as well as from failing response *in vivo* to methylene blue or ascorbic acid (reduction of MHb to ferrohemoglobin). The abnormal hemochromogen could not be transferred to MHb by ferricyanid, nor could it be reduced by hydrosulfite or transferred to COHb, as judged from absorption spectral curves, whereas it could be transformed quantitatively to acid hematin. The peroxidase activity of the blood was the same as for normal blood with the same iron content. No difference from normal hemoglobin in electrophoretic mobility (paper electrophoresis) was found at pH 8.6.

It is concluded that the abnormal hemochromogen assumingly is a Fe-chromogen with peroxidase activity but not able to combine with oxygen and responsible for a new form of hereditary cyanosis. The hemochromogen is called Norin-hemochromogen referring to the name of the cyanotic family.

Cholyl-CoA as an Intermediate in Taurocholic Acid Formation by Rat Liver Microsomes

Jon Bremer

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

We have previously shown that both the microsomic fraction and the particle-free supernatant of rat liver homogenates are required for the conjugation of cholic acid with taurine [1,2].

The mechanism of this conjugation has been subjected to further studies.

Rat liver microsomes were isolated as follows: The liver was homogenized in 4 parts 10 % sucrose. Nuclei and mitocondria were sedimented at 16 000 g for 15 min, the microsomes at 25 000 g for 110 min. The fraction was washed once.

The taurocholic acid formation was followed by means of ^{35}S-labelled taurine as previously described [1]. It appears from Table 1 that the particle free supernatant could be substituted by CoA and ATP. The conjugation was also stimulated by Mg^{++} (though not in the published experiment) and fluoride. Cysteine or glutathion was required when a crude CoA was used, but when a reduced, 70 % pure CoA was used, the effect of cysteine or glutathion was slightly inhibiting.

Table 1. Complete system: Phosphate buffer pH 7.4 0.022 M; *KF 0.215* M; *Cysteine 0.007* M; *CoA 0.001* M; *ATP 0.007* M; *Mg^{++} 0.001* M; *Sucrose 0.035* M; *Microsomes from approximately 500 mg liver per vessel.*

Experiment I 2 *μmoles taurine/vessel*
2 *μmoles cholic acid/vessel*
Total volume 1.5 ml/vessel

Experiment II 50 *μmoles hydroxylamine/vessel*
1 *μmole cholic acid/vessel*
Total volume 1.75 ml/vessel

All solutions were adjusted to pH 7.4. Gas phase air, Incubation time 120 min, temp. 37° C.

Factor *excluded* from the complete system	*Experiment I* Taurocholate formed μmoles/vessel		*Experiment II* Hydroxamic acid formed μmoles/vessel	
None	0.31	0.27	0.71	0.69
Cholic Acid	0	0	0.03	0.03
KF	0.12	0.12	0.42	0.41
ATP	0	0	0	0
CoA	0	0	0.14	0.07
Cysteine	0.34	0.35	0.85	0.82
Mg^{++}	0.41	0.36	0.74	0.73
Hydroxylamine	—	—	0	0.05

These findings make it probable that cholic acid is conjugated with taurine with cholyl-CoA as an intermediate. This was further supported by the formation of cholic hydroxamic acid when hydroxylamine was added to the incubation mixture instead of taurine.

The cholic hydroxamic acid formed was isolated by a modification of Eriksson and Sjövall's [3] quantitative paper chromatography for conjugated bile acids. A mixture of 1 part *n*-butanol and 2 parts petroleum spirit (b. p. above 120° C) was used as a moving phase. The hydroxamic acids spots were eluted with ethanol, the eluate was blown to dryness and the residue dissolved in conc. H_2SO_4. After 1 hour the density at 389 mμ was measured.

By this method the formation of cholic hydroxamic acid was shown to be dependent on the presence of ATP, CoA and fluorid. As hydroxylamine represents a trapping agent for CoA-activated carboxyl groups, it is concluded that cholyl-CoA represents the "activated cholic acid" which conjugates with taurine.

The ^{35}S-labelled taurine was generously supplied by L. Eldjarn, Norsk Hydro's Institute for Cancer Research, Oslo.

This work has been supported by *The Norwegian Cancer Society*, Oslo.

1. Bremer, J. *Acta Chem. Scand.* **9** (1955) *In press.*
2. Bremer, J. and Gloor, U. *Acta Chem. Scand.* **9** (1955) *In press.*
3. Eriksson, S. and Sjövall, J. *Acta Chem. Scand.* **8** (1954) 1099.

Observations on Biosynthesis of Lecithins

Donald J. Hanahan* and Martin Rodbell

University of Washington, Department of Biochemistry, Seattle, Washington, U.S.A.

Studies have been carried out on the metabolism of lecithin and certain of its derivatives in the subcellular fractions of the liver cell. Although lecithin was apparently not oxidized and had no obvious effect on the isolated mitochondria, lysolecithin, glyceryl phosphorylcholine, phosphorylcholine and glycorophosphate plus choline had a marked stimulatory effect on the oxidation rate of the mitochondrial systems. One of the most active compounds in this stimulatory action was phosphoryl choline which was very actively incorporated as an intact unit into lecithin. The implications of these observations and possible synthetic routes for lecithin will be discussed.

* Temporary address: Department of Physiological Chemistry, University of Lund, Lund, Sweden.

The Metabolism of Cysteamine Sulphinic Acid (Hypotaurine) in Rats, Investigated by Means of Radioactive Sulphur (^{35}S)

L. Eldjarn and A. Sverdrup*

Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

Cysteamine sulphinic acid is formed by the mammalian organism and is an intermediate in the degradation of cysteine [1]. The compound is formed by the decarboxylation of cysteine sulphinic acid [2] as well as by the oxidation of cysteamine and cystamine [3].

In order to study the metabolism of cysteamine sulphinic acid, the compound was synthesized and labeled with radioactive sulphur. ^{35}S-labeled cystamine was prepared as previously described [3]. Cysteamine sulphinic acid was prepared from cystamine according to the following dismutation reactions:

$$(HCl \cdot NH_2CH_2CH_2S)_2 + Hg^{++} \longrightarrow NH_2CH_2CH_2SOH + NH_2CH_2CH_2SHgCl$$

$$NH_2CH_2CH_2SOH \longrightarrow NH_2CH_2CH_2SO_2H + (NH_2CH_2CH_2S)_2$$

The mercaptide was precipitated by 10 % ethyl alcohol. When 8 equivalents of $HgSO_4$ was used, a yield of 23 % of cysteamine sulphinic acid admixed 9 % cystamine. was obtained. The cystamine was removed by adsorbtion on Dowex 50. The cysteamine sulphinic acid was crystallized from water-ethanol ether. M. p. 170° C.

One mg of ^{35}S-labeled cysteamine sulphinic acid was administered to male rats (250 g). After fractionation of the urine [3] the greater part of the radioactivity was recovered as sulphate and as taurine. Most probable the metabolism of cysteamine sulphinic acid in the rat organism is confined to these two reactions. Taurine is known not to be converted to sulphate by the rat organism. These findings should provide good opportunity to study the mechanism of sulphate formation in mammals by means of cysteamine sulphinic acid labeled with radioactive sulphur.

1. Awapara, J. *J. Biol. Chem.* **203** (1953) 183.
2. Bergeret, B. and Chatagner, F. *Biochem. et Biophys. Acta* **9** (1952) 141.
3. Eldjarn, L. *Scand. J. Clin. & Lab. Invest.* **6** (1954) Suppl. 13.

* Fellow of the "Norwegian Cancer Society", Oslo, Norway.