Experimental Studies of Biliary Excretion of Piperacillin

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The nonrecirculating isolated perfused rat liver was used to study biliary antibiotic excretion by the liver in a steady-state, controlled environment in which bile flow, bile salt output, and antibiotic delivery were maintained under constant conditions. The effects of piperacillin, ampicillin, and gentamicin on bile flow and bile salt output were analyzed; none altered bile salt output, and only high concentrations of piperacillin (100 μg/mL) increased bile flow. The ratio of antibiotic concentration in bile and perfusate depended on the type of antibiotic and perfusate concentration. Piperacillin infusions at perfusate concentrations of 50 or 100 μg/mL (in the presence of 60 μM taurocholate) yielded bile to perfusate ratios of 112 ± 10 *versus* 49 ± 3, respectively. Using similar perfusate, the concentration ratios for ampicillin (20 μg/mL) and gentamicin (10 μg/mL) were only 3.4 ± 0.5 and 0.5 ± 0.1, respectively. By altering the perfusate to contain either 60 μM or 240 μM taurocholate, we found variance in bile salt output from 27 ± 1 to 115 ± 2 μmol/h, yet this alteration had little effect on the output of ampicillin (perfusate concentration of 20 μg/mL), 73 ± 7 *versus* 74 ± 12 μg/h, or piperacillin (perfusate concentration 100 μg/mL), 10 ± 1 *versus* 11 ± 2 mg/h. Thus, it appears ampicillin and piperacillin are excreted into bile at high concentrations by bile salt-independent pathways. Partial biliary obstruction (6 cm H_2O) results in significant decreases in bile volume. Infusion of 50 μg/mL of piperacillin resulted in increased biliary flow that approached nonobstructed values. Obstruction resulted in significant decreases in bile piperacillin concentration. Whether the choleretic effect of high concentrations of piperacillin has any clinical significance in nonobstructed or obstructed conditions remains to be established.

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THE PHYSIOLOGY OF ANTIBIOTIC EXCRETION by the liver is an area of clinical relevance,[1] yet little is known of the relationship between antibiotic excretion and bile flow or composition. Most studies of biliary antibiotic excretion have depended on T-tube sampling, in which the antibiotic levels in bile and serum were in constant flux and the bile salt output was diminished.[2,3] Such studies make assumptions about the relationship between biliary antibiotic excretion and serum antibiotic concentration *in vivo;* however, the presence of extrahepatic metabolism and excretion, fluctuating hormonal influences on the liver, and variable hepatic blood flow make final results difficult to interpret. In addition, these variables make analysis of the effects of antibiotic excretion on bile flow and composition hard to determine.[4-6] To address these problems, we used a nonrecirculating isolated perfused rat liver (IPRL) to investigate the relationship of bile flow and bile salt output to bile antibiotic concentration. Such studies allow a more detailed analysis of biliary excretion of antibiotics in a manner not previously described. The IPRL can achieve a stable, well-controlled sytem in which the effects of antibiotics on bile flow and composition as well as the effect of varying bile composition on antibiotic output into bile could be assessed.

We chose to evaluate ampicillin and gentamicin for several reasons: (1) their potent *in vitro* activity against *Streptococcus faecalis* and *Escherichia coli,* important pathogens in biliary tract sepsis[7]; (2) their frequent use in cholangitis and acute cholecystitis; and (3) their representation of two important classes of antimicrobial agents, β-lactams and aminoglycosides. We also selected piperacillin, a newer, extended β-lactam, due to claims of the

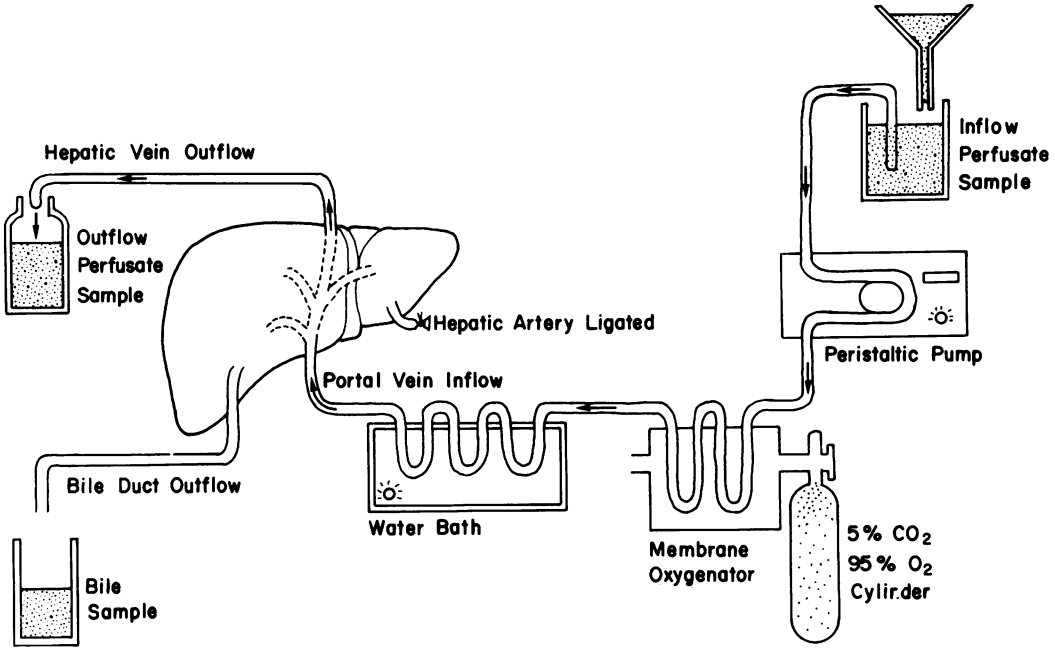


FIG. 1. Liver perfusion apparatus: the nonrecycling rat liver perfusion apparatus is depicted. Inflow perfusate is pumped *via* peristaltic pump through a membrane oxygenator. The water bath is effective in maintaining a constant 37 C inflow perfusate temperature. Hepatic vein outflow is then used to collect the effluent, and the bile duct is cannulated for continuous biliary samples.

high biliary concentrations attained during therapy[8–10] and its increased activity *in vitro* against the same members of the Enterobacteriaceae involved in biliary infections.[11,12]

We hypothesized that ampicillin, gentamicin, and piperacillin might alter bile flow or bile salt output, and conversely that varying bile salt output and bile flow might alter antibiotic output or concentrations. We sought to determine changes in antibiotic concentration and output over time during constant bile salt output using the IPRL. Finally, we sought to evaluate the consequences of partial biliary obstruction on biliary excretion of piperacillin and compare those effects with the nonobstructed state.

Materials and Methods

The IPRL System

The liver perfusion apparatus used in these studies is a nonrecirculating system, as depicted in Figure 1. A perfusate mixture with fixed concentrations of antibiotic and bile salt was oxygenated using a Silastic tubing membrane oxygenator[13] with 95% O_2 + 5% CO_2, heated in a water bath to 37 C,[14] and perfused *in situ* in rat livers *via* the portal vein using a peristaltic pump. The perfusate consists of 3% albumin (Fraction V Bovine albumin, #A-8022 Sigma Chemical, St. Louis, MO), 10% washed human erythrocytes, and 100 mg/dL dextrose in Krebs bicarbonate solution adjusted to pH 7.4 with 1 N NaOH, along with a fixed concentration of radiolabeled ^{14}C taurocholate (Sigma Chemical, St. Louis, MO) and antibiotic. This mixture is associated with negligible histologic changes and enzyme release.[15–18] Perfusate ^{14}C taurocholate concentrations of either 60 or 240 μM were studied; flow rates of 1.1–1.4 mL/min · g^{-1} liver weight (approximately 10 mL/min) were used to approximate the physiologic range of rat portal flow.[19–23] The concentrations of antibiotic in the perfusate were: gentamicin, 10 μg/mL; ampicillin, 20 μg/mL; and piperacillin, 50 and 100 μg/mL, respectively, representative of serum concentrations in the range achieved after standard parenteral doses used to treat infections in humans.[8–10] Although some authors have suggested using perfusion media with higher hematocrits, the lower hematocrit in our perfusate is similar to that used by others[15–18,24] and minimizes the effects of erythrocytes on antibiotic metabolism or binding without compromise of adequate tissue oxygenation.

The livers of groups of 10 or more adult female Wistar rats weighing 300–400 g were perfused *via* the portal vein after hepatic artery occlusion.[15] The rats were weighed, anesthetized with intraperitoneal pentobarbital (30 mg/kg), and placed supine on wooden plates. PE10 tubing was used to cannulate the bile duct to collect all hepatic bile in 3-mL glass tubes. The hepatic artery was ligated and the portal vein perfused through an 18-gauge catheter. The inferior vena cava was cut to avoid hepatic congestion, and the superior vena cava was cannulated through the right atrium with PE240 tubing, which allowed the hepatic effluent to be collected in graduated beakers. The inferior vena cava was then ligated. The total hepatic ischemic time was never in excess of 5–10 seconds. The liver was covered *in situ* with plastic wrap to prevent dessication and was placed in an insulated, temperature-controlled box. Portal vein perfusion pressures were 10–12 cm H_2O.

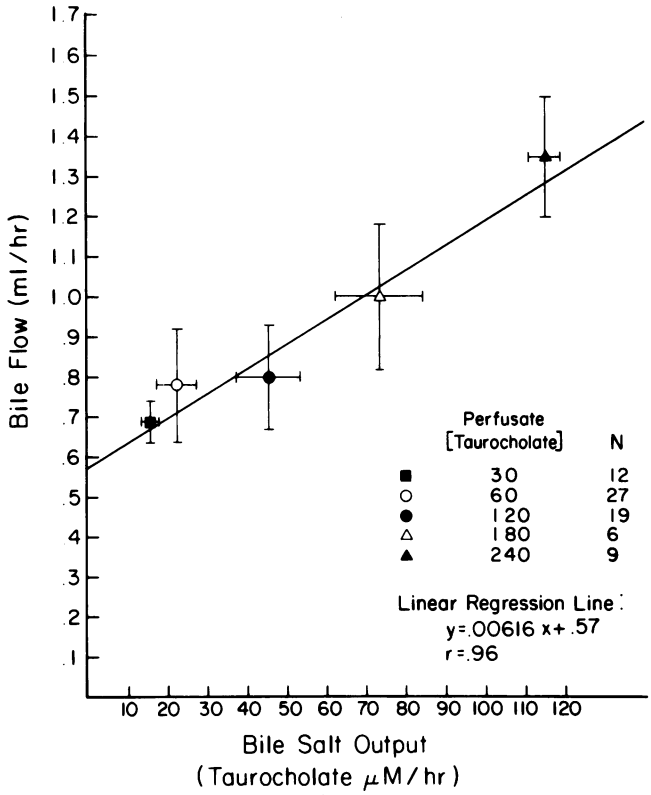


FIG. 2. The effect of different perfusate bile salt concentrations on bile flow: over a range of taurocholate concentrations in the perfusate (30–240 μM), bile flow was graphed against bile salt output and was linearly related with a correlation coefficient of 0.96. (Mean ± SEM.)

Bile and perfusate outflows were collected over three 30-minute periods, and inflow and outflow perfusate as well as bile were analyzed for antibiotic concentrations using standard bioassays. Standard curves were constructed in perfusate and rat bile. Selected samples of bile and perfusate were also analyzed using gas liquid chromatography to exclude biologically active metabolites. Bile and perfusate bile salt concentrations were calculated using a Beckman liquid scintillation counter (model #LS-100c, Beckman Instruments, Fullerton, CA). Bile salt concentration was calculated from a ratio of the radioactivity in the sample to the radioactivity in the perfusate inflow sample with known bile salt concentration. Antibiotic delivery to the liver was determined from the inflow concentration of antibiotic, and the perfusate flow rate was measured directly in the graduated beakers used for collecting the total outflow during each consecutive 30-minute interval. Antibiotic uptake by the liver was calculated using the formula:

$$\text{Ab uptake } (\mu g/h) = (PV - HV) \times 0.9 \times Q,$$

where PV = inflow antibiotic concentration (μg/mL), HV = outflow antibiotic concentration (μg/mL), and Q = perfusate volume (mL/h).

Antibiotic biliary output was calculated using the formula:

$$\text{Ab output } (\mu g/h) = V \times C,$$

where V = bile volume (mL/h), and C = bile antibiotic concentration (μg/mL). At the end of each experiment, the rat livers were removed and weighed wet, and a sample was fixed in formalin and examined histologically.

To evaluate the effect of partial biliary obstruction on hepatic excretion of piperacillin, we used the model and calculations as described above. Partial obstruction was accomplished by elevating the biliary catheter on a post attached to each perfusion platform to give an obstruction to biliary flow equal to 6 cm H_2O. Four groups of animals were then measured (N = 8 each group), two groups without piperacillin, with or without obstruction. Two additional groups with 50 μg of piperacillin with or without obstruction were then completed.

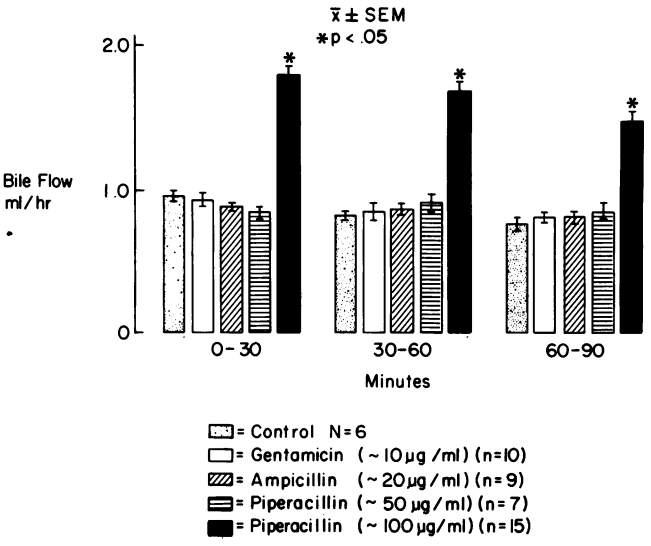


FIG. 3. The effect of antibiotic on bile flow (60 μM of taurocholate): gentamicin, ampicillin, and piperacillin (50 μg/mL) result in stable bile flows that are not significantly different from each other over all three periods of perfusion. Piperacillin (100 μg/mL) results in significantly increased bile flow in each period.

Results

IPRL Bile Flow, Bile Salt Output, and Histology

During 90 minutes of perfusion, bile flow and bile salt output remained constant when either 60 or 240 μM of taurocholate was added to the perfusate. Mean ± SEM bile flow was 0.85 ± 0.03 mL/h and 1.35 ± 0.10 mL/h when 60 or 240 μM of taurocholate was used, respectively. Mean ± SEM bile salt output increased from 26.8 ± 0.8 to 115.2 ± 2.1 μM/h when 60 or 240 μM of taurocholate was used in the perfusate. Bile flow plotted against bile salt output when the perfusate contained varying concentrations of ^{14}C-labeled taurocholate is graphically depicted in Figure 2. Bile flow (mL/h) was calculated to be equal to $6.6 \times 10^{-3} \times$ bile salt output (μM/h) + 0.57, with a correlation coefficient of 0.96. Partial pressures of oxygen

were stable over 90 minutes, averaging more than 200 mmHg inflow and 6 mmHg outflow over all three 30-minute intervals. The liver sections showed minimal histologic evidence of ischemic damage, consistent with our previous experience,[15] after 90 minutes of perfusion in both nonobstructed and obstructed livers.

The Effect of Piperacillin, Ampicillin, and Gentamicin on Bile Flow and Bile Salt Output

Figures 3 and 4 demonstrate the effect of varying antibiotics on bile flow or bile salt output. Piperacillin (50 μg/mL), gentamicin (20 μg/mL), and ampicillin (20 μg/mL) all resulted in similar bile flow and bile salt output. Stable inflow concentrations suggested a lack of binding to albumin and a lack of absorption by erythrocytes. When these values were compared with controls over the same period, no statistically significant differences were noted for any period. Higher perfusate piperacillin concentrations (100 μg/mL), resulted in bile flows that varied from 1.79 ± 0.07 to 1.46 ± 0.07 mL/h (N = 15). These were significantly higher statistically ($p < 0.001$) than bile flows in control and lower concentration piperacillin (50 μg/mL) perfusate. Bile salt output was unchanged at the higher concentration of piperacillin, suggesting enhancement of bile salt independent bile flow.

The Effect of Altering Bile Salt Output on Antibiotic Excretion

By increasing the perfusate concentration of taurocholate from 60 to 240 μM, we could increase bile salt output almost fourfold. However, when the output of ampicillin given at 20 μg/mL was calculated, we found ampicillin output to be unrelated to bile salt output. Ampicillin output averaged 73 ± 7 μg/h *versus* 74 ± 12 μg/h when 60 or 240 μM of taurocholate, respectively, was added to the perfusate. Similarly, no significant alteration in piperacillin output occurred when piperacillin was given at 100 μg/mL, 10 ± 1 *versus* 11 ± 2 mg/h.

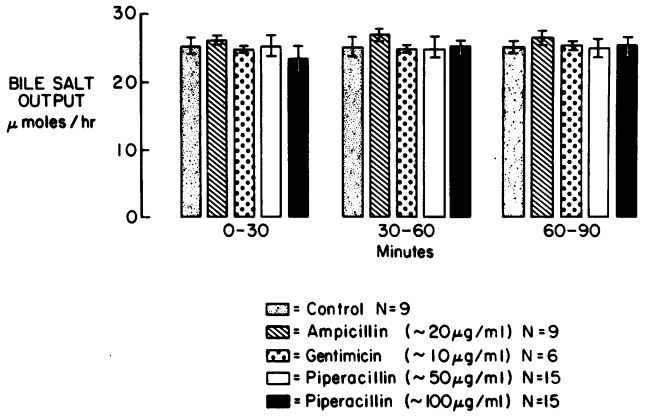
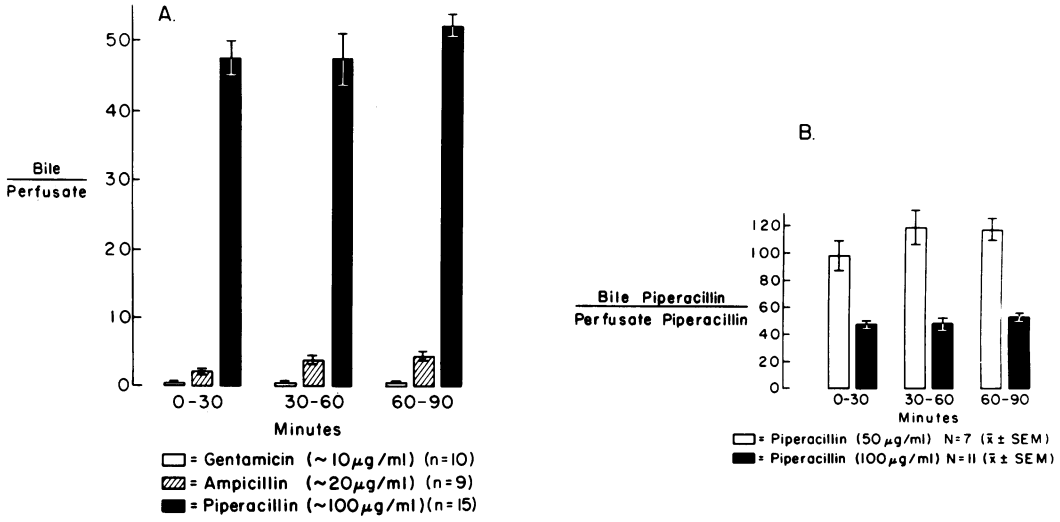


FIG. 4. Bile salt output as a function of antibiotic (6 μM of taurocholate) comparing ampicillin (20 μg/mL), gentamicin (10 μg/mL), and piperacillin (50 μg/mL and 100 μg/mL): stable bile salt output is demonstrated in each 30-minute period over the entire 90-minute perfusion. (Mean ± SEM.)

Biliary and Perfusate Antibiotic Concentrations

Figure 5 represents the bile to perfusate antibiotic concentration ratio in the presence of 60 μM of taurocholate. Gentamicin ratios varied from 0.32 ± 0.06 to 0.59 ± 0.10 during the 90-minute perfusion period. Ampicillin ratios varied from 2.07 ± 0.03 to 4.37 ± 0.55. The piperacillin bile to perfusate ratios were significantly greater. Piperacillin (50 μg/mL) ratios varied from 99 ± 11 to 117 ± 7, and piperacillin (100 μg/mL) ratios varied from 47 ± 2.2 to 52 ± 1.6. Absolute piperacillin concentrations in bile were nearly identical when either 50 or 100 μg/mL of piperacillin was used in the perfusate (Fig. 6).



FIGS. 5A and B. *A.* Bile/serum antibiotic concentration: the bile/serum ratio for piperacillin (100 μg/mL) shows ratios far in excess of those of gentamicin and ampicillin, suggesting maximal concentration. *B.* Bile piperacillin/serum piperacillin ratios suggest that piperacillin is maximally concentrated in the bile at 50 μg/mL. (Mean ± SEM.)

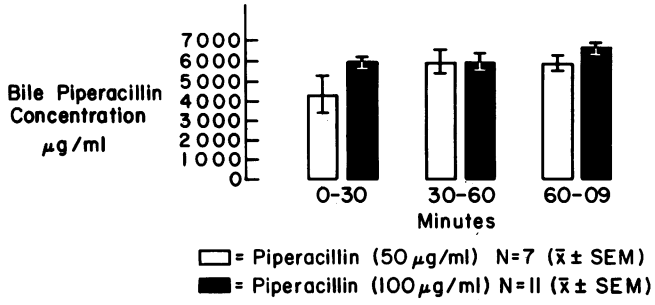


FIG. 6. Bile piperacillin concentrations for 50 μg/mL perfusate and 100 μg/mL perfusate were stable and not statistically different from each other in each of the 30-minute periods.

Hepatic Uptake and Excretion of Piperacillin (50 or 100 μg/mL) as a Function of Concentration and Delivery

When 50 or 100 μg/mL of piperacillin was added to the perfusate, the hepatic extraction of piperacillin averaged about 50% of the delivered dose (Fig. 7). In each case, only one third of the piperacillin taken up by the liver was excreted in a biologically active form; this varied little over 90 minutes of perfusion. No metabolites of piperacillin were found in bile by gas liquid chromatography, and the levels measured by bioassay were equal to those determined by chromatography. Since the proportion of delivered piperacillin taken up or excreted by hepatocytes was independent of perfusate piperacillin concentrations, it is likely that the variations observed in the ratio of bile to perfusate piperacillin concentration depended on the choleretic effect of piperacillin at higher doses.

Single-pass uptake of gentamicin and ampicillin by the liver was low in these experiments, and differences in inflow concentrations were too small to measure. For this reason, extraction and excretion data for these antibiotics could not be calculated, and only the single doses of gentamicin and ampicillin were studied.

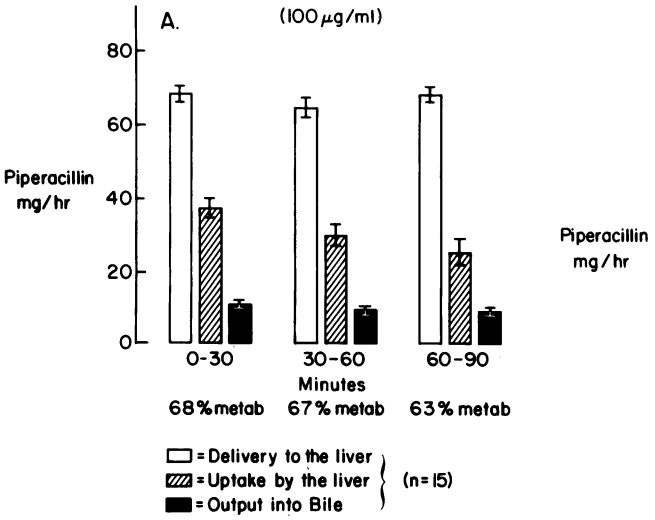
The Effect of Obstruction on Bile Flow, Bile Salt Output, and Bile Piperacillin Concentration

Figure 8 shows the effect of obstruction on bile flow with and without piperacillin (50 μg/mL) (N = 8 all groups). With no piperacillin, 6 cm H_2O obstruction results in a 50% decrease in bile flow in the first 30-minute period (0.88 ± 0.03 *vs.* 0.47 ± 0.05), which persists through 90 minutes of perfusion. Interestingly, when piperacillin is added, bile flow values approach those of the unobstructed state.

Figure 9 shows the effect of obstruction on bile salt output. Without piperacillin, obstruction results in a significant decrease in bile salt output in the first 30 minutes (26.4 ± 0.5 *vs.* 20.0 ± 2.5 μM/h), which persists over the 90-minute perfusion. With piperacillin, obstruction results in a slowly declining bile salt output that becomes significantly reduced by the last 30 minutes. Figure 10 shows that partial obstruction results in significantly decreased bile piperacillin levels in the first 30 minutes (5100 ± 413 *vs.* 4412 + 382 μg/mL), which persists for the 90-minute perfusion.

Discussion

Piperacillin is a penicillin derivative with broad-spectrum activity against Enterobacteriaceae, enterococci, and anaerobes. Its activity, along with high reported biliary levels, has prompted its recent clinical use in treating biliary infections[8,9,12] with encouraging early results. Although its main source of excretion is renal, the levels in bile greatly exceed the simultaneous serum concentrations.[8,12,25] Our preliminary findings agree with these reports.[26] Although the clinical relevance of obtaining ad-



FIGS. 7A and B. *A.* Piperacillin delivery to, uptake, and excretion by the perfused rat liver: piperacillin at 100 μg/mL in the perfusate demonstrates a 63–68% metabolism by the liver. The hepatic extraction of piperacillin averaged about 50% of the delivered dose. *B.* The same approximate numbers exist when piperacillin is perfused at 50 μg/mL in the perfused liver; the perfused liver appears to metabolize 60–65% of the antibiotic. (Mean ± SEM.)

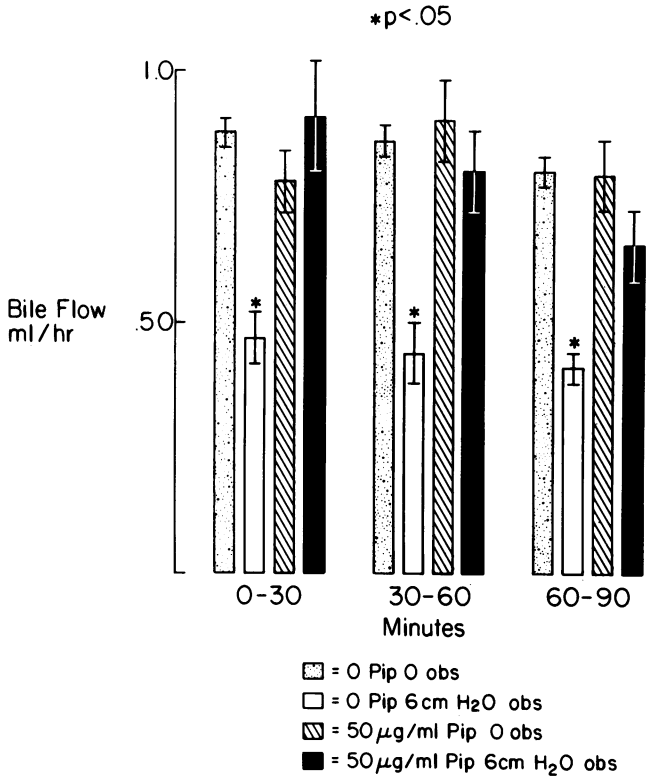


FIG. 8. The effect of piperacillin and obstruction on bile flow. Without piperacillin, 6 cm H_20 obstruction causes approximately 50% reduction in bile flow. When 50 μg/mL of piperacillin is added, bile flow is increased in obstruction to values that compare with unobstructed controls in all three periods.

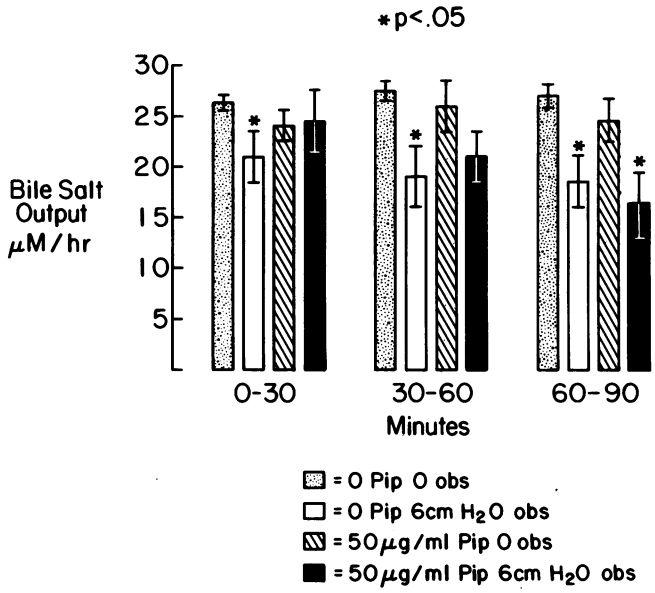


FIG. 9. Effect of piperacillin and obstruction on bile salt output. Without piperacillin, obstruction causes a significant decrease in bile salt output. With piperacillin and obstruction, there is an initially normal bile salt output that falls significantly by 60–90 minutes.

equate biliary levels of antibiotics in biliary infections is controversial,[27,28] analogies in other clinical settings, such as urinary tract infections[29] and disorders of the central nervous system,[30,31] suggest the importance of maintaining adequate antibiotic concentrations in body fluids. Indirect evidence suggests that antibiotics with high biliary penetration more rapidly eliminate the septic focus from bile.[25,32] Although most antibiotic concentrations are reduced in bile by the presence of biliary obstruction,[9,33–37] cholangitis most often occurs in the setting of partial rather than complete obstruction,[28] and antibiotics achieving high penetration into bile are more likely also to achieve therapeutic concentrations during partial obstruction.[34] We believe, our studies are unique because of the finding that piperacillin, at a relevant clinical dose, significantly alters bile flow in both obstructed and nonobstructed states.

Study of the physiology of hepatic excretion of antibiotics has clinical and physiologic relevance.[1–3] The pharmacokinetics of hepatic antibiotic excretion, even in the absence of biliary or renal disease, are not well understood.[38,39] Most data come from models in which bile salt depletion occurs secondary to biliary fistula, and antibiotic excretion and bile flow and composition vary. Using the IPRL, we could stabilize bile salt output, oxygen consumption, portal flow, and antibiotic delivery to the liver. Over the 90 minutes of perfusion, we maintained these parameters in a closely controlled range. We found that piperacillin is highly concentrated in bile, exerts a choleretic effect not related to alteration in bile salt output,

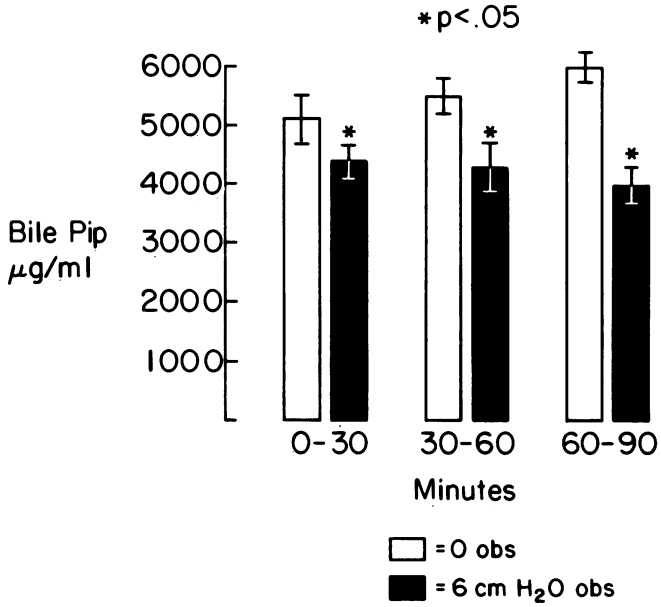


FIG. 10. Effect of obstruction on bile piperacillin concentration. Obstruction is associated with biliary concentration of greater than 4000 μg/mL for 90 minutes. This is significantly less than unobstructed controls.

and has an excretion little altered by variations in bile flow mediated by changes in bile salt output. With two concentrations of piperacillin in the perfusate, the ratio of delivery to uptake or excretion appeared constant. *In vivo* studies are the obvious next step in the evaluation of these *in vitro* findings.

The mechanism of antibiotic transport into bile is largely unknown. Concentrations of antibiotics in bile have been shown to exceed serum concentrations both *in vivo*[9,33-37] and in isolated perfused liver preparations.[4,5,40] The mechanisms of transport of other anions have likewise been controversial. Organic compounds are believed to enter bile both *via* transcellular routes across sinusoidal and canalicular membranes and *via* a paracellular route across tight junctions between hepatocytes. Organic anions appear to be restricted in movement across tight junctions, however, and excretion is almost entirely transcellular.[41,42] Bile salts, which are anionic compounds, appear to be actively transported *via* a carrier-mediated protein, which may depend at least in part on NaK-ATPase. Whether other anions are transported *via* similar mechanisms is controversial.[43] Our system showed that bile salt output was not decreased by piperacillin excretion and, conversely, that piperacillin output was unaltered by increased taurocholate output. A noncompetitive interaction between bile salts and organic ions has been shown in some studies[44] but not in others.[45] Our system showed a similar lack of interaciton between ampicillin output and bile salt excretion. This contradicts the data of Mandiola et al., whereby infusions of increasing doses of sodium taurocholate in dogs was associated with increased biliary ampicillin output.[6]

The mechanism and clinical applicability of the choleretic effect of 100 μg/mL, but not 50 μg/mL, concentrations of piperacillin remains to be determined. Analysis of bile piperacillin output suggests that the choleretic effect of piperacillin does not depend solely on its osmotic effect in bile. Since hepatic bile is isosmotic with serum, piperacillin can draw only about 1 kg of water per 300 mOsm secreted. Given a molecular weight of 540 daltons, the maximum choleretic response of 100 μg/mL of piperacillin would be 0.13 mL/h, which is much less than the 0.70 mL/h observed. By calculating the osmotic effect exerted by piperacillin output, we were unable to explain the marked increase in bile flow observed. This is not surprising, in that the choleretic effect of infused bile salts, which are also anions, is not primarily related to their osmotic effect in bile, but rather to the simultaneous transport of inorganic ions.[46] The absence of other osmotically demonstrated metabolites by chromatography may be explained by the cotransport of other inorganic ions that accompanied piperacillin excretion. We used gas liquid chromatography to help us understand hepatic piperacillin excretion, and the results give only partial conclusions. It appears that only approximately one third of the piperacillin extracted by the liver appeared in the bile ostensibly in a biologically active form. We did not prove this conclusively by gas liquid chromatographic analysis of venous effluent or hepatic parenchyma, and those studies could help elucidate the metabolism more exactly. It is clear, however, that approximately two thirds of the antibiotic is removed by the liver in the IPRL and the remaining one third appears in bile in high concentrations with an associated effect on bile flow.

We realize that the use of the IPRL begs the important question about similarities between hepatic metabolism in rats and humans. We have reported here and in preliminary reports[26] that we achieved similar bile concentrations of piperacillin that occur in humans.[9] We further realize that extrapolations about increased biliary flow can only be suggestive and grounds for clinical studies because of differences in our *ex vivo* preparation including such "nonphysiologic" entities as low hematocrit perfusate and occlusion of the hepatic artery.

Of additional clinical relevance is that cholangitis often occurs in the face of at least partial biliary obstruction. We believe our studies show a unique response to antibiotic excretion during partial obstruction in that high biliary concentrations and altered biliary flow occur. Previous studies with ampicillin suggested that antibiotics had no such effect.[6] Therefore, the choice of a newer, more highly penetrant biliary antibiotic mandates a careful clinical decision in the face of partial obstruction.

In conclusion, we have used a nonrecirculating IPRL model that allowed stable bile flow, bile salt output, and O_2 consumption over 90 minutes, to study biliary antibiotic excretion. Commonly used concentrations of gentamicin and ampicillin did not alter flow, but 100 μg/mL concentrations of piperacillin resulted in significant increases in bile flow and a lower dose altered biliary flow in which partial biliary obstruction existed. Alterations in bile salt output did not affect ampicillin or piperacillin output; conversely, none of the antibiotics, *per se,* studied altered bile salt output. This model can be used to compare bile to serum antibiotic concentration ratios, serum extraction fractions, and bile output for a variety of antibiotics at different concentrations in an otherwise equivalent environment. Piperacillin undergoes much higher excretion into bile than does ampicillin or gentamicin and achieves levels many times higher than concomitant serum concentrations.

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