Identification of bile acid coenzyme A synthetase in rat kidney

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Abstract Bile acid CoA synthetase has been discovered in rat kidney. Incubation of kidney microsomes with [^{14}C]chenodeoxycholic acid and CoA produced a single peak with the high performance liquid chromatography (HPLC) retention time of CDC-CoA. This peak, when incubated with purified bile acid CoA:amino acid N-acyltransferase (BAT) from human liver and either taurine or glycine, led to the formation of CDC-taurine or CDC-glycine, respectively. Kinetic analysis revealed apparent K_ms for CDC and CoA of 2.5 μM and 2.6 μM, respectively. This activity appeared specific for bile acids as it was not inhibited by benzoic acid or salicylic acid, known substrates for other rat kidney CoA synthetases. This demonstrates that the kidney has the potential for bile acid metabolism and may have a role in bile acid physiology.—**Kwakye, J. B., S. Barnes, and R. B. Diasio.** Identification of bile acid coenzyme A synthetase in rat kidney. *J. Lipid Res.* 1993. **34:** 95–99.

Supplementary key words microsomes • chenodeoxycholyl-CoA • chenodeoxycholyltaurine • chenodeoxycholylglycine

Bile acids are synthesized in the liver and conjugated with either taurine or glycine to form bile acid N-acyl amidates prior to secretion into bile (1). It had been previously thought that conjugation of bile acids occurs solely in the liver; however, we recently demonstrated a unique source of bile acid CoA:amino acid N-acyltransferase (BAT) (E.C. 2.3.1.65) activity in rat kidney (2), suggesting that the kidney may have a role in bile acid physiology.

The conjugation of bile acids in the liver has been shown to occur in two distinct steps (3). In an initial reaction, the bile acid is activated to a coenzyme A thioester:

$$\text{Bile acid} + \text{ATP} + \text{CoA} = \text{bile acid CoA} + \text{AMP} + \text{PPi}.$$

This reaction is catalyzed by the microsomal enzyme, bile acid CoA synthetase (E.C. 6.2.1.7) (4). The bile acid CoA subsequently reacts with taurine or glycine in a reaction catalyzed by BAT (5, 6) to form a bile acid N-acyl amidate:

$$\text{Bile acid CoA} + \text{amino acid} = \text{bile acid N-acyl amidate} + \text{CoA}.$$

In order for BAT to form bile acid N-acyl amidates in the kidney, there is an absolute requirement for bile acid CoA synthetase activity to be present in this organ. Furthermore, bile acid CoA synthetase would be expected to be rate-limiting, as it is in the liver (7), and therefore the more important of the two enzymes. As the kidney is a very unusual location for a bile acid conjugating enzyme, the question of whether it has the capacity to form bile acid N-acyl amidates cannot be resolved without also demonstrating bile acid CoA synthetase activity.

The purpose of the present study was to determine whether bile acid CoA synthetase activity is also present in rat kidney and to characterize its kinetic and substrate properties.

MATERIALS AND METHODS

Materials

[Carboxyl-^{14}C]chenodeoxycholic acid (50.5 mCi/mmol) and [2,4-^{3}H]cholic acid (25 Ci/mmol) were purchased from New England Nuclear Corporation, Boston, MA. They were purified by HPLC before use. Unlabeled bile acids, benzoic acid, salicylic acid, and ATP were purchased from Aldrich Chemical Company, Milwaukee, WI. Coenzyme A, CTP, and GTP were purchased from Sigma Chemical Co., St. Louis, MO. Diethyl ether was purchased from Fisher Scientific, Norcross, GA. Chenodeoxycholyl-CoA was synthesized by the method of Shah and Staple (8) and purified as previously described (9). All other solvents and reagents were purchased in the highest grade possible.

Preparation of microsomes

Microsomes were prepared as described by Suzue and Marcel (10). Kidneys were removed from male Sprague-

Abbreviations: CoA, coenzyme A; CDC, chenodeoxycholic acid; CDC-CoA, chenodeoxycholyl CoA; CDC-taurine, chenodeoxycholyltaurine; CDC-glycine, chenodeoxycholylglycine; BAT, bile acid CoA:amino acid N-acyltransferase; HPLC, high performance liquid chromatography.

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Dawley rats after cervical dislocation and washed in cold physiological saline. All subsequent procedures were done at 4°C. Kidneys were homogenized in 4 volumes of 0.25 M sucrose solution and centrifuged at 20,000 *g* in a Beckman Type 50.2 Ti rotor for 20 min. The resulting supernatant was centrifuged at 105,000 *g* in a Beckman Type 50.2 Ti rotor for 1 h. The microsomal pellet was resuspended in the same volume of sucrose solution and centrifuged again at 105,000 *g* for 1 h. The washed microsomes were homogenized in 0.25 M sucrose solution containing dithiothreitol (1 mg/ml). Small aliquots were stored at −70°C.

Bile acid CoA synthetase activity

Bile acid CoA synthetase activity was measured as described previously (11). The standard assay contained 0.02 μCi [^{14}C]chenodeoxycholic acid, 5 μM unlabeled chenodeoxycholic acid, 0.2 mM CoA, 5 mM ATP, 50 mM sodium fluoride, 200 μg enzyme protein, and 100 mM Tris-HCl buffer in a volume of 0.5 ml at a final pH of 8.5. The reaction mixture without the enzyme was incubated at 37°C in a shaking water bath for 3 min; then, enzyme was added to initiate the reaction. After a further 10 min incubation, the reaction was terminated by addition of 0.5 ml of ice-cold methanol and samples were centrifuged to remove the precipitated protein. The supernate (0.7 ml) was added to 0.5 ml of 6% (v/v) perchloric acid. It was extracted twice with 10 ml diethyl ether saturated with water, to remove unreacted bile acid. Excess diethyl ether was removed from the aqueous phase by evaporation under nitrogen. The extract was neutralized with 50 μl of 11.5 N ammonium hydroxide and transferred into scintillation vials for determination of radioactivity. Assays were performed in triplicate and blanks were carried out in the presence of heat-denatured kidney microsomes.

Identification of product

The reaction mixture was concentrated by evaporation under dry nitrogen and analyzed by reversed-phase HPLC. Chemically synthesized chenodeoxycholyl-CoA (CDC-CoA) was added to the concentrated sample and injected on a Hewlett-Packard 1050 HPLC fitted with a 250 × 4.6 mm reversed-phase C_{18} column (Jones Chromatography, Littleton, CO). The column was eluted at a flow rate of 1 ml/min with 75% methanol–2 mM potassium phosphate, pH 3.0, as the mobile phase. The unlabeled CDC-CoA was monitored by UV absorbance at 259 nm.

To confirm that the product was CDC-CoA, purified BAT (50 μg) obtained from human liver (12) and unlabeled taurine or glycine (final concentration, 10 mM) were added to the reaction mixture after 30 min incubation and further incubated for 30 min. The reaction was stopped with 0.5 ml of ice-cold methanol, concentrated, and injected on an HPLC column under the conditions described above. The elution pattern was compared to that obtained with chenodeoxycholyltaurine (CDC-taurine) and chenodeoxycholylglycine (CDC-glycine), synthesized as described elsewhere (9).

Kinetic experiments

Initial rates of reaction were determined at various concentrations of CDC (1–10 μM) and coenzyme A (CoA) (1.5–8 μM) at saturating concentrations of CoA (200 μM) and CDC (5 μM), respectively. The protein concentration and incubation times were chosen such that not more than 10% of the limiting substrate was consumed in the reaction. The kinetic parameters were calculated by fitting these data to the Michaelis-Menten equation by nonlinear regression analysis.

To determine whether other organic acids were alternate substrates for this enzyme, formation of bile acid CoA was determined at various concentrations of benzoic acid or salicylic acid (0–400 μM).

Assay optimization

Optimization of bile acid CoA synthetase activity was carried out in 0.1 M Tris-HCl buffer at 37°C, varying the pH between 6.5 and 9.8 in the presence of 5 mM $MgCl_2$. Optimum magnesium requirement of this enzyme was determined in 0.1 M Tris-HCl buffer at pH 8.5; 1 mM EDTA was added to one group of samples to yield a Mg^{2+}-free solution. Linearity of the enzyme-stimulated reaction with time was examined using 200 μg of microsomal protein; at the optimized time (10 min), the range of protein concentration over which the reaction was linear was determined.

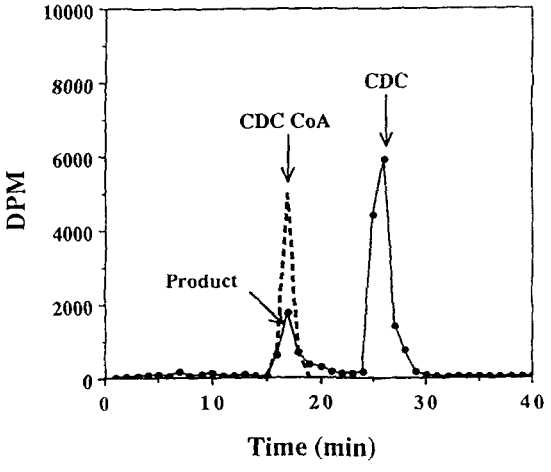

Fig. 1. HPLC profile of radioactivity (●—●) in reaction mixture. Incubation conditions were described under Materials and Methods. The incubation mixture was concentrated under a stream of dry nitrogen and chemically synthesized chenodeoxycholyl-CoA was added as a standard. The latter was monitored by UV absorbance at 259 nm (----).

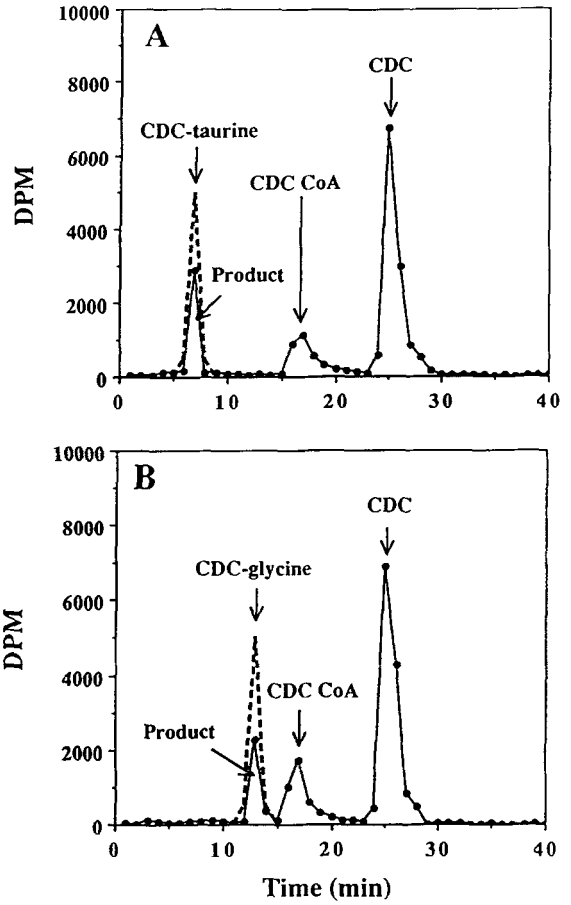

Fig. 2. *HPLC profile of radioactivity (●—●) after further incubation* of the metabolite shown in Fig. 1 with purified BAT and unlabeled taurine (A) or unlabeled glycine (B). Conditions were as described in Materials and Methods. Elution times of authentic standards are indicated by dashed lines (----).

BAT was purified as described previously (12). Protein concentration was determined by the method of Lowry et al. (13).

RESULTS

Identification of product

Bile acid CoA synthetase activity in rat kidney was initially demonstrated using a radioassay developed for this enzyme in rat liver (11). Identity of the product was verified by reversed-phase HPLC analysis. In addition to the unreacted CDC peak, a new peak of radioactivity was observed that coeluted with chemically synthesized CDC-CoA (**Fig. 1**). On further incubation with purified human liver BAT and either unlabeled taurine or glycine, a third peak appeared which had the chromatographic properties of CDC-taurine or CDC-glycine, respectively (**Fig. 2 A & B**), confirming that the original product was CDC-CoA.

Assay conditions

Rat kidney bile acid CoA synthetase showed maximum activity at pH 8.5 (**Fig. 3A**). Increasing magnesium concentration stimulated enzyme activity until it reached a maximum at 5 mM (Fig. 3B). With no added Mg^{2+}, enzyme activity was 33% of the maximum activity; however, on addition of 1 mM EDTA, enzyme activity dropped to zero. Formation of bile acid CoA was directly related to the incubation time up to 20 min (**Fig. 4A**). At 10 min incubation, the reaction rate was also linearly related to the amount of microsomal protein added up to 400 μg (**Fig. 4B**).

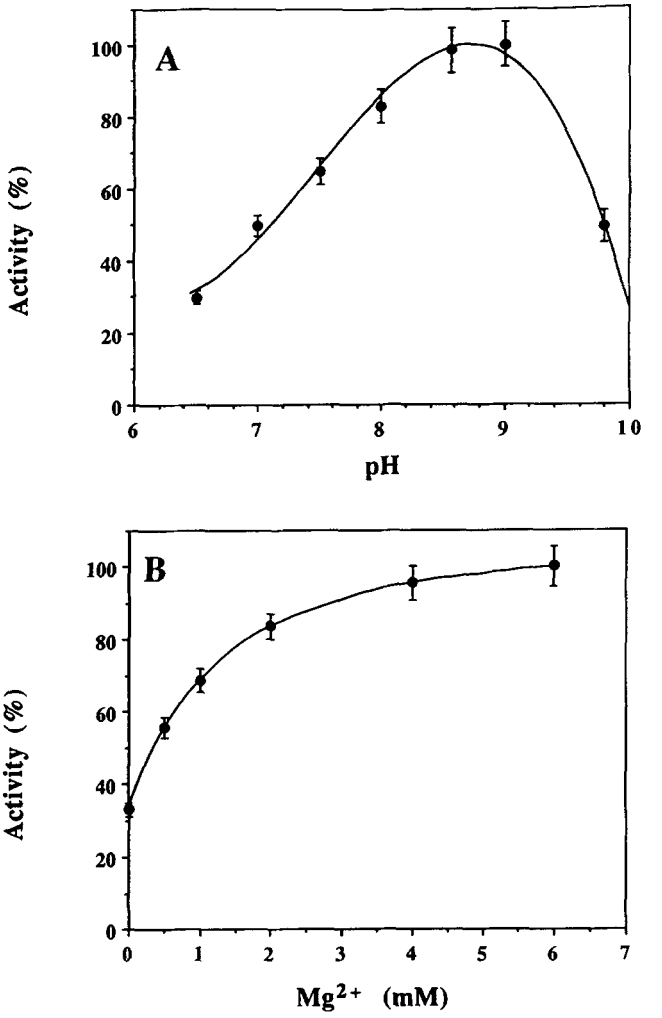

Fig. 3. Effect of pH (A) and magnesium concentration (B) on rat kidney bile acid CoA synthetase activity. Enzyme activity was measured as described for the standard assay except for the pH and magnesium concentration. In A, 5.0 mM $MgCl_2$ was used and pH was varied; in B incubation was carried out in 0.1 M Tris-HCl, pH 8.5, and magnesium concentration was varied. Enzyme activity was expressed as a percentage of the maximum observed activity (3.5 pmol/min per mg). Each value is the mean ± SEM for three experiments.

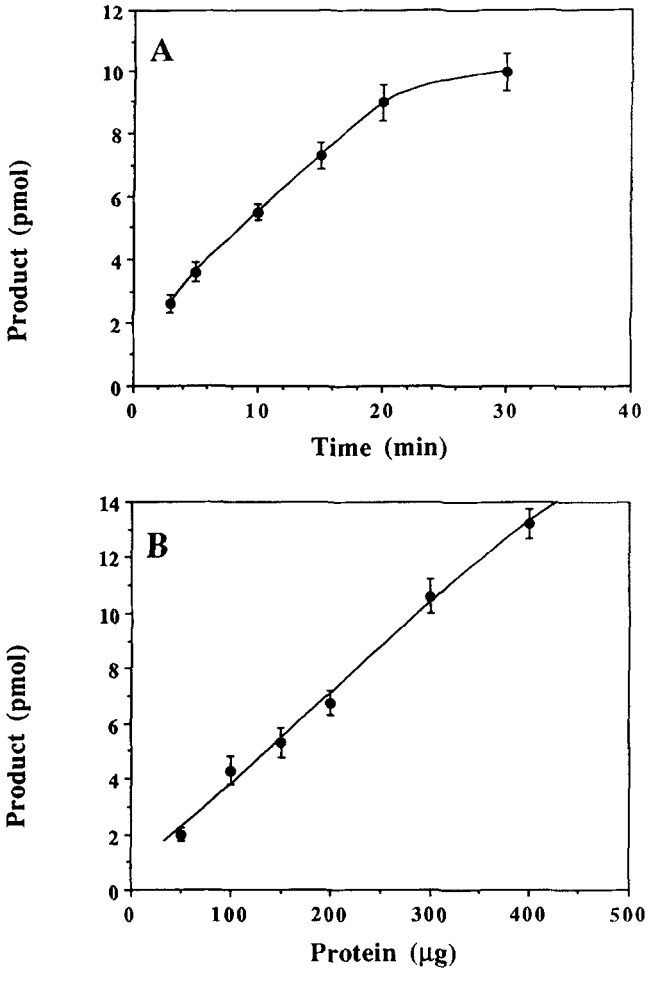

Fig. 4. Variation of rat kidney bile acid CoA synthetase activity with incubation time (A) and protein concentration (B). The assay mixture contained 0.02 μCi[^{14}C]CDC, 5 μM unlabeled CDC, 200 μM CoA, 5 mM ATP, 5 mM $MgCl_2$, 50 mM NaF, and 0.1 M Tris-HCl, pH 8.5. In A, 200 μg of microsomal protein was used. In B, incubation was carried out for 10 min. Each value is the mean ± SEM for three experiments.

Substrate specificity

ATP was essential for enzyme activity. Substitution of ATP with an equivalent amount of GTP or CTP did not yield any product. Enzyme activity was also partially inhibited (33%) by 5 mM pyrophosphate, a product of the reaction. In addition to CDC, [2,4-^{3}H]cholate was demonstrated to be a substrate of the enzyme (data not shown). Unlabeled deoxycholate (2 μM) and lithocholate (3 μM) inhibited the formation of CDC-CoA by 50%, suggesting that they are also substrates.

Kinetic studies

At a saturating concentration of CoA, the initial rates of reaction increased with increasing concentrations of CDC, conforming to Michaelis-Menten kinetics (**Fig. 5**). The apparent K_m of CDC was 2.5 ± 0.38 μM and the

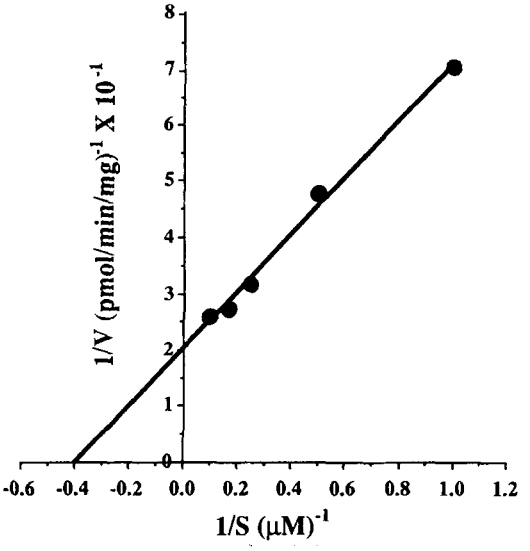

Fig. 5. Double-reciprocal plot of the rate of reaction as a function of CDC concentration. Initial rates of CDC-CoA formation were determined at various concentrations of CDC (1.0, 2.0, 4.0, 6.0, and 10.0 μM) in the presence of a saturating concentration of CoA (200 μM).

V_{max} was 5.0 ± 0.2 pmol/min per mg. Increasing concentrations of CoA at a fixed concentration of CDC also produced increasing initial rates of reaction up to 4.5 μM CoA (**Fig. 6**), although at higher concentrations of CoA, substrate inhibition was observed. The apparent K_m and V_{max} of CoA were 2.6 ± 0.17 μM and 2.8 ± 0.12 pmol/min per mg, respectively.

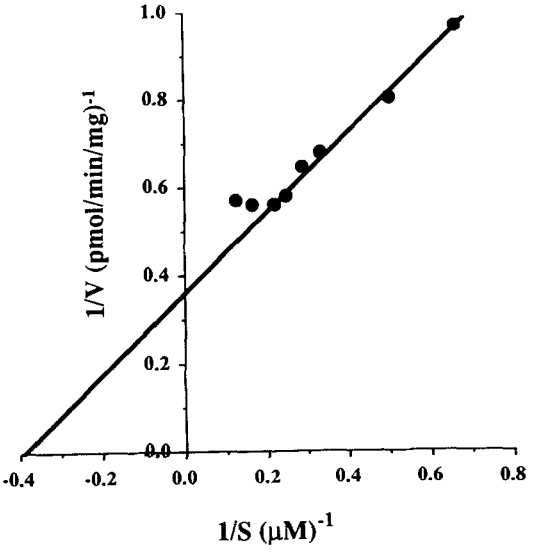

Fig. 6. Double-reciprocal plot of rat kidney bile acid CoA synthetase activity as a function of CoA concentration. Initial rates of reaction were determined at various concentrations of CoA (1.5, 2, 3, 3.5, 4, 4.5, 6, and 8 μM) in the presence of 5 μM CDC. Incubation time and protein concentration were chosen such that no more than 10% of the limiting substrate was consumed in the reaction.

Substrate competition experiments

Benzoic acid and salicylic acid over the concentration range of 0–400 μM did not inhibit CDC-CoA formation.

DISCUSSION

We have shown in this study, using a radioassay with HPLC analysis of the product, that bile acid CoA synthetase, a key enzyme in the formation of bile acid N-acyl amidates, is present in the rat kidney microsomal fraction. The product of the reaction was shown to be CDC-CoA on the basis of: *1)* its coelution from HPLC with chemically synthesized CDC-CoA; and *2)* formation of taurine and glycine conjugates of CDC when it was incubated with taurine and glycine, respectively, in the presence of purified human liver BAT.

The kidney is known to have other organic acid CoA synthetase activities, for instance, the ability to form CoA esters of benzoic acid (14) prior to conjugation with glycine in the synthesis of hippurate. CoA esters of other drug acids such as salicylic acid are also known to be formed in the kidney (15). The inability of rat kidney bile acid CoA synthetase to be inhibited by either of these alternate substrates suggests that it is distinct from other rat kidney CoA synthetases.

It is presently unknown whether the same form of bile acid CoA synthetase exists in rat liver and kidney; however, they have several properties in common. The rat kidney enzyme has a pH optimum of 8.5 compared to 9.0 for the hepatic bile acid CoA synthetase (16). Magnesium is essential for both enzymes to function, with EDTA completely eliminating enzyme activity in both rat kidney and liver (17). Like bile acid CoA synthetase from rat liver (16, 17), the rat kidney enzyme has a very high affinity for its bile acid substrate. The rate of reaction increased as a function of CoA only at low concentrations (1.5–4.5 μM), but there was very little increase in reaction rate at CoA concentrations above 4.5 μM suggesting inhibition by CoA. A similar property has been demonstrated with bile acid synthetase from rat liver (17). Purification and determination of the molecular weight of both enzymes will further clarify whether they represent the same or different proteins.

We recently demonstrated BAT activity in rat kidney (2). Our present finding that bile acid CoA synthetase activity is also present in rat kidney, demonstrates that the kidney has the full capacity to form bile acid N-acyl amidates. Most bile acids filtered by the kidney are reabsorbed (over 95%), with minimal amounts being lost in the urine (18). Conjugation of bile acids in the kidney may serve as a protective mechanism by which the renal cell avoids injury from circulating unconjugated bile acids. In light of these findings, it is possible that our knowledge of bile acid physiology is not complete, as the kidney may have a role in bile acid metabolism.

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REFERENCES

1. Hofmann, A. F. 1988. Bile acids. *In* The Liver: Biology and Pathology. I. M. Arias, W. B. Jakoby, H. Popper, D. Schacter and D. A. Shafritz, editors. Raven Press, New York. 553–573.
2. Kwakye, J. B., M. R. Johnson, S. Barnes, and R. B. Diasio. 1991. Identification of bile acid CoA:amino acid N-acyltransferase in rat kidney. *Biochem. J.* **280:** 821–824.
3. Bremer, J. 1956. Choloyl-S-CoA as an intermediate in the conjugation of cholic acid with taurine by rat liver microsomes. *Acta Chem. Scand.* **10:** 56–71.
4. Schérten, T. 1971. Bile acid conjugation. *In* Metabolic Conjugation and Metabolic Hydrolysis. W. H. Fishman, editor. Vol. II. Academic Press, New York. 75–121.
5. Schérten, T. 1967. The synthesis of taurocholic and glycocholic acids by preparations of human liver. 1. Distribution of activity between subcellular fractions. *Biochim. Biophys. Acta.* **141:** 144–154.
6. Bremer, J., and U. Gloor. 1955. Studies on the conjugation of cholic acid with taurine in cell subfractions. *Acta Chem. Scand.* **9:** 689–698.
7. Vessey, D. A. 1978. The biochemical basis for the conjugation of bile acids with either glycine or taurine. *Biochem. J.* **174:** 621–626.
8. Shah, P. P., and E. Staple. 1968. Synthesis of coenzyme A esters of some bile acids. *Steroids.* **12:** 571–576.
9. Johnson, M. R., S. Barnes, and R. B. Diasio. 1989. Radioassay of bile acid CoA:glycine/taurine N-acyltransferase using n-butanol solvent extraction procedure. *Anal. Biochem.* **182:** 360–364.
10. Suzue, G., and Y. L. Marcel. 1972. Specificity of long-chain acyl coenzyme A synthetase from rat liver microsomes. Influence of the position of double bonds in octadecadienoic acids. *Biochemistry.* **11:** 1704–1708.
11. Killenberg, P. G. 1978. Measurement and subcellular distribution of choloyl-CoA synthetase and bile acid-CoA:amino acid *N*-acyltransferase activities in rat liver. *J. Lipid Res.* **19:** 24–31.
12. Johnson, M. R., B. J. Kwakye, S. Barnes, and R. B. Diasio. 1991. Purification and characterization of bile acid CoA:amino acid N-acyltransferase from human liver. *J. Biol. Chem.* **266:** 10227–10233.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
14. Schacter, D., and J. V. Taggart. 1953. Benzoyl coenzyme A and hippurate synthesis. *J. Biol. Chem.* **203:** 925–934.
15. Killenberg, P. G., and J. R. Webster. 1980. Conjugation by peptide bond formation. *In* Enzymatic Basis of Detoxification. W. B. Jakoby, editor. Vol. II. Academic Press, New York. 141–167.
16. Schepers, L., M. Casteels, K. Verheyden, G. Parmentier, S. Asselberghs, H. J. Eyssen, and G. P. Mannaerts. 1989. Subcellular distribution and characteristics of trihydroxycoprostanoyl-CoA synthetase in rat liver. *Biochem. J.* **257:** 221–229.
17. Vessey, D. A., and D. Zakim. 1977. Characterization of microsomal cholyl CoA synthetase. *Biochem. J.* **163:** 357–362.
18. Barnes, S., J. L. Gollan, and B. H. Billing. 1977. The role of tubular reabsorption in the renal excretion of bile acids. *Biochem. J.* **166:** 65–73.