PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **155**, 55-58 (1977)

Quantification of Taurine Synthesis in the Intact Rat and Cat Liver (39744)[1]

WILLIAM G. M. HARDISON,[2] CRAIG A. WOOD, AND JOHN H. PROFFITT

Division of Gastroenterology, Department of Medicine, Veterans Administration Hospital, San Diego and Department of Medicine, University of California, San Diego, California 92161

Data from studies with liver homogenates indicate livers of different species differ in their ability to synthesize taurine from various substrates. Rat liver synthesizes taurine rapidly from cystine sulfinic acid (CSA) (1, 2), whereas cat and human livers synthesize taurine slowly (1, 2) or not at all (3, 4) from CSA. Recently a system utilizing inorganic sulfate for taurine synthesis has been described and it has been suggested that this pathway may be important in species which lack an active CSA pathway (5, 6). No data exist concerning rates or pathways of taurine synthesis in the intact liver of any species. We studied the taurine synthetic capacity of isolated perfused rat and cat livers to quantify total taurine synthesis and to estimate the relative importance of taurine synthesis from CSA and from inorganic sulfate.

Materials and methods. Male Sprague-Dawley rats, 250-350 g, and young cats, 350-500 g, male and female, were used. In perfusions involving [^{14}C]taurine, 2 μCi of isotope was injected intravenously into the fasted animal 30-60 min before hepatectomy. Under ether (rats) or pentobarbital (cats) anesthesia, the inferior vena cava was ligated, cannulas were placed in the superior vena cava, portal vein, and common bile duct, and livers were removed and transferred to a standard perfusion apparatus.[3] In the cat, the cystic duct was ligated. Perfusate composition was: Ringer's bicarbonate solution containing 4% bovine serum albumin to which washed bovine erythrocytes were added to a final hematocrit of 33%.[4] Perfusate was gassed with 95% O_2-5% CO_2. Perfusion was by gravity at 1-2 ml/g of liver/min and lasted 300-360 min. Except where noted, sodium cholate was infused into the perfusate at a rate of 0.46 (rat) or 0.91 (cat) μmole/min in order to deplete liver taurine. Livers were biopsied at 1 hr and at the end of perfusion for measurement of hepatic taurine concentration by methods previously described (7). Bile was collected hourly and analyzed for total bile acids, taurocholate, glycocholate, and unconjugated cholic acids as previously described (7). Total taurine synthesis was calculated by taurine balance as the difference between decrease in hepatic taurine pool and taurine excreted as biliary taurocholate (7). In perfusions involving [^{35}S]sulfate,[5] 5 μCi of isotope was injected into the perfusate 15-45 min after the start of perfusion. The protocol was modified for perfusions involving [^{14}C]CSA. CSA, 0.35 μCi, was injected into the perfusate 15-45 min after the start of perfusion. To avoid dilution of evolved CO_2 and ensure its complete collection, CO_2 was eliminated from the gassing mixture and Tricine[6] buffer was substituted for bicarbonate. Osmolality and pH of the perfusate were unchanged.

Sulfate was measured by the method of Berglund and Sorbo (9). The barium sulfate precipitate from this reaction was counted in a 1:3 water: Aquasol system. All other samples were counted in Bray's solution in a Nuclear-Chicago Mark II scintillation counter with quench corrected by the external standards ratio method. In perfusions involving [^{35}S]sulfate, the taurocholate band from thin-layer chromatography of biliary

[1] This study was supported by Grant AM 18289 from the National Institute of Arthritis, Metabolism and Digestive Diseases and by the Medical Research Service of the Veterans Administration.

[2] Send reprint requests to William G. M. Hardison, M.D., Section of Gastroenterology, Veterans Administration Hospital, 3350 La Jolla Village Drive, San Diego, California 92161.

[3] Medical Research Apparatus, Inc., Boston, Mass.

[4] Bovine blood was a gift from Talone Packing Co., Escondido, Calif.

[5] New England Nuclear, Boston, Mass.

[6] Calbiochem, La Jolla, Calif.

Copyright © 1977 by the Society for Experimental Biology and Medicine
All rights reserved.

ISSN 0037-9727

bile acids was eluted and hydrolyzed in 2 *N* NaOH for 3 hr at 15 psi. The free taurine was then assayed according to the method for tissue taurine (7) and its specific activity was determined.

Results. Taurine synthesis was assessed directly by measuring the amount of newly synthesized taurine appearing in the system and indirectly by measuring decline in specific activity of taurine and taurocholate over the course of perfusion. Table I summarizes data from perfusions involving [^{14}C]taurine. Rat livers, whether or not depleted of taurine by sodium cholate infusion, all synthesized taurine. Mean taurine synthesis rate of infused livers was not significantly different from that of noninfused livers. Only three of six cat livers appeared to synthesize taurine at a low rate and the negative mean synthesis rate was not significantly different from zero.

Measurement of decline in specific activity of taurine and taurocholate during perfusion, although not a quantitative index of taurine synthesis in this system, is probably a sensitive qualitative index of taurine synthesis. Thus, an easily measurable decline in taurine specific activity towards the end of perfusion when the system is relatively taurine depleted could be caused by synthesis of an amount of taurine corresponding only to a few percent of the original taurine pool. Synthesis of this amount of taurine might well not be detected by our taurine balance methods. Taurine specific activity fell substantially in all rat livers. The greater fall in rat livers infused with sodium cholate (mean decline 72%) than in livers not so infused (mean decline 40%) is probably the result of greater loss of [^{14}C]taurine as taurocholate in bile. The fall in taurine specific activity in the cat livers (mean decline 21%) suggests that taurine synthesis was occurring at a low rate. This decline, however, was short of significance ($0.1 > P > .05$ by paired *t* test). Changes in biliary taurocholate specific activities, Fig. 1, generally reflected the change in hepatic taurine specific activities. Taurocholate in bile from cholate-infused rat livers showed the greatest fall in specific activity. Taurocholate specific activities in bile from cat livers declined late in perfusion but, again, this was not significant ($0.1 > P > .05$). The delayed rise in biliary taurocholate specific activity in the cat livers probably reflects lower bile flow in the cat and dilution of the first-hour bile sample with unlabeled gallbladder bile.

Table II shows data derived from perfusions in which [^{14}C]CSA was injected into the perfusate. Because CSA specific activity in liver tissue could not be measured, no absolute figure for taurine synthesis from CSA can be derived. If one assumes equal hepatic concentrations of CSA in the two species, however, one may derive from the known taurine pools the ratio of taurine- and taurocholate-specific activities one would expect if hepatic taurine synthesis rate from CSA were equal in the two spe-

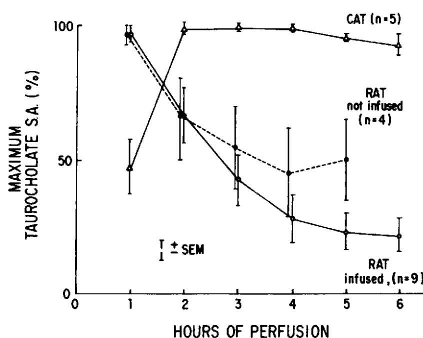

FIG. 1. Specific activity of biliary taurocholate, expressed as percentage of maximum specific activity, for each hour of perfusion.

TABLE I. DATA FROM RAT AND CAT LIVER PERFUSIONS UTILIZING [^{14}C]TAURINE.

Animal group	Fall of hepatic taurine sp act (%)	Hepatic taurine concentration 1 hr (μmole/g)		Taurine, synthesis rate (nmole/g/min)
		1 hr	6 hr	
Rats, cholate infused (n = 9)	72 ± 10.8[a]	3.4 ± 1.82	0.9 ± .13	7.6 ± 2.19
Cats, cholate infused (n = 5)	21 ± 20.9	8.4 ± 2.31	3.3 ± 1.53	−0.7 ± 2.29
Rats, not infused (n = 4)	40 ± 14.0	2.8 ± 0.91	5.3 ± 2.91	10.9 ± 6.75

[a] x Values ± SD.

TABLE II. DATA FROM RAT AND CAT LIVER PERFUSIONS UTILIZING [^{14}C]CSA.

Animal	Initial hepatic taurine sp act (dpm/μmole)	Maximum biliary TC sp act (dpm/μmole)	Percentage of dpm as[a]	
			CO_2	TC
Rat 1	5420	24150	0.73	1.17
Rat 2	10520	16100	0.79	3.30
Cat 1	810	1140	0.13	0.75
Cat 2	1050	2930	0.10	0.42

[a] Percentage of administered radioactivity appearing as carbon dioxide or as biliary taurocholate during perfusion per gram of liver.

cies. This ratio of rat:cat would be 4:8. The actual ratios of hepatic taurine and of maximal biliary taurocholate specific activities in the first hour of perfusion were 8:9 and 9:6, respectively. This suggests that hepatic taurine synthesis rate from CSA was about twice as rapid in the rat as in the cat liver. About six times as much [^{14}C]CSA was decarboxylated and about four times as much [^{14}C]CSA was converted to taurocholate per gram of liver in the rat as in the cat liver. Taken together these data suggest taurine synthesis via the CSA decarboxylase pathway is more active in the rat than in the cat liver, but without knowledge of CSA specific activities this cannot be proven. Conversely, the data do demonstrate taurine synthesis via the CSA decarboxylase pathway in the intact cat liver, a finding we did not anticipate.

In perfusions involving [^{35}S]sulfate, taurine synthesis occurred in the rat liver (8.9 nmole/g/min) and in the cat liver (1.7 nmole/g/min) as measured by taurine balance. Measured hepatic sulfate specific activity at the end of perfusion was 8230 dpm/μmole in the rat and 9020 dpm/μmole in the cat. However, no measurable radioactivity appeared either in hepatic or biliary taurine. By our methods we could have detected synthesis rates by this pathway at least as low as 0.1 nmole/g/min. The intact perfused liver, at least over the short period of 5 hr, did not synthesize taurine from inorganic sulfate.

Discussion. From the taurine balance data alone, one would have to say that the intact rat liver actively synthesized taurine but the cat liver did not. In fact, the cat liver probably synthesized taurine slowly. Although the fall in specific activity of taurine in cat liver and bile was not statistically significant, the cat liver to some extent decarboxylated CSA and produced taurine from it. The known greater activity of CSA decarboxylase in rat than in cat liver homogenates (1, 2) and the present data on CSA conversion to taurine in the intact rat and cat livers, suggest that the different activities of the CSA decarboxylase pathway of taurine synthesis accounts for the differences in taurine synthetic rates between cat and rat livers. This is supported by the absence of any contribution to taurine synthesis by the inorganic sulfate pathway. The possibility, however, exists of still other pathways for hepatic taurine synthesis. Jacobsen and Smith in their extensive review (10) summarized evidence for another pathway involving oxidation of cysteamine to hypotaurine by a persulfurase widely distributed in animal tissues. Its quantitative significance is unknown but its theoretical presence prevents our attributing all taurine synthesis not occurring via the inorganic sulfate pathway to the CSA decarboxylase pathway.

Our inability to demonstrate taurine synthesis from inorganic sulfate does not mean that this pathway never contributes significantly to taurine synthesis in mammals. Our animals were on no special diets and studies lasted only 5 to 6 hr. Long-term taurine deprivation, especially in animals with low hepatic CSA decarboxylase activity, might stimulate this pathway. Certain dietary components have been described which stimulate or inhibit this pathway. In the chick liver, methionine stimulates and cysteine inhibits the pathway (11). In the rat, the pathway is sensitive to potassium concentrations (12). Under certain circumstances, then, taurine synthesis from inorganic sulfate might become important.

The study raises questions about factors which control taurine synthesis but does not answer them. In our experiments on the rat liver, acute taurine depletion by sodium cholate infusion did not stimulate taurine synthesis. Livers not infused with sodium cholate synthesized taurine as rapidly as infused livers and actually increased hepatic taurine concentration over the course of

perfusion. Possibly taurine synthesis would be suppressed at higher liver taurine concentrations than those we observed after an 18-hr fast, or perhaps feeding per se may suppress taurine synthesis. Alternatively, taurine synthesis may be constant in the rat with taurine in excess of hepatic requirements being stored in extrahepatic tissue or excreted in the urine. The requirement of pyridoxine for taurine synthesis via the CSA decarboxylase pathway has been defined (3) but this is probably not an important physiological control mechanism.

Our results help explain why in the rat, bile acid is conjugated predominantly with taurine and why the cat is susceptible to taurine depletion (13). The requirement for bile acid conjugation in the rat is about 20–23 nmole/min (14, 15). Our data indicate rat liver synthesizes more than enough taurine to meet this need and may explain why the rat liver, although capable of conjugating bile acid with glycine (16), *in vivo* conjugates 80–95% of its bile acid with taurine. The data also explain why the cat, which conjugates bile acid only with taurine, is susceptible to taurine deficiency. Without dietary taurine, the cat liver would expend its taurine in bile acid conjugation. Detection of unconjugated cholic acid in the gallbladder bile of taurine-deficient cats supports this concept (17).

Human liver is more similar to cat than to rat liver in its taurine synthetic mechanisms (10). In man, disorders which diminish ileal reabsorption of bile acid are associated with high bile acid, and hence, taurine loss. One protective mechanism, however, exists in man. Human liver can conjugate bile acids well with glycine when bile acid conjugation requirements are increased. Whether this mechanism is sufficient in man to conserve the taurine pool and prevent deficiency over often many years of increased taurine loss is unknown.

1. Jacobsen, J. G., and Smith, L. H., Jr., Nature (London) **200,** 575 (1963).
2. Jacobsen, J. G., Thomas, L. L., and Smith, L. H., Jr., Biochim. Biophys. Acta **85,** 103 (1964).
3. Hope, D. B., Biochem. J. **59,** 497 (1955).
4. Blaschko, H., Biochem. J. **36,** 571 (1942).
5. Martin, W. G., Sass, N. L., Hill, L., Tarka, S., and Truex, R., Proc. Soc. Exp. Biol. Med. **141,** 632 (1972).
6. Martin, W. G., Truex, C. R., Tarka, S. M., Hill, L. J., and Gorby, W. J., Proc. Soc. Exp. Biol. Med. **147,** 563 (1974).
7. Hardison, W. G. M., and Proffitt, J. H., Amer. J. Physiol., in press.
8. Rassin, D. K., and Gaull, G. E., J. Neurochem. **24,** 969 (1975).
9. Berglund, F., and Sorbo, F., Scand. J. Clin. Lab. Invest. **12,** 147 (1960).
10. Jacobsen, J. G., and Smith, L. H., Jr., Physiol. Rev. **48,** 424 (1968).
11. Martin, W. G., Poultry Sci. **51,** 608 (1972).
12. Groby, W. G., and Martin, W. G., Proc. Soc. Exp. Biol. Med. **148,** 544 (1975).
13. Hayes, K. C., Carey, R. E., and Schmidt, S. Y., Science **188,** 949 (1975).
14. Cronholm, T., and Sjovall, J., Eur. J. Biochem. **2,** 375 (1967).
15. Bergstrom, S., Fed. Proc. **21,** 28 (1962).
16. Bremer, J., Biochem. J. **63,** 507 (1956).
17. Rabin, B., Nicolosi, R. J., and Hayes, K. C., J. Nutr. **106,** 1237 (1976).

Received October 11, 1976. P.S.E.B.M. 1977, Vol. 155.