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On the Formation of Deoxycholic Acid from Cholic Acid in the Rabbit

Bile Acids and Steroids 48

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The excretion of glycocholic acid and glycodeoxycholic acids in rabbits with a bile fistula has been studied. 30—40 h after operation glycodeoxycholic acid has almost disappeared. In rabbits with a bile fistula administered cholic acid-24-^{14}C is excreted unchanged in the fistula. In "whole" animals labelled cholic acid gives rise to labelled deoxycholic acid probably through the action of intestinal micro-organisms.

The main bile acids in rabbit bile are glycocholic and glycodeoxycholic acids [1-4]. When rabbit fistula bile collected immediately after surgery was fractionated by chromatography the amount of glycodeoxycholic acid was about ten times that of glycocholic acid [4]. On the other hand Okamura and Okamura were not able to isolate deoxycholic acid from a large amount of fistula bile [1].

In normal human bile, collected by duodenal tubage, cholic and deoxycholic acids are regularly present together with chenodeoxycholic acid [5]. The acids occur conjugated with glycin or taurin. When human bile from post-operative choledochal drainage was examined by paper chromatography no deoxycholic acid could be detected [6], nor could it be found in the duodenal contents of newborn babies [7].

The absence of deoxycholic acid from bile that has not been subjected to the action of intestinal microorganisms suggested the possibility that this acid is not primarily formed in the liver but arises from the attack of the microorganisms on some other bile acid, *e. g.* cholic acid. It has been found, that when ^{14}C-labelled cholic acid is administered to normal human subjects, and the bile acids fractionated a few days later, significant label is found in the dihydroxycholanic acid fraction [8].

In the present work the relationship between cholic and deoxycholic acids in the rabbit has been studied.

EXPERIMENTAL

Cholic acid-24-^{14}C had been prepared according to Bergström, Rottenberg and Voltz [9].

Bile fistula rabbits. Animals weighing 2—3 kg were anesthetized with ether and cannulation of the common bile duct was performed with a polythene tube just above the entrance of the common duct into the duodenum. No attempt was made to remove the gall-bladder. After operation the rabbits were given a subcutaneous infusion of 0.9 % sodium chloride at a rate of 10—20 ml per hour. This was found to be necessary as the animals otherwise died. The rabbits started eating after about one day and were fed on cabbage, carrots and oats and had access to 0.9 % sodium chloride as drinking water. When the rabbits were found to eat normally the infusion was no longer necessary. They were kept in cages that somewhat restricted their movements and could be kept in seemingly good condition for at least two weeks.

Chromatographic separation of the bile acids was carried out with reversed phase column chromatography [10] and by paper chromatography, by which technique the quantitative composition could also be determined [11,12]. Glycocholic and glycodeoxycholic acids were separated with ascending chromatography of the bile using ethylene chloride/heptane 70/30 (v/v) saturated with 70 % acetic acid as moving phase. After localization the acids were eluted and the amounts calculated from the absorbancy in 65 % sulfuric acid [13]. For identification purposes sulfuric acid absorption spectra were recorded with a self-recording spectrophotometer (Perkin-Elmer Spectracord 4 000).

RESULTS

Excretion of glycocholic and glycodeoxycholic acids in bile fistulas. The concentration of glycocholic and glycodeoxycholic acids in bile was followed in three animals. The values are given in Table 1 and show a marked decrease in the quotient between glycodeoxycholic and glycocholic acids.

A more detailed analysis of the course of the excretion was performed in animal No. 3 from which bile was collected in 2-h fractions with a fraction collector for the first 42 h following the operation and the bile acid concentration was determined in each fraction. These data have been used for con-

Table 1. Concentration of glycocholic and glycodeoxycholic acids in small samples of fistula bile collected at different times after operation.

Animal	Hours after operation	Glycocholic acid (GC) mg/100 ml	Glycodeoxycholic acid (GD) mg/100 ml	GD/GC
1.	0	33	655	19.9
	24	162	14	0.09
	72	407	8	0.02
2.	0	24	192	8.0
	12	8	30	3.8
	24	62	55	0.9
3.	0	30	420	14.0
	12	19	56	2.9
	24	186	65	0.35
	36	223	16	0.07

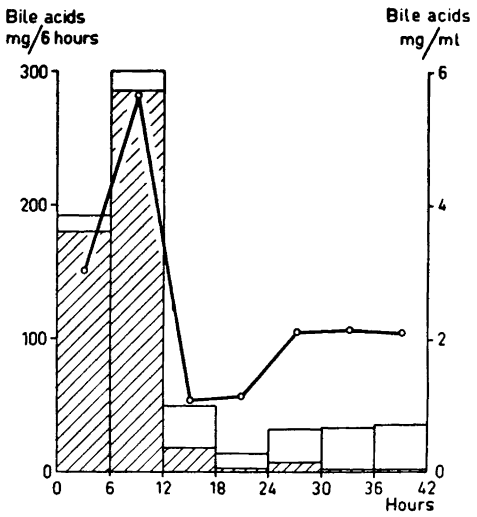

Fig. 1. Excretion of bile acids in a bile fistula rabbit. ▭ glycodeoxycholic acid □ glycocholic acid. O····· O glycocholic + glycodeoxycholic acid mg/ml.

structing Fig. 1 which shows the amounts of glycocholic and glycodeoxycholic acids excreted during each 6-h interval and also the mean bile acid concentration in these periods. A gradual shift in the proportion between the excreted amounts of glycocholic and glycodeoxycholic acids is obvious. Thus during the first periods glycodeoxycholic acid constitutes more than 90 % of the total bile acids excreted, while glycocholic acid is by far dominating in the later periods. The quotient between the amount of glycodeoxycholic acid and glycocholic acids excreted in different periods reached a maximum of 35 at 6—8 h after operation — in this period highly concentrated bile probably originating from the gall-bladder was obtained — and then decreased attaining a plateau level of 0.05 from 32 h onwards. The absorption spectra in 65 % sulfuric acid of material eluted from the glycodeoxycholic acid spot and of authentic glycodeoxycholic acid were recorded. The two spectra showed a good agreement in the first periods, while in the later a discrepancy was noted. Recordings from the first and the last 2-h intervals are shown in Figs. 2 and 3. Descending paper chromatograms in a medium suitable for the separation of glycodeoxycholic and glycochenodeoxycholic acids were run and showed that most of the acid excreted in small amount at the end of the experiment was not identical with glycodeoxycholic acid.

Fig. 1 further shows that the excreted amount of bile acids as well as their concentration fell during the experimental period. In the first 18 h a total of 550 mg of glycine conjugated bile acids was excreted. Most of this amount probably represents bile salts taking part in the enterohepatic circulation at the time of the operation.

Administration of cholic acid-24-^{14}C to rabbits with a bile fistula. Bile fistulas were made on two rabbits and cholic acid-24-^{14}C injected intraperitoneally 6 h and 36 h after the operation, respectively. The bile was collected for 3 h after the injection and hydrolyzed for 8 h at 120° C in a sealed glass tube.

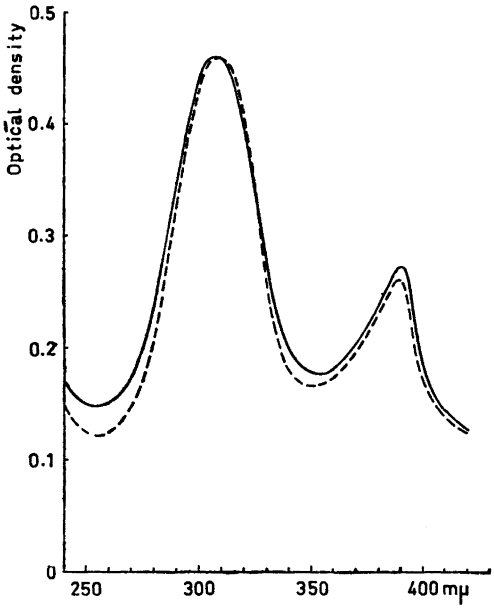

Fig. 2. Absorption spectra of glycodeoxycholic acid (——) and material from the glycodeoxycholic acid spot (0—2 hours) (- - -). Samples heated simultaneously for 10 minutes at 60° C in 65 per cent sulfuric acid.

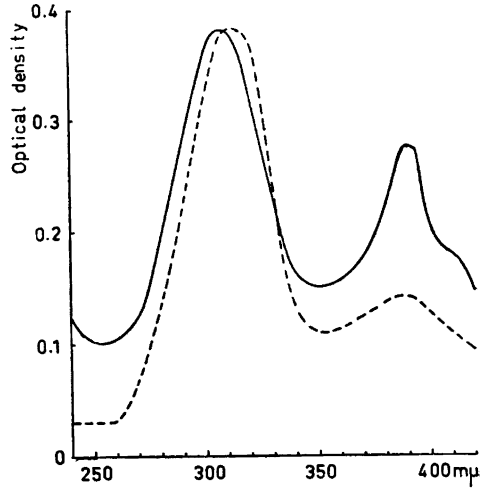

Fig. 3. Absorption spectra of glycodeoxycholic acid (——) and material from the glycodeoxycholic acid spot (40—42 hours) (- - -). Samples heated as in Fig. 2. The difference between the spectra of authentic glycodeoxycholic acid in Fig. 2 and Fig. 3 is due to minor variations in the concentration of the sulfuric acid.

A chromatogram of the free acids from the first rabbit is shown in Fig. 4. One titration peak is seen at 15—30 ml caused by cholic acid and one at 50—80 ml due to deoxycholic acid. All the activity is confined to the cholic acid peak whereas none is present in the deoxycholic acid. In the second rabbit the same result was obtained except for the almost complete absence of a titration peak at the position of deoxycholic acid.

Administration of cholic acid-24-^{14}C to "whole" animals. Two rabbits were injected intraperitoneally with labelled cholic acid. Three days later a bile fistula was made on the animals. The bile was collected, hydrolyzed and subjected to chromatography as above. The results from one of the rabbits is shown in Fig. 5. Only traces of radioactivity remain at the position of cholic acid, whereas practically all of it is found in the deoxycholic acid band. A minor amount appeared as a less hydrophilic compound. The deoxycholic acid was crystallized from acetic acid/water and gave 30 mg of crystals, m. p. 169—70 °C and a specific activity of 1 200 c.p.m. which was found to remain unchanged on further recrystallization from different solvents. The less hydrophilic compound has not been further investigated.

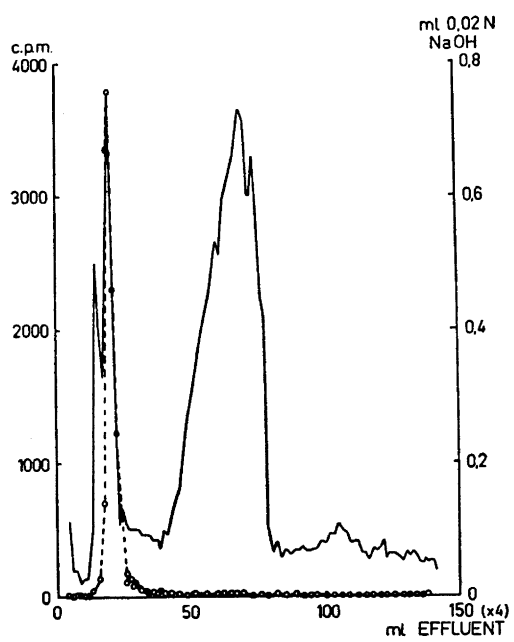

Fig. 4. Chromatography of hydrolyzed bile from a bile fistula rabbit injected intraperitoneally with cholic acid-24-^{14}C 6 hours after operation. Phase system: Moving phase: methanol/water 55/45. Stationary phase: chloroform/heptane 9/1. Solid line: titration values. Broken line: radioactivity.

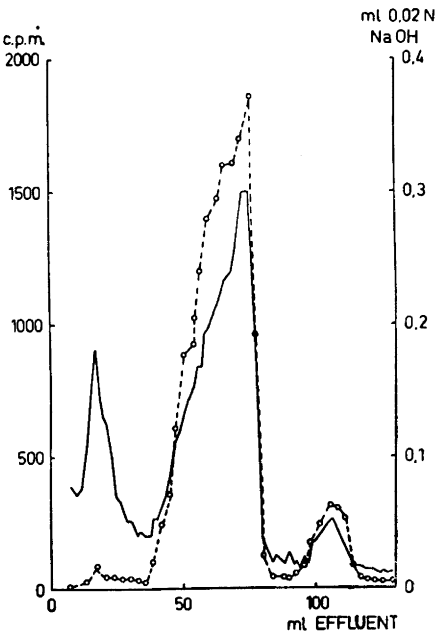

Fig. 5. Chromatography of hydrolyzed bile from a bile fistula rabbit, injected intraperitoneally with cholic acid-24-^{14}C 3 days before operation. Phase system: same as in Fig. 4. Solid line: titration values. Broken line: radioactivity.

DISCUSSION

The transformation of cholic acid into deoxycholic acid has been postulated by Japanese workers. Their conclusions, however, are mainly based on experiments in which as much as 30 g of the bile acid was fed and small amounts of the metabolites isolated from the urine (for a summary see Refs.[14,15]).

The disappearance of deoxycholic acid from bile fistula bile in 30—40 h clearly suggests that this acid is not an excretory metabolic product of the rabbit liver. The possibility remains, however, that a change in the proportion between the bile acids formed in the liver is induced by the continuous withdrawal of the bile. The very rapid disappearance of the deoxycholic acid makes this assumption less likely as do also experiments with rats where the proportion between cholic and chenodeoxycholic acid remains mainly unchanged when a bile fistula is made [16].

When labelled cholic acid is administered intraperitoneally into a rabbit with a bile fistula it is absorbed from the peritoneal cavity into the portal circulation passing through the liver into the bile fistula. If the liver was able to transform cholic acid into deoxycholic acid one would expect the label to

appear in the deoxycholic acid, even though only one passage through the liver has taken place. In the rat, *e. g.*, where certain transformations of the bile acids have been studied (*e. g.* deoxycholic acid → cholic acid; chenodeoxycholic acid → hydroxylated metabolites; *cf.* Ref.[17]) one passage through the liver suffices to give a high percentage of conversion.

When cholic acid is given to whole animals the acid will take part in the enterohepatic circulation and thus be subjected to the action of the intestinal microorganisms. It is well known that they effectively attack bile acid molecules resulting in the formation of various metabolites (*cf.* Refs.[18,19]). They may therefore be responsible for the conversion of cholic acid into deoxycholic acid or into some intermediate which is then converted to cholic acid in the liver. This is made very probable by the experiments in which labelled cholic acid was allowed to circulate for three days in the intact animal. Practically all of the recovered activity was then found in the deoxycholic acid.

The results of this work once more show the necessity of considering the influence of intestinal microorganisms in studies of cholesterol and bile acid metabolism.

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REFERENCES

1. Okamura, T. and Okamura, T. *Hoppe-Seylers Z. physiol. Chem.* **199** (1930) 11.
2. Kishi, S. *Ibid.* **238** (1936) 210.
3. Sekitoo, T. *Ibid.* **199** (1931) 225.
4. Ekdahl, P. H. and Sjövall, J. *Acta Physiol. Scand.* **34** (1955) 287.
5. Sjövall, J. *To be published.*
6. Ekdahl, P. H. and Sjövall, J. *To be published.*
7. Enkrantz, J. C. and Sjövall, J. *To be published.*
8. Lindstedt, S. *To be published.*
9. Bergström, S., Rottenberg, M. and Voltz, J. *Acta Chem. Scand.* **7** (1953) 481.
10. Sjövall, J. *Acta Physiol. Scand.* **29** (1953) 232.
11. Sjövall, J. *Arkiv Kemi* **8** (1955) 317.
12. Sjövall, J. *Acta Chem. Scand.* **10** (1956) 1051.
13. Eriksson, S. and Sjövall, J. *Arkiv Kemi* **8** (1955) 311.
14. Haslewood, G. A. D. *Physiol. Rev.* **35** (1955) 178.
15. Tayeau, F. *Exposés ann. biochim. méd.* **10** (1949) 251.
16. Eriksson, S. *Acta Chem. Scand.* **10** (1956) 156.
17. Bergström, S. *Record Chem. Progr. Kresge-Hooker Sci. Lib.* **16** (1955) 63.
18. Lindstedt, S. and Norman, A. *Acta Physiol. Scand.* **34** (1955) 1.
19. Ekdahl, P. H. and Sjövall, J. *Ibid.* **34** (1955) 329.

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