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Metabolism of Chenodeoxycholic Acid in the Rabbit

Bile Acids and Steroids 104

KJELL HELLSTRÖM and JAN SJÖVALL

Department of Medicine, Serafimerlasarettet, and
Department of Chemistry, Karolinska Institutet, Stockholm, Sweden

Two rabbits, one with a permanent duodenal fistula and the other with a permanent stomach fistula, were given chenodeoxycholic acid-24-^{14}C intravenously. Bile samples were taken intermittently for three days through the fistulas which were closed except during the sampling periods. Unchanged chenodeoxycholic acid could not be positively identified. Lithocholic acid was the main metabolite in the feces and bile of one rabbit. In addition to lithocholic acid, ursodeoxycholic acid and small amounts of 7-ketolithocholic acid were found in both the bile and feces of the other animal. The excretion of isotope in feces indicated a slow turnover of the acids.

Cholic and deoxycholic acids are the main bile acids in rabbit bile (for references see Ref.[1]). Cholic acid is formed from cholesterol in the liver whereas deoxycholic acid is secondarily formed from cholic acid by intestinal microorganisms [1]. Chenodeoxycholic acid has not been found in rabbit bile but lithocholic acid, which can be formed from this acid in the rat intestine [2], has been isolated in minor amounts [3]. The rabbit has been extensively used for studies of atherosclerosis and in connection with an investigation of bile acid production in this animal we found it necessary to acquire more information on the qualitative metabolism of chenodeoxycholic acid in the rabbit. Results obtained with the aid of labelled chenodeoxychclic acid are presented in this paper.

EXPERIMENTAL

Chenodeoxycholic acid-24-^{14}C was synthesized according to Bergström, Rottenberg and Voltz [4]. The specific activity was about 10 μC per mg. Ursodeoxycholic acid (m.p. 202–203°) was prepared according to Samuelsson [5].

Animal experiments. Two rabbits weighing about 2.5 kg were used. One of these was provided with a permanent fistula between the duodenum and the abdominal wall (rabbit DK), the other one was provided with a similar fistula between the distal part of the stomach and the abdominal wall (rabbit VK). The fistula consisted of a plexi-glas tube (length 5 cm) that could be closed with a plexi-glas stopper occupying the whole

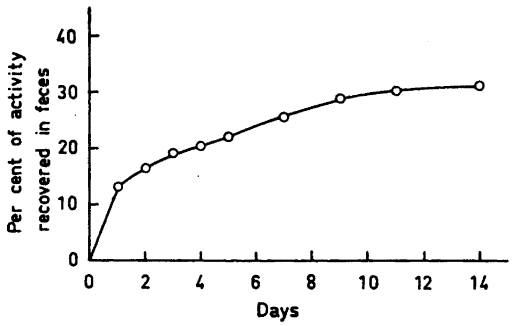

Fig. 1. Cumulative excretion in the feces of rabbit VK of activity not removed through the fistula.

volume of the tube up to the level of the mucosa [6]. Bile samples were taken directly *via* the duodenal fistula by removing the stopper, or by introducing a rubber tube through the stomach fistula into the duodenum. Except when samples were being taken, the plexiglas tubes were stoppered and the animals were allowed to move freely in their cages. This technique was adopted in order to follow specific activities of bile acids *in vivo* and details will be described later. The experiments were started about one month after the operation. The animals had free access to cabbage and carrots, were apparently healthy and increased in weight. Rabbit VK is still in good health 10 months after the operation.

2.8 mg of the sodium salt of the labelled chenodeoxycholic acid was given intravenously in 4 ml of saline. 24 h later duodenal contents was collected after i.v. injection of cholecystokinin-pancreozymin (kindly supplied by Prof. E. Jorpes). Collection was continued for 6 h and the procedure repeated on the following two days. Feces were collected every day.

Extraction and chromatography. About 10 volumes of ethanol was added to the duodenal contents. After filtration, most of the solvent was evaporated on the water bath and the bile acids were extracted with butanol from the acidified aqueous solution. The homogenized daily portions of feces were extracted 4—5 times with ethanol by refluxing for 2 h. Complete extraction of the isotope was ascertained by radioactivity measurements on each extract. After concentration of the combined extracts, the bile acids were extracted with ether from an acidified aqueous solution. Reversed phase chromatography was performed as previously described [7,8]. The following phase systems were used:

Phase system	Moving phase (ml)	Stationary phase (ml)
C 1	Methanol-water 150:150	Chloroform-*iso*octanol 15:15
F 1	Methanol-water 165:135	Chloroform-heptane 45:5
F 2	Methanol-water 180:120	Chloroform-heptane 45:5
F 3	Methanol-water 180:120	Chloroform-heptane 40:10

4.5 g of hydrophobic Supercel or Hostalen (Hoechst) [9] was used to support 4 and 3 ml, respectively, of the stationary phase. When larger columns were needed these amounts were proportionally increased. Radioactivity was determined by the counting of an infinitely thin layer in a Frieseke-Hoepfner (FH 51) methane gas-flow counter.

RESULTS

A total of about 15 % of the isotope injected was recovered by collection of duodenal contents for 6 h during each of the 3 days following the injection of chenodeoxycholic acid-24-^{14}C. The fecal excretion of isotope was extremely

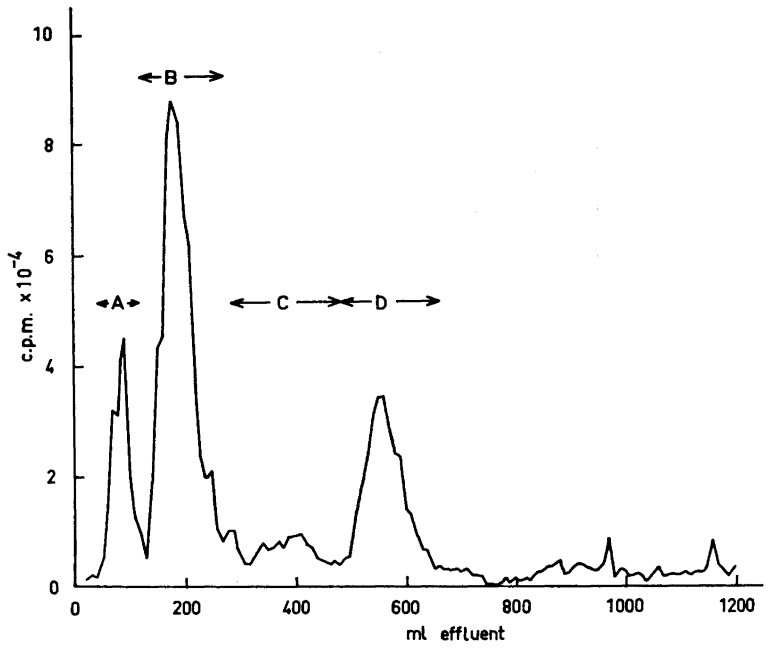

Fig. 2. Chromatogram of labelled compounds in the bile of rabbit VK. Column: 36 g Hostalen, phase system F 1.

slow. Only 22 % of the activity not recovered from the duodenum was excreted during the first 4 days in rabbit DK. Rabbit VK was therefore studied for 14 days and the same result was obtained as shown in Fig. 1.

Radioactive compounds in duodenum. The radioactive compounds extracted from the bile obtained from the duodenum were separated on Hostalen columns with phase system F 1. The result for rabbit VK is shown in Fig. 2. Conjugated bile acids appear with the front in this phase system but, as seen in the figure, only a minor part of the activity is eluted with the front (A, Fig. 2). The main part of the isotope is present in two peaks (B and D, Fig. 2) which appear at places corresponding to ursodeoxycholic and lithocholic acids, respectively. Only about 2 % of the total activity remained in the stationary phase. The active bands A, B, C, D (Fig. 2) were further purified by rechromatography on hydrophobic Supercel.

When peak A was chromatographed with phase system C 1, one main peak was found (Fig. 3, left curve). Chromatography, with inactive cholic acid added as carrier, showed that no radioactive cholic acid was present. The nature of the compounds separated with phase system C 1 has not been further investigated.

The rechromatography of peak B (Fig. 2) on Supercel columns with phase system F 1 is shown in Fig. 3 (middle curve). The isotope appears just before the large titration peak of the deoxycholic acid normally found conjugated in

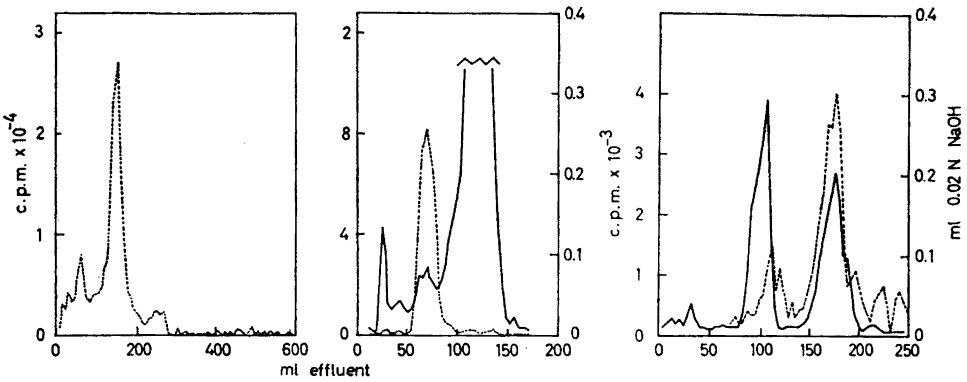

Fig. 3. Left curve: Rechromatography of peak A (Fig. 2) on a 18 g Hostalen column with phase system C 1. Middle curve: Rechromatography of peak B (Fig. 2) on a 9 g Supercel column with phase system F 1. Right curve: Rechromatography of band C (Fig. 2) together with unlabelled 7-ketolithocholic acid. 9 g Supercel, phase system F 1. Solid line: Titration. Broken line: Radioactivity.

rabbit bile. There is a small but definite titration peak coinciding with the radioactive one. No isotope is seen at the place of chenodeoxycholic acid. The radioactive fractions were re-run together with inactive ursodeoxycholic acid and the activity coincided with the titration peak of the added carrier (Fig. 4, left curve). The small titration peak which can be seen after the main peak is due to incomplete removal of the deoxycholic acid originally present in the bile. The identity of the labelled compound with ursodeoxycholic acid was further established by isotope dilution.

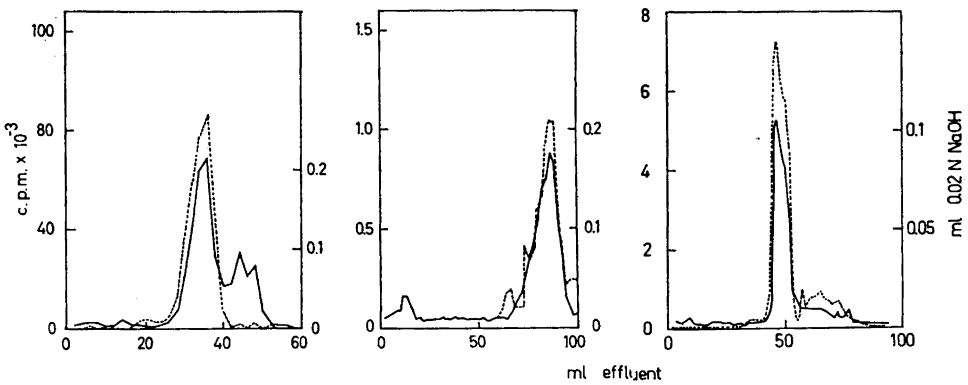

Fig. 4. Left curve: Rechromatography of peak B (Fig. 2) together with inactive ursodeoxycholic acid. 4.5 g Supercel, phase system F 1. Middle curve: Rechromatography of peak C from feces (Fig. 5) together with inactive 7-ketolithocholic acid. 4.5 g Supercel, phase system F 1. Right curve: Rechromatography of part of the "lithocholic acid band" from feces of rabbit DK together with inactive lithocholic acid. Solid line: Titration. Broken line: Radioactivity.

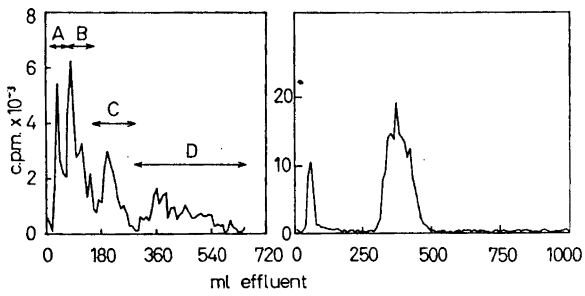

Fig. 5. Chromatograms of labelled fecal acids obtained from rabbit VK (left curve, 18 g Hostalen) and from rabbit DK (right curve, 27 g Hostalen). Phase system F 1 was used in both cases.

After one rechromatography with phase system F 1, band C (Fig. 2) was re-run with unlabelled 7-ketolithocholic acid. Most of the isotope appeared together with the carrier (Fig. 3, right curve) and the identity was established by isotope dilution. As was the case with the rechromatography of ursodeoxycholic acid, not all of the very large amounts of inactive deoxycholic acid had been removed. Therefore, a titration peak of this acid is still seen in Fig. 3 at 100 ml effluent.

The last radioactive band seen in Fig. 2 (peak D,) was rechromatographed with phase system F 2 and a titration peak coincided with the isotope peak. Chromatography with carrier lithocholic acid and determinations of specific activity upon recrystallization with lithocholic acid, showed that the labelled compound (Fig. 2, D) was identical with this acid.

Analysis of the duodenal bile from rabbit DK gave essentially the same result as above but, in this animal, lithocholic acid was by far the dominating metabolite (74 %) whereas only about 10 % of the total activity appeared in the ursodeoxycholic acid band. Furthermore, 7-ketolithocholic acid could not be found but this acid also accounted for only 5—10 % of the total metabolites in rabbit VK.

Radioactive compounds in feces. The analysis of the metabolites in feces was carried out in the same way as described for the duodenal bile. The main bands of radioactivity present in the chromatograms of feces were the same as those of the duodenal bile. The first chromatographies of a fecal sample from both rabbit VK and rabbit DK on Hostalen columns with phase system F 1 are shown in Fig. 5 (left and right curves resp.). Lithocholic acid was even more dominating in the feces of rabbit DK than in the duodenal contents of this animal (Fig. 5, right curve). It was identified by rechromatography with carrier lithocholic acid (Fig. 4, right curve) and by isotope dilution. Chromatograms of fecal extracts from the first, second and third days after the injection of the labelled chenodeoxycholic acid were very similar.

The labelled bands B, C, and D from rabbit VK shown in Fig. 5 (left curve) were rechromatographed with inactive ursodeoxycholic, 7-ketolithocholic and lithocholic acids, respectively. In all cases most of the isotope appeared

together with the reference compounds. This is shown for 7-ketolithocholic acid in Fig. 4 (middle curve). No isotope dilution experiments were made. The small amount of isotope appearing with the front (A, Fig. 5) was not studied further.

DISCUSSION

A striking finding is the very slow excretion of isotope in the feces of both the rabbits. This indicates a much longer half-life time of the chenodeoxycholic acid in the rabbit than in the rat. In this connection it is interesting to note that the amount of bile acids taking part in the enterohepatic circulation seemed to be very large in the rabbits previously studied (about 550 mg) [1]. In view of the sensitivity of the rabbit for hypercholesteremia, these quantitative aspects of the bile acid metabolism are now being studied.

Another finding differing from that found in rat and man is the occurrence of large amounts of unconjugated bile acids in the duodenal contents. In the bile all bile acids are conjugated [1]. This indicates a bacterial hydrolysis of bile acids already in the duodenum. In order to find out whether this was due to the presence of a fistula, four normal rabbits were killed and the duodenal contents was analyzed with paper chromatography. Large but variable amounts of free bile acids were found.

Unpublished findings by S. Lindstedt indicate that minute amounts of chenodeoxycholic acid are formed from cholesterol in bile fistula rabbits. We have now shown that chenodeoxycholic acid is rapidly and almost completely metabolized mainly to lithocholic and ursodeoxycholic acids. It is therefore probable that any chenodeoxycholic acid formed will be transformed and escape detection in normal bile. Lithocholic acid has been isolated from rabbit bile [3] and we have seen titration peaks in the chromatograms coinciding with both the labelled lithocholic acid and the labelled ursodeoxycholic acid.

Although we have not determined whether the transformation of chenodeoxycholic acid takes place in the liver or in the intestine, or in both, it seems most probable that lithocholic acid is formed by microorganisms, as is the case in the rat [3]. The transformation of chenodeoxycholic acid during the enterohepatic circulation is very extensive in the rabbit, which is also true for cholic acid in this animal [1]. The ursodeoxycholic acid found is probably formed *via* the 7-ketolithocholic acid also found. Whether the 7-ketolithocholic acid has been formed both by liver enzymes [10] and in the intestine by the action of *E. coli* [11] is not known.

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