ACTA CHEMICA SCANDINAVICA 9 (1955) 268—271

The Conjugation of Glycine with Cholic Acid and Benzoic Acid in Rat Liver Homogenate. Bile Acids and Steroids 21

JON BREMER*

Department of Physiological Chemistry, University of Lund, Sweden, and Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway

The formation of glycocholic and taurocholic acid has been shown to take place in a system containing microsomes and supernatant from rat liver, whereas the hippuric acid formation also requires the presence of mitochondria.

The taurine present in the rat liver has been shown to be quantitatively conjugated with cholic acid in spite of the presence of considerably greater amounts of glycine.

We have recently shown that both the "microsome fraction" and the "supernatant fraction" of rat liver homogenate are essential for the conjugation of cholic acid with taurine[1,2]. We have now been able to separate the system responsible for the taurocholic and glycocholic acid formation from that forming hippuric acid.

Taurocholic acid was formed in preference to glycocholic acid when both taurine and glycine were present.

EXPERIMENTAL

Liver homogenate of male rats (weight 200—250 g) was separated into "mitochondria fraction", "microsome fraction" and "supernatant fraction" as described elsewhere [2]. The homogenate fractions tested for enzyme activity were incubated with cholic acid, benzoic acid, ^{14}C-labeled glycine and ^{35}S-labeled taurine as shown in Fig. 1. The incubates were then extracted with 1/3 volume *n*-butanol [1], but the contents of the vessels had to be acidified to pH 1 to make also the hippuric acid extractable. This procedure has been shown to leave the free taurine in the water phase whereas taurocholic acid is extracted by the butanol [1]. Fig. 1 shows that also the glycine stays almost quantitatively in the water phase whereas the glycocholic and hippuric acids are extracted.

* Fellow of the *"Norwegian Cancer Society"*, Oslo, Norway. Present address: Norsk Hydro's Inst. for Cancer Research.

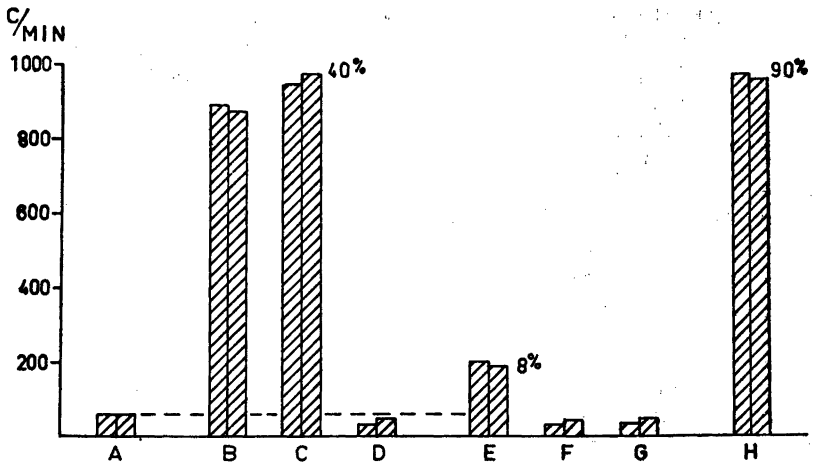

Fig. 1. Total volume of the vessels 1.75 ml. 1 ml 20 % homogenate added to each vessel. Concentrations: Potassium phosphate buffer pH 7.7 0.037 M, *sucrose 0.080* M, *nicotinamide 0.017* M, *magnesium chloride 0.00065* M. *ATP 1.2 μmole/vessel, versene 0.5 mg/vessel.*

A. 1 ml homogenate centrifuged at 400 g for 5 min. 1.8 μmole glycine-^{14}C representing 15 000 c/min/vessel.
B. As A + 2.5 μmole cholic acid.
C. 1 ml homogenate centrifuged at 12 000 g for 10 min. 1.8 μmole glycine-^{14}C + 2.5 μmole cholic acid.
D. 1 ml homogenate centrifuged at 100 000 g for 30 min. 1.8 μmole glycine-^{14}C + 2.5 μmole cholic acid.
E. As A + 2.5 μmole benzoic acid.
F. As C, but with benzoic acid instead of cholic acid.
G. As D, but with benzoic acid instead of cholic acid.
H. 1 ml homogenate centrifuged at 12 000 g for 10 min. 0.4 μmole taurine-^{35}S representing 7 250 c/min/vessel. + 2.5 μmole cholic acid.

Each vessel acidified with 0.1 ml conc. HCl and extracted with 0.62 ml of butanol. 0.1 ml butanol plated and counted. The double columns represent parallels.

The distribution of hippuric acid between the butanol/*N* hydrochloric acid phases in the concentration range used was estimated spectrophotometrically at 2 700 Å. The partition coefficient butanol/*N* hydrochloric acid was found to be 5.2. After equilibration of the acidified reaction mixture the volume of the aqueous phase was about 1.94 ml and that of the butanol phase 0.54 ml.

The distribution of glycocholic acid between the butanol/*N* hydrochloric acid phase was assumed to be the same as for taurocholic acid [1].

The amounts of the conjugates extracted with butanol was determined by measuring the radioactivity of aliquots plated directly under standard conditions [1].

RESULTS AND DISCUSSION

The two separate experiments done gave similar results. One of the experiments is shown in Fig. 1.

The liver homogenate used in this experiment was nearly five times as efficient in conjugating cholic acid with glycine (Fig. 1 B) as in conjugating

benzoic acid with glycine (Fig. 1 E). The 1.8 μmole glycine-^{14}C added to the systems gave under identical conditions rise to 0.72 μmole glycocholic acid and 0.15 μmole hippuric acid, respectively. Since some free glycine was presumably present in the liver homogenate, these amounts must represent the minimal amounts of newly formed conjugated acids. A content of 0.2 mg of free glycine per g rat liver has been reported by Wu[8].

Furthermore, there is a distinct difference in the localization of rat liver systems responsible for the formation of the glycocholic and hippuric acids. The "microsome" plus "supernatant" fractions retained in full the activity to synthesize glycocholic acid (Fig. 1 C), but were unable to bring about the formation of hippuric acid (Fig. 1 F). The finding that the mitochondria are essential for the hippuric acid synthesis is in accordance with the results of Kielly and Schneider[3] in mouse liver homogenate.

Both the "microsome" fraction and the "supernatant" are essential for the formation of glycocholic acid (Fig. 1 C, D). This localization is similar to that of the system forming taurocholic acid[2].

In order to compare the conjugation of cholic acid with taurine and with glycine, the concentration of preformed taurine in the homogenate had to be determined. Two vessels with homogenate from the same liver were incubated with 0.4 μmole taurine labeled with ^{35}S (Fig. 1 H). The activity recovered as taurocholic acid correspond to 90 % of the amount added. Spectrophotometric determination according to Sjövall [4,5,1] showed, however, that a total amount of 0.70 μmole taurocholic acid had been formed, *i.e.*, the total amount of taurine present at the start of the experiment must have been about 0.78 μmole, of which only 0.4 μmole was the labeled material added.

Similarly spectrophotometric determination of the formation of taurocholic acid when the system in addition to cholic acid and preformed taurine also contained ^{14}C-labeled glycine (1.8 μmole) (Fig. 1 C) showed the taurine to be almost quantitatively conjugated in spite of the glycine present. This finding agrees with the earlier observations of Bergström and Gloor[6] in human liver homogenates.

The enzymic systems conjugating cholic acid with taurine and glycine in the rat must therefore have greater preference for taurine; the cited results give a good explanation of the fact that rat bile contains only 5—10 % of the cholic acid conjugates in the form of glycocholic acid[7] in spite of the relatively high glycine content in rat liver. The glycine content of rat liver has been reported to be three times that of taurine on a molar base[8].

Whether the conjugation of cholic acid with glycine and taurine is performed by the same enzyme system is as yet an open question.

The author wishes to express his appreciation to L. Eldjarn and P. Fritzson, Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway, for the synthesis of ^{35}S-labeled taurine.

The author is also indebted to J. Sjövall, Department of Physiological Chemistry, University of Lund, Sweden, for the use of his unpublished method for the quantitative determination of bile acids by paper chromatography.

This work is in part supported by *Statens Medicinska Forskningsråd*, Sweden, and by *Knut och Alice Wallenbergs Stiftelse*, Sweden.

REFERENCES

1. Bremer, J. *Acta Chem. Scand.* **9** (1955). *(In press).*
2. Bremer, J. and Gloor, U. *Acta Chem. Scand.* **9** (1955). *(In press).*
3. Kielley, R. K. and Schneider, W. C. *J. Biol. Chem.* **185** (1950) 869.
4. Sjövall, J. *Acta Chem. Scand.* **8** (1954) 339.
5. Eriksson, S. and Sjövall, J. *Acta Chem. Scand. (To be published.)*
6. Bergström, S. and Gloor, U. *Acta Chem. Scand.* **8** (1954) 1109.
7. Norman, A. *Acta Physiol. Scand.* **32** (1954) 1.
8. Wu, C. J. *J. Biol. Chem.* **207** (1954) 775.

Received October 30, 1954.