Regulation of Hepatic Transport Systems Involved in Bile Secretion During Liver Regeneration in Rats

THERA A. VOS,[1] JENNY E. ROS,[1] RICK HAVINGA,[2] HAN MOSHAGE,[1] FOLKERT KUIPERS,[2] PETER L. M. JANSEN,[1] AND MICHAEL MÜLLER[1]

We investigated the expression of hepatic transport systems involved in bile secretion during liver regeneration after partial hepatectomy (PH) in rats. Initial studies showed maximal BrdU incorporation 24 hours after PH. Therefore, transporter expression and bile secretion were analyzed in detail at this time. The mRNA levels of the multidrug resistance genes *mdr1a* and *mrp1* slightly increased, whereas *mdr1b* mRNA levels showed an extensive increase after PH. The mRNA levels of the conjugate transporter, mrp2, decreased slightly, whereas mrp2 protein levels did not change. Bilirubin secretion did not change, but the biliary glutathione secretion markedly decreased and the hepatic GSH content increased. The messenger RNA levels of the bile salt uptake transporters ntcp, oatp1, and oatp2 and the bile salt exporter, bsep/spgp, all decreased with ntcp showing the most prominent decrease. Protein levels of ntcp dramatically decreased whereas oatp2 only slightly decreased. Oatp1 protein expression slightly increased and bsep/spgp protein levels did not change. Decreased levels of bile salt uptake systems were associated with a 10-fold increase in the plasma bile salt concentration, yet, bile flow and bile salt secretion were increased when expressed per gram liver and unaffected when expressed on the basis of body weight. In conclusion, during the initial phase of rat liver regeneration ntcp is down-regulated whereas other transporter proteins involved in bile secretion are only slightly affected. Despite increased serum bile salt levels the remnant liver is not cholestatic: bile flow is maintained by uptake of bile salts probably via oatp isoforms and their secretion via bsep/spgp. (HEPATOLOGY 1999;29:1833-1839.)

Under normal circumstances hepatocytes are quiescent cells that fulfill a variety of tissue-specific functions such as xenobiotic biotransformation and bile formation. The latter is crucial for maintenance of cholesterol homeostasis, absorption of dietary fats, and removal of waste products and their metabolites. Bile formation is an osmotic process that critically depends on active secretion of osmotically active compounds from liver into the bile canalicular lumen. Bile salts and glutathione (GSH) are considered the major contributors to bile flow generation in rodents.[1,2] After removal of part of the liver, the remaining hepatocytes undergo a synchronized process of DNA synthesis and cell division. DNA synthesis starts in periportal hepatocytes with a first peak 22 to 24 hours after partial hepatectomy (PH). DNA replication of nonparenchymal cells follows 24 to 36 hours later. Within a few weeks the liver has grown to its original mass and the hepatocytes become quiescent again.[3,4]

Although one would expect a decreased hepatic excretory and secretory function during cell division because of partial loss of cell polarity, earlier investigations have actually shown increased bile flow and increased secretion of bile salts, when expressed per gram remaining tissue, during liver regeneration in rodents.[5-8] The relationship between bile formation and the expression of hepatic transport systems involved herein have not been defined under these conditions. The uptake of cholephilic compounds from sinusoidal blood into hepatocytes is largely dependent on the Na+/taurocholate cotransporting polypeptide ntcp[9] and the Na+-independent organic anion transporting polypeptides oatp1[10] and oatp2.[11] Ntcp transports conjugated bile salts (*e.g.*, taurocholate) and, to a lesser degree, unconjugated bile salts (*e.g.*, cholate). Both oatp1 and oatp2 can transport a wide variety of structurally unrelated compounds including bromosulfophthalein, taurocholate, cholate, leukotriene C₄, *S*-dinitrophenyl glutathione, and steroid conjugates (*e.g.*, estradiol-17β-glucuronide).[12,13] Recently, it has been shown that hepatic uptake of organic anions by oatp1 can be driven by the exchange with intracellular GSH.[13]

Secretion of cholephilic compounds from hepatocytes into bile is largely dependent on members of two subfamilies of the ATP-binding cassette protein superfamily[1,2]: the P-glycoprotein (Pgp) subfamily and the multidrug resistance protein (MRP) subfamily. Until now, four members of the Pgp subfamily have been cloned. Mdr1a and mdr1b are present at low levels at the canalicular membrane of normal rodent liver. Overexpression of mdr1a/mdr1b confers multidrug resistance against a broad variety of natural toxins and drugs. In contrast to mdr1, the canalicular expression of mdr2 is high. This transporter functions as a flippase that translocates

phosphatidylcholine from the inner to the outer leaflet of the canalicular membrane for secretion into bile. Finally, the sister of Pgp (spgp) has recently been identified as the major canalicular bile salt export pump (bsep).[14]

The expression of four members of the MRP subfamily has been shown in rat liver so far. In normal liver, mrp1 is present at low levels. Mrp1 is able to transport mostly multivalent anionic conjugates such as bilirubin diglucuronide and GSH S-conjugates, including the lipid peroxidation product leukotriene C_4. In contrast to mrp1, its homologue mrp2 is highly expressed in the liver and is located at the canalicular membrane. Mrp2 has the same substrate specificity as mrp1. Two other mrp homologues are also present in the liver, mrp3 (= mlp2)[15,16] at low levels and mrp6 (= mlp1)[16,17] at relatively high levels. Mrp6 is located at the lateral plasma membrane of hepatocytes and transports the anionic cyclopentapeptide endothelin receptor antagonist BQ123.[17] The endogenous substrate of mrp6 is not yet known.

Earlier studies have shown a strongly decreased expression of ntcp[18] and a clearly increased expression of mdr1,[19-21] especially mdr1b,[22] after PH in rats. However, in these studies, neither the flow of bile nor the secretion of bile constituents and the expression of other transporters located at the basolateral and canalicular membrane were determined. For normal production of bile, the organic anion transporter mrp2 and the bile salt transporter bsep/spgp are essential. Therefore, we examined the expression of these transporters and related this to bile flow and secretion of bile salts, bilirubin conjugates, and GSH. Because under conditions where basolateral transporters are down-regulated, sinusoidal uptake rather than canalicular secretion may become rate-limiting for overall bile formation. Therefore, we also investigated the expression of the basolateral uptake transporters ntcp, oatp1, and oatp2 during liver regeneration.

MATERIALS AND METHODS

Animals. Pathogen-free male Wistar rats (220-260 g) were purchased from Harlan-CPB, Zeist, the Netherlands. They were kept under routine laboratory conditions at the Central Animal Laboratory of the University of Groningen. The rats received standard laboratory chow and had free access to food and water. This study was approved by the Local Committee for Care and Use of Laboratory Animals.

Experimental Design. PH was performed according to the technique of Higgins and Anderson.[23] Sham-operated animals received the same treatment as partial hepatectomized rats, including manipulation of the liver, but without hepatectomy. For measurement of transporter mRNA levels at different time points after PH (sham, 3, 12, 24, 48, and 216 hours, n = 4), livers were perfused with phosphate-buffered saline (PBS) under sodium pentobarbital anesthesia (60 mg/kg, intraperitoneally), removed, cut into small pieces, snap-frozen in liquid nitrogen and stored at −80°C until further use. In another experiment, rats underwent PH (n = 3) or sham operation (n = 3) and rat hepatocytes were isolated 24 hours later by two-step collagenase perfusion as described previously.[24] The hepatocytes were used immediately for isolation of mRNA and plasma membranes. Bile was collected 24 hours after PH (n = 5) or after sham operation (n = 5) by cannulation of the common bile duct under sodium pentobarbital anesthesia (60 mg/kg, intraperitoneally). After a calibration time of 5 minutes, bile was collected for 5 minutes and immediately frozen in liquid nitrogen. For determination of bile GSH levels, a second fraction of bile was collected for 15 minutes in a preweighted tube with 50 µL 10% 5-sulfosalicylic acid (Sigma, St. Louis, MO), weighted again, centrifuged for 5 minutes at 13,000 rpm and frozen in liquid nitrogen. Blood was obtained by

cardiac puncture, mixed with ethylenediaminetetraacetic acid and centrifuged. Samples of 600 µL plasma were frozen in liquid nitrogen. For GSH determination, 800 µL plasma was immediately mixed with 200 µL ice-cold 10% 5-sulfosalicylic acid and centrifuged for 5 minutes at 13,000 rpm. Supernatant was frozen in liquid nitrogen.

Analytical Methods. Bile flow was determined gravimetrically, assuming a density of 1.0 mg/µL. Total bile salts, phospholipids, and cholesterol in plasma and bile were measured as described.[25] Total GSH levels (GSH+GSSG) in plasma, liver, and bile were determined according to Griffith.[26] Aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), and total bilirubin were determined by routine clinical chemistry.

Reverse-Transcriptase Polymerase Chain Reaction. Because the expression of the mdr1a, mdr1b, and mrp1 genes is very low in normal rat liver, we performed semiquantitative reverse-transcriptase polymerase chain reaction (RT-PCR) to detect these and other transporter gene products. Total RNA was isolated from frozen rat liver and isolated hepatocytes using TRIzol Reagent (Life Technologies, Grand Island, NY) according to manufacturer's instructions. Subsequently, messenger RNA (mRNA) was isolated using the Oligotex mRNA mini-kit (Qiagen GmbH, Hilden, Germany). Single stranded cDNA was synthesized from 2.5 µg mRNA using 0.5 nmol random primers (Pharmacia, Uppsala, Sweden) and 50 U AMV Reverse-Transcriptase (Promega, Madison, WI) in a buffer containing 50 mmol/L Tris-HCl, 50 mmol/L KCl, 10 mmol/L DTT, 10 mmol/L $MgCl_2$, 0.5 mmol/L spermidine (Promega), 50 U RNA guard (Pharmacia), and 1.25 mmol/L of each dNTP (Pharmacia) in a total volume of 100 µL. RT was performed for 10 minutes at 25°C and for 1 hour at 50°C and the samples were subsequently heated for 5 minutes at 95°C to terminate the RT reaction. With the complementary DNA (cDNA) obtained, a PCR reaction was performed using 3 µL of the RT reaction mixture supplemented with 2.5 U Taq polymerase (Pharmacia), 50 pmol/L sense and 50 pmol/L antisense primer. The final reaction volume was 50 µL. The tubes were incubated in a Gene Amp PCR system 2400 (Perkin-Elmer, Norwalk, CT) at 95°C for 5 minutes to denature the primers and cDNA. The cycling program was 95°C for 30 seconds, 54°C to 60°C for 30 seconds, 72°C for 30 seconds, and for 5 minutes in the last cycle and comprised 20 to 33 cycles. For each primer set, an increasing number of PCR-cycles with otherwise fixed conditions was performed to determine the optimal number of cycles to be used. This was determined to be halfway the exponential phase. β-Actin was used as internal control. Primers specific for ntcp[9] (sense: 5′-ATG CCC TTC TCT GGC TTT CT-3′; antisense: 5′-GCT CCA TGG TTC TGA TGG TT-3′), oatp1[10] (sense: 5′-AAG CGA AGA AGC TGG AAA CA-3′; antisense: 5′-CAC CAC AGG TCT GTG CAG TT-3′), oatp2[11] (sense: 5′-TGC ACA CTT AGC ATT CTG GC-3′; antisense: 5′-TGC ATG TAA CCC AAC TCC AA-3′), and β-actin[27] (sense: 5′-CCT AAG GCC AAC CGT GAA AAG-3′; antisense: 5′-TCT TCA TGG TGC TAG GAG CCA-3′) were used resulting in amplified products of 500, 302, 496, and 646 bp, respectively. All other PCR-primers used in this study were described previously.[28] All PCR-products were sequenced to confirm specificity of the PCR-primers. In each experiment, water was used as a negative control. Ten microliters of each PCR product was loaded on a 2.5% agarose gel and stained with ethidium bromide.

Isolation of Crude Plasma Membranes From Isolated Hepatocytes. Freshly isolated hepatocytes were washed in Krebs buffer (118 mmol/L NaCl, 5 mmol/L KCl, 1.1 mmol/L $MgSO_4$, 2.5 mmol/L $CaCl_2$, 1.2 mmol/L KH_2PO_4, 25 mmol/L $NaHCO_3$, 10 mmol/L D[+]glucose, 10 mmol/L HEPES, 1% bovine serum albumin [pH 7.42]) and pelleted at 500 *g* for 8 minutes. For permeabilization, cells were stirred in 35 mL 1 mmol/L $NaHCO_3$/0.1 mmol/L PMSF for 1 hour at 4°C. After centrifugation at 90,000*g* for 30 minutes at 4°C, the pellet was resuspended in 15 mL 250 mmol/L sucrose/0.1 mmol/L PMSF and homogenized 50 times with a tight Dounce homogenizer. A centrifuge tube was loaded with 15 mL 38% (wt/wt) sucrose and 15 mL

hepatocyte homogenate, respectively. After centrifugation at 90,000g for 90 minutes at 4°C, the 38% fraction was washed in 1 mmol/L NaHCO$_3$/0.1 mmol/L PMSF and centrifuged at 47,000g for 30 minutes at 4°C. The pellet was resuspended in 1 mmol/L NaHCO$_3$/Complete protease inhibitor cocktail (1 tablet/50 mL, Boehringer Mannheim GmbH, Mannheim, Germany) by 15 times in-and-out suctioning through a 25-gauge needle and stored at −80°C until further use.

Antibodies. Antibodies against mrp2 and bsep/spgp were described before.[28] Antibodies against ntcp,[29] oatp1,[30] and oatp2[31] were kind gifts from Dr. Bruno Stieger and Dr. Peter J. Meier (Zürich, Switzerland). Mouse mAb C219 (Signet Laboratories Inc., Dedham, MA) was used to detect all pgps. Mouse mAb against dipeptidyl peptidase IV (dppIV/CD26) was purchased from Endogen (Woburn, MA). Na$^+$K$^+$-ATPase antibodies raised in goats against the α and β subunits of Na$^+$K$^+$-ATPase were kindly provided by Dr. Wilbert Peters, Nijmegen, The Netherlands.

Western Blot Analysis. The protein concentrations in membrane fractions were determined with the DC Protein Assay from Bio-Rad (Bio-Rad Laboratories, Hercules, CA) using bovine serum albumin as standard. Fifty micrograms of membrane proteins was fractionated on a 7.5% (mrp2, bsep/spgp, C219, and dppIV) or 10% (ntcp, oatp1, oatp2, and Na$^+$K$^+$-ATPase) sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to nitrocellulose (Amersham International plc, Buckinghamshire, England), using a tankblotting system according to the manufacturer's instructions (Bio-Rad Laboratories). For dppIV detection, samples were boiled in sample buffer for 5 minutes before loading on SDS-PAGE. BDH molecular-weight standards (42,700-200,000 molecular-weight; BDH Ltd., Dorset, England) were used as marker proteins. The blots were stained with Ponceau S-solution (0.1% Ponceau S [wt/vol] in 5% acetic acid [vol/vol]; Sigma) to confirm similar protein concentrations in every lane. The blots were incubated with the first antibody diluted in PBS containing 4% SKIM milk (Fluka BioChemica, Buchs, Switzerland) and 0.1% polyoxyethylene sorbitan monolaurate (Tween-20, Sigma), washed in PBS/0.1% Tween-20, subsequently incubated with horseradish peroxidase–labeled swine anti-rabbit IgG or rabbit anti-mouse IgG diluted in PBS/4% SKIM milk/0.1% Tween-20 (dilution 1:2,000; DAKO A/S, Glostrup, Denmark) and finally developed using Pierce SuperSignal Chemiluminescent Substrate Luminol/Enhancer (Pierce, Rockford, IL).

Statistical Analysis. The data resulting from the two experimental groups were expressed as the mean ± SEM. An unpaired Student's t test was used to compare the means between the two groups. A P value less than .05 was considered significant.

RESULTS

Time-Dependent Changes in Transporter mRNA Levels After Partial Hepatectomy. In an initial experiment we characterized the expression of several hepatic transport systems at 3, 12, 24, 48, and 216 hours after PH using semiquantitative RT-PCR (Fig. 1, n = 4). In the Pgp subfamily, mRNA levels of both *mdr1a* and *mdr2* slightly increased after PH. The increased expression of *mdr1a* was maximal 24 hours after PH, whereas *mdr2* expression was maximal 48 hours after PH. *Mdr1b* expression increased after PH, with a maximum at 24 hours, whereas *bsep/spgp* mRNA levels showed a slight decrease (Fig. 1). In the MRP subfamily, *mrp1* mRNA levels were increased 3 hours after PH and remained increased until at least 48 hours. *Mrp2* mRNA levels were dramatically decreased 12 hours after PH, but returned to almost normal levels at later time points. The expression of the gene encoding γ-glutamylcysteine synthetase (γ-gcs), the key enzyme in glutathione synthesis, was maximally elevated 24 hours after PH. The mRNA levels of the basolateral uptake
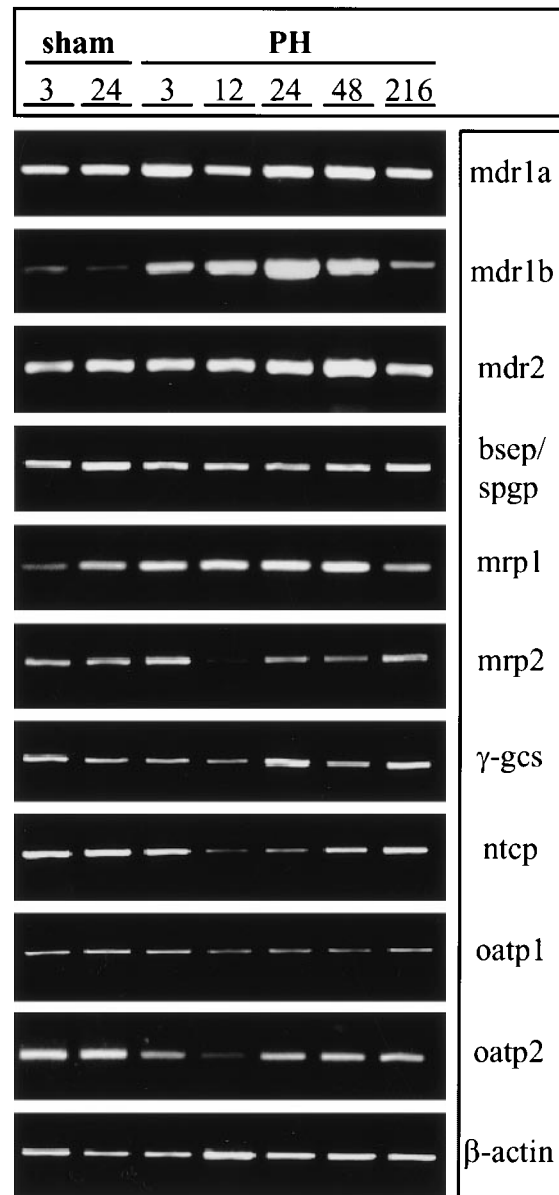

FIG. 1.   Time-dependent expression of transporter mRNA in total rat liver after partial hepatectomy. Total rat liver RNA was isolated and RT-PCR was performed as described in Materials and Methods. RT-PCR-products are indicated on the *right side* of each gel and time points after partial hepatectomy *above* the figure. Data are representative for n = 4 per time point.

transporters *ntcp, oatp1,* and *oatp2* were reduced 12 hours after PH and recovered thereafter.

After this initial study, we chose 24 hours after PH to investigate in more detail the bile flow and secretion of bile salts, cholesterol, phospholipids, GSH, and conjugated bilirubin after PH. This time point was chosen because 24 hours after PH a peak in DNA synthesis was found, as measured by BrdU incorporation (data not shown).

Characteristics of Partial Hepatectomized Rats. The body weight of sham-operated rats was 243 ± 11.7 g before and 244 ± 8.7 g 24 hours after surgery. The body weight of PH rats was 241 ± 9.2 g before and 227 ± 9.5 g 24 hours after surgery. On average, 7.7 ± 0.7 g of liver were removed. Liver weights from sham-operated animals were used to calculate the %

liver weight/body weight (4.8%). From this, the percentage of liver removed from the partial hepatectomized group was estimated to be 66%.

Plasma Parameters in Partial Hepatectomized Rats. Compared with sham-operation, PH led to significantly increased AST, ALT, and ALP plasma levels 24 hours after operation (AST: 93 ± 12 vs. 383 ± 47 U/L, ALT: 52 ± 8 vs. 203 ± 32 U/L, and ALP: 139 ± 13 vs. 256 ± 26 U/L, all $P < .05$). The concentration of bile salts in plasma increased 10-fold after PH (Table 1). Also bilirubin levels in plasma increased significantly, although less pronounced (1.6-fold, Table 1). Plasma cholesterol levels decreased to 76% of control levels ($P < .05$) and plasma phospholipids to 89% (not significant). Total GSH in plasma did not change after PH (Table 1).

Bile Secretion in Partial Hepatectomized Rats. In bile neither the bile salt nor the bilirubin concentration changed significantly after PH (Table 2). Cholesterol and phospholipid concentrations both decreased significantly to 55% and 54% of control values. The biliary GSH concentration decreased to 23% (Table 2), whereas GSH levels in the liver increased significantly from 6.6 ± 1.2 to 11.5 ± 1.6 μmol/g liver. When expressed per gram liver, bile flow was increased 1.75-fold after PH (Table 3). The secretion of bile salts and bilirubin was also increased (1.9 [$P < .05$] and 1.5-fold [ns], respectively). Cholesterol and phospholipid secretion did not change significantly but the secretion of GSH decreased to 39% of control levels. When calculated per 100 g body weight (Table 3), bile flow and the secretion of bile salts and bilirubin did not change significantly, whereas the secretions of cholesterol, phospholipids, and GSH were decreased.

Transporter mRNA Levels in Hepatocytes Isolated From Remnant Livers 24 Hours After Partial Hepatectomy. To be certain that the observed changes in transporter mRNA levels as shown in Fig. 1 are taking place in hepatocytes rather than in other liver cell types, RT-PCR was performed on RNA obtained from hepatocytes that were isolated 24 hours after PH (Fig. 2). Twenty-four hours after PH, a very strong increased expression of *mdr1b* mRNA was seen, whereas *mdr1a* and *mdr2* mRNA levels only slightly increased. The mRNA levels of the bile salt transporter bsep/spgp and the major conjugate transporter mrp2 slightly decreased. The mRNA levels of *mrp1* and γ-gcs were increased. Twenty-four hours after PH, *ntcp* mRNA was dramatically decreased. *Oatp1* and *oatp2* mRNA levels were also decreased, but less pronounced then that of *ntcp*. β-Actin mRNA was slightly increased 24 hours after PH, as reported before.[21,32-34] This was confirmed by Northern blot with 28S as control for equal RNA loading (data not shown).

Transporter Protein Levels in Hepatocytes Isolated From Remnant Livers 24 Hours After Partial Hepatectomy. Hepatocytes isolated 24 hours after PH were used to obtain crude plasma membrane fractions for Western blot analysis (Fig. 3). After PH, no changes of mrp2 and bsep/spgp protein levels were observed. The C219 signal, detecting all pgps, was dramatically increased after PH. Protein levels of the uptake transporter ntcp dramatically decreased, whereas oatp2 protein levels only slightly decreased (Fig. 3). The appearance of ntcp protein bands as doublets has been described previously and is probably because of partial deglycosylation of the protein.[29] Twenty-four hours after PH, oatp1 protein levels were slightly increased, despite decreased oatp1 mRNA levels. As controls for the membrane isolation procedure, dppIV (canalicular protein) and Na^+K^+-ATPase (α and β subunits, basolateral protein) were used and no significant changes in their expression were found (Fig. 3).

DISCUSSION

In this study we compared the effects of PH on bile secretion with the expression of a number of transporters involved in bile formation. Expressed per g liver, PH led to an increase in bile flow, an increased secretion of bile salts, an

TABLE 1. Plasma Composition in Sham-Operated and Partial Hepatectomized Rats

	Sham	24-hr PH
Bile salts‡	36.3 ± 2.7	380.3 ± 99.0*
Total bilirubin‡	3.60 ± 0.12	5.73 ± 0.55*
Cholesterol§	1.05 ± 0.06	0.80 ± 0.06*
Phospholipids§	2.18 ± 0.08	1.95 ± 0.08†
Total GSH‡	12.8 ± 1.0	13.1 ± 2.1†

NOTE. Data represent the mean ± SEM of n = 6 rats per group.
*$P < .05$.
†Not significant.
‡μmol/L.
§mmol/L.

TABLE 2. Bile Composition in Sham-Operated and Partial Hepatectomized Rats

	Sham	24-hr PH
Bile salts‡	54.5 ± 3.5	59.4 ± 1.9†
Total bilirubin§	41.6 ± 1.4	34.9 ± 3.5†
Cholesterol‡	0.73 ± 0.06	0.40 ± 0.03*
Phospholipids‡	6.7 ± 0.4	3.6 ± 0.4*
Total GSH‡	3.6 ± 0.8	0.83 ± 0.23*

NOTE. Data represent the means ± SEM of n = 5 rats per group.
*$P < .05$.
†Not significant.
‡mmol/L.
§μmol/L.

TABLE 3. Bile Flow and Biliary Secretion Rates in Sham-Operated and Partial Hepatectomized Rats

	Sham	24-hr PH
Secretion per gram liver		
Bile flow‡	1.5 ± 0.1	2.6 ± 0.4*
Bile salt§	80 ± 4	152 ± 22*
Total bilirubin§	0.06 ± 0.001	0.09 ± 0.02†
Cholesterol§	1.1 ± 0.1	1.0 ± 0.2†
Phospholipid§	9.9 ± 0.7	9.4 ± 1.6†
Total GSH§	5.4 ± 1.3	2.1 ± 0.6*
Secretion per 100 gram body weight		
Bile flow‖	7.1 ± 0.3	6.3 ± 1.2†
Bile salt¶	383 ± 18	370 ± 59†
Total bilirubin¶	0.29 ± 0.01	0.22 ± 0.04†
Cholesterol¶	5.2 ± 0.6	2.5 ± 0.4*
Phospholipid¶	47.7 ± 3.2	22.6 ± 3.9*
Total GSH¶	26.4 ± 6.7	5.2 ± 2.3*

NOTE. Data represent the means ± SEM of n = 5 rats per group.
*$P < .05$.
†Not significant.
‡μL/g liver/min.
§nmol/g liver/min.
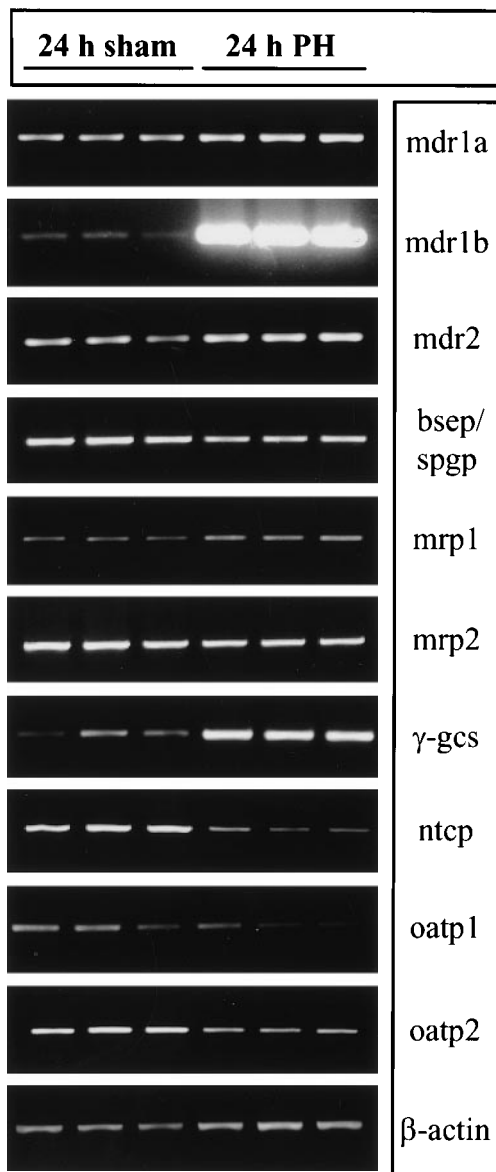‖μL/100 g b.wt./min.
¶nmol/100 g b.wt./min.

FIG. 2. Expression of transporter mRNA in isolated rat hepatocytes 24 hours after partial hepatectomy. RNA was isolated from freshly isolated rat hepatocytes and RT-PCR was performed as described in Materials and Methods. RT-PCR-products are indicated on *the right side* of each gel and treatment group *above* the figure. Results from hepatocyte isolations from three sham-operated and three PH rat livers are shown.

hepatocytes against products of cellular oxidative stress reactions such as lipid peroxidation products.[28]

As shown in a study from Green et al.[18] and confirmed in our study, PH leads to drastically decreased ntcp mRNA and protein levels. Therefore, the Na^+-dependent uptake of bile salts will be seriously decreased. Twenty-four hours after PH, oatp1 protein levels were slightly increased, despite decreased mRNA levels. This could be the result of a decreased
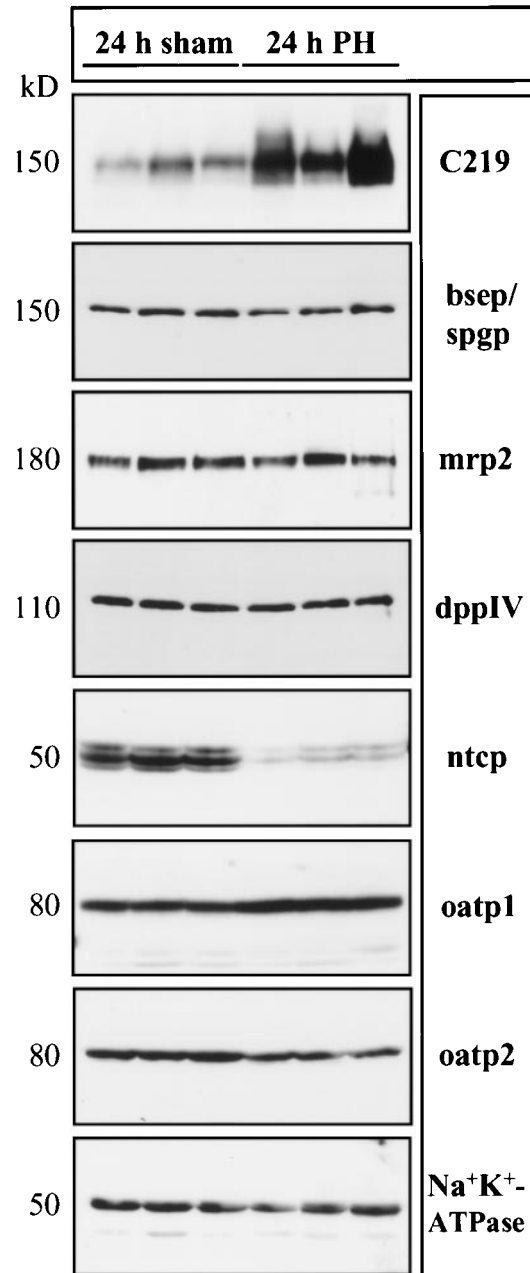

FIG. 3. Western blots of transporter proteins in crude plasma membranes of isolated rat hepatocytes 24 hours after sham operation or partial hepatectomy. Fifty micrograms of crude plasma membranes was separated on 7.5% (mrp2, bsep/spgp, C219, and dppIV) or 10% (ntcp, oatp1, oatp2, and Na^+K^+-ATPase) SDS-PAGE gel and transferred to nitrocellulose. The proteins were visualized using the corresponding antibodies, as indicated on the *right side* of each blots. C219 recognizes all Pgps. Apparent molecular weights are indicated on the *left side* of each blot, the treatment group *above* the blot. Results from hepatocyte isolations from three sham-operated and three PH rat livers are shown.

unchanged secretion of conjugated bilirubin and a decreased secretion of GSH. PH had no effect on the secretion of cholesterol and phospholipids per g liver. Despite a significantly increased secretion of bile salts and a decreased secretion of GSH, the mRNA and protein levels of bsep/spgp and mrp2, the transporters thought to be involved in bile salt[14] and GSH secretion,[35] showed no major changes after PH. The strong up-regulation of *mdr1b* mRNA and the minor increase in *mdr1a* and *mdr2* mRNA are in agreement with earlier studies.[19-22] In this study we show that *mrp1* mRNA is up-regulated after PH. This is in agreement with the increased levels of mrp1 seen in proliferating hepatocyte-derived cells.[36] Up-regulation of *mrp1* and *mdr1b*, which are normally expressed at very low levels, may increase the resistance of

breakdown of the oatp1 protein during liver regeneration. It has to be noted however, that little information is yet available on the mechanism of degradation of the various transporters. Oatp2 protein levels were only slightly decreased 24 hours after PH. This preservation of oatp1 and oatp2 protein expression could compensate, at least in part, for the markedly decreased ntcp expression (Fig. 3) and ensure ongoing basolateral uptake of bile salts and other organic anions. However, because of their similar K_M values for taurocholate (ntcp $\sim$ 25 μmol/L[37]; oatp1 $\sim$ 50 μmol/L[38]; oatp2 $\sim$ 35 μmol/L[11]), oatp1 and oatp2 would be fully saturated in the absence of ntcp expression and can obviously not prevent a marked increase in the plasma concentration of bile salts. Nevertheless, we postulate that after PH bile salts are taken up mainly via the oatp isoforms. oatp2 is an especially good candidate because it is expressed at high levels in rat liver. Uptake of bile salts via oatp1 and oatp2 will be facilitated because of the 10-fold increased serum bile salt levels. Moreover, oatp1-mediated uptake of bile salts is also stimulated by higher intracellular GSH concentrations,[13] as found during liver regeneration by us and others.[39]

Under normal circumstances only periportal hepatocytes are involved in uptake (by ntcp) and secretion (by bsep/spgp) of bile salts. Because of the decreased ntcp expression and the high serum bile salt levels, oatp-expressing midzonal and pericentral hepatocytes are likely to participate in bile salt uptake during liver regeneration. This would explain the increased bile salt secretion capacity per g liver we observed. Furthermore, participation of pericentral hepatocytes in bile salt clearance has been shown under conditions of high serum bile salts.[40-43] Moreover, Baumgartner et al.[44] reported a decreased metabolic zonation of bile salt processing after PH in the isolated perfused rat liver. Such a shift of bile salt processing toward the pericentral hepatocytes in regenerating liver could protect periportal hepatocytes against the high levels of toxic bile salts that have to be handled by a decreased liver mass. Apparently, high plasma bile salt levels are less toxic to the organism than high intracellular bile salt levels, probably because plasma bile salts are bound to plasma components.

This study also shows that the secretion of GSH is significantly decreased after PH per g liver, whereas the secretion of conjugated bilirubin did not change. Since the GSH concentrations and the mRNA of γ-gcs, the rate-limiting enzyme in GSH synthesis, are increased after PH, these findings can either be explained by a down-regulation of the implicated transporters or by competitive inhibition of GSH transport by other intracellular solutes. It has been reported that canalicular GSH secretion might be mediated by mrp2.[35] Mrp2 mRNA levels are decreased at early, but not later time points, whereas no changes in mrp2 protein levels were observed. Therefore, competition between the low-affinity substrate GSH and some high-affinity substrates such as bilirubin glucuronides[45] is the most likely explanation for the reduced biliary GSH secretion.

In summary, the remnant liver is exposed to increased bile salt concentrations after PH. Down-regulation of ntcp impairs the uptake of bile salts by this Na^+-dependent, high-affinity bile salt uptake transporter. This will prevent or reduce the toxic accumulation of bile salts in periportal hepatocytes, which constitutes the main proliferating compartment in the regenerating liver. Oatp isoforms may take over the predominant role of ntcp in bile salt uptake, thereby recruiting midzonal and pericentral hepatocytes for bile salt uptake and maintaining unchanged bile salt secretion expressed per 100 g body weight or, in other words, the small remnant liver is fully capable to maintain hepatic flux of the circulating bile salt pool. By adapting the expression of uptake systems, the remnant liver can handle the increased bile salt load per cell without overloading periportally located proliferating hepatocytes.

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