiloride). A $Cl^-/HCO_3^-$ exchanger was demonstrated in these IBDUs by the effects of $Cl^-$ removal and readmission, which increased and decreased $pH_i$, respectively (Table 3). These effects were abolished by pretreatment for 40–60 min with 1 mM DIDS ($n = 5$) or by acute exposure to 0.5 mM $H_2DIDS$ ($n = 4$; Fig. 4), confirming the presence of this exchanger.

Together these studies establish the presence of $Na^+/H^+$ exchange, $Na^+$, $HCO_3^-$ symport, and $Cl^-/HCO_3^-$ exchangers in IBDUs, as reported in isolated bile duct epithelial cells (2, 4). These findings also indicate that there is no statistical difference in the activities of these transporters in bile duct cells from these two different preparations, although the activity of the $Na^+/H^+$ exchanger in the IBDU was 25% less than in bile duct cells isolated from normal liver, whereas the $Na^+$, $HCO_3^-$ symport was 44% greater (2). Consistent with these findings, basal $pH_i$ in Hepes was lower in IBDU than in isolated cells, whereas basal $pH_i$ in KRB was higher.

Table 3. Effect of secretin on activity of the $Cl^-/HCO_3^-$ exchanger

| Condition | $pH_i$, mean ± SD | |
|---|---|---|
| | Control | Secretin |
| Basal $pH_i$ | 7.17 ± 0.06 | 7.16 ± 0.06 |
| $Cl^-$ removal | | |
| $pH_i$, Δ | 0.24 ± 0.06 | 0.28 ± 0.07 |
| pH, max units/min | 0.13 ± 0.05 | 0.26 ± 0.14* |
| $H^+$ flux, mM/min | 6.43 ± 2.57 | 13.17 ± 7.93* |
| $Cl^-$ readmission | | |
| pH, max units/min | 0.19 ± 0.06 | 0.31 ± 0.15* |
| $H^+$ flux, mM/min | 12.58 ± 5.38 | 25.13 ± 18.61** |

Two sequential $Cl^-$ removal/readmission maneuvers were done ($n = 10$), the second during perfusion with 200 nM secretin. Data are expressed as means ± SDs and compared by using the paired Student's $t$ test. max, Maximum. *, $P < 0.03$; **, $P < 0.05$.

Studies of luminal pH revealed a baseline pH of 7.63 ± 0.33 in $HCO_3^-$-containing medium, which increased significantly after forskolin by 0.21 ± 0.12 (SD) pH units ($n = 8$; $P < 0.05$) compared with a pH decrease of 0.04 ± 0.22 pH unit ($n = 8$) in controls perfused with the dimethyl sulfoxide carrier.

Secretin had no effect on basal $pH_i$ but stimulated both the maximal rate of alkalinization after $Cl^-$ removal (0.26 ± 0.14 pH unit/min) and of $pH_i$ recovery after $Cl^-$ readmission (0.31 ± 0.15 pH unit/min) with respect to control values ($Cl^-$ removal = 0.13 ± 0.05 pH unit/min, $P < 0.03$; $Cl^-$ readmission, 0.19 ± 0.06 pH unit/min, $P < 0.05$), indicating significant stimulation of $Cl^-/HCO_3^-$ exchanger activity (Table 3) comparable with values previously demonstrated in preparations of isolated bile duct epithelial cells (2).

## DISCUSSION

We report the successful isolation of intact small polarized bile duct units that can be stimulated to secrete in response to physiologic stimuli. The isolation procedure is done relatively quickly in 2–3 hr and does not depend on fastidious microdissection techniques, as required for the isolation of larger bile duct segments (6). Immediately after isolation, the IBDU appear as elongated tubules with diameters of ≈15–25 μm. Subsequently they round up in culture, forming aggregates of cells with easily recognized luminal spaces when viewed with Nomarski optics. Most of these aggregates are sealed from the external medium, as shown by exclusion of dextran-40, and accumulate fluid within this enclosed space. Generally a single layer of epithelial cells line the lumen, as demonstrated by electron micrographs that also reveal the characteristic appearance of bile duct epithelial cells with large lobulated nuclei and sparse mitochondria. These findings resemble previous morphological descriptions of isolated bile ducts in cell culture from both normal (15) and bile duct-obstructed livers (16). The identity of bile duct epithelial cells is confirmed by positive histochemical staining for γGT and immunocytochemistry for cytokeratins 7 and 19, as shown in isolated bile duct epithelial cell preparations (2, 4).

The most important feature of these IBDUs is the ability to directly assess the secretory properties of this portion of the biliary epithelium by recording cross-sectional images of the bile duct lumen. With time in culture or after a secretory stimulus, changes in luminal volume can be measured. Using this approach, we have demonstrated that activation of adenylyl cyclase by forskolin or the addition of a cell-permeant form of cAMP to the medium ($Bt_2cAMP$), as well as addition of the hormone secretin, all independently stimulate fluid secretion from this portion of the biliary epithelium. These small bile duct units have the largest surface area within the biliary epithelium and are therefore probably of primary importance in the modification of hepatic bile as it passes along the bile duct epithelium in the biliary tree. These secretory responses are in keeping with previous observations which indicate that

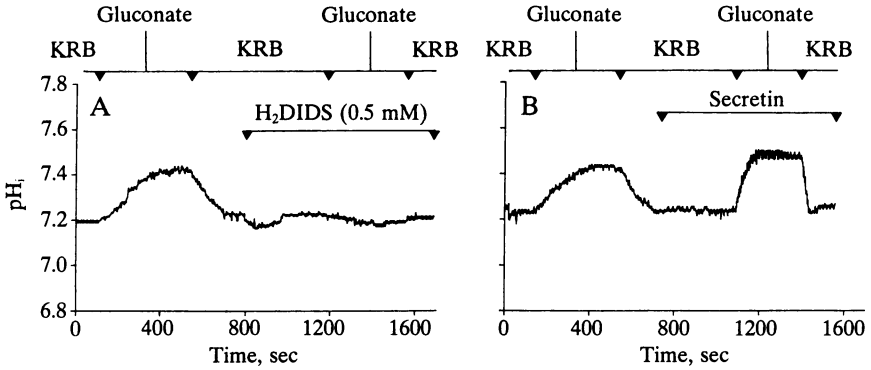


FIG. 4. (*A*) Effects of $Cl^-$ removal/readmission maneuvers on $pH_i$ measured with BCECF before and after exposure to 0.5 mM $H_2DIDS$ ($n$ = 4). $H_2DIDS$ completely abolished the alkalinization promoted by the acute $Cl^-$ removal produced by equimolar substitutions with gluconate, indicating that the $pH_i$ increase induced by $Cl^-$ removal depends on $HCO_3^-$ transport across the cell membrane. Preincubation with 1 mM DIDS for 40–60 min also abolished the effect of $Cl^-$ removal ($n$ = 5, data not shown). (*B*) Secretin (200 nM, $n$ = 10) significantly increased both maximal rate of alkalinization after $Cl^-$ removal and $pH_i$ recovery after $Cl^-$ readmission with respect to control values (see Table 3), indicating stimulation of $Cl^-/HCO_3^-$ exchange activity.

secretin increases cAMP levels in isolated bile duct epithelial cells (17).

Measurements of luminal pH using microinjection of BCECF–dextran confirm that the duct secretion is alkaline and that stimulation of secretion is associated with a further increase in luminal pH. Together the parallel findings of increased fluid secretion, increased $Cl^-/HCO_3^-$ activity, and increased luminal pH provide direct evidence that these small bile duct epithelial cells are a primary source of $HCO_3^-$-enriched secretion, as proposed from classical studies in intact animals (18). Our findings are also consistent with an apical location for the $Cl^-/HCO_3^-$ exchanger, an observation confirmed in human liver by recent immunocytochemical studies with antibodies to the anion-exchange 2 transporter (19).

As reported by Roberts *et al.* (6), forskolin significantly increases the luminal pH consistent with activation of a $Cl^-/HCO_3^-$ exchanger on the luminal membrane, as demonstrated (2) for secretin in isolated nonpolarized bile duct epithelial cells. The present study not only confirms this finding by showing that forskolin increases biliary luminal pH after microinjection of BCECF–dextran but also shows that secretin directly activates $Cl^-/HCO_3^-$ exchange in polarized IBDUs and that $Cl^-$ removal blocks the stimulatory response from forskolin.

Determination of the origin of these bile duct segments is based on size fractionation by filtering fragments through a 100-$\mu$m nylon mesh and then collecting the filtrate on a 30-$\mu$m nylon mesh. Although portions of larger bile ducts could have been fragmented during enzymatic digestion and also passed through the 100-$\mu$m mesh screen, the smaller bile ducts comprise most of the epithelial cells in the biliary tree and, thus, should represent the largest proportion of the collected isolated bile duct tubules. Furthermore, the width of $\gamma$GT-positive ducts measured in fixed specimens immediately after their isolation averaged only 20.8 $\mu$m, consistent with the known size of small interlobular ducts and ductules. In addition, the nuclei are centrally located in these IBDU, rather than basally located, as observed in larger bile ducts. Nevertheless, until markers are available that allow a more precise determination of the site of origin within the biliary tree, we cannot exclude the possibility that the preparation described here is heterogeneous in origin. Similarly, perhaps regions exist within the smaller bile duct segments that are more or less responsive to the stimulatory effects of secretin, and the ability to form aggregates with expanding luminal spaces is a function of only a portion of the small bile duct segments. Until these issues are resolved, it remains possible that these preparations are not necessarily representative of all small-sized interlobular bile ducts.

In addition, the ability to micropuncture the lumens of these IBDU should enable studies to be done that directly assess the characteristics of bile duct secretions. This advantage should also facilitate the application of electrophysiologic studies analogous to those applied previously to the isolated hepatocyte couplet (20).

Finally, the ability to isolate physiologically intact polarized bile duct units from small interlobular bile ducts of a size comparable to the site of injury of many "vanishing bile duct" disorders in man, suggests that these techniques could be adapted to study the pathophysiology of these cholestatic liver disorders (8).

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