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Metabolism of Bile Acids in Rat Liver Slices and Homogenates. Bile Acids and Steroids 15

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The conjugation and 7 α-hydroxylation of desoxycholic acid to taurocholic acid [1] found to take place in the rat *in vivo* has also been demonstrated in rat liver slices [2].

These reactions have now been studied both in rat liver slices and homogenates. The techniques used and some results will be reported in this paper. For the quantitative estimation of the reaction products the paper chromatographic procedures of Sjövall [3] for free and conjugated bile acids have been used extensively.

EXPERIMENTAL

Animals. The white female rats used throughout all experiments had an average weight of 150 g. The average liver fresh weight was 5—6 g. The experiments were always started in the morning to get as uniform conditions as possible. The animals, which had free access to their normal diet up to this time, were killed by a blow on the head, the liver excised and immediately placed in ice cold buffer solution or homogenizing medium.

Labelled bile acids. The acids were prepared according to Bergström, Rottenberg and Voltz [4]. The desoxycholic acid-24-^{14}C used in the experiments reported here, was 99 % pure as measured by paper chromatography. The rest of the activity was within the limits of normal deviation from the background, with no definite spot on the paper. The stock solutions contained 1 mg per ml of the different bile acids as sodium salts in water adjusted to pH 7.5.

Buffer solution. The buffer solution was an isotonic Krebs-Ringer phosphate buffer pH = 7.4 [5].

Homogenizing medium. The homogenizing medium consisted of a 0.08 *M* Sørensen phosphate buffer pH = 7.4 and 0.12 *M* sucrose solution, the whole being approximately isotonic. (10.1 g Na_2HPO_4 + 1.4 g KH_2PO_4 + 43 g sucrose per liter.) The solution was freshly prepared every month and stored at 4—6° C.

Preparation of slices. The liver (5—6 g) was cut in 5—7 pieces, each of them placed on a cooled Petri dish and cut vertically by means of a razor blade by hand. Each section had an approximate thickness of 0.5 mm. The slices were kept under ice cold buffer solution until they were used. Average time between killing the rat and beginning of the incubation, 20 minutes.

Preparation of homogenates. The liver was first cut in slices as mentioned above but in the homogenizing medium instead of the Krebs-Ringer buffer. To the slices was then added three times the amount (v/w) of homogenizing medium at 0° C. The mixture was transferred to a homogenizer with a plastic pestle (loose fitting, width between glass and

pestle 0.8 mm) and homogenized for 30 sec. with a motor of 1/6 HP and 1 500 RPM. In order to keep the speed constant, a motor of this strength is desirable. The whole was then poured into a precooled centrifuge tube and centrifuged at 400 × g for 5 minutes. The supernatant was used and the precipitate discarded. The average time between killing the rat and finishing the homogenization was 15 minutes.

After extensive experimentations of which some data are recorded in Table 1, these conditions were found to give optimal results. Preliminary slicing of the liver before homogenizing was found to be necessary as it was impossible to use a tissue press. The necessary pressure, even during a very short time (2—5 sec), resulted in an inactive preparation. Miller and Warren [7] have made similiar observations. In accordance with Bucher *et al.*[8] it was found essential to have a loose fitting pestle but in this work addition of nicotinamide to the homogenizing medium did not influence the results.

Dry weights and N-content. 1 g liver fresh weight corresponds to 300 mg dry weight. 1 ml of the supernatant homogenate = 78 mg dry weight, (54 mg thereof from homogenizing medium). Corrected dry weight, 24 mg per ml supernatant. Nitrogen content about 3.4 mg N per ml.

Incubation and extraction of the reaction products. Slices (1—2 g) or homogenates were shaken (100 strokes per min.) with the appropriate additions in 25 ml Erlenmeyer flasks or centrifuge tubes at 37° C for 2 hours in air unless otherwise stated. A bile acid/tissue ratio of 1 : 10 000 was generally used. The reaction was stopped by addition of 5 volumes of ethanol and the mixture was acidified with a few drops of glacial acetic acid.

After 1 hour the mixture was filtered or centrifuged. The residue was washed 3 times with 2 ml of ethanol. The combined filtrates were then blown to dryness in a stream of air at 50° C. From 2 ml of homogenate (800 mg fresh weight liver) about 100 mg dry weight residue was obtained of which 75 % was sucrose or inorganic salts from the medium. About 90 % of the activity was recovered with no selective loss of any one of the reaction products.

The residue was dissolved in 1 or 2 ml of 70 % aqueous ethanol and used without further purification for paper chromatography.

Paper chromatography. We used essentially the methods developed by Sjövall [3] and added about 1/50 of the incubation residue to each strip. This gave us about 2 mg substance with an activity of 300—1 000 cpm. The strips were saturated in the chromatographic vessels overnight and run 12 hours for the butanol and 4 hours for the isopropylether/heptane chromatograms. They were dried for five min. at 80° C and cut at the upper and lower ends so that they could be put on a metal sheet for counting of radioactivity.

Table 1. Different experimental conditions for preparing rat liver homogenates.

2 ml rat liver homogenate corresponding to 800 mg fresh weight + 125 μg desoxycholic acid-24-^{14}C, pH = 7.4, 2 hrs 37°. Total conversion to tauro acids = 100 %. Average values each out of 2 similiar experiments.

No.	Variations in the preparation of the homogenate	% Conversion of desoxycholic acid to tauro acids
1	13 000 RPM, motor strength 1/80 HP	0
2	4 000 » » » »	90
3	2 500 » » » »	90
4	1 700 » » » »	75
5	1 500 » » » 1/6 HP	95
6	Same as 5, homogenizing time 5 seconds	5
7	» » 5, » » 20 »	100
8	» » 5, » » 40 »	100
9	» » 5, » » 60 »	95
10	Same as 8, glass pestle; centrifuged 5 minutes at 400 × g	95
11	» » 8, » » » 5 » » 2 000 × g	40
12	» » 8, plastic pestle; » 5 » » 400 × g	100
13	» » 8, » » » 5 » » 2 000 × g	45

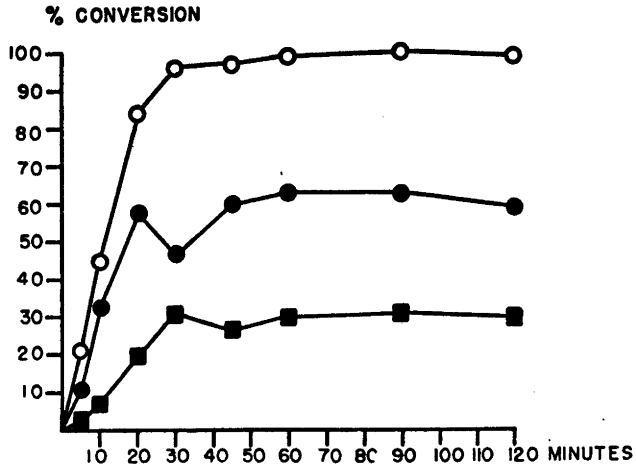

Fig. 1. Incubation of [^{14}C] *desoxycholic acid (125 μg) with 2 ml of rat liver homogenate for different times. Taurine conjugates (○), taurodesoxycholic acid (●) and taurocholic acid (■).*

The counting of the strips. We used an automatic scanner for measuring the radioactivity of the various strips with a mica-end window, lead shielded G. M. tube (Tracerlab. Inc.). Attached was a printing time counter and an automatic strip feeder. The apparatus was designed in the workshop of this institute and will be published elsewhere in due course. The strip was advanced 1 cm at each step and the same length was counted by the G. M. tube through an appropriate mask.

We normally used 200 preset counts, the time required being printed on a paper strip by means of the printing time meter. The standard error of the results from the average with a preset of 200 counts was about 12 %. The background was 35 ± 4 c/m. With higher preset counts the deviation decreases. (Preset 400 ± 10 %; preset 600c ± 8 %; preset 1 000c ± 6 %). The time for measuring a 30 cm strip with 200 preset counts was about 3 hours.

The counts obtained under these conditions (Whatman No. 1 filter paper) were about 35 % of those obtained when the same amount of labelled compound was plated on copper planchets in infinitely thin layer.

Reaction products. The identity of the formed cholic acid was verified after hydrolysis. *via* chromatography and isotope dilution, as described earlier[1,2].

RESULTS

A) *Influence of different experimental conditions on the rate of conjugation and oxidation.* Some results of preparing the homogenates under different conditions are shown in Table 1. The best method so far is No. 12 as described in detail in the experimental part. We also made some preliminary experiments on the inhibition of the reaction and found that the homogenate was totally inactivated by heat (5 min., 60° C), pressure (forcing through a tissue press) and heavy metalions (Cu, 10^{-3} *M*). Cyanide inhibited 50 % at 10^{-4} *M* concentration.

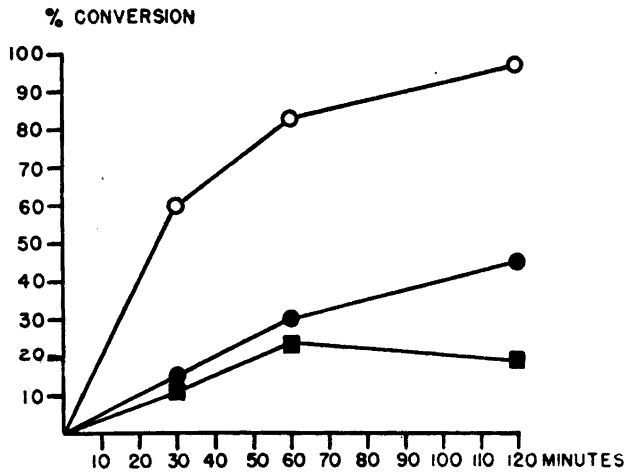

Fig. 2. Incubation of [^{14}C] *desoxycholic acid (250 μg) with rat liver slices (2 g) for different times. Taurine conjugates (○), taurodesoxycholic acid (●) and taurocholic acid (■).*

B) *The effect of the incubation time.* The influence of the incubation time is shown in Fig. 1 and 2. It is to be noticed in Fig. 1 that taurodesoxycholic acid reaches the plateau value 10 min. earlier than taurocholic acid, indicating that the reaction proceeds from free desoxycholic acid *via* taurodesoxycholic acid to taurocholic acid. This of course cannot be regarded as definitely proven by this experiment. Another observation supporting this course for the reaction is that in all our experiments we were unable to detect free cholic acid.

In a similiar experiment with rat liver slices (Fig. 2) the time for the completion of the reaction is somewhat longer. This is to be expected because of the slow diffusion of the added bile acid into the cells of the slices.

C) *Variation of the amount of desoxycholic acid.* In order to get data on the capacity of rat liver homogenates to convert desoxycholic acid into taurodesoxy and taurocholic acid increasing amounts of this bile acid were added to a fixed amount of rat liver homogenate, (2 ml homogenate corresponding to 800 mg liver fresh weight). In Fig. 3 the results of a typical experiment are shown. A practically complete conjugation of the added bile acid is obtained up to 250 μg per vessel. An inhibition is seen when 400 μg or more of the bile acid was added to the incubation mixture. This gives a minimum inhibition concentration of about 11 mg/100 ml (average for 4 experiments). Essentially the same data were obtained in 2 runs with rat liver slices. The content of free taurine present in these experiments corresponds to three times this amount [9]. Addition of free taurine did not change the results. Data on the influence of other bile acids and of the reaction products will be published in a later communication.

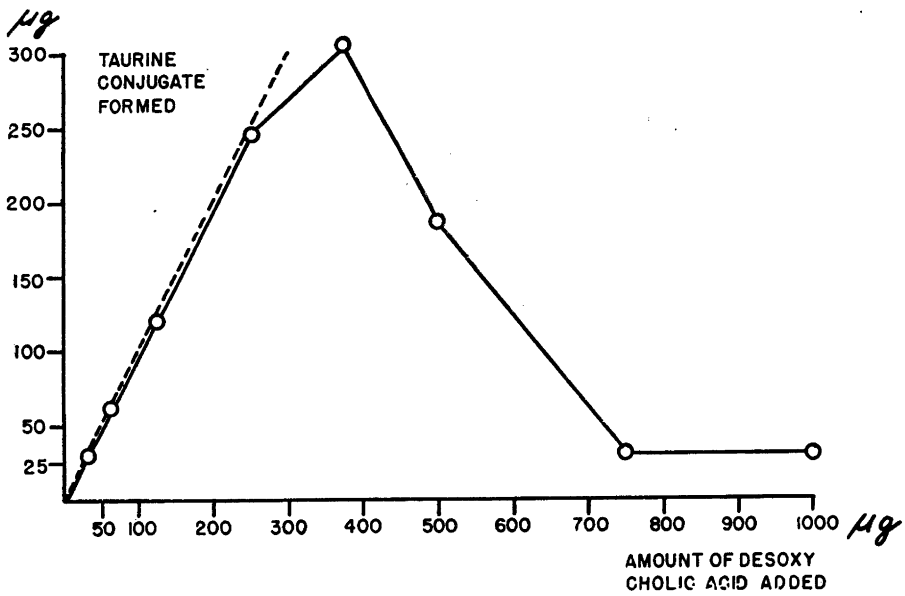

Fig. 3. Incubation of different amounts of [^{14}C] *desoxycholic acid with 2 ml of rat liver homogenate. Taurine conjugates (○). Interrupted line corresponds to total conjugation of bile acid present.*

SUMMARY

1) The experimental conditions for the conversion of desoxycholic acid-24-^{14}C into taurodesoxy and taurocholic acid by rat liver slices and homogenates have been studied.

2) The time curves of these processes and also the capacity of the rat liver to conjugate have been investigated.

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