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Research Article

In Vivo Microdialysis Sampling in the Bile, Blood, and Liver of Rats to Study the Disposition of Phenol

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Methods for continuous *in vivo* sampling in the bile, blood, and liver extracellular fluid are described. These methods are based on microdialysis sampling in anesthetized rats. A new flow-through microdialysis probe is described for sampling bile while maintaining normal bile flow. All three sites are simultaneously and continuously sampled to provide concentration–time profiles at multiple sites in a single experimental animal. This technique is demonstrated by studying the hepatic metabolism and biliary excretion of phenol in rats. Following an i.v. infusion of phenol, the major hepatic metabolite was found to be phenyl-glucuronide. Hydroquinone and 2-glutathionyl–hydroquinone were also detected but at lower concentrations. A similar pattern of metabolites was found in the bile and blood. For all of the metabolites, bile concentrations are higher than liver concentrations, indicating that the metabolites are actively excreted into the bile.

KEY WORDS: microdialysis; bile sampling; disposition studies; *in vivo* sampling.

INTRODUCTION

Microdialysis sampling is a technique originally developed for following neurotransmitter release in the brain (1,2). The neurochemical applications have recently been reviewed (3). More recently, microdialysis sampling has been expanded to other sites including the blood (4–7), liver (5,8), adipose tissue (9,10), eye (11), and muscle (12). These reports have also extended the technique's application to pharmacokinetic and metabolic investigations in addition to the established neurochemical applications. Microdialysis sampling is a promising technique for absorption, distribution, metabolism, and excretion (ADME) studies because metabolic events can be followed *in vivo* simultaneously in a variety of tissues and fluids without the need for actually removing samples of those tissues and fluids (13).

Microdialysis sampling is accomplished by implanting a short length of dialysis fiber at the site of interest. This fiber is slowly perfused with a sampling solution. Small molecules in the sample diffuse through the dialysis membrane into the sampling solution. The sampling solution flows through the dialysis probe and into a collection vessel for subsequent analysis. While microdialysis sampling is an invasive technique in that a probe must be inserted into the experimental animal, perturbations to the physiology of the organism are minimized relative to the removal of tissue or fluid samples.

This report describes the initial evaluation of the use of *in vivo* microdialysis for the study of hepatic metabolism. The metabolism of phenol was used as a test system for microdialysis sampling in the liver and bile of living animals. Sampling of the liver extracellular fluid and of the blood was performed with a previously described flexible microdialysis probe (7). Sampling of the bile was performed with a new flow-through microdialysis probe. Localized hepatic metabolism was studied by directly administering phenol to the liver through the dialysis probe. Multiple compartment profiling was done by administering phenol i.v. Microdialysis sampling provided concentration–time profiles for phenol and its metabolites at multiple, simultaneously sampled sites in a single experimental animal.

MATERIALS AND METHODS

Chemicals. Hydroquinone, phenol, glutathione, benzoquinone, and phenyl-glucuronide were purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of reagent grade or better and were used as received.

Microdialysis Probe Construction. Microdialysis probes were fabricated using regenerated cellulose dialysis fibers of 232-μm i.d and 250-μm o.d. (Dow Chemical Corporation, Midland, MI). These fibers have a molecular weight cutoff of 5000. The flexible microdialysis probe was constructed, as previously described (7), by inserting two pieces of fused silica tubing, 75-μm i.d. and 147-μm o.d. (Polymicro Technologies Inc., Phoenix, AZ), into a length (less than 5 mm) of polyethylene tubing, 0.28-mm i.d. and 0.61-mm o.d. One of the pieces of fused silica was inserted into but not through the polyethylene tubing. The other piece of fused silica was inserted through the polyethylene tubing such that 5 mm was exposed. The exposed piece of fused silica tubing was covered with the dialysis fiber, which was then attached to the polyethylene tubing with UV curable adhesive (UVEXS, Inc., Sunnyvale, CA). The other end of the dialysis fiber was sealed with the epoxy.

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Bile Microdialysis Probe Construction. The flow-through microdialysis probe (Fig. 1) for use in the common bile duct was constructed by inserting a piece of fused silica tubing into both ends of a 7-cm length of dialysis tubing and sealing them into place using UV curing adhesive. The dialysis tubing was then inserted into a 6-cm piece of polyethylene tubing (PE-50; 580-μm i.d × 965-μm o.d.). To the ends of the PE-50 tubing were connected 5-cm lengths of PE-10 tubing (280-μm i.d., 610-μm o.d.) and the connections were sealed with UV-curable adhesive and cured under UV light for ca. 1 min. Hot-melt glue was used to make O-rings 5 mm from both ends of the PE-10 tubing. These O-rings were used to secure the probe once ligated into the bile duct.

In Vivo *Metabolism Experiments.* Male Sprague–Dawley rats weighing between 300 and 400 g were used in all experiments. The rats were anesthetized using the inhalation anesthetic isoflurane. An i.v. cannula (PE-10 tubing) was inserted into the right external jugular vein. When blood sampling was conducted a flexible microdialysis probe was also inserted into the right jugular vein alongside the cannula as described previously (7). The liver was exposed by making a midline incision beginning at the xiphoid cartilage and extending for ca. 6 cm. The liver was moved aside and the bile duct was isolated from the surrounding tissues using jeweler's forceps. The duct was then ligated with silk suture and placed under tension using artery forceps. A small V-shaped cut was made in the bile duct, approximately 1 cm from where it joins the liver, and one end of the flow-through microdialysis probe inserted through the proximal cut toward the liver. The first end of the probe was then ligated into place on either side of the O-ring. By ligating the probe into the bile duct on either side of an O-ring, the probe will not slip out of the bile duct. The dialysis probe was allowed to fill with bile and the second end of the probe was inserted into the distal part of the bile duct through a second V-shaped cut. The distal end of the dialysis probe was ligated into the bile duct in the same manner as before.

For comparison of whole bile to bile dialysate, the distal end of the dialysis probe was placed into a microcentrifuge tube to collect bile samples. Liver tissue sampling was conducted by making a small hole in the median lobe of the liver using a 25-G syringe needle. A flexible microdialysis probe was then inserted into the liver through the hole made by the needle. After insertion of the microdialysis probe, the incision was closed using tissue staples. The microdialysis probe was perfused with Ringer's solution at a flow rate of 1 μL/minute and samples of the dialysate were collected over 10-min intervals. Blank dialysate samples were collected for 1 h, after which phenol was administered to the animal. Dialysate samples were continuously collected over 10-min intervals for 2.5 hr after administration of phenol.

To examination systemic administration, a phenol solution was infused into the jugular vein cannula at a rate of 941 μg/min (10 μmol/min) for 20 min (19 mg delivered). To examine direct administration into the liver, phenol was delivered directly by the hepatic dialysis probe. This probe was perfused with a 500 μ*M* phenol solution for 90 min. This resulted in a constant delivery of 0.181 nmol/min into the tissue and 16 μmol delivered. The rate of phenol delivery was determined by comparison of residual phenol in the dialysate to the initial phenol concentration in the perfusate.

After collection, dialysate samples were analyzed by liquid chromatography. Dialysate samples from the liver microdialysis probe were analyzed immediately after they were collected. Bile samples, bile dialysate, and blood dialysate samples were immediately frozen on dry ice and kept frozen until they were analyzed to avoid decomposition of the analytes. All dialysis samples were injected directly into the chromatographic system without cleanup.

Microdialysis Probe Calibration. It is necessary to determine the relationship of the concentration in the dialysate to the concentration in the sample. The recovery of a dialysis probe is defined as the ratio of the dialysate concentration to the sample concentration as a function of the perfusion rate, temperature, probe geometry, sample matrix, and analyte. Recoveries of all dialysis probes were determined from Ringer's solution consisting of 155 m*M* NaCl, 5.5 m*M* KCl, and 2.3 m*M* $CaCl_2$. Dialysis probes were perfused at a rate of 1 μL/min for all experiments. The average recovery of phenol, phenyl-glucuronide, hydroquinone, and 2-glutathionyl–hydroquinone was 30.9 ± 2.5, 16.4 ± 2.6, 38.4 ± 1.3, and 11.4 ± 3.1%, respectively, through flexible microdialysis probes. The recovery of each dialysis probe was determined both before and after implantation. The recovery determined after the implantation was used to calculate *in vivo* concentrations, although the two recoveries never differed by more than 5% relative. The relative recovery for phenol, phenyl-glucuronide, hydroquinone, and 2-glutathionyl–hydroquinone through the flow-through microdialysis probe was determined to be 94.4 ± 0.8, 89.5 ± 1.5, 101.8 ± 2.6, and 75.2 ± 3.8%, respectively. Recoveries were determined by perfusing Ringer's solution through the dialysis fiber at a flow rate of 1 μL/min and pumping standard solutions of analyte in Ringer's solution through the outer cannula at a flow rate of 50 μL/min, which approximates bile flow.

Chromatographic Analysis. Separation of phenol and its metabolites was achieved using a liquid chromatographic system consisting of a PM-60 LC pump, a LC-4B dual-electrode electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, IN), and a SPD-6AV variable-wavelength UV/vis detector (Shimadzu Scientific Instruments, Inc., Columbia, MD) operated at a wavelength of 220 nm for

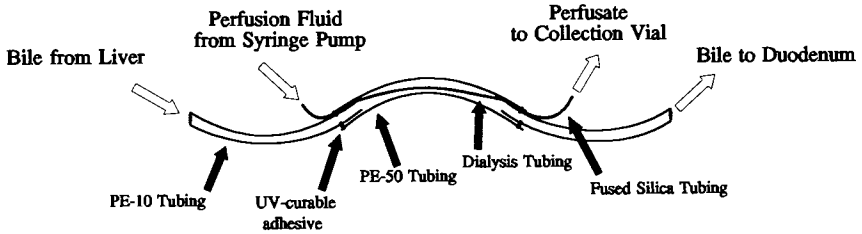

Fig. 1. Flow-through microdialysis probe used for bile sampling.

all experiments. The electrochemical detector was operated in the series configuration using glassy carbon electrodes. The upstream electrode was operated at a potential of +700 mV versus Ag/AgCl and the downstream electrode was operated at 0.0 V. The separation was achieved using a Sep Stick 3-μm (1.0 × 100-mm) ODS column (Bioanalytical Systems, Inc.). The separation conditions included a mobile phase of 0.05 *M* ammonium phosphate buffer (pH 2.5) with 5% (v/v) acetonitrile, a flow rate of 100 μL/min, and an injection volume of 1 μL. All dialysis samples were directly injected into the chromatograph without pretreatment. Detection limits using electrochemical detection were 50 n*M* for hydroquinone and 20 n*M* for HQ-SG. Detection limits using UV detection were 1.0 μ*M* phenol and 1.5 μ*M* phenyl-glucuronide.

UV spectra of phenol and its metabolites in bile dialysate samples were obtained using an LC system consisting of a LC-6A pump (Shimadzu Scientific Instruments) and a PF-1 diode array detector (Groton Technology, Inc., Groton, CT). The separation was achieved using a Hypersil 5-μm ODS column (4.6 × 150 mm), a mobile phase of 0.05 *M* ammonium phosphate buffer (pH 2.5) with 10% (v/v) acetonitrile, and a flow rate of 1 mL/min.

Preparation of Hydroquinone–Glutathione Adducts. A mixture of 5 mmol 1,4-benzoquinone in 100 mL THF was added dropwise to a mixture of 5 mmol glutathione in 100 mL water, with constant stirring. The reaction was evaporated *in vacuo* at 40°C to a volume of about 10 mL, to which was added 50 mL of acetone. The solution was cooled to 5°C for 12 hr, at which time large crystals had formed. The crystals were filtered, then washed with cold acetone, and the residual acetone evaporated *in vacuo* at room temperature. The yield obtained using this procedure was 90.5%. The structure of the product was confirmed by FAB-MS and by [^{1}H] and [^{13}C] NMR in D_2O. Multiple hydroquinone glutathione adducts were prepared using 2-glutathionyl–hydroquinone as the starting material. To 1 mmol 2-glutathionyl–hydroquinone in water was added 2 mmol 1,4-benzoquinone in THF. The hydroquinone formed in the reaction and the excess benzoquinone were extracted with ethyl acetate and 5 mmol glutathione was added to the solution. The resulting solution was rotary evaporated to about 10% of its original volume. The hydroquinone–glutathione adducts were isolated from the mixture using semipreparative liquid chromatography. The chromatographic conditions included a mobile phase of water with 10% (v/v) acetonitrile and a YMC Pack ODS column (10 × 300 mm). The glutathione adducts were detected using a SPD-6AV UV/vis detector (Shimadzu Scientific Instruments, Inc.) operated at a wavelength of 220 nm. Fractions which contained conjugates were collected and lyophilized. Structures of the conjugates were confirmed using ^{13}C and ^{1}H NMR (in D_2O), FAB mass spectrometry, and UV spectroscopy. Hydroquinone–cysteine and hydroquinone–NAC adducts were prepared in a similar manner.

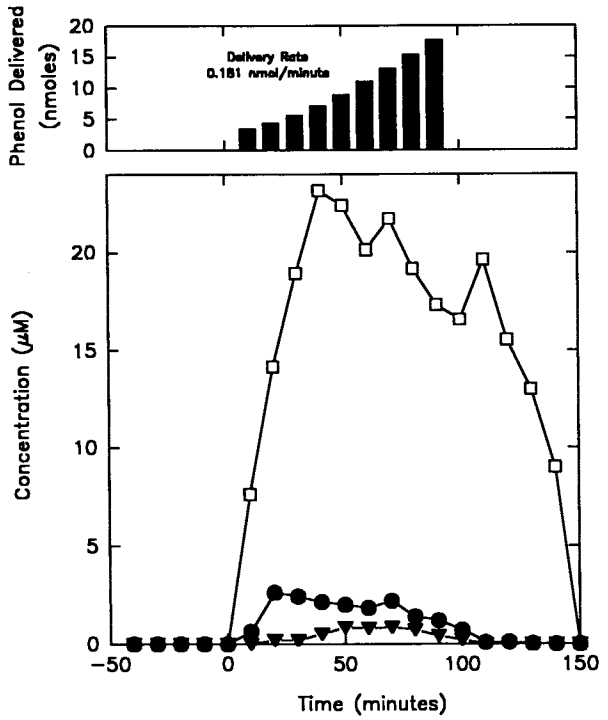

Fig. 2. Phenol metabolism in liver tissue during localized delivery of phenol through the microdialysis probe. (□) Phenyl-glucuronide; (●) hydroquinone; and (▼) 2-glutathionyl–hydroquinone.

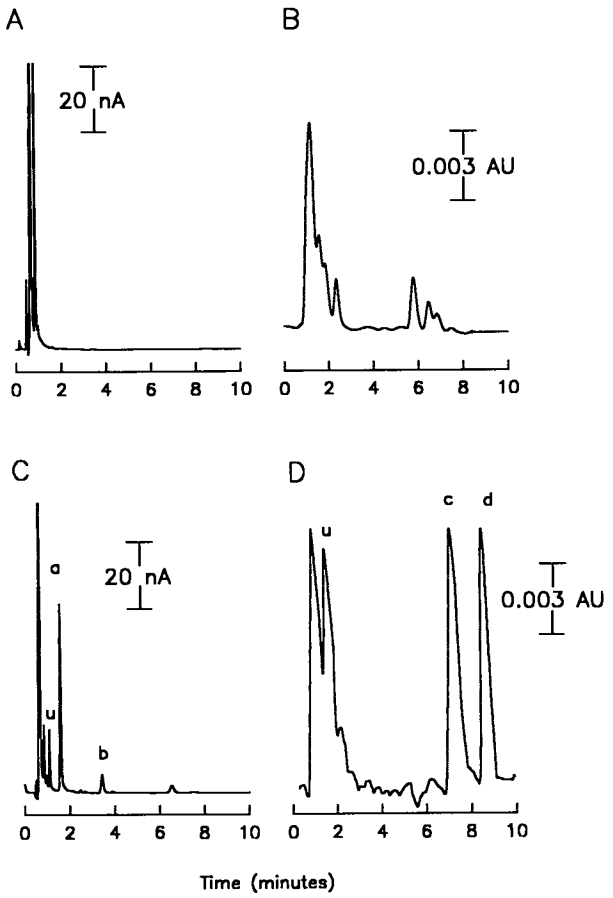

Fig. 3. Chromatograms of liver dialysate. Prior to dosing using electrochemical (A) and UV detection (B) and after dosing with phenol using electrochemical (C) and UV detection (D). Peaks are (a) hydroquinone, (b) 2-glutathionyl–hydroquinone, (c) phenyl-glucuronide, (d) phenol, and (u) unidentified.

RESULTS AND DISCUSSION

Direct Administration of Phenol Through the Dialysis Probe. Following implantation of a flow-through microdialysis probe into the bile duct and a flexible microdialysis probe into the liver, the flexible probe in the liver was perfused with a Ringer's solution which contained 500 μ*M* phenol as described above. Hydroquinone, 2-glutathionyl–hydroquinone, and phenyl-glucuronide were detected in the liver dialysate, while the microdialysis probe was perfused with phenol (Fig. 2), however no metabolites were seen in the bile dialysate. Phenol is delivered to the tissue only directly at the site of the microdialysis probe. The concentration of phenol drops very rapidly as the distance from the probe increases. While the concentration of phenol at the dialysis probe is high (ca. 200 μ*M*), the total amount delivered to the liver as a whole is very small. As a consequence, metabolite concentrations are high near the probe and decrease to zero concentration a short distance (ca. 1 to 2 mm) from the probe. No phenol or phenol metabolites were detected at a second microdialysis probe implanted 5 mm from the delivery probe and perfused with only Ringer's solution. No metabolites are detected in the bile during direct infusion into the liver because of the large dilution effect. Even if all of the phenol delivered to the liver were excreted in the bile, total bile phenol metabolites would only be a few picomoles per milliliter.

Using the microdialysis probe to deliver a compound into the tissue, metabolism can be investigated in localized areas without contribution from other tissues. Although metabolism in specific regions can be studied by delivering a compound through the microdialysis probe, the routes of elimination from the tissue cannot be studied due to the low concentrations encountered away from the environment around the probe.

Intravenous Infusion of Phenol. After implanting a dialysis probe into the liver and allowing the system to recover for an hour, collection of dialysis blanks was started. Figures 3A and B show chromatograms of a blank dialysate sample from the liver using electrochemical and UV detection respectively. After the blank dialysate samples were collected, infusion with phenol was started at a rate of 10 μmol/min and dialysate samples were continued to be collected over 10-min intervals. Figures 3C and D are chromatograms of liver dialysate collected 100 min following the start (80 min after completion) of infusion with phenol. One major metabolite is apparent in the EC chromatogram (Fig. 3C) and two are apparent in the chromatogram using UV detection (Fig. 3D). The major metabolite observed in the EC chromatogram (peak a) was determined to be hydroquinone. A minor metabolite in the EC chromatogram (peak b) has been determined to be 2-glutathionyl–hydroquinone. The metabolites observed in the UV chromatogram (peaks c and d) were determined to be phenyl-glucuronide and phenol, respectively. All metabolite identities were confirmed by coelution with authentic standards, comparison of UV spectra, and their electrochemical characteristics.

The UV spectra of standards and of the metabolites

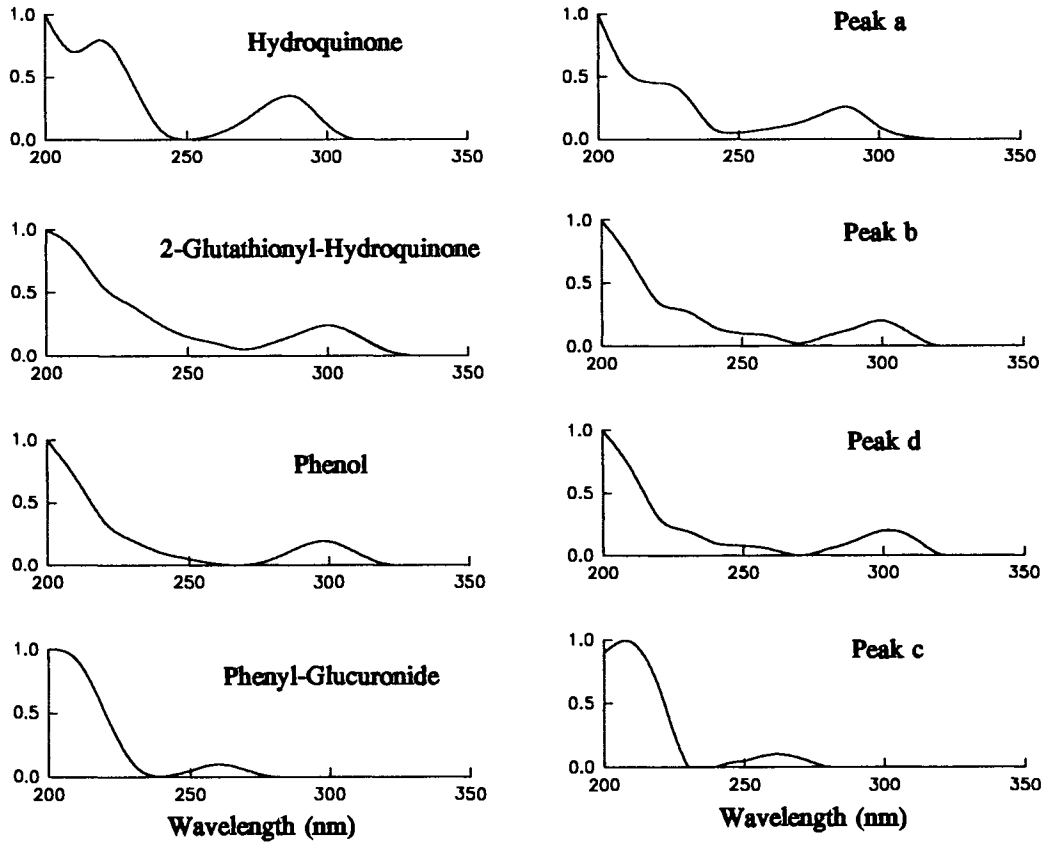

Fig. 4. UV spectra of standards and phenol metabolites detected in bile.

Table I. Current Ratios of Metabolites

	Current ratio[a] (E_2/E_1)[b]		
	0/+500 mV	0/+1100 mV	−200/+1100 mV
Hydroquinone	0.71	0.72	0.76
Peak a	0.71	0.73	0.75
$HQ(SG)_1$	0.57	0.53	0.55
Peak b	0.55	0.54	0.57
Phenol	ND[c]	0.11	0.16
Peak d	ND	0.09	0.16

[a] Ratios are downstream electrode response/upstream electrode response.
[b] Potentials are expressed as millivolts versus Ag/AgCl.
[c] Not detected.

observed after infusion with phenol are shown in Fig. 4. It can be seen that the spectra of the standards compare well with the spectra of the metabolites. The electroactive metabolites were further characterized by their electrochemical behavior. Both their oxidation potential and their chemical reversibility were assessed (Table I). Because of their quinone structure, both hydroquinone and 2-glutathionyl–hydroquinone are much easier to oxidize and exhibit much higher degrees of reversibility than phenol. Phenyl-glucuronide was not observed in the EC chromatograms due to its high oxidation potential. This is consistent with the structure of phenyl-glucuronide since there are no easily oxidizable groups. An unidentified metabolite (peak u) is also seen in both chromatograms and appears to be present in substantial amounts. This metabolite elutes very close to the void, indicating a high hydrophilicity. It is also hard to oxidize and irreversible, indicating a phenol as opposed to quinone structure. It is speculated that this metabolite is phenyl-sulfate, which is reported to be one of the major metabolites of phenol. Unfortunately, no authentic compound was

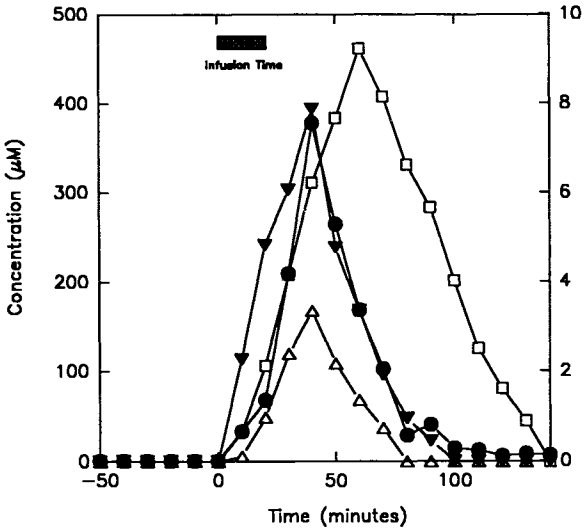

Fig. 5. Concentration–time profile of phenol and its metabolites in the liver following infusion with phenol. Left axis: (□) phenyl-glucuronide; (△) phenol. Right axis: (●) hydroquinone; (▼) 2-glutathionyl–hydroquinone.

available to confirm this assignment. The concentration–time profiles of the major metabolites found in the liver after infusing with phenol are shown in Fig. 5. It can be seen that the phenol concentration in the liver peaks at 40 min after the infusion is begun. Hydroquinone and 2-glutathionyl–hydroquinone are formed at rates which appear to be governed by the rate of distribution of phenol into the liver, i.e., their kinetics are governed by their rates of formation. The formation of phenyl-glucuronide proceeds at a rate which is less than the rate of distribution of phenol into the liver and peaks at a time of 60 min. This type of behavior indicates that the kinetics of phenyl-glucuronide is governed by its

Table II. Pharmacokinetics of Phenol and Metabolites in Bile, Blood and Liver[a]

	HQ	$HQ(SG)_1$	Phenyl-glucuronide	Phenol
		Liver		
t_p (min)	34 ± 10	36 ± 15	50 ± 10	40 ± 10
C_p (μM)	6.5 ± 0.98	11.6 ± 3.8	756 ± 161	225 ± 35
k_β (min^{-1})	0.046 ± 0.003	0.048 ± 0.003	0.024 ± 0.003	0.047 ± 0.002
		Bile		
t_p (min)	33 ± 15	46 ± 15	50 ± 10	33 ± 6
C_p (μM)	29.3 ± 9.8	132 ± 47	5801 ± 462	261 ± 27.4
k_β (min^{-1})	0.024 ± 0.003	0.033 ± 0.003	0.018 ± 0.003	0.048 ± 0.011
		Blood		
t_p (min)	35 ± 7	35 ± 7	55 ± 7	20 ± 5
C_p (μM)	6.7 ± 1.4	3.5 ± 2.2	137 ± 34	8543 ± 3534
k_β (min^{-1})	0.038 ± 0.002	0.023 ± 0.001	0.022 ± 0.001	0.083 ± 0.002
		Ratio of C_p in various compartments		
Bile/liver	5.3 ± 1.1	11.5 ± 5.4	7.9 ± 1.5	1.1 ± 0.15
Bile/blood	4.3 ± 1.0	44.2 ± 12.6	46.3 ± 15.7	0.03 ± 0.01

[a] Values are reported as mean ± standard deviation; $n = 3$. t_p is the time of maximal concentration, C_p is the maximal concentration, and k_β is the elimination rate constant.

elimination rate, i.e., its rate of formation is faster than its rate of elimination. Terminal rate constants for elimination from the liver are listed in Table II.

Biliary Elimination of Phenol Metabolites. Figure 6A is a chromatogram of dialysate blank collected from a flow-through dialysis probe implanted in the common bile duct prior to dosing. After dosing with phenol a number of metabolites are observed in the dialysate. A chromatogram of dialysate collected 100 min after beginning infusion with phenol is shown in Fig. 6B. The major metabolites which could be positively identified in the EC chromatogram are hydroquinone and 2-glutathionyl-hydroquinone. 2-Glutathionyl-hydroquinone, phenyl-glucuronide, and phenol are observed in the UV chromatogram of bile dialysate (Fig. 6C). The large, unidentified peak at 2 min is again possibly phenyl-sulfate. The concentration-time profiles for the identified metabolites in the bile and liver are shown in Fig. 7. Immediately after the start of infusion, phenol was present in the bile and reached its peak concentration after approximately 30 min. The metabolites of phenol were seen in the bile after

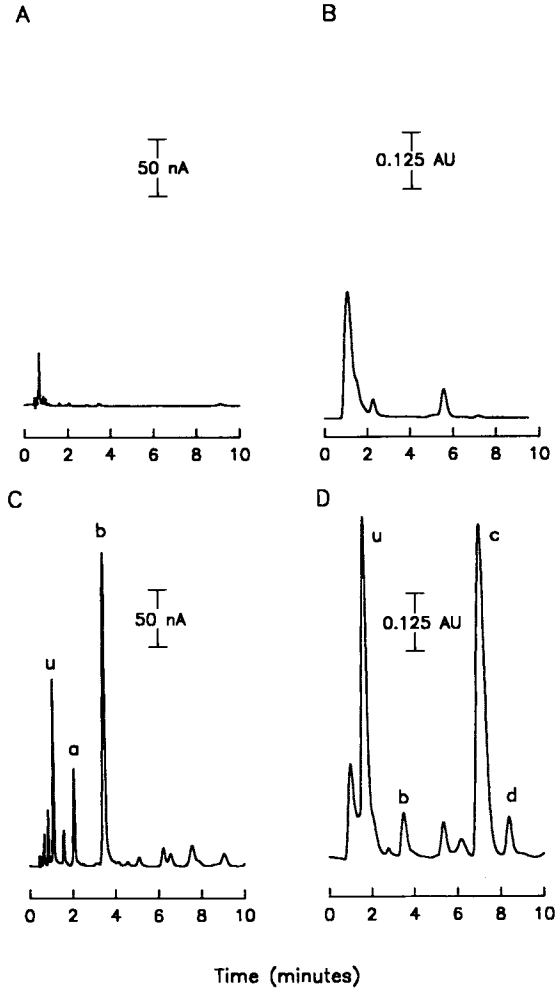

Fig. 6. Chromatograms of bile dialysate after intravenous infusion with phenol. Prior to dosing using electrochemical (A) and UV detection (B) and after dosing with phenol using electrochemical (C) and UV detection (D). Peaks are (a) hydroquinone, (b) 2-glutathionyl-hydroquinone, (c) phenyl-glucuronide, (d) phenol, and (u) unidentified.

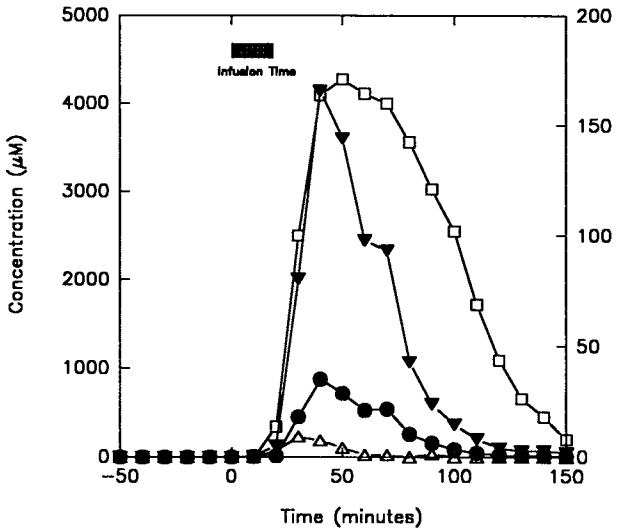

Fig. 7. Concentration-time profiles of phenol and its metabolites in the bile following infusion with phenol. Left axis: (□) phenyl-glucuronide; (△) phenol. Right axis: (●) hydroquinone; (▼) 2-glutathionyl-hydroquinone.

10 min; however, peak concentrations were not reached until 30 min for hydroquinone, 40 min for 2-glutathionyl-hydroquinone, and after 50 min for phenyl-glucuronide.

While the unidentified metabolite cannot be quantitated, the relative kinetics can still be determined. Concentration (based on chromatographic peak area) vs time profiles for this metabolite in the liver and bile are shown in Fig. 8. The terminal half-life of elimination was found to be 19.3 ± 2.6 min from the liver and 28.9 ± 3.1 min from the bile.

Intravenous Sampling. Microdialysis sampling was carried out in the jugular vein simultaneously with sampling in the liver and the bile. After implanting the microdialysis probes, the animal was allowed to recover and phenol was infused into the jugular vein. Blood dialysate chromatograms are shown in Fig. 9. Figures 9A and B are blood dialysate chromatograms prior to dosing with phenol using electro-

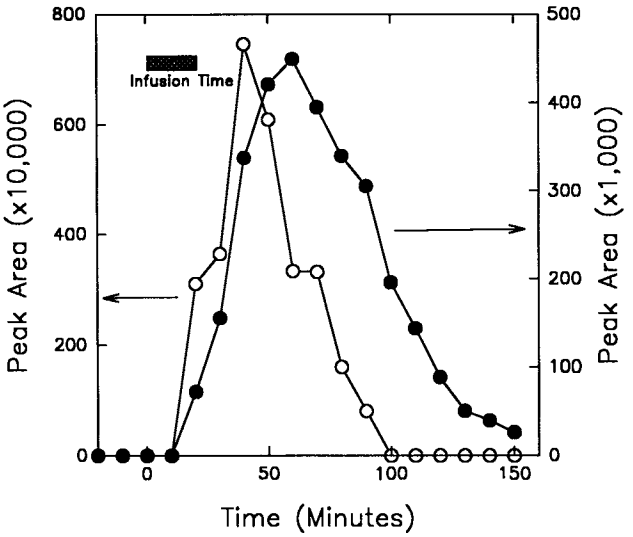

Fig. 8. Concentration-time profiles of the unidentified metabolite in liver (○) and in bile (●) based on chromatographic peak areas.

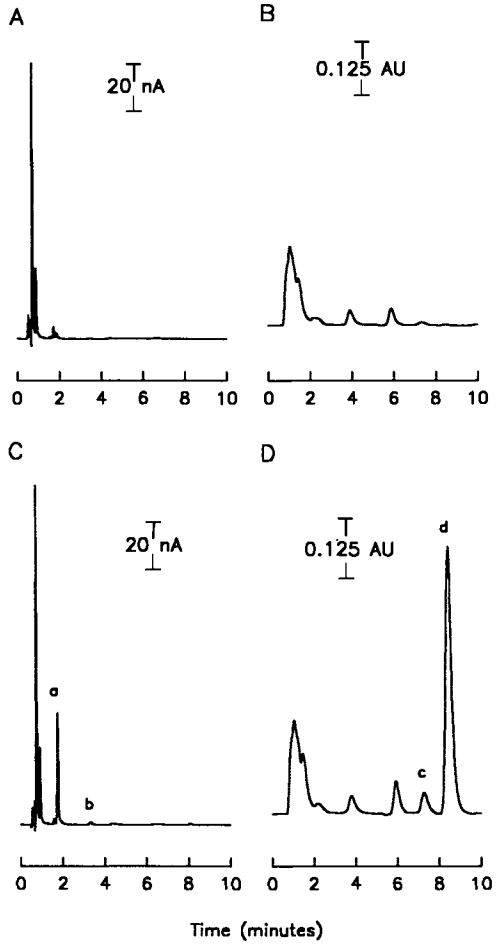

Fig. 9. Chromatograms of blood dialysate. Prior to dosing using electrochemical (A) and UV detection (B) and after dosing with phenol using electrochemical (C) and UV detection (D). Peaks are (a) hydroquinone, (b) 2-glutathionyl–hydroquinone, (c) phenyl-glucuronide, and (d) phenol.

chemical detection and UV detection respectively. Figures 9C and D are blood dialysate chromatograms collected after infusion with phenol using electrochemical detection and UV detection respectively. The major metabolite observed in the chromatogram using electrochemical detection is hydroquinone (peak a); 2-glutathionyl–hydroquinone (peak b) is also seen in very small quantities. Phenyl-glucuronide (peak c) and phenol (peak d) are observed in the UV chromatograms. The concentration–time profiles of phenol and its metabolites which were observed in the blood are shown in Fig. 10. It can be seen that the concentrations of metabolites observed in the liver and the blood were significantly less than the concentrations in the bile. Liver extracellular fluid and blood concentrations were slightly different; however, both liver and blood reached peak concentrations at the same time. The unidentified metabolite observed in the liver and bile was not detected in the blood. The peak concentration of phenol is higher in the blood than in the liver. This may be an artifact due to sampling the blood very near the site of infusion. The concentrations of 2-glutathionyl–hydroquinone and phenyl-glucuronide are significantly higher in the liver than in the blood, while the concentration

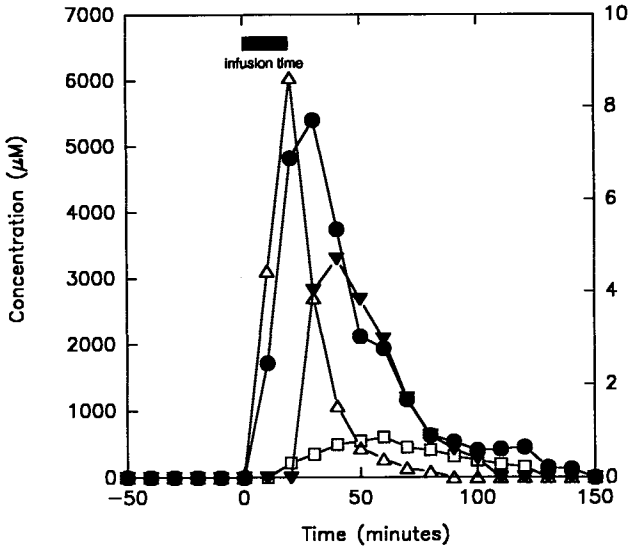

Fig. 10. Concentration–time profiles of phenol and its metabolites in the blood following infusion with phenol. Left axis: (□) phenyl-glucuronide; (△) phenol. Right axis: (●) hydroquinone; (▼) 2-glutathionyl–hydroquinone.

of hydroquinone is the same at the two sites. Since the liver is the major site of conjugation the concentrations of 2-glutathionyl–hydroquinone and phenyl-glucuronide are expected to be higher in the liver than in the blood.

Most compounds are readily excreted into the bile. A ratio of bile-to-plasma concentrations greater than unity indicates that the compound is concentrated in the bile by an active transport mechanism or by the formation of micelles in the bile. Table II gives the ratios of peak concentrations for phenol and its metabolites observed in the blood, bile, and liver after infusing with phenol. It can be seen that the bile/liver ratios of the peak concentrations are all greater than unity, indicating that these compounds are actively secreted into the bile from the liver. The bile/plasma ratios are also greater than one for all metabolites; however, since the blood concentration of phenol at the site of the probe is much higher than in the liver, the bile/plasma ratio is less than one.

Conclusions. Based on these data, it is evident that microdialysis can be used to demonstrate differences in disposition of metabolites between the bile and the extracellular fluid of the liver, where the metabolite can be removed by the flow of blood to the systemic circulation. The use of the flow-through microdialysis probe for bile sampling allows metabolite concentrations to be determined without removing bile samples and without perturbing normal bile flow. In addition, microdialysis probes can be implanted into tissues for the determination of tissue distribution of compounds and their metabolites without the need for removing tissue samples or sacrificing large numbers of animals to construct concentration time profiles.

Microdialysis sampling provides a method for continuously monitoring *in vivo* events in both fluids and tissues. This technique provides several advantages for monitoring of biochemical reactions. Protein-free samples amenable to direct injection into a chromatographic system for analysis are obtained. Multiple sites can be monitored continuously

in a single animal. Finally, test compounds can be administered locally through the probe as well as by systemic methods. There do remain several concerns with the use of *in vivo* microdialysis sampling. Microdialysis is an invasive technique in which some tissue damage occurs during probe implantation. The affect of this damage on subsequent experiments is not completely clear at this time. The small sample volumes may be difficult to analyze and the time resolution of the experiment depends upon the detection limits of the analytical method. Intercellular material is not sampled; only the extracellular fluid is directly in communication with the microdialysis probe. Finally, because microdialysis represents a new method, complete interpretation of the results may depend upon further insight into the biochemical and microdialysis processes.

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