ACTA CHEMICA SCANDINAVICA 11 (1957) 414—416

On the Metabolism of Taurine Conjugated 3α, 7α, 12α-Trihydroxycoprostanic Acid in the Rat

Bile Acids and Steroids 45

SVEN LINDSTEDT and ARNE NORMAN

Department of Physiological Chemistry, University of Lund, Lund, Sweden

The taurine conjugate of tritium labelled 3α,7α,12α-trihydroxycoprostanic acid has been prepared. In experiments on rats it was found that no splitting of the conjugate occurs in the liver but that it is split to a small extent in the intestine.

It has been shown that all bile acids naturally occurring in the rat bile are conjugated with taurine, only traces of glycine conjugates being found [1]. When the bile acids in the feces are examined by chromatography one finds only a small amount of conjugated acids [2]. When, however, the bacterial flora in the intestine has been depressed with chemoterapeutics practically all the bile acids in the feces are in the conjugated form [3]. This has led to the view that the microbial enzymes are mainly responsible for the normally occurring hydrolysis, an opinion which is further supported by the observation that certain anaerobic bacteria have a high capacity of splitting conjugated bile acids [4].

It has not, however, been definitely settled whether the digestive enzymes or enzymes in the liver are capable of splitting bile acid conjugates. Portman and Mann [5] have fed ^{35}S-labelled taurocholic acid to bile fistula rats and found 2—18 % of the radiosulfur in the urine. The hydrolysis in this case may well have been caused by bacterial enzymes attacking bile salts. Norman [6] has injected glycocholic acid-24-^{14}C intraperitoneally into bile fistula rats and into rats on which the bile fistula was made 6 h after injection. In the first case no taurocholic acid was found in the bile while in the latter about 50 % of the given amount appeared as taurocholic acid. The results indicated that no hydrolysis of the glycocholic acid occurs in the liver.

We have made similar experiments with tauro-trihydroxycoprostanic acid. The free trihydroxycoprostanic acid is known to be rapidly degraded to cholic acid in the liver [7]. If any hydrolysis of the taurine conjugates occurs in the liver one would thus expect to find activity in the cholic acid excreted in the bile.

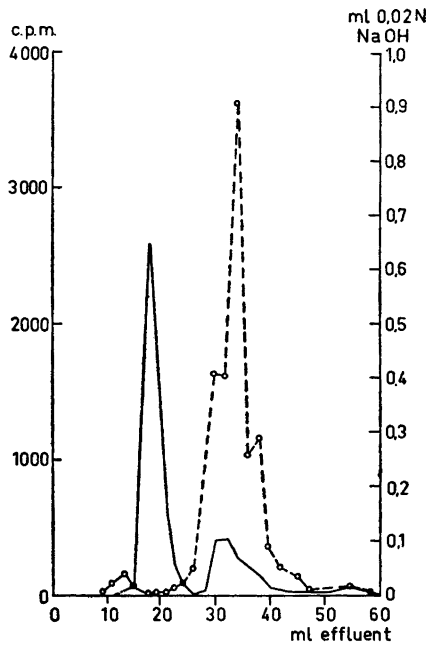

Fig. 1. Chromatography of conjugated bile acids excreted in a bile fistula rat after intraperitoneal injection of tritium labelled taurotrihydroxycoprostanic acid. Phase system D. Moving phase: water. Stationary phase: butanol. Solid line: Titration. Broken line: Radioactivity.

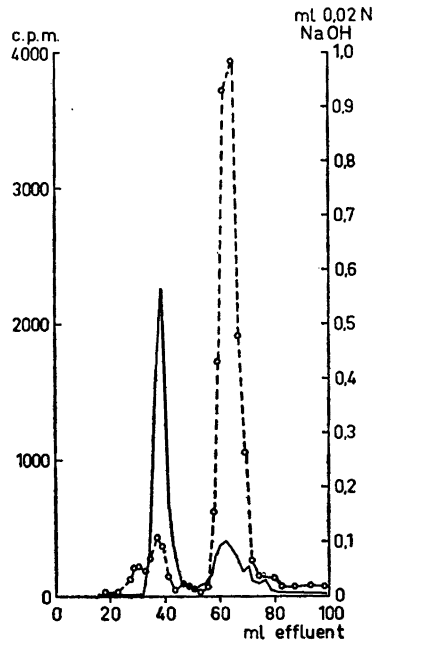

Fig. 2. Chromatography of conjugated bile acids in the bile fistula of a rat that had received an intraperitoneal injection of tritium labelled tauro-trihydroxycoprostanic acid 6 hours prior to operation. Phase system D. Solid line: Titration. Broken line: Radioactivity.

EXPERIMENTAL

Tritium-labelled 3*a*,7*a*,12*a*-25-L-trihydroxycoprostanic acid prepared by Bridgwater and Lindstedt [7] was coupled to taurine by the micromethods described by Norman [8]. The acid was purified by chromatography with phase system D (*cf.* Ref.[7]). It had a specific activity of about 250 000 c.p.m. per mg when counted in an "infinitely thin" layer in a Tracerlab gas-flow counter.

One mg of the sodium salt of the taurine-conjugated acid was administered intraperitoneally to bile fistula rats or normal rats on which a bile fistula was made 6 h after the administration. For details of the fractionation procedure see the preceding paper [7].

RESULTS AND DISCUSSION

Fig. 1 shows a chromatogram of an unhydrolyzed butanol extract of the bile excreted during a 24 h period after intraperitoneal administration of taurotrihydroxycoprostanic acid. The titration peaks at 15—25 ml and 30—40 ml are caused by taurocholic and taurochenodesoxycholic acid, respectively. No activity is present at the place of taurocholic acid, all of it being confined to a peak immediately after taurochenodesoxycholic acid at the place where tauro-

trihydroxycoprostanic acid is known to appear. For further identification the fractions containing the labelled material were hydrolyzed and rerun with phase system F (*cf.* Ref.[7]). The labelled compound now appeared at the place of free trihydroxycoprostanic acid added as carrier.

In Fig. 2 a chromatogram is shown of the bile acids excreated in a bile fistula made 6 h after injection of tauro-trihydroxycoprostanic acid. In this case about 10 % of the activity appears at the place of taurocholic acid and the rest at that of tauro-trihydroxycoprostanic acid. After hydrolysis the free bile acids were identified as cholic and trihydroxycoprostanic acid.

The results show that taurotrihydroxycoprostanic acid is excreted unchanged by the liver and indicate that the conjugating reaction in general is to no noticeable extent reversible. A slight hydrolysis of a minimum of 10 % must have occurred when the injected taurotrihydroxycoprostanic acid had taken part in the enterohepatic circulation for 6 h as this percentage of the tauro-conjugated C_{27}-acid was then recovered as the C_{24} cholic acid that was excreted as its taurine conjugate.

This work is part of investigations supported by *Statens medicinska forskningsråd* and *Knut och Alice Wallenbergs Stiftelse.*

REFERENCES

1. Norman, A. *Acta Physiol. Scand.* **32** (1954) 1.
2. Lindstedt, S. and Norman, A. *Acta Physiol. Scand.* **34** (1955) 1.
3. Norman, A. *Acta Physiol. Scand.* **33** (1955) 99.
4. Norman, A. and Grubb, R. *Acta Pathol. et Microbiol. Scand.* **36** (1955) 537.
5. Portman, O. W. and Mann, G. V. *J. Biol. Chem.* **213** (1955) 733.
6. Norman, A. *Proc. Roy. Physiograph. Soc. Lund* **25** (1955) No. 2.
7. Bridgwater, R. J. and Lindstedt, S. *Acta Chem. Scand.* **11** (1957) 409.
8. Norman, A. *Arkiv Kemi* **8** (1955) 331.

Received February 1, 1957.