THE EFFECT OF PANTETHINE, A PRECURSOR OF COENZYME A, ON BILE ACIDS AND LIPIDS IN RATS FED WITH A CHOLESTEROL DIET[*]

By

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ABSTRACT

The effect of pantethine on bile acids and lipids was studied by the washout technique in Wistar female rats fed for four weeks with a 1% cholesterol diet supplemented with 0.5% cholic acid, either containing or not containing 1% pantethine.

Pantethine caused a slight increase in the biliary cholesterol concentration with a significant decrease in the serum cholesterol, β–lipoprotein and triglyceride levels. An increase in the pool size, secretion rate and enterohepatic circulation rate of primary bile acids was at the same time observed in the animals. The production of lithogenic bile was, therfore, inhibited in rats fed with a cholesterol diet treated with pantethine.

An analysis of conjugated bile acids revealed a decrease in the glycine/taurine ratio of the whole bile acids as detected by high performance liquid chromatography as a result of pantethine administration.

INTRODUCTION

Complete β-oxidation of a long-chain fatty acid involves condensation of fatty acid with the thiol group of coenzyme A (CoA)[1].

CoA is closely related to amino acids, lactic acid and glucose synthesis through pyruvic acid and oxaloacetate, and also to conjugation of bile acids[1]. Mitochondria contains an enzyme that catalyzes condensation of acetoacetyl-CoA with acetyl-CoA, leading to the formation of β–hydroxy–β–methylglutaryl CoA (HMG CoA)[1]. HMG CoA is further metabolized in reactions leading to synthesis of cholesterol.

This coenzyme is known to retain residues of both pantethenic acid and adenine nucleotide. Pantethine, being a precursor of CoA, may play an important role in the above metabolic processes.

This has already been more or less proven by the fact that pantethine decreases hepatic glycogen but increases hepatic ATP and acetoacetate concentration in rabbits fed with a ranolin added diet[2].

In the present experiment, the influence of pantethine upon bile acid and lipid metabolism was investigated in rats fed with a cholesterol diet.

MATERIALS AND METHODS

Wistar female rats fed with a 1% cholesterol diet supplemented with 0.5% cholic acid, either containing or not containing 1% pantethine

(Daiichi Pharmaceutical Co. Tokyo) for four weeks were examined by the experimental procedures designed basically according to the washout technique proposed by Mok et al[3].

After feeding the experimental diets for 4 weeks, animals were fasted from the previous night till the termination of surgical operation on the following day. Changes in diurnal rates of cholesterol and bile acid synthesis was avoided by performing each experiment between 9 : 00 and 10 : 00 am. After intraperitoneal injection of 0. 4 ml/kg somnopentyl, the abdomen was opened, the common bile duct firmly ligated and a fine polyethylene cannula (0. 11 inch I. D.) was inserted into the proximal end of bile duct, whereupon the abdomen was closed and the animal was kept in a Bollman restraining cage. During the experiment, the effect of starvation on bile acid metabolism was avoided by keeping animals in non-fasting state in the cage. Bile was collected at intervals of three hours for a total of 24 hours.

According to the method described by Mok et al.[3] the bile acid secretion rate per day (output) was defined as the whole bile acid output, as calculated by extrapolation from the output discharged during the first three hours after the bile duct cannulation. When the pool was washed out, the secretion rate fell to reach the lowest point when any primary bile acid that emerged was replaced with what was newly synthesized by the liver. This was defined as the basal rate of hepatic primary bile acid synthesis.

The primary bile acid pool (trihydroxy bile and chenodeoxycholic acids) was calculated by subtracting the basal rate of hepatic bile acid synthesis from the total amount of bile acids obtained from the time of cannulation until the lowest point on the washout curve. The secondary bile acid pool (lithocholic acid and deoxycholic acid) was defined as the total amount of bile acid secreted for 24 hours. The enterohepatic circulation frequency of primary bile acids per day was obtained by dividing the secretion rate by the pool size.

Biliary cholesterol, lecithin and total bile acid concentrations were determined in bile secreted in initial three hours (total bile acids were defined as the sum of four primary and secondary bile acid peaks determined by gaschromatography.) and lithogenic index was cal-

culated by Thomas and Hofmann's formula[4] based on the triangular coordinate reported by Admirand and Small[5].

Bile acid and bile lipid determinations:

Determination of bile acids was performed by the method reported by Kawamoto et al[6].

The rat bile contains several trihydroxy bile acids, of which cholic acid and muricholic acids have equal retention volumes in the above gaschromatographic condition. The amount of cholic acid was obtained by subtracting the amount of muricholic acids determined by the combined methods of gas-chromatography and thin layer chromatography from the total amount of trihydroxy bile acids determined by the above method[6].

Biliary cholesterol and lecithin were determined by the method described in the previous study[6].

Serum lipids and serum GOT were determined by the standard laboratory methods. β-lipoprotein was determined by immuno-nephelometry fundamentally modified from the method of Heiskel et al[7].

High performance liquid chromatographic procedures:

Individual unconjugated and glycine and taurine conjugated bile acids were analyzed by a high performance liquid chromatograph-TRIROTOR II (Nippon Bunko Co.) equipped with a solvent programmer GP-A 30 and UV-spectrophotometer UVIDEC-100 III (Ex : 365, Em : 470 nm). Separation was performed in JASCO Bile pack column (4. 6 mm I. D. × 25 cm). The enzyme solvent systems were A: $CH_3CN/10$ mM KH_2PO_4 pH 7. 8 : 40/80 v/v) and B : $CH_3CN/(30$ mM KH_2PO_4 pH 7. 8 : 20/80 v/v).

RESULTS

1. Pool size of bile acids.

Table 1 shows the average pool sizes of primary and secondary bile acids. Pantethine caused an increase in the two primary bile acid (cholic and chenodeoxycholic acids) pool sizes of rats fed with a cholesterol diet, with a statistic significance. The pool sizes of two secondary bile acids, lithocholic and deoxycholic acids, showed no change in contrast to the increase in the primary bile acid pools as above mentioned. Therefore, the ratio of the pool

sizes of primary to secondary bile scids was increased in rats to which pantethine was given. The increase especially in the ratio of pool size of chenodeocholic to lithocholic acid was statistically significant.

Table 1. Pool size of bile acids and ratio of primary to secondary bile acid pool in control and pantethine-treated rats.

Group	Control	Pantethine
Number of rat	5	5
Trihydroxy bile acid		
Cholic acid (μg/100 gRat)	1669.8±208.2	2008.4±179.8*
Muricholic acid (μg/100 gRat)	1038.2±150.0	1196.0±134.2N S
Chenodeoxycholic acid (μg/100gRat)	668.2± 48.1	788.4± 59.8**
Deoxycholic acid (μg/100 gRat)	619.4±214.5	584.8±124.9N S
Lithocholic acid (μg/100 gRat)	41.4± 10.9	31.6± 5.9N S
Chendeoxycholic acid/Lithocholic acid pool	17.0± 4.3	25.8± 6.1*
Cholic acid/ Deoxycholic acid pool	4.7± 1.3	5.7± 1.4N S

M±S D

2. Synthesis rate of primary bile acids.

As shown in Table 2, the synthesis rate of trihdroxy bile and chenodeoxycholic acids per day slightly rose in pantethine given rats, although statistic significance was non-existent between rats with and without pantethine.

Table 2. Daily synthesis rate of primary bile acids: Trihydroxy bile (cholic and muricholic) acids and chenodeoxycholic acid. (μg/100 gRat/24 hours)

Group	n	Trihydroxy bile acid	Chenodeoxycholic acid
Control	5	642.7±215.8	104.6±13.2
Pantethine	5	802.9±138.5N S	150.4±46.7N S

M±S D   n: number of rat

3. Sceretion rate and circulation frequency of total primary bile acids.

Both the secretion rate (output) and circulation frequency of primary bile acids also tended to elevate in rats treated with pantethine.

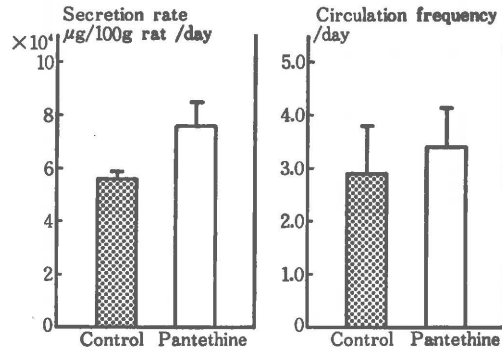4. Biliary cholesterol and lecithin concentra-

Fig. 1. Secretion rate and circulation frequency of total primary bile acids (trihydroxy bile acids and chenodeoxycholic acid) in bile of control and pantethine-treated rats

tions of initial three hours.

As shown in Figure 2, the biliay cholesterol concentration slightly rose but no elevation of the lecithin concentration was observed in animals treated with pantethine.
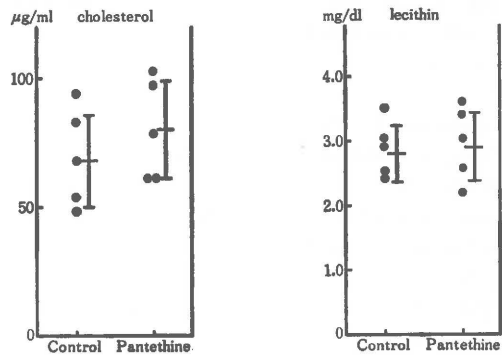

Fig. 2. Biliary cholesterol and lecithin concentration of bile in control and pantethine-treated rats

5. Lithogenic index of bile secreted in initial three hours.

As shown in Figure 3, the lithogenic index of bile did not change in rats treated with pantethine.

6. Individual unconjugated and conjugated bile acids, and glycine and taurine ratio (G/T) of bile.

As shown in the upper half of Figure 4, the decrease in glycine conjugated cholate and chenodeoxycholate and the slight increase in taurocholate were revealed in bile of rats treated with pantethine.

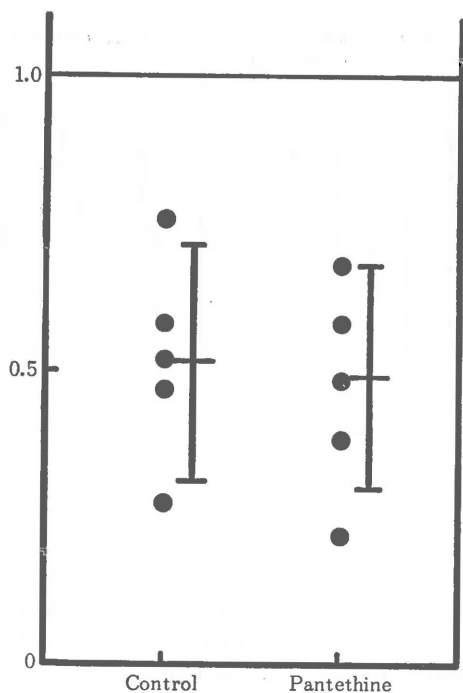Therefore, the glycine/taurine ratio tended

Fig. 3. Lithogenic index of bile (Admirand-Small)[14] calculated by Thomas and Hofmann's formula

A : Unconjugated-cholic acid   B : Glycocholate
C : Glycochenodeoxycholate   D : Taurocholate
E : Taurochenodeoxycholate   F : Taurodeoxycholate
                                   (※ P<0.05)

Fig. 4. Individual taurine and glycine-conjugated bile acids and taurine/glycine ratio (T/G) of bile

to decrease in animals treated with pantethine although unconjugated cholate also increased and remaining two taurine-conjugated bile acids remained unchanged in these rats as shown in the lower half of Figure 4.

7. Serum lipid and lipoproteins.

Table 3 shows serum lipids and lipoproteins in rats treated with and without pantethine. The significant decrease in serum cholesterol, triglycerides and β-lipoprotein are obvious in rats treated with pantethine. However, pantethine did not influence other serum lipid concentrations including HDL-cholesterol in the present experimental conditions.
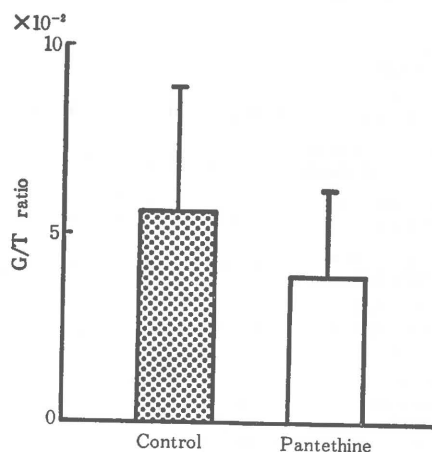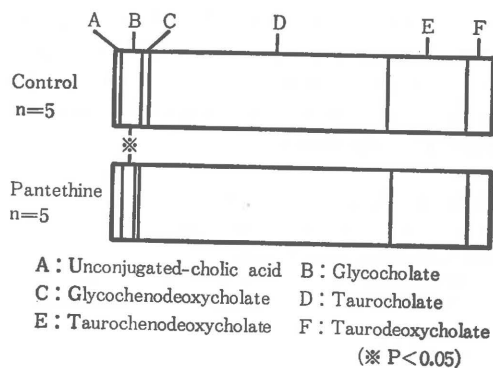
DISCUSSION

The mechanism of the action of pantethine to lower serum triglycerides can be estimated to be effectuated by accelerated β-oxidation of fatty acids through the aid of CoA derived from pantethine[2]. This hypothesis is supported by the fact that a large number of CoA molecules is required for β-oxidation reaction of fatty acids in progress in the liver[1].

From the present experiment, cholesterol mainly in serum β-lipoprotein seems either to have been incorporated into a catabolic

Table 3. Serum lipids and lipoproteins in control and pantethine-treated rats. (mg/dl)

Group	n	Cholesterol	Triglyceride	Phospholipid	HDL-Cholesterol	HDL-phospholipid	β-lipoprotein
Control	5	73.3±17.5	58.2±16.7	66.4±11.0	20.6±2.3	40.2± 7.9	487.3±101.9
Pantethine	5	42.4±11.3**	40.3±3.7N S	62.2±22.6N S	21.3±5.8N S	40.0±13.0N S	203.7±106.7**

M± S D   n: number of rat

pathway or to have been directly excreted into bile as free cholesterol in the liver of rats treated with pantethine.

The enlarged pool size of primary bile acids is usually caused by their increased synthesis in the liver[8] or by the supply of bile acids[9].

On the other hand, it is also possible to consider that disturbance or inhibition of conversion of primary bile acids into secondary bile acids causes expansion of primary bile acid pool sizes.

Some antibiotics seems to inhibit dehydroxylation by microorganism in the intestine and reduce synthesis of lithocholic acid from chenodeoxycholic acid[10].

However, the pool sizes of two secondary bile acids produced in the intestine did not almost differ between the rats treated with and without pantethine. Therefore, the increased pool sizes of two primary bile acids are considered to have been caused by the increased bile acid synthesis alone which was induced in the excess substrate (cholesterol) by pantethine.

To lower the serum cholesterol level, however, results sometimes in inducing excessive cholesterol excretion into bile and producing lithogenic bile in human beings and experimental animals[6,11]. Lithogenic bile frequently seen existing in gallstone patients seems to be due to the decreased pool size of bile acids[12,13] or the increased cholesterol concentraion in bile[14].

In the present experiment, pantethine brought about a slight increase in the biliary cholesterol concentration in contrast to biliary lecithin in rats fed with a cholesterol diet. At the same time, it increased the primary bile acid pool sizes (cholic and chenodeoxycholic acids) with an increase in their synthesis rate in the liver, resultantly elevating the ratio of primary bile acid pool sizes to their respective secondary bile acid pool sizes (chenodeoxycholic acid/lithocholic acid, $p < 0.01$: cholic acid/deoxycholic acid, N. S.).

The above results may indicate, therefore, that pantethine does not induce the formation of lithogenic bile, though it significantly reduced the serum cholesterol and β–lipoprotein levels in rats fed with a cholesterol diet.

The frequency of enterohepatic bile acid circulation if accerelated increases the secretion rate of bile aicd into bile instantaneously, contributing to the prevention of lithogenic bile. Therefore, the mean lithogenic index was in fact not increased in rats treated with pantethine.

Synthesis of cholic acid and chenodeoxycholic acids is immediately followed by conjugation with glycine or taurine. Similarly free bile acids (deoxycholic and lithocholic acids) upon arrival at the liver in the portal blood are rapidiy conjugated with those amino acids.

CoA participates in the process of conjugation to form "active" bile acids acyl CoA[15].

Glycine conjugation is withheld in the liver diseases and glycine/taurine (G/T) ratio is of diagnostic value in ileal disorders[11].

The present experiment showed that the majority of bile acids was conjugated to taurine in rats fed with a cholesterol diet and pantethine altered the G/T ratio to decrease in the whole bile acids in bile through the decrease in the glycocholate ($p < 0.05$) and glycochenodeoxycholate percentage and the slight increase in the taurocholate precentage.

In conclusion, contrary to the dramatic changes in serum cholesterol and other lipids, pantethine may be incapable of accerelating lithogenesity under the present experimental condition.

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