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Studies of feedback suppression of bile salt synthesis in the bile-fistula rat

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Abstract We have previously reported that intravenous infusion of taurocholate at 10 μmol/(100 g · hr) into bile-fistula rats suppressed bile salt synthesis by 85% (Pries et al. 1983. *J. Lipid Res.* **24:** 141-146). Recently, however, infusion rates twice this high have been reported not to suppress synthesis (Davis et al. 1984. Falk Symposium 42. MTP Press Ltd., Boston. 37-45). Because the only major difference in design of these two studies was supplementation with sodium bicarbonate to replace biliary losses induced by bile salt choleresis, we have repeated our studies with and without bicarbonate supplementation. Without bicarbonate, as before, we found suppression of synthesis during infusion of taurocholate at 10 μmol/(100 g · hr). With bicarbonate, no suppression of synthesis occurred at these infusion rates. These data indicate that bicarbonate supplementation is essential when testing physiological effects of infused bile salt in the bile-fistula rat. —**Duane, W. C., A. P. McHale, and J. N. Hamilton.** Studies of feedback suppression of bile salt synthesis in the bile-fistula rat. *J. Lipid Res.* 1988. **29:** 212-214.

Supplementary key words cholesterol • liver

Feedback inhibition has been widely accepted as the major regulator of bile salt synthesis. The first evidence of this regulatory mechanism was obtained 30 years ago by Erikssen (1) who demonstrated in rats that diversion of bile through a fistula increased bile salt synthesis. Subsequently several laboratories, including our own, presented evidence that infusion of bile salts into animals with bile fistulae reduced synthesis (2-5).

Recently, however, reports questioning feedback inhibition have appeared. Two independent laboratories have reported that addition of bile salts to liver cell cultures did not inhibit bile salt synthesis (6, 7). Moreover, both of these laboratories have extended their work to in vivo infusions of taurocholate into bile-fistula rats. One found no inhibition of synthesis (8, 9). The other found inhibition at intestinal infusion rates of >24 μmol/(100 g · hr), a rate considerably higher than that which we found to inhibit synthesis (10).

When the first of these reports appeared (8), we contacted its senior author, Dr. Roger Davis, to compare our previous experimental design with his. The only apparent difference was that Davis' laboratory supplemented their rats with sodium bicarbonate, while we had not done so. We therefore, have attempted to repeat our experiments with bicarbonate supplementation.

METHODS

Sodium taurocholate was purchased from Calbiochem, Los Angeles, CA. Thin-layer chromatography of 100 μg of this bile salt revealed a single band by sulfuric acid charring. [^{14}C]Taurocholate was purchased from New England Nuclear, Boston, MA and found to be $>98\%$ radiochemically pure by thin-layer chromatography.

Male Sprague-Dawley rats weighing between 275 and 325 g were purchased from Biolab Corporation, White Bear Lake, MN. They were maintained in a light- and temperature-controlled environment and fed standard rat chow for at least 3 days prior to use. Bile and jugular venous cannulae were placed as previously described (5). Both cannulae were run subcutaneously and brought out through a small interscapular incision. Outside the rat, cannulae were run through a spring harness which was attached to the top of the rat cage. Rats so harnessed could freely obtain food and water provided in the cage.

Rats supplemented with bicarbonate were continuously infused into the jugular cannula with a solution of 75 mM NaCl, 30 mM $NaHCO_3$, and 8 mM KCl at a rate of 0.84 ml/hr from placement of the fistula until the termination of the experiment. Rats not supplemented with bicarbonate were infused similarly except that no $NaHCO_3$ was included in the infusate.

Bile salt synthesis was measured as bile salt output from a chronic bile fistula. Bile salt was measured by an automated procedure based on the hydroxysteroid dehydrogenase technique (5). Any exogenous taurocholate infused

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during these measurements was labeled with [^{14}C]taurocholate. The precise output of exogenous bile salt into bile was determined from output of radioactivity into bile corrected for the specific activity of the infusate. The calculated exogenous bile salt output was subtracted from total bile salt output to calculate synthesis. This method, which might be termed "isotope correction," has been described in detail previously (5).

Biliary bicarbonate was measured by the Clinical Chemistry Core of the Core Center for the Study of Advanced Liver Disease of the University of Minnesota. Bile samples for this analysis were collected under oil.

RESULTS

Fig. 1 shows the effect of intravenous infusion of taurocholate at 10 μmol/(100 g · hr) on bile salt synthesis in chronic bile-fistula rats. Except for the variable of sodium bicarbonate supplementation, these studies were designed to duplicate experiments previously reported from our laboratory (5). Without bicarbonate, bile salt synthesis fell to about 25% of control levels within 24 hr, results very similar to those we reported previously (5). However, when bicarbonate supplementation was provided, infusion of taurocholate at this rate did not alter bile salt synthesis. Also shown in Fig. 1A is an increase of about 20 μeq/hr in biliary output of bicarbonate during infusion of taurocholate in the two rats not supplemented with bicarbonate.

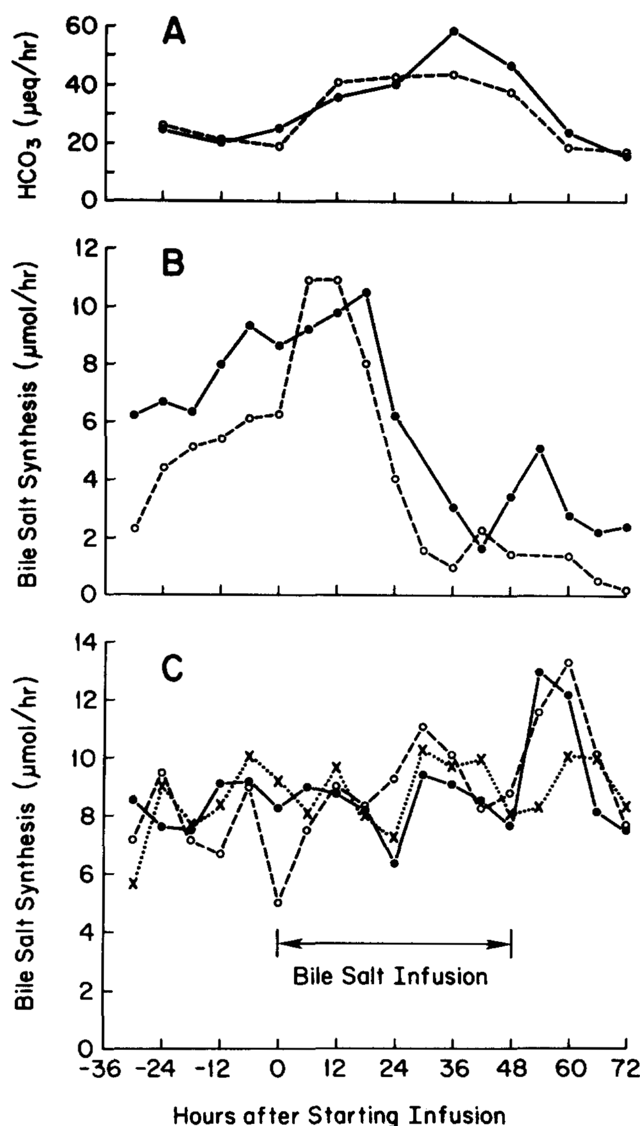

Fig. 1. Biliary output of bicarbonate in two bile fistula rats not supplemented with bicarbonate (A); bile salt synthesis by isotope correction in two rats not supplemented with bicarbonate (B); and three rats given continuous intravenous bicarbonate supplementation (C). Horizontal axis is time following the start of an intravenous infusion of taurocholate at 10 μmol/(100 g · hr). This infusion produced a bicarbonate choleresis and suppressed bile salt synthesis in rats not supplemented with bicarbonate. In rats supplemented with bicarbonate, taurocholate at this infusion rate had no effect on bile salt synthesis.

DISCUSSION

Four studies prior to 1985 reported inhibition of bile salt synthesis during infusion of exogenous bile salt into chronic bile-fistula rats (2–5). In the earliest of these, Bergstrom and Danielsson (2) found that intraduodenal infusion of taurochenodeoxycholate at approximately 5 μmol/(100 g · hr), when the infusion was continued for at least 24 hr, inhibited synthesis of cholic acid to about 20% of baseline. Later, two separate studies by Shefer and colleagues (3, 4) demonstrated that intraduodenal infusion of taurocholate at 19–26 μmol/(100 g · hr) induced an hepatic flux of 60–80% of the infusion rate and inhibited synthesis as measured both by output of taurochenodeoxycholate and by incorporation of radioactive precursors into bile salt. Subsequently, using isotopic bile salt to correct output of total bile salt from biliary fistulae (isotope correction method), we found that infusion of 10–12 μmol/(100 g · hr) intravenously induced an hepatic flux virtually identical to the infusion rate and inhibited total bile salt synthesis to about 15% of baseline (5). In 1983, prompted by failure of added bile salt to suppress synthesis in isolated hepatocytes, Davis et al. (6) attempted to reproduce these experiments. They found that intravenous infusion of taurocholate at rates up to 20 μmol/(100 g · hr) into bile fistula rats did not suppress bile salt synthesis measured by isotope correction (8, 9). Verbal comparison of our study design with that of Davis indicated only one major difference: they had supplemented their rats with sodium bicarbonate to replace biliary losses while we had not. The reports by Bergstrom and Danielsson (2) and Shefer et al. (3, 4) made no mention of bicarbonate supplementation.

The data presented in Fig. 1 indicate that bicarbonate supplementation is critical. Without bicarbonate, bile salt

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synthesis was suppressed by an intravenous infusion of 10 μmol/(100 g·hr), both in this study (Fig. 1) and in our previous study (5). With bicarbonate, this rate of infusion did not suppress synthesis. Moreover, the suppression of synthesis in rats lacking bicarbonate supplementation was probably a nonspecific, rather than a physiologic, effect because when the bile salt infusion was stopped, no rebound in synthesis occurred.

While these data indicate that feedback inhibition of bile salt synthesis in the rat does not occur at hepatic flux rates of 10-15 μmol/(100 g·hr), they do not eliminate feedback inhibition as an important physiological regulator of bile salt synthesis. Two recent studies from independent laboratories have presented strong evidence that the normal physiological hepatic flux of bile salt in the rat is 28-42 μmol/(100 g·hr) (11, 12). It seems clear, therefore, that feedback inhibition cannot be dismissed as a physiologic mediator of bile salt synthesis until fluxes in this range have been studied. We have made extensive attempts to study these higher flux rates over the past 4 years. For several reasons, including the difficulty of distinguishing toxic from physiologic effects of infused bile salts, these attempts have been unsuccessful.

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REFERENCES

1. Eriksson, S. 1957. Biliary excretion of bile acids and cholesterol in bile fistula rats. *Proc. Soc. Exp. Biol. Med.* **94:** 518-582.
2. Bergstrom, S., and H. Danielsson. 1958. On the regulation of bile acid formation in the rat liver. *Acta Physiol. Scand.* **43:** 1-7.
3. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1969. Feedback regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **10:** 646-655.
4. Shefer, S., S. Hauser, I. Bekersky, and E. H. Mosbach. 1970. Biochemical site of regulation of bile acid biosynthesis in the rat. *J. Lipid Res.* **11:** 404-411.
5. Pries, J. M., A. Gustafson, D. Wiegand, and W. C. Duane. 1983. Taurocholate is more potent than cholate in suppression of bile salt synthesis in the rat. *J. Lipid Res.* **24:** 141-146.
6. Davis, R. A., P. M. Hyde, J-C. Kuan, M. Malone-McNeal, and J. Archambault-Schexnayder. 1983. Bile acid secretion by cultured rat hepatocytes. *J. Biol. Chem.* **258:** 3661-3667.
7. Kubaska, W. M., E. C. Gurley, P. B. Hylemon, P. S. Guzelian, and Z. R. Vlahcevic. 1985. Absence of negative feedback control of bile acid biosynthesis in cultured rat hepatocytes. *J. Biol. Chem.* **260:** 13459-13463.
8. Davis, R. A., P. M. Hyde, M. M. McNeal, and J. A. Schexnayder. 1983. In vivo demonstration that bile acids do not directly induce biosynthetic feedback regulation. *Arteriosclerosis.* **3:** 490a.
9. R. A. Davis, C. A. Musso, and G. R. Lattier. 1985. Regulation of bile acid synthesis by microsomal cholesterol. *In* Falk Symposium 42: Enterohepatic Circulation of Bile Acids and Sterol Metabolism. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press Ltd., Boston. 37-45.
10. Heuman, D. M., M. D. Law, C. Hartman, W. M. Kubaska, P. B. Hylemon, and Z. R. Vlahcevic. 1986. Effect of taurocholate infusion (TCI) on bile acid synthesis (BAS) and cholesterol 7α-hydroxylase (C7αH) activity. *Gastroenterology.* **90:** 1734.
11. Weis, E. E., and C. A. Barth. 1978. The extracorporeal bile duct: a new model for determination of bile flow and bile composition in the intact rat. *J. Lipid Res.* **19:** 856-862.
12. Kuipers, F., R. Havinga, H. Bosschieter, G. P. Toorop, F. R. Hindriks, and R. J. Vonk. 1985. Enterohepatic circulation in the rat. *Gastroenterology.* **88:** 403-411.

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