The Importance of Balancing Bile Salt Concentration to Avoid Fluid Loss When Using the Microdialysis Shunt Probe

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The microdialysis shunt probe is implanted in the bile duct of animals to monitor the concentration of drugs and metabolites in the bile. Bile salts can mediate the loss of fluid across the probe membrane, affecting dialysate flow rate and the amount of sample collected. Bile salts added to the perfusate maintained normal fluid flow through the test probe.

The microdialysis shunt probe is implanted in the bile duct to monitor the concentration of drugs in the bile of intact animals (1-3). A shunt probe ***(F1)*** is composed of two channels. The dimensions of the shunt channel, which carries the bile flow, are sized to allow insertion within the bile duct of an adult rat. The perfusate channel consists of a linear microdialysis probe suspended inside the shunt. The membrane of this suspended probe continuously samples low molecular weight compounds from the bile. Once implanted in the rat, dialyzed samples can be obtained for analysis without disruption of bile flow or removal of bile. The probe can be used in awake, freely moving animals for an extended period of time (2).

It is important to understand how bile constituents might influence both fluid loss and the transport of drugs across the dialysis probe membrane. Fluid loss across the membrane can critically decrease the total volume of sample collected. In on-line studies, variation in fluid flow rate can cause irregularities in the injected sample volume and affect the quantitation of the dialysate and the calibration of the microdialysis probe. Other research has shown that fluid loss can occur when using microdialysis in skeletal muscle and adipose tissue. Adding a colloid of dextran-70 to the perfusate balanced the fluid flow across the membrane in these tissues (4).

In a functioning microdialysis probe, ultrafiltration or osmotic flux can cause fluid loss. Ultrafiltration is the flow of solution across a membrane due to a pressure differential. Osmotic flux occurs when an imbalance of non-diffusable particles causes water to diffuse across the membrane. In the microdialysis shunt probe, when Ringer's solution is used as the perfusate against rat bile in the shunt, an osmotic flux causes water to flow to the shunt side of the membrane. This causes a substantial decrease in the volume of dialysate collected. In this study, the effect of bile salt concentration on the volumetric flow rate is shown. It is also shown that a 2% solution of bile salts in the perfusate is sufficient to maintain normal fluid flow.

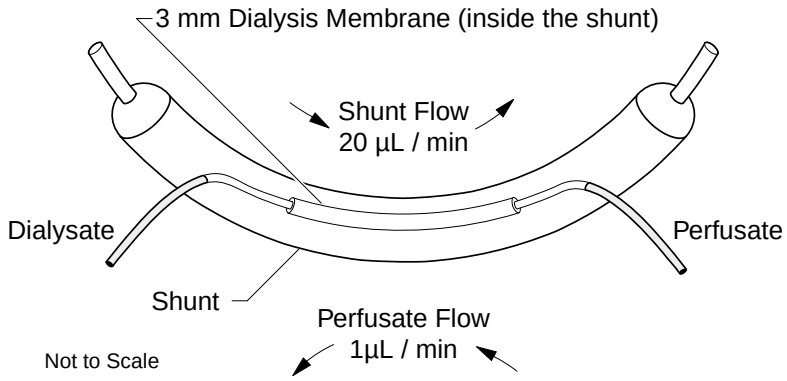
Methods

Materials

A bile shunt probe as shown in ***F1***, having a 3.0 cm PAN microdialysis membrane (polyacrylonitrile, MW cut-off 30,000 daltons), was used in this study. The following reagents were used as obtained from Sigma (St. Louis, Missouri): bile salts (approximately 50% sodium cholate and 50% deoxycholate, Sigma # B-8756), sodium dodecylsulfate (SDS), KCl, $CaCl_2$ and NaCl. Ringer's solution was prepared by mixing NaCl (155 mM, 9.0580 g/L), KCl (5.5 mM, 0.4100 g/L) and $CaCl_2$ (2.3 mM, 0.2553 g/L). Bile salts Ringer's solution (BSR) is a 2% (w/v) solution of Sigma bile salts (Sigma # B-8756) in Ringer's. Rat

F1

Bile shunt probe.



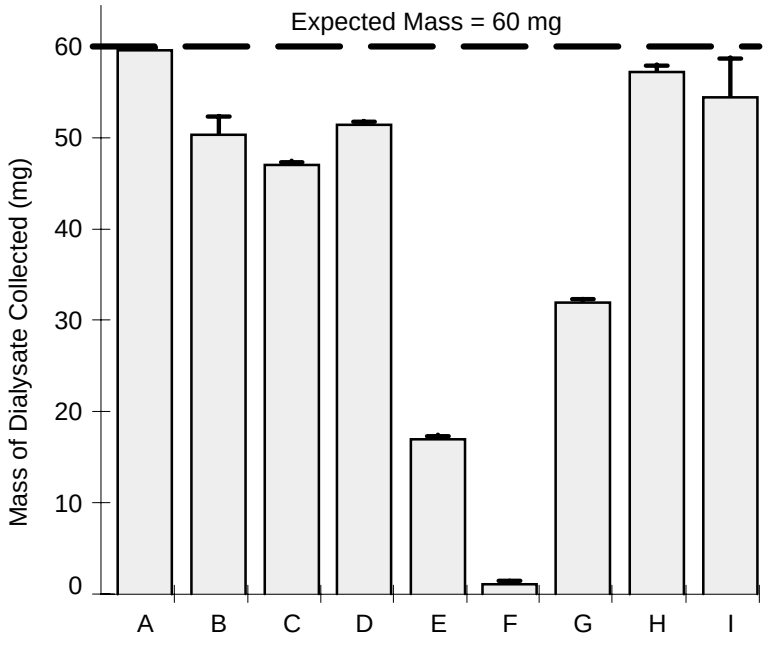
F2

Effect of shunt-perfusion fluid imbalance on volume of sample collected.

Bar A = no probe, water through tubing only.

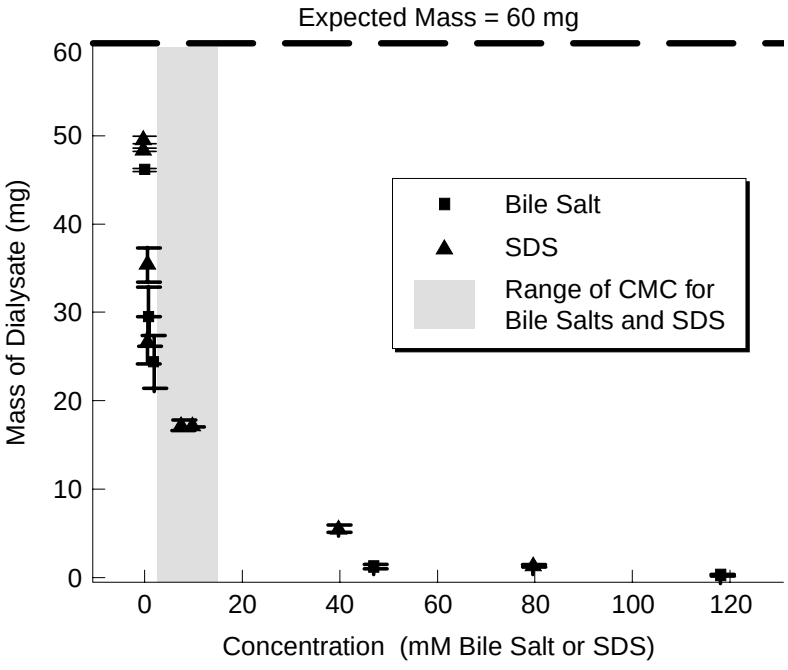
Perfusate composition:
B-G = water
H = rat bile
I = BSR

Shunt composition:
B = water (control)
C = Ringer's
D = 1 M NaCl
E = 10 mM SDS
F = BSR
G-I = rat bile



F3

Relationship between the mass of dialysate collected and the concentration of either bile salts or SDS.



bile was obtained by cannulation of the bile duct of an anesthetized rat and freezing the bile at -5° C until use. All solutions were made using NANOpure deionized water (Barnstead Co., Boston, Mass.) filtered through a 0.2 µm nylon syringe filter.

Volumetric Flow Measurements (Weight Method)

The flow measurements were all done at room temperature. The bile shunt probe was perfused using a BAS Bee syringe pump (MF-1001) and 1 mL syringe operating at 1 µL/min. The shunt flow was set at 20 µL/min using a 5 mL syringe and BAS Bee syringe pump. The probe was allowed to equilibrate for 30 min with each sample. Dialysate samples were collected using a BAS Honeycomb fraction collector (MD-1200) into pre-weighed, capped 300 µL autosampler vials refrigerated at 4° C. Three 60-min samples were collected for each determination. A decrease in mass collected is synonymous with a decrease in sample volume or a decrease in volumetric flow through the perfusate channel. Between each experiment the probe was flushed with water. A blank sample, containing water in both the shunt and the perfusate, was measured at the end of each day to monitor the integrity of the probe. The syringe flow rate was checked by pumping water through a piece of probe tubing (without membrane) at 1 µL/min for 60 min.

Results and Discussion

F2 shows how varying the composition in the shunt affects the amount of sample collected through the perfusate channel. In this figure, Bar B is the control sample where water was dialyzed against water in the shunt. Notice that bars C and D, with water dialyzed against 1 M NaCl (Bar D) and Ringer's (Bar C) in the shunt, are not significantly different from the control. These small ions appear to be capable of crossing the membrane and achieving steady state rapidly. When 10 mM SDS (Bar

T1

Critical micelle concentrations and aggregation number of SDS and various bile salts at 25° C (5).

Compound	Forumla Weight g/mol	Critical Micelle Concentration x 10^{-3} M	Aggregation Number
sodium dodecyl sulfate (SDS)	288.4	8.1	62
sodium cholate	430.6	13–15	2–4
sodium deoxycholate	414.4	4–6	4–10
sodium taurocholate	537.7	10–15	5

E) or BSR (Bar F) are in the shunt dialyzed against water, there is a significant loss of sample collected.

The relationship between the sample volume and the concentration of SDS and bile salts is shown in ***F3***. Bile salts and SDS may be prohibited from crossing the membrane due to either their hydrophobicity or their size. Considering only molecular weight, individual molecules of bile salts and SDS would be capable of crossing the membrane. These molecules tend to form large aggregates and micelles with the critical micelle concentrations (CMCs) in the range of 5 - 15 mM. From ***F3*** it can be seen that the sample mass collected decreases dramatically above the CMC for these compounds. This indicates that the larger aggregates have greater difficulty crossing the membrane and achieving a steady state on the time scale of the microdialysis. The concentration gradient created by these micelles can cause loss of water from the perfusate to the shunt due to osmosis. This water loss accounts for the observed decrease in sample volume collected.

In rat bile, the bile acids are generally present as mixed micelles containing around 500 bile acid molecules (27,000 daltons) (6). The formation of micelles reduces the overall osmolarity of the bile compared to the presence of individual bile acid molecules. These large aggregates, however, would not be expected to cross through the membrane, and therefore they can create the osmotic driving force for water to cross into the shunt. ***F2*** shows that when rat bile in the shunt is dialyzed against water (Bar G), the expected loss of dialysate sample is observed. It also shows that when BSR in the perfusate is dialyzed against rat bile (Bar I), the flow rate matches what is obtained when rat bile is dialyzed against itself (Bar H). Therefore, a 2% solution of bile salts in Ringer's in the perfusate should be used to maintain flow and prevent fluid loss when using a microdialysis shunt probe.

Conclusion

In the microdialysis shunt probe, bile salts can influence the loss of fluid across the membrane, affecting the dialysate flow rate and amount of sample collected. A solution of 2% bile salts is sufficient to maintain normal fluid flow through the probe.

References

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