THYROID FUNCTION IN RELATION TO BILE LIPIDS AND BILE ACIDS*

AN EXPERIMENTAL APPROACH BY CANNULATION OF THE BILE DUCT OF RATS SO THAT THE ENTERO-HEPATIC CIRCULATION IS MAINTAINED

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It is known that the main metabolic end-products of cholesterol in rats are cholic acid and deoxycholic acid, and that rats with biliary fistulae secrete these bile acids as their taurine conjugates, whereas most faecal bile acids are unconjugated. In rabbits and man, however, deoxycholic acid is formed from cholic acid during the entero-hepatic circulation and appears in bile together with cholic acid and chenodeoxycholic acid.[3] In rats treated with thyroid hormone the cholic acid in bile decreases rapidly,[5] whereas the bile cholesterol and chenodeoxycholic acid secretions are increased.[2]

A great deal of work on bile acids has been performed in rats with biliary fistulae, and consequently, with no entero-hepatic circulation. Moreover, most methods for quantitative bile-acid analysis measure trihydroxycholanic and dihydroxycholanic acids separately, but do not differentiate between chenodeoxycholic acid and deoxycholic acid. Under these conditions, and also because of the absence of a gall-bladder in the rat, it would be impossible to demonstrate the presence of deoxycholic acid in the rat, even if it were formed from cholic acid by the action of intestinal micro-organisms as has been suggested is the case in man and the rabbit.

The purpose of the present investigation was, firstly, to develop a technique for bile-duct cannulation in rats in such a way that the entero-hepatic circulation was maintained; secondly, to devise a simple method for the routine quantitative analysis of deoxycholic acid and, thirdly, to compare the influence of thyroid hormones on the bile volume, bile lipids and bile acids of rats with biliary fistulae as against those of rats in which the bile re-circulates.

In rats whose entero-hepatic circulation was maintained, 2 cannulae were inserted into the common bile duct—one into the peripheral end and the other into the central end. The cannulae were connected on the backs of the rats, allowing free movement of the animals. Bile was collected for 6 hours every 2nd day and was compared with bile from rats with continuous biliary drainage as described earlier.[5] Bile cholesterol, lipid phosphorus, cholic acid and deoxycholic acid were measured in thyroidectomized, normal, and hyperthyroid rats. For the analysis of deoxycholic acid the ultra-violet absorption technique of Mosbach[4] was modified and applied to unhydrolyzed bile. The following equation was derived for the estimation of the 'true' bile cholic and deoxycholic acid:

$$x = 153{\cdot}39\ E_1 - 28{\cdot}00\ E_2$$
$$y = -32{\cdot}78\ E_1 + 111{\cdot}70\ E_2$$

where x = pure cholic acid; y = pure deoxycholic acid; E_1 = optical density measured at 320 mμ after 15 minutes of incubation at 60°C with 65% H_2SO_4; E_2 = optical density measured at 385 mμ after 60 minutes of incubation at 60°C with 65% H_2SO_4. The mean recovery for this method on unhydrolyzed bile was 80% (range 71-88%) for cholic acid, and 82% (range 72-90%) for deoxycholic acid.

Bile samples (6 hourly every 2nd day) of thyroidectomized rats in which the entero-hepatic circulation was maintained, were pooled; part of the bile was hydrolyzed, incubated with H_2SO_4 and the absorption spectra (200-440 mμ) of the unhydrolyzed bile samples compared with those of hydrolyzed bile. The results were then compared with those obtained from rats treated with tri-iodothyronine (20 μg. T_3 per day). A decrease of 30% in the cholic-acid concentration was observed in the bile of thyroidectomized rats during hydrolysis, whereas the dihydroxycholanic acids as measured at 385 mμ, increased by 59% in the bile of both thyroidectomized rats and rats treated with tri-iodothyronine during hydrolysis of the bile. The drop in cholic acid concentration, as affected by hydrolysis, is ascribed partly to the liberation of taurine from chenodeoxycholic acid, and partly to the destruction of cholic acid by alkaline hydrolysis. The increase in dihydroxycholanic acids during hydrolysis is due to the shift of the free chenodeoxycholic acid peak from 305 mμ (for taurochenodeoxycholic acid) to 385 mμ (for free chenodeoxycholic acid). At the same time hydrolysis causes a shift of the taurodeoxycholic acid peak at 389 mμ to that of free dihydroxycholanic acids at 385 mμ demonstrating the existence of taurodeoxycholic acid in the unhydrolyzed bile of rats in which the entero-hepatic circulation is maintained. In the bile of rats treated with tri-iodothyronine hydrolysis causes a shift of the cholic acid peak at 320 mμ to that of 350 mμ, thereby demonstrating the decrease of cholic acid and the simultaneous increase in taurochenodeoxycholic acid in hyperthyroidism.

In group studies on rats it was demonstrated that treatment with tri-iodothyronine and tri-iodothyroacetic acid produced an increase in concentration of bile cholesterol, lipid phosphorus, chenodeoxycholic acid and deoxycholic acid as well as an increase in their respective quantities secreted for 6 hours every 2nd day. The bile concentration and total output of deoxycholic acid were increased to a greater extent the longer the bile was allowed to re-circulate. In every case the cholic acid concentration decreased by at least 1/3rd of its original value during treatment with thyroid hormones. It was possible to demonstrate these changes repeatedly on the same thyroidectomized rats with bile in re-circulation in experiments lasting as long as 1 month. The volume of bile secreted was markedly increased by treatment with thyroid hormone.

On the basis of these findings it is postulated that thyroid hormone stimulates the whole metabolic pathway from acetate to cholesterol, and all the catabolic pathways of cholesterol to bile acids and ketocholanic acids. In rats in which the entero-hepatic circulation is maintained, such stimulation will result in the decrease of cholic acid and the increase of chenodeoxycholic acid and deoxycholic acid, as follows:

1. Chenodeoxycholic acid is formed from cholesterol but, since the resulting chenodeoxycholic acid is not a precursor of cholic acid, it will accumulate in bile.

2. Deoxycholic acid is formed from cholesterol as an intermediate stage in the formation of cholic acid. If, however, the entero-hepatic circulation is maintained, the cholic acid is reconverted back to deoxycholic acid. Part of the remaining cholic acid is further metabolized to ketocholanic acids.

3. Lithocholic acid is formed from cholesterol which, in rats, may be changed to chenodeoxycholic acid.

4. Chenodeoxycholic acid is formed from 3α, 7α-dihydroxycoprostane and cholic acid from 3α, 7α, 12α-trihydroxycoprostane. The cholic acid is again either converted to dihydroxycholanic acids, or further oxidized to ketocholanic acids.

All these reactions will lead to the disappearance of cholic acid and the accumulation of chenodeoxycholic and deoxycholic acids in bile when the thyroid is hyperactive. The reverse, i.e., the increase of cholic acid in hypothyroid rats, will inhibit cholesterol synthesis.[1]

Since the blood-cholesterol and lipid-phosphorus concentrations are inversely related to the bile-cholesterol and lipid-phosphorus secretion in hypo- and hyperthyroidism, the blood lipids appear to be controlled to a greater extent by endocrine factors affecting mobilization and water metabolism than by metabolic synthesis as influenced by the thyroid.

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REFERENCES

1. Beher, W. T. and Baker, G. D. (1958): Proc. Soc. Exp. Biol. Med., **98**, 892.
2. Eriksson, S. (1957): *Ibid.*, **94**, 582.
3. Lindstedt, S. and Sjövall, J. (1957): Acta chem. scand., **11**, 42.
4. Mosbach, E. M., Kalinsky, H. J., Halpern, E. and Kendall, F. E. (1954): Arch. Biochem., **51**, 402.
5. Van Zyl, A. (1957): J. Endocr., **16**, 213.