buffer homogenate by the differential centrifugation method of Schneider and Hogeboom (*2*). The microsomes were washed once each with isotonic KCl and distilled water. The supernatant was dialyzed for 24 hours against distilled water and then lyophilized. Mitochondria were isolated from an 0.88*M* sucrose homogenate (*2*). Taurocholic acid was routinely determined with the use of the furfural-$H_2SO_4$ reaction of Pettenkofer (*3*) after free cholic acid had been removed by extraction with acetone and ethyl ether in the proportion of 1 to 2 and ethyl ether. The identity of the product as taurocholic acid was confirmed by paper chromatography.

Incubation of 50 mg of washed, lyophilized guinea pig liver microsomes with 4 μmoles of coA, 10 μmoles of cholic acid, 44 μmoles of adenosine triphosphate (ATP), and 30 μmoles of $MgCl_2$ in 1.0 ml of 0.1*M* $K_2HPO_4$ buffer at *p*H 7.6 for 60 min resulted in the formation of 2.2 μmoles of a cholic acid derivative. When the compound was chromatographed on Whatman No. 1 filter paper, an ultraviolet absorbing band having the $\lambda_{max.}$ of adenine was noted at an $R_f$ of 0.62 (60-percent propanol in $H_2O$) or at an $R_f$ of 0.81 (50-percent ethanol in $H_2O$). Spraying a portion of the paper with the $SbCl_3$-acetic acid spray, that has been described previously (*4*) revealed a single rose-colored band that is characteristic of cholic acid. This band coincided exactly in position and shape in both solvent systems with the position and shape of the 260-mμ absorbing band; the compound was further identified as cholyl coA by means of color reactions listed in Table 1. Finally, hydrolysis of the compound resulted in the appearance of two bands on paper chromatograms, one having the $R_f$ value and color reaction of cholic acid, the other having the $R_f$ value and color reaction of coA.

Table 1. Identification of cholyl coA.

| Test | Result |
|---|---|
| Cholic acid ($SbCl_3HAc$) (*4*) | + |
| Adenine ($\lambda_{max.}$ 260 mμ) | + |
| RSH (nitroprusside) (*8*) | – |
| RS—C—R′ (NaOH-nitroprusside) (*8*) | + |
| RS—C—R′ (hydroxylamine-$FeCl_3$) (*9*) | + |
| Ester phosphate (ammonium molybdate-$HClO_4$) (*10*) | + |

Table 2. Role of cholyl coA in taurocholic acid synthesis. The figures in parentheses in column 1 are (i) amount, in millimicromoles, of cholyl coA and cholic acid and (ii) amount, in micromoles, of taurine added to the supernatant. The final volume was 0.6 ml in 0.03*M* $K_2HPO_4$ buffer at *p*H 7.6.

| Compound(s) added to 20 mg of dialyzed liver supernatant | Taurocholic acid synthesized (mμ mole) |
|---|---|
| Cholyl coA (118) | 0.0 |
| Cholyl coA (118) pretreated with base* + taurine (24) | 0.0 |
| Cholic acid (166) + taurine (24) | 0.0 |
| Cholyl coA (118) + taurine (24) | 85.3 |

* Cholyl coA in 0.1 ml water, hydrolyzed by the addition of 0.1 ml 4*N* NaOH and incubation for 2 min at 37°C. The mixture was neutralized with $H_2SO_4$ before addition of the liver supernatant.

If chromatographically pure cholyl coA is incubated together with taurine in the presence of dialyzed guinea pig liver supernatant, taurocholic acid is produced (Table 2); this demonstrates that cholyl coA is an intermediate in the synthesis of the conjugated bile salt.

Taurocholic acid can also be readily synthesized by combining the reactions given in Eqs. 1 and 2. Microsomes, dialyzed supernatant, cholic acid, coA, ATP, and taurine are all required for taurocholic acid production in this overall reaction. Either microsomal or supernatant fraction alone is inactive.

The enzyme responsible for cholic acid activation would appear to be distinct from those that catalyze the formation of the coA derivatives of fatty acids (*5*, *6*), benzoic acid (*6*) and *p*-aminobenzoic acid (*7*) in that the intracellular distribution of the other activating enzymes and, in one case, the tissue localization as well, are quite different from that of the cholyl coA-forming enzyme. The bile acid activating enzyme is found only in the microsomes of liver. Liver mitochondria are inactive and no kidney fraction will carry out the reaction. The enzyme that activates short-chain fatty acids, benzoic acid, and *p*-aminobenzoic acid, on the other hand, is known to be localized in the mitochondria of both liver and kidney (*6*, *7*). Likewise, the long-chain fatty acid-activating enzyme of liver supernatant (*5*), which in our studies readily synthesized palmityl coA, is incapable of so activating cholic acid.

The activating system was also found to be capable of catalyzing the formation of other bile acid-coA derivatives. Desoxycholyl coA and $C^{14}$-labeled lithocholyl coA have been prepared in micromole quantities. Their roles in the scheme of bile acid metabolism, with regard to both conjugation and interconversions, are now under study.

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### References and Notes

1. Since completion of this paper, Elliott [*Biochim. et Biophys. Acta* 17, 440 (1955)] has reported that optimal formation of cholyl hydroxamic acid by liver microsomes required the presence of ATP, coA, and cysteine. This finding is consistent with the observation, reported here, that cholyl coA is produced by such a system.
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16 August 1955

### Correction

The names of the compounds in the last three lines of Table 1 in "Inhibition of the effect of some carcinogens by their partially hydrogenated derivatives" by Kotin, Falk, Lijinsky, and Zechmeister [*Science* **123**, 102 (20 Jan. 1956)] should read dihydrodibenzanthracene, decahydrodibenzanthracene, and perhydrodibenzanthracene.

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3 February 1956

*The beauty of electricity, or any other force, is not that the power is mysterious and unexpected . . . but that it is under law and that the taught intellect can even now govern it largely.*—M. FARADAY.