Organic anion transporting polypeptide (oatp) is an integral membrane protein cloned from rat liver that mediates Na\(^+\)-independent transport of organic anions such as sulfobromophthalein and taurocholic acid. Previous studies in rat hepatocytes suggested that organic anion uptake is associated with base exchange. To better characterize the mechanism of oatp-mediated organic anion uptake, we examined transport of taurocholate in a HeLa cell line stably transfected with oatp under the regulation of a zinc-inducible promoter (Shi, X., Bai, S., Ford, A. C., Burk, R. D., Jacquemin, E., Hagenbuch, B., Meier, P. J., and Wolkoff, A. W. (1995) J. Biol. Chem. 270, 25591–25595). Whereas noninduced transfected cells showed virtually no uptake of \(^{3}H\)taurocholate, taurocholate uptake by induced cells was Na\(^+\)-independent and saturable \((K_m = 18.4 \pm 3.5 \mu M; V_{\text{max}} = 62.2 \pm 1.4 \text{ pmol/min/mg protein}; n = 3)\). To test whether organic anion transport is coupled to HCO\(_3\)\(^-\) extrusion, we compared the rates of taurocholate-dependent HCO\(_3\)\(^-\) efflux from alkali-loaded noninduced and induced cells. Monolayers grown on glass coverslips were loaded with the pH-sensitive dye 2,7'-bis(carboxyethyl)-5(6)-carboxyfluorescein; intracellular pH \((pH_i)\) was measured by excitation ratio fluorometry.

In the absence of extracellular Cl\(^-\) and taurocholate, isohydric reduction of superfusate HCO\(_3\)\(^-\) concentration from 50 to 25 \(\mu M\) resulted in an insignificant change in \(pH_i\) over time \((dpH_i/dt)\) in both groups. Addition of 25 \(\mu M\) taurocholate to the superfusate led to a rapid fall in \(pH_i\) in induced \((-0.037 \pm 0.011\text{ pH units/min to } pHi, 7.41 \pm 0.14)\) but not in noninduced \((0.003 \pm 0.006 \text{ pH units/min to } pHi, 7.61 \pm 0.08)\) cells \((p < 0.03)\). These data indicate that oatp-mediated taurocholate transport is \(Na^+\)-independent, saturable, and accompanied by HCO\(_3\)\(^-\) exchange. We conclude that organic anion/base exchange is an important, potentially regulatable component of oatp function.

The liver is the primary organ responsible for removal of organic anions such as bilirubin and sulfobromophthalein (BSP)\(^1\).

\(^*\) This work was supported by a Grant-in-Aid from the American Heart Association (to L. M. S.) and National Institutes of Health Grants DK29296 and DK41296 (to A. W. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

\(^\dagger\) To whom correspondence should be addressed: Rm. 721 RFK, Albert Einstein College of Medicine, 1410 Pelham Pkwy. South, Bronx, NY 10461. Tel.: 718-430-2503; Fax: 718-824-2392; E-mail: satlin@acem.yu.edu.

\(^\ddagger\) The abbreviations used are: BSP, sulfobromophthalein; oatp, organic anion transporting polypeptide.

Organic anion transporting polypeptide (oatp) is an integral membrane protein cloned from rat liver that mediates Na\(^+\)-independent transport of organic anions such as sulfobromophthalein and taurocholic acid. Previous studies in rat hepatocytes suggested that organic anion uptake is associated with base exchange. To better characterize the mechanism of oatp-mediated organic anion uptake, we examined transport of taurocholate in a HeLa cell line stably transfected with oatp under the regulation of a zinc-inducible promoter (Shi, X., Bai, S., Ford, A. C., Burk, R. D., Jacquemin, E., Hagenbuch, B., Meier, P. J., and Wolkoff, A. W. (1995) J. Biol. Chem. 270, 25591–25595). Whereas noninduced transfected cells showed virtually no uptake of \(^{3}H\)taurocholate, taurocholate uptake by induced cells was Na\(^+\)-independent and saturable \((K_m = 18.4 \pm 3.5 \mu M; V_{\text{max}} = 62.2 \pm 1.4 \text{ pmol/min/mg protein}; n = 3)\). To test whether organic anion transport is coupled to HCO\(_3\)\(^-\) extrusion, we compared the rates of taurocholate-dependent HCO\(_3\)\(^-\) efflux from alkali-loaded noninduced and induced cells. Monolayers grown on glass coverslips were loaded with the pH-sensitive dye 2,7'-bis(carboxyethyl)-5(6)-carboxyfluorescein; intracellular pH \((pH_i)\) was measured by excitation ratio fluorometry.

In the absence of extracellular Cl\(^-\) and taurocholate, isohydric reduction of superfusate HCO\(_3\)\(^-\) concentration from 50 to 25 \(\mu M\) resulted in an insignificant change in \(pH_i\) over time \((dpH_i/dt)\) in both groups. Addition of 25 \(\mu M\) taurocholate to the superfusate led to a rapid fall in \(pH_i\) in induced \((-0.037 \pm 0.011\text{ pH units/min to } pHi, 7.41 \pm 0.14)\) but not in noninduced \((0.003 \pm 0.006 \text{ pH units/min to } pHi, 7.61 \pm 0.08)\) cells \((p < 0.03)\). These data indicate that oatp-mediated taurocholate transport is \(Na^+\)-independent, saturable, and accompanied by HCO\(_3\)\(^-\) exchange. We conclude that organic anion/base exchange is an important, potentially regulatable component of oatp function.

The liver is the primary organ responsible for removal of organic anions such as bilirubin and sulfobromophthalein (BSP). From the circulation. These compounds are bound tightly to albumin, from which they are rapidly extracted by hepatocytes (1, 2). Functional studies in short term cultured rat hepatocytes (3, 4) and isolated perfused rat liver (1, 2) revealed carrier-mediated kinetics for this uptake process. Recently, using a functional expression cloning strategy, a rat liver cDNA encoding a Na\(^+\)-independent organic anion transport protein, oatp, was isolated (5, 6). Stable transfection of this cDNA into HeLa cells using a vector containing a zinc-inducible promoter has allowed for characterization of transporter activity in the absence of other hepatocyte cell membrane transporters that may mediate and/or modify organic anion uptake (7). Whereas parent and noninduced transfected HeLa cells show no significant base line BSP transport, zinc-induced transfected cells avidly take up the organic anion in a manner identical to that previously described in cultured rat hepatocytes (7).

The mechanism by which oatp mediates organic anion transport has not been established. Studies in short term cultured rat hepatocytes showed that BSP uptake is stimulated by an outwardly directed pH gradient \((pH_i > pHi)\) (3), suggesting that a component of BSP uptake is associated with H\(^+\) cotransport or OH\(^-\) (or HCO\(_3\)\(^-\)) exchange. The purpose of the present study was to determine whether oatp-mediated organic anion transport is coupled to base exchange. To this end, we used a fluorescence assay to examine the capacity of organic anions to stimulate HCO\(_3\)\(^-\) extrusion in alkali-loaded HeLa cells transfected with oatp cDNA under regulation of a zinc-inducible promoter.

Whereas previous analyses of oatp-mediated organic anion uptake were performed with \(^{35}S\)BSP (7), we found that the intense purple color of this compound in alkaline solutions limited visibility in the fluorescence functional assays described below. We thus chose to use the colorless organic anion taurocholate, also a substrate for oatp (8), throughout this investigation. Initial studies were performed to validate that the characteristics of taurocholate transport mediated by oatp in these cells were similar to those previously described for BSP (7).

**EXPERIMENTAL PROCEDURES**

**HeLa Cell Culture and Transfection**—HeLa cells (ATCC) that had been stably transfected with a plasmid in which oatp expression was regulated by a zinc-inducible promoter were maintained as described previously (7). For induction of expression, 100 \(\mu M\) ZnSO\(_4\) was added to the culture medium for 24 h, and an additional 50 \(\mu M\) ZnSO\(_4\) was added for the final 18–20 h before use (7). For fluorescence assays, cells were plated on 2 \(\times\) 5 mm pieces of No. 1 glass coverslips (Corning Glass, Corning, NY) and grown to confluence.

\(^{3}H\)Taurocholate Uptake by HeLa Cells—Uptake of 22,34-\(^{3}H\)-sodium taurocholate (specific activity 58 Ci/mmol; gift from Dr. Alan Hofmann) by HeLa cells was determined in the absence of bovine serum albumin as described previously for studies of BSP transport (7) utilizing modified serum-free medium containing (in mM): 135 NaCl, 1.2 MgCl\(_2\), 0.81 MgSO\(_4\), 27.8 glucose, 2.5 CaCl\(_2\), and 25 HEPES adjusted to pH 7.2 with NaOH. Cell protein was determined in replicate plates by

---

*This paper is available online at [http://www.jbc.org](http://www.jbc.org)*
the method of Lowry et al. (9) using bovine serum albumin as standard.

Saturation of [3H]Taurocholate Uptake—These studies were performed at taurocholate concentrations of 1–200 μM. Preliminary studies showed that uptake of [3H]taurocholate by zinc-induced cells was linear for at least 5 min. Uptake of [3H]taurocholate was determined at 4 and 37 °C over the initial 5 min of linear uptake. Data were analyzed by nonlinear least squares regression (SigmaPlot version 5.0, Jandel Scientific, San Rafael, CA), and $K_m$ and $V_{max}$ were calculated.

$Na^+$-Independent Initial Uptake of [3H]Taurocholate—Medium was prepared in which NaCl was substituted isomotically by KCl. Initial uptake of [3H]taurocholate was determined over 5 min in KCl-substituted medium.

Effect of pH Gradients on Initial Uptake of [3H]Taurocholate—Modified serum-free medium was prepared as above except that the pH was adjusted to 6.0 or 8.0 with 1M NaOH. Cells were washed twice with 1.5 ml of either pH 6.0 or 8.0 medium in which they were then incubated for 30 min at 37°C. Unidirectional pH gradients were generated by rapidly incubating the cells in medium of the opposite pH, as described previously (3). Initial uptake of [3H]taurocholate was determined over 5 min after addition of the organic anion to the opposite medium.

Measurement of Intracellular pH ($pHi$)—$pHi$ was estimated by excitation ratio fluorometry using a silicon-intensified target video camera (Leitz, Wetzler, Germany) attached to the cine port of a Nikon Diaphot inverted microscope, as described previously (10, 11). Cells were identified using a 50× Leitz water objective. The green fluorescence of 2',7'-bis(carboxyethyl)-5(6)-carboxyfluorescein (BCECF; Molecular Probes) was visualized with a Nikon B filter cassette. There was no detectable autofluorescence of the cells or any of the solutions used.

Sequential fluorescence images of BCECF-loaded cells were obtained at 10-s intervals at excitation wavelengths of 490 and 440 nm (emission at 530 nm) and stored on a Digital Instruments computer. Data were analyzed with a commercially available digital video image analysis system (MetaFluor; Universal Imaging, West Chester, PA).

An intracellular calibration was performed at the conclusion of each experiment using the nigericin technique, as described previously (10, 11). High-$K^+$ intracellular calibration buffers (Table I, solution 4) containing 10 μM nigericin (Molecular Probes) were adjusted to $pH$ values of 6.8, 7.3, and 7.8.

Kinetic Analysis of Taurocholate/HCO$_3^-$ Exchange—Coverslips to which cell monolayers were attached were placed on the floor of a temperature-controlled specimen chamber, bathed with standard perfusate (Table I, solution 1) at 37 °C, and gassed with 95% O$_2$, 5% CO$_2$. Basal exchanges were performed manually by rapidly replacing the 1.5 ml volume of bathing solution three times. After equilibration, cell monolayers were exposed to 20 μM BCECF acetoxyethyl ester for 10–15 min. After rinsing and obtaining base line $pHi$ measurements, cells were alkali-loaded by isohydric application of a 50 mM HCO$_3^-$, CI$^-$-free solution (Table I, solution 3). This solution was prepared by replacing 25 mM sodium gluconate in the standard CI$^-$-free solution (Table I, solution 2) with an additional 25 mM NaHCO$_3$ and gassing with 90% O$_2$, 10% CO$_2$. After obtaining a stable 490 nm/450 nm fluorescence signal over a given cell, cells were alkali-loaded (10, 12) by abruptly reducing the HCO$_3^-$ concentration in the superfusate to 25 mM and CO$_2$ to 5% at a constant $pH$ of 7.4 (Table I, solution 2). In the continued absence of Cl$^-$, the 490 nm/450 nm fluorescence ratios were monitored. Thereafter, taurocholate (2.5 or 25 μM) and finally Cl$^-$ (Table I, solution 1) were added to the superfuse and 490 nm/450 nm emissions monitored.

For each cell studied, the initial rate of change in $pHi$ ($d$pHi$/dt$) observed in response to a change in superfuse was calculated by linear regression analysis, as described previously (10, 11). At least 5 data points were used for each slope determination. As buffer capacity measurements of HeLa cells were not performed, cellular OH$^-$ (HCO$_3^-$) fluxes were not determined.

Statistical Analysis—Results are expressed as mean ± S.E.; n represents the number of monolayers. In fluorescence studies, data from multiple (2–5) cells per plate were averaged to provide a mean value. Significant differences between paired data were determined by paired t test. Comparisons of unpaired data were performed by t test or analysis of variance and multiple range test, as appropriate. The software programs SigmaPlot (version 5.0) and SigmaStat (Jandel Scientific) were used for all statistical analyses. Significance was asserted if $p < 0.05$.

RESULTS

Characteristics of [3H]Taurocholate Uptake by HeLa Cells

Taurocholate uptake by induced oatp-transfected HeLa cells was rapid and linear over 5 min at 37 °C (Fig. 1). In contrast, little ligand associated with induced cells at 4 °C (Figs. 1 and 2). The initial rate of taurocholate uptake in induced cells exceeded the rates of nonspecific association of the ligand with noninduced cells measured at 37 °C and both induced and noninduced cells studied at 4 °C (Fig. 2).

Initial uptake of taurocholate was saturable (Fig. 3). The mean $K_m$ was 19.4 ± 3.3 μM and $V_{max}$ was 62.2 ± 1.4 pmol/min/mg protein ($n = 3$ experiments).

As previously reported for BSP uptake in rat hepatocytes (3), substitution of extracellular Na$^+$ by K$^+$ had no effect on the initial rate of [3H]taurocholate uptake (102% of control; $n = 2$), confirming that oatp-mediated taurocholate uptake is Na$^+$-independent. These studies also suggest that this process is not electrogenic, as exposure to a high K$^+$ bathing solution that should result in cell depolarization did not alter the rate of taurocholate uptake.

### TABLE I Composition of solutions

| Solution | 1 | 2 | 3 | 4 |
|----------|---|---|---|---|
| NaCl     | 115 | 115 | 90 | 45.0 |
| Na gluconate | 25 | 25 | 50 | 99.5 |
| NaHCO$_3$ | 2.5 | 2.5 | 2.5 | 0.5 |
| KCl      | 20 | 2.5 | 2.5 | 0.2 |
| K$_2$HPO$_4$ | 1.2 | 1.2 | 1.2 | 1.6 |
| MgCl$_2$ | 0.3 | 0.3 | 0.3 | 13.3 |
| Na$_2$PO$_4$ | 2.5 | 2.5 | 2.5 | 13.3 |
| Na lactate | 4.0 | 4.0 | 4.0 | 2.5 |
| Na$_2$ citrate | 1.0 | 1.0 | 1.0 | 1.0 |
| L-alanine | 6.0 | 6.0 | 6.0 | 6.0 |
| Glucose  | 5.5 | 5.5 | 5.5 | 5.5 |
| O$_2$ (%) | 95 | 95 | 95 | 95 |
| CO$_2$ (%) | 5  | 5  | 5  | 5  |
| pH       | 7.4 | 7.4 | 7.4 | Variable |
The experimental data (means of duplicate determinations) and the nonlinear least squares linear regression method. The results were computer fit to a single class of binding sites by a kinetic model. The initial rate of taurocholate uptake determined. With an outwardly directed OH\(^-\) gradient (pHi/pHo = 6/8), initial uptake of taurocholate over 5 min was >2-fold higher than uptake observed in the presence of an inwardly directed pH gradient (pHi/pH\(_o\) = 6/8). Results are expressed as means ± S.E. of three studies.

We exploited the activity and reversibility of this Cl\(^-\)/HCO\(_3\) exchange to load HeLa cells with HCO\(_3\). Exposure to a 50 mM HCO\(_3\), Cl\(^-\)-free solution led to a significant increase in pH\(_i\) in noninduced cells (7.42 ± 0.07 to 7.64 ± 0.07; n = 8; p < 0.05) over ~10 min. Induced cells, characterized by a resting pH\(_i\) of 7.59 ± 0.14, showed insignificant additional alkalinization (to pH\(_i\) 7.76 ± 0.10; n = 7; p = 0.3) in response to this maneuver. Reduction of the extracellular HCO\(_3\) concentration from 50 to 25 mM in the continued absence of Cl\(^-\) led to no significant change in pH\(_i\) in either noninduced or induced cells (Figs. 5 and 7).

Effect of Taurocholate on pH\(_i\) Recovery in Noninduced HeLa Cells—Fig. 5A depicts a representative tracing of taurocholate-dependent pH\(_i\) recovery in a single alkali-loaded noninduced HeLa cell. In this experiment, the cell was alkali-loaded from a resting pH\(_i\) of 7.4 to a maximum pH\(_i\) of 7.6. pH\(_i\) recovery (i.e. extrusion of the HCO\(_3\)\(^-\) load) was followed initially in the absence of Cl\(^-\) and taurocholate and then following the sequential addition of 2.5 and 25 µM taurocholate to the bathing medium. Note that pH\(_i\) did not return toward baseline until Cl\(^-\) was restored to the superfusate.

We found that the rate of Cl\(^-\)-independent pH\(_i\) recovery in noninduced cells did not differ from zero on addition of 2.5 or 25 µM taurocholate to the bathing medium (Fig. 6). The absence of significant taurocholate-dependent pH\(_i\) recovery in noninduced cells was reflected in the absence of change in pH\(_i\) observed in response to exposure to either 2.5 µM (7.56 ± 0.06 to 7.59 ± 0.06) or 25 µM (Fig. 7A) taurocholate. Restoration of extracellular Cl\(^-\) to the bathing medium led to significant (p < 0.05) pH\(_i\) recovery in cells exposed to 2.5 µM (~0.041 ± 0.009 pH units/min to a final pH\(_i\) of 7.35 ± 0.08) and 25 µM (~0.43 ± 0.011 pH units/min to a final pH\(_i\) of 7.31 ± 0.09) taurocholate.

Effect of Taurocholate on pH\(_i\) Recovery in Induced HeLa Cells—A representative tracing of taurocholate-dependent pH\(_i\) recovery of a single alkali-loaded zinc-induced HeLa cell is shown in Fig. 5B. In this experiment, the cell was alkali-loaded from a resting pH\(_i\) of 7.5 to a maximum pH\(_i\) of 7.8. In the absence of Cl\(^-\) and taurocholate, pH\(_i\) remained high on reduc-
tion of the extracellular HCO₃⁻ concentration from 50 to 25 mM. Addition of 2.5 μM taurocholate, however, led to a prompt, albeit partial, reduction in pHᵢ. On exposure to 25 μM taurocholate, pHᵢ rapidly fell to baseline. Readoption of Cl⁻ to the extracellular medium had no further effect on pHᵢ.

The rate of Cl⁻ independent pHᵢ recovery in induced cells was \(-0.024 \pm 0.010\) pH units/min in the presence of 2.5 μM taurocholate (Fig. 6); pHᵢ fell from its maximum of \(7.81 \pm 0.08\) to \(7.69 \pm 0.07 \) (p = 0.005) in this group of cells. Restoration of Cl⁻ to the medium bathing these cells led to a further reduction in pHᵢ, at a rate of \(-0.037 \pm 0.007\) pH units/min to stabilize at pHᵢ of 7.40 ± 0.04 (p < 0.05 compared with pHᵢ in absence of Cl⁻).

In a separate group of induced cells, addition of 25 μM taurocholate caused cell pHᵢ to fall at a rate of \(-0.037 \pm 0.011\) pH units/min (Fig. 6) from a maximal pHᵢ of 7.69 ± 0.11 to 7.41 ± 0.14 (Fig. 7B). In this group of cells, restoration of cell Cl⁻ led to no further decrease in pHᵢ, which had already reached baseline (d(pHᵢ)/dt = \(-0.014 \pm 0.015\) pH unit/min to 7.37 ± 0.16; p = NS compared with pHᵢ in 25 μM taurocholate, Cl⁻-free medium and initial resting pHᵢ).

Effect of ZnSO₄ on Resting pHᵢ and Cl⁻/HCO₃⁻ Exchange in Parent HeLa Cells—Cumulative evidence suggests that a variety of cells possess a zinc-sensitive H⁺ conductance (13). To determine whether exposure to ZnSO₄ altered H⁺/HCO₃⁻ transport in HeLa cells, we compared resting pHᵢ and Cl⁻/HCO₃⁻ exchange activity in parent HeLa cells grown in the absence or presence of 100 μM ZnSO₄ for 48 h. Our observation of statistically similar resting pHᵢ values (7.16 ± 0.08 versus 7.27 ± 0.07; p = 0.36) and increases in pHᵢ (0.21 ± 0.08 versus 0.26 ± 0.10; p = 0.74) in response to extracellular Cl⁻ replacement with gluconate in cells grown in the presence (n = 5) and absence (n = 5), respectively, of ZnSO₄ suggested it unlikely that this compound systematically altered pHᵢ regulatory pathways or capacity for Cl⁻/HCO₃⁻ exchange.

**DISCUSSION**

Functional characterization of individual transport pathways mediating uptake of organic anions in the rat liver has been complicated by the presence in hepatocytes of multiple endogenous organic anion transport proteins (3, 15). To selectively characterize the mechanism of oatp-mediated taurocholate transport, we used HeLa cells permanently transfected with a eukaryotic expression vector containing an inducible oatp cDNA (7).

The results of the present study indicate that oatp-mediated transport of taurocholate is Na⁺-independent, saturable, and temperature-dependent. These transport characteristics are similar to those previously described for oatp-mediated BSP transport (7). Similarly, we observed that taurocholate uptake...
is stimulated by imposition of an outwardly directed pH gradient, as previously reported for BSP transport in cultured hepatocytes (3).

Coupling of organic anion transport to pH gradients could arise from anion/OH\(^-\) (or HCO\(_3^-\)) exchange, a process equivalent to anion-H\(^+\) cotransport. Indeed, cholate uptake in the intact liver has been postulated to be mediated, at least in part, by bile acid/OH\(^-\) exchange (16, 17). To test the hypothesis that oatp-mediated taurocholate uptake is coupled to base exchange, we examined the capacity of extracellular taurocholate to stimulate base efflux in HCO\(_3^-\)-loaded noninduced and induced HeLa cells. As shown in Figs. 5 and 6, extracellular taurocholate stimulated HCO\(_3^-\) extrusion in alkaline-loaded induced cells alone. These results indicate that oatp expression confers on HeLa cells the capacity for taurocholate/HCO\(_3^-\) exchange. The recovery of pH\(_i\) in induced cells to a value below their initial resting pH\(_i\) may represent “overshoot” of taurocholate uptake. The cessation of taurocholate/HCO\(_3^-\) exchange upon reduction of pH\(_i\) may explain, at least in part, the reduction in organic anion uptake previously observed in cells depleted of ATP (4, 7). Although not examined in the present study, pH\(_i\) would be expected to fall with ATP depletion, reducing the driving force for organic anion/HCO\(_3^-\) exchange.

Our results indicate that oatp mediates Na\(^+\)-independent, high affinity transport of taurocholate. A Na\(^+\)-dependent bile acid transporter (ntcp) cloned from rat liver (18) has also been recently characterized. COS-7 cells transfected with ntcp cDNA revealed Na\(^+\)-dependent taurocholate transport with a K\(_m\) of 29 \(\mu\)M (19). Although under normal conditions the majority of bile acid uptake by the liver is thought to be Na\(^+\)-dependent (20), the relative contributions of oatp and ntcp to conjugated bile acid transport in various pathobiologic states remain to be determined.

Immunocytochemical studies indicate that oatp is localized to the basolateral (sinusoidal) plasma membrane of hepatocytes, a location consistent with its presumed role in clearing ligands from the circulation (21). As such, the transporter would be expected to extrude cellular HCO\(_3^-\), if that were the physiologic substrate extruded in exchange for organic anion uptake, into the circulation. In rat liver, both a Na\(^+\)/H\(^+\) antiporter (22) and Na\(^+\)-HCO\(_3^-\) symporter (23, 24) are localized to the basolateral membrane; the latter transporter is believed to function as a base loader under resting conditions (25). Thus, the presence of a basolateral organic anion/base exchanger might provide a pathway for OH\(^-\) (or HCO\(_3^-\)) exit from the hepatocyte. Although the pH of the hepatocyte is ~7.2 (26) and thus presumably slightly more acidic than the portal blood (pH 7.4), the outward movement of base may be favored by local outwardly directed gradients established by the basolateral Na\(^+\)-HCO\(_3^-\) symporter and the negative cell potential (~40 mV) (27, 28).

It should be noted that our demonstration of taurocholate/HCO\(_3^-\) exchange does not prove that HCO\(_3^-\) is the physiologic substrate for oatp in vivo. Other potential substrates capable of driving uphill oatp-mediated organic anion transport include \(\alpha\)-ketoglutarate and metabolic intermediates that accumulate to relatively high concentrations within the hepatocyte. While beyond the scope of the present study, identification of the physiologic substrate(s) for oatp remains an important goal for future investigation.

REFERENCES
1. Scharschmidt, B. F., Waggner, J. G., and Berk, P. D. (1975) J. Clin. Invest. 56, 1280–1292
2. Stollman, Y. R., Gartner, U., Theilmann, L., Ohmi, N., and Wolkoff, A. W. (1983) J. Clin. Invest. 72, 718–723
3. Min, A. D., Johansen, K. L., Campbell, C. G., and Wolkoff, A. W. (1991) J. Clin. Invest. 87, 1496–1502
4. Wolkoff, A. W., Samuelson, A. C., Johansen, K. L., Nakata, R., Withers, D. M., and Soasia, A. (1987) J. Clin. Invest. 79, 1259–1268
5. Jacquemin, E., Hagenbuch, B., Stieger, B., Wolkoff, A. W., and Meier, P. J. (1991) J. Clin. Invest. 88, 2146–2149
6. Jacquemin, E., Hagenbuch, B., Stieger, B., Wolkoff, A. W., and Meier, P. J. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 133–137
7. Shi, X., Bai, S., Ford, A., C., Burk, R. D., Jacquemin, E., Hagenbuch, B., Meier, P. J., and Wolkoff, A. W. (1985) J. Biol. Chem. 260, 25581–25586
8. Kanai, N., Lu, R., Bao, Y., Wolkoff, A. W., and Schuster, V. L. (1986) Am. J. Physiol. 270, F319–F325
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
10. Satlin, L. M., Matsumoto, T., and Schwartz, G. J. (1992) Am. J. Physiol. 262, F539–F547
11. Silver, R. E., Mennit, P. A., and Satlin, L. M. (1996) Am. J. Physiol. 270, F550–F557
12. Ganz, M. B., Boyarsky, G., Sterzel, R. B., and Boron, W. F. (1989) Nature 337, 545–551
13. Lukacs, G. L., Kapus, A., Nanda, A., Romanek, R., and Grinstein, S. (1993) Am. J. Physiol. 265, C3–C14
14. Holsey, C., Cracue, E. J., Jr., and Nair, C. N. (1990) J. Cell Physiol. 142, 586–591
oatp Mediates Organic Anion/HCO$_3^-$ Exchange

15. Wolkoff, A. W. (1994) in *The Liver: Biology and Pathobiology* (Arias, I. M., Boyer, J. L., Fausto, N., Jakoby, W. B., Schachter, D., and Shafritz, D. A., eds) 3rd Ed., pp. 179–188, Raven Press, Ltd., New York
16. Blitzer, B. L., Terzakis, C., and Scott, K. A. (1986) *J. Biol. Chem.* 261, 12042–12046
17. Veith, C. M., Thalhammer, T., Felberbauer, F. X., and Graf, J. (1992) *Biochim. Biophys. Acta* 1103, 51–61
18. Hagenbuch, B., Steiger, B., Foguet, M., Lubbert, H., and Meier, P. J. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 88, 10629–10633
19. Boyer, J. L., Ng, O. C., Ananthanarayanan, M., Hofmann, A. F., Schteingart, C. D., Hagenbuch, B., Steiger, B., and Meier, P. J. (1994) *Am. J. Physiol.* 266, G382–G387
20. Meier, P. J. (1995) *Am. J. Physiol.* 269, G801–G812
21. Bergwerk, A. J., Shi, X., Ford, A. C., Kanai, N., Jacquemin, E., Burk, R. D., Bai, S., Novikoff, P. M., Steiger, B., Meier, P. J., Schuster, V. L., and Wolkoff, A. W. (1996) *Am. J. Physiol.* 271, G231–G238
22. Moseley, R. H., Meier, P. J., Aronson, P. S., and Boyer, J. L. (1986) *Am. J. Physiol.* 250, G35–G43
23. Gleeson, D., Smith, N. D., and Boyer, J. L. (1989) *J. Clin. Invest.* 84, 312–321
24. Renner, E. L., Lake, J. R., Scharschmidt, B. F., Zimmerli, B., and Meier, P. J. (1989) *J. Clin. Invest.* 83, 1225–1235
25. Fitz, J. G., Lidofoisky, S. D., Weisger, R. A., Xie, M. H., Cochran, M., Grotmol, T., and Scharschmidt, B. F. (1991) *J. Membr. Biol.* 122, 1–10
26. Benedetti, A., Strazzabosco, M., Corasanti, J. G., Haddad, P., Graf, J. and Boyer, J. L. (1991) *Am. J. Physiol.* 261, G512–G522
27. Fitz, J. G., and Scharschmidt, B. F. (1987) *Am. J. Physiol.* 252, G56–G64
28. Graf, J., Henderson, R. M., Krumpholz, B., and Boyer, J. L. (1987) *J. Membrane Biol.* 95, 241–254