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Studies on the 7α-Hydroxylation of Taurodesoxycholic Acid in Rat Liver Homogenates. Bile Acids and Steroids 18

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Fractionation of rat liver homogenate into a particle free supernatant (1 hr, 100 000 × g) and a crude residue containing microsomes, mitochondria *etc.* has been carried out. It is shown that the particle-free medium is capable of hydroxylating taurodesoxycholic acid in the 7 α-position yielding taurocholic acid. About 40 % of the amount hydroxylated in total homogenates is hydroxylated by supernatant alone.

The addition of ATP greatly stimulates the hydroxylation. Supernatant from 1 g liver can hydroxylate almost 1 mg of taurodesoxycholic acid in 2 hours. Versene increases the yield at a certain concentration range but inhibits in greater concentrations.

The conversion of desoxycholic acid into taurocholic acid has been demonstrated to occur in the rat *in vivo* [1] as well as *in vitro* in liver slices [2] and homogenates [3,4,5]. We are now presenting some results of experiments on the 7α-hydroxylation of taurodesoxycholic acid-24-^{14}C in rat liver homogenates. These homogenates have also been fractionated and the effect of different additions on the hydroxylation has been investigated.

EXPERIMENTAL

Taurodesoxycholic acid-24-^{14}C was prepared according to Bergström and Norman [6] from desoxycholic acid-24-^{14}C[7]. We are indebted tc Dr. A. Norman and fil.kand. K. Pääbo, respectively, for preparing these compounds.

The method used for paper chromatography of free and conjugated bile acids has been described by Sjövall [8] and the measurement of the radioactivity of the paper strips has been described earlier [3]. The homogenizing technique used has been described earlier but we have now used the medium proposed by Bucher [9,10] with only minor changes. One litre of the medium contained 10.8 g of monobasic potassium phosphate, 3.2 g of potassium hydroxide, 1 g of magnesium chloride (6 H_2O) and 3.6 g of nicotinic acid amide. The pH was 7.5 ± 0.1. The incubation vessel, a small pyrex test tube containing 1 ml of homogenate or homogenate fraction with the additions indicated was shaken in a water bath at 37° for 2 hours. The reaction was stopped by the addition of five volumes of ethanol and the resulting mixture was filtered and worked up as described earlier [3].

The crude homogenates were centrifuged in a precooled tube of an ordinary centrifuge at 400 × g for 10 minutes, yielding what is referred to as "total homogenate" in the following. To prepare a particle-free supernatant of this homogenate it was centrifuged at 100 000 × g for 60 min. in a preparative Spinco ultracentrifuge. The fatty top layer was then carefully pipetted off and the rest of the supernatant used for the incubations. In some experiments the whole sediment (mitochondria, microsomes, "fluffy" layer) was stirred up in the appropriate amount of homogenizing medium and used without further differentiation and is referred to as "crude residue". The supernatant contained about 2 mg of nitrogen per ml (corrected for the content of nicotinamide) whereas the total homogenate contained about 3.4 mg of N per ml.

RESULTS

The total homogenate was first separated into sediment and particle-free supernatant at 100 000 × g. The 7α-hydroxylation of taurodesoxycholate to taurocholate and the conjugation of cholic acid with taurine was then investigated with the supernatant and with the resuspended sediment.

The results of 4 representative experiments of each type are shown in Tables 1 and 2.

Table 1. 7α-hydroxylation of 50 μg taurodesoxycholic acid-24-^{14}C in rat liver homogenate and fractions thereof. 1 ml incubation medium corresponding to about 200 mg rat liver fresh weight. Incubation: 2 hours; pH 7.5; air.

Expt. No.	Hydroxylated amount in μg			
	total homogenate	supernatant	total sediment	supernatant + total sediment
1	22	16	8	22
2	13	6	9	26
3	35	9	7	41
4	38	12	6	24
Average values	27	10.8	7.5	28

Table 2. Conjugation of cholic acid with taurine in rat liver homogenate and fractions thereof. 1 ml incubation medium corresponding to about 200 mg of liver fresh weight. Each vessel contained 50 μg of cholic acid, no other additions. 2 hrs 37° in air, pH = 7.5.

Expt. No.	Taurocholic acid formed in μg			
	total homogenates	supernatant	total sediment	supernatant + total sediment
1	39	7	7	29
2	38	6	9	38
3	38	6	7	41
4	41	6	3	42
Average values	39	6.3	6.5	38

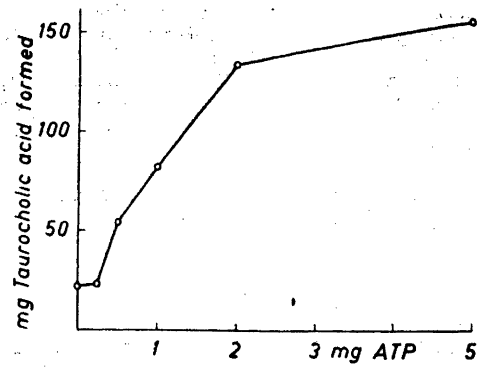

Fig. 1. Effect of increasing amounts of ATP on 7 α-hydroxylation of taurodesoxycholate-24-^{14}C. (0.2 mg). Reaction time: 2 hours at 37°, air.

These results show that the supernatant without any additions has approximately 40 % of the hydroxylating capacity of the total homogenate whereas the conjugation only occurred to about 15 % of that of the total homogenate or of the recombined fractions. Further data on the conjugation of bile acids in fractionated liver homogenates are being published by Bremer [18].

The effect of adenosinetriphosphate (ATP) on hydroxylation is illustrated in Fig. 1. In this experiment one ml of the supernatant hydroxylated about 22 μg taurodesoxycholate whereas this amount increased to a maximum of 134 μg after addition of 2 mg of ATP.

The effect of ATP on the hydroxylation was more marked in the supernatant than in the total homogenate where presumably the structural element contains both energy yielding and consuming systems. In one experiment similar to that shown in Fig. 1, 5 mg of ATP caused a seven fold increase to

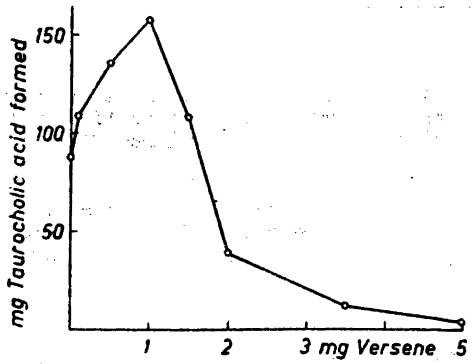

Fig. 2. Effect of versene on hydroxylation of taurodesoxycholate. Each vessel: Rat liver supernatant (1 ml) + taurodesoxycholic acid-24-^{14}C + ATP (1 mg). Incubation 2 hours at 37° in air.

Table 3. Influence of ATP, DPN and fumarate on 7 α-hydroxylation of taurodesoxycholate (50 μg) in 1 ml of supernatant. Incubation at 37° in air for 2 hours.

	μg taurocholic acid found
Total homogenates	19
Supernatant	17
» + 2 μmoles ATP	37
» + 1 μmole DPN	15
» + 1 μmole fumarate	14
» + DPN + fumarate	13
» + ATP + DPN	36
» + ATP + fumarate	35
» + ATP + DPN + fumarate	34

188 μg of hydroxylated product in 1 ml of the supernatant whereas the total homogenate only reached 92 μg with the same addition.

We found earlier that the hydroxylating system is very sensitive to some heavy metal ions, and have therefore investigated the influence of a chelating agent, versene, *i. e.* ethylenediamine tetraacetic acid.

A typical experiment is shown in Fig. 2 in which increasing amounts of versene were added to 1 ml of the supernatant to which, furthermore, 2 mg of ATP had been added.

There was almost a doubling of the yield after the addition of 1 mg (3 μmoles) of versene but larger amounts rapidly caused an almost complete inhibition. Sweat [13] reported some inhibition of the 11 α-hydroxylase with versene.

The effect of fumarate and diphosphopyridine nucleotide (DPN) either alone or in combination with ATP is shown in Table 3. From these results it can be concluded that in the present system ATP is the only compound showing any noticeable effect. Some experiments with addition of TPN, reduced DPN and cytochrome c did not cause any noticeable changes in the results. Work is now in progress to fractionate the supernatant in order to facilitate further studies of the enzymic mechanisms involved.

The 7 α-hydroxylase system studied is thus contained mainly in the particle-free supernatant and the activity is greatly stimulated by ATP.

This is thus at variance with the 11 α-hydroxylase [12-13] from adrenals that has been shown to be contained in the particles isolated between 2 000 × g and 19 000 × g [13] or at 5 000 × g [14] whereas the supernatant has been found to be practically inactive in this respect.

The 17α- and 21-hydroxylases are, however, apparently contained mainly in the supernatant (*cf.*[14], page 175, footnote 2).

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