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A Method for the Estimation of the Taurine Content and its Conjugation with Cholic Acid in Rat Liver. Bile Acids and Steroids 22

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A method for the determination of the taurine content of rat liver and for the taurine conjugation with cholic acid in rat liver homogenates is described. The taurine content of fresh rat liver was found to be 0.1—0.6 mg/g in male rats, and 0.7—1.1 mg/g in female rats.

Under the conditions described 2.2 μmole taurocholic acid was formed per g fresh liver. Due to the relatively high taurine content of the rat liver, addition of taurine to the reaction mixture had only an insignificant effect on the yield of taurocholic acid.

Homogenates centrifuged free from cell debris and nuclei were twice as effective as the total homogenates.

The taurine conjugation and the α-hydroxylation of desoxycholic acid have been effected by rat liver slices [1] and homogenates [2].

The present paper is primarily concerned with a simplified procedure to measure the taurine concentration and taurine conjugation in rat liver homogenates with the aid of ^{35}S-labelled taurine **.

The measurements of taurocholic acid formed was based on a selective extraction of taurocholic acid with *n*-butanol while the free taurine remained almost quantitatively in the water phase. Some of the factors influencing the synthesis of taurocholic acid from taurine have also been investigated and are reported here.

EXPERIMENTAL

The ^{35}S-labelled taurine had an activity of approximately 400 000 c/min./mg as measured in infinite thin layer with a TG 2 Tracerlab tube.

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Albino rats, weighing 200—250 g, were used. The animals were killed by a blow on the neck. The livers were immediately removed and dropped into ice-cold isotonic KCl solution and weighed by differences.

The homogenate was prepared in a glass homogenizer with a loose-fitting pestle according to the method of Bergström and Gloor [3]. The homogenizing medium consisted of sucrose, potassium phosphate buffer of pH 7.4, magnesium chloride and nicotinamide [4]. One part of liver and three parts of homogenizing medium were homogenized for 45 seconds. The resulting homogenate was either used as such, or centrifuged free from cell debris and nuclei at 400 × g for 5 minutes.

In the incubation experiments 2 ml of the homogenate were added to each vessel with the appropriate additions. The total volume per vessel was 2.5—2.8 ml. The following concentrations were maintained in the incubation experiments: Potassium phosphate buffer (pH 7.4) 0.034—0.037 *M*, nicotinamide 0.016—0.018 *M*, $MgCl_2$ 0.004 *M*, sucrose 0.068—0.076 *M*, KCl 0—0.022 *M*.

The vessels were shaken in a Warburg apparatus at 37—38° C with a frequency of 100 strokes per minute. The gas phase was air and the incubation time was two hours.

The reaction was stopped by immersing the vessels for half a minute in boiling water. Much of the proteins was precipitated by this treatment. After cooling to room temperature, 1/3 volume of *n*-butanol was added and the vessels were shaken for 15 minutes in a shaking machine to establish equilibrium between the phases. The contents of the vessels were then centrifuged in narrow tubes at 400 × g for two minutes in order to obtain a clear separation of the two phases with the precipitated proteins at the interphase.

A suitable aliquot of the upper butanol layer (0.05—0.1 ml) was finally pipetted off for paper chromatography or plating. In the latter case empirical corrections of the counting data for self-absorbtion were made.

Analytical methods. The analytical technique was essentially based on the advantageous distribution of the taurocholic acid between the water and butanol phases. As the butanol and the water are partially miscible, it was found convenient to determine empirically the correlation between the original concentration in the water phase before extraction, and the concentration in the butanol phase after extraction with 1/3 volume *n*-butanol.

The analytical technique was tested by means of the quantitative paper chromatographic method as described by Eriksson and Sjövall [5,6].* In this system the taurine migrates with a speed approximately 1/3 of that of taurocholic acid. These compounds could therefore easily be separated.

The butanol phase could be applied directly on the paper, since the dry matter was only 5—6 mg/ml butanol. A sample of 0.05—0.1 ml was placed on the paper in small portions under a stream of warm air. After chromatography the spots were eluted with absolute alcohol and the eluate evaporated to a small volume for plating, or to dryness for spectrophotometric measurement in concentrated sulphuric acid at 389 mμ, using a Beckman quartz spectrophotometer.

Table 1 shows the results of some of these control experiments. It appears that the addition of radioactive taurine corresponding to 30 000 c/min. to 2.8 ml gave rise to only 15—20 c/min./0.05 ml butanol phase. It is also evident that no detectable amount of any extractable radioactive compound other than taurocholic acid had been formed. The slightly higher activity of the chromatographed sample was due to the smaller self-absorbtion, as most of the impurities in the butanol disappeared during the chromatographic procedure.

Further there is a close proportionality between the taurocholic acid formed and the amount of labelled taurine conjugated, the former determined spectrophotometrically, the latter by plating and counting of the butanol phase.

The effect of the pH on the distribution of the taurocholic acid between the water- and the butanol phases was studied. For this purpose ^{35}S-labelled taurocholic acid was prepared biologically as described above. Aliquots were dissolved in phosphate buffers of varying pH and shaken with 1/3 volume of butanol. Table 2 shows the resulting distri-

* The author is indebted to S. Erihsson and J. Sjövall, Department of Physiological Chemistry, University of Lund, for the use of their unpublished method for the quantitative determination of bile acids by paper chromatography.

Table 1. Total volume 2.8 ml. Vessels A—C, 2 ml 25 % homogenate centrifuged at 400 × g for 5 minutes. Vessel D, 2 ml 25 % uncentrifuged homogenate.

Vessel	Additions	c/min. in 0.05 ml butanol phase, directly plated	c/min. in 0.05 ml butanol, chromatographed, eluted and plated	μmole of added taurine ^{35}S conjugated	μmole taurocholate formed determined spectrophotometrically
A	0.8 μmole taurine-^{35}S = 30 000 c/min.	18	1	0	0
B	0.8 μmole taurine-^{35}S 2 μmole cholic acid	381	421	0.31	1.1
C	0.8 μmole taurine-^{35}S 2 μmole cholic acid	401	—	0.32	1.1
D	0.8 μmole taurine-^{35}S 2 μmole cholic acid	213	—	0.17	0.55

Table 2. The distribution of taurocholic acid between water and butanol at varying pH. 1.75 ml H_2O (0.75 ml 0.11 M phosphate buffer + 1 ml 1.15 % KCl) and 0.58 ml butanol (1/3 volume).

pH	c/min. added to each vessel	c/min. recovered in 0.1 ml butanol	Partition coefficient butanol/water	Concentration in butanol phase/initial conc. in H_2O
1	3 000 = 171/0.1 ml	468	~ 12	2.75
6.5	3 000	336	~ 8	2.0
7.2	3 000	344	~ 8	2.0
8	3 000	353	~ 8	2.0
14	3 000	342	~ 8	2.0

Table 3. The recovery of increasing amounts of taurocholic acid by butanol extraction. 0.75 ml potassium phosphate pH 7.2, 1 ml 20 % centrifuged homogenate, radioactive taurocholic acid corresponding to 1 600 c/min./vessel. 0—3 μmole inactive taurocholic acid added.

μmole inactive taurocholate added	c/min. recovered per 0.1 ml butanol phase
0	186
0.1	169
0.3	197
0.5	197
1.0	171
2.0	175
3.0	162

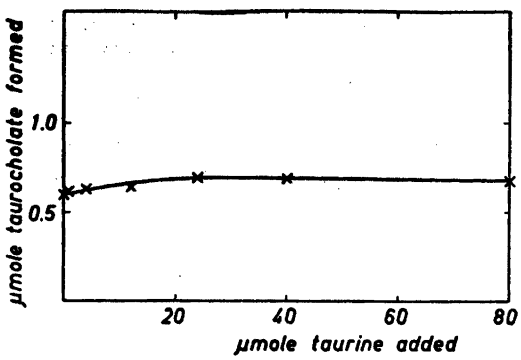

Fig. 1. Total volume 2.5 ml. 2 ml 25 % homogenate centrifuged at 400 g for 5 min. Substrate additions 2.5 µmole cholic acid, 0—80 µmole taurine. 0.05 ml butanol phase chromatographed, eluted and measured spectrophotometrically in concentrated H_2SO_4.

bution. By acidifying the solution, a greater concentration of taurocholic acid was achieved in the butanol phase. Nevertheless, we preferred to extract without acidifying the solution, as the addition of acid greatly affected the rate of migration of the bile acids on the paper chromatograms.

Table 3 shows the control of the distribution coefficient in the concentration interval used in the experiments to be described. Homogenate and a constant amount of ^{35}S-labelled taurocholic acid (0.2—0.3 µmole per vessel) were added to the vessels. Further there were added increasing amounts of inactive taurocholic acid. It appears that a constant concentration relationship between the butanol and water phases was obtained. Consequently there was no trapping of taurocholic acid in the precipitated proteins, and the partition coefficient also remained constant within the concentration range studied.

THE ESTIMATION OF FREE TAURINE IN RAT LIVER

As we could follow the formation of taurocholic acid in the rat liver homogenate both spectrophotometrically and by means of the ^{35}S-labelled taurine, we had the opportunity to calculate the taurine content of rat liver. Thus in the experiment shown in Table 1, only 0.32 µmole of the added 0.8 µmole labelled taurine had been conjugated, although the total amount taurocholic acid formed was 1.1 µmole, as determined spectrophotometrically (Table 1, B and C). To each vessel there were added 2 ml of a 25 % homogenate, *i. e.* 500 mg fresh liver. The amount of preformed taurine in the liver could consequently be calculated as follows:

$$\frac{(1.1-0.32) \times 0.8}{0.32} = 1.95 \text{ µmole/500 mg liver} = 0.49 \text{ mg taurine/g fresh liver.}$$

Similar calculations were performed in nine experiments with male rats. The taurine content was found to range between 0.1—0.6 mg/g liver (average 0.35 mg/g).

Three experiments with female rats gave a taurine content of 0.7—1.1 mg/g liver (average 0.9 mg/g). There were great variations in the values, but a sex difference seemed to be present.

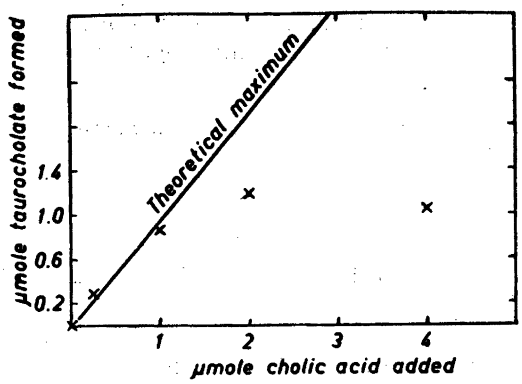

Fig. 2. Total volume 2.5 ml, 2 ml 25 % homogenate centrifuged at 400 g for 5 min. Substrate additions 1.6 µmole taurine, 0—4 µmole cholic acid. 0.05 ml butanol phase chromatographed, eluted and measured spectrophotometrically in 1 ml concentrated sulphuric acid.

This result seems to be in contrast with the findings of Sloan-Stanley [7] and of Blaschko *et al.*[8] who found the cysteic acid decarboxylase activity to be much higher in the male rat liver than in the female rat liver. This discrepancy may be another indication that taurine is formed through other metabolic channels than by the decarboxylation of cysteic acid [9-11].

The taurine content in male rat liver was reported by Wu [12] to be 0.104 ± 0.09 mg/g liver on a standard diet. After nine dayes fasting the content had increased to 0.6 ± 0.14 mg/g.

The relative high taurine content of the rat liver may also explain the insignificant effect of added taurine on the yield of taurocholic acid in the experiment shown in Fig. 1. This experiment was performed with inactive taurine and spectrophotometric determination of the taurocholic acid formed. Addition of 20 µmole taurine or more had only a weakly stimulating effect. This indicates that the preformed 2—3 µmole taurine present in the homogenate was enough to give optimal conditions for the reaction.

Fig. 2 illustrates the effect of increasing amounts of cholic acid on the synthesis of taurocholic acid. The amount formed levelled off at about 1 µmole cholic acid added. No taurine-conjugated or free cholic acid seemed to be present in the liver.

COMMENTS ON THE TAURINE CONJUGATION

The taurine conjugation and 7-hydroxylation of desoxycholic acid in liver slices and liver homogenates have been reported by Bergström *et al.*[1,2]

In preliminary experiments we were able to show that also cholic acid was conjugated in liver slices when incubated in Ringer buffer solution. In Table 1 is shown that rat liver homogenates effected conjugation of cholic acid with taurine, and that this conjugation was doubled when the homogenates were

centrifuged free from cell debris and nuclei at 400 × g for five minutes (Table 1, C and D). This result warrants further investigations on the localization of the process within the cell.

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