

LIPID METABOLISM IN THE DEVELOPMENT OF CHOLESTEROL GALLSTONES IN HAMSTERS

III. THE EFFECT OF DIETARY CHOLESTEROL ON ITS BILIARY CONCENTRATION AND LITHOGENESITY^{*)}

By

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ABSTRACT

The effect of dietary cholesterol on the lithogenesis of bile in female hamsters was investigated. Biliary and serum lipids and serum lipoproteins of the hamsters fed with the normal diet supplemented with 1% cholesterol for 10, 20 and 30 days were analyzed. The synthesis rate of phospholipids and triglycerides were determined with (1-¹⁴C) palmitate incorporated into these lipids in the livers of the animals.

The biliary cholesterol output slightly but significantly increased with a small and temporary increase in phospholipids 20 and 30 days after feeding the cholesterol diet. But these animals failed to show a significant change in the total and individual bile acids.

Though the lithogenic index (Holzbach) gradually increased in accordance with the above change in the biliary lipid composition, it stayed far below the saturation point (1.0) even 30 days after feeding the cholesterol diet, not causing the gallstone formation in the animal gallbladder. The striking increase in serum lipids including total cholesterol, phospholipids and triglycerides, and serum lipoproteins was, on the other hand, observed so shortly as 10 days after the cholesterol diet feeding. The cholesterol diet feeding also produced the increase in the synthesis of phospholipids and triglycerides contributing to the fatty acid composition of biliary phospholipids.

These results suggest that dietary cholesterol brings about the increase in the biliary cholesterol output but that phospholipids synthesized in the liver maintain the lithogenesis far below the saturation point in the normal female hamsters.

INTRODUCTION

The gallstone production in hamsters by

lithogenic diet containing 72.3% glucose had been reported in the previous studies by the authors to be resultant of the relative decrease

^{*)} 梶山梧朗, 久保田茂夫, 佐々木 博, 川本敏雄, 三好秋馬: ハムスターにおけるコレステロール胆石形成過程の脂質代謝 Ⅲ. 食餌性コレステロールの胆汁および胆石形成度に対する効果

in the proportion of phospholipids to cholesterol in bile instead of the relative decrease in the proportion of bile acids to cholesterol in bile¹¹.

The phospholipids synthesized in the liver seemed to have been consumed for the production of pre- β -lipoprotein which was simultaneously stimulated by the excess synthesis of triglycerides in the liver.

The failure of chenical acid in dissolving gallstone or the adverse effect, i.e., the deterioration of gallstone by a large dosage of chenical acid in these animals was likely to support the above hypothesis^{2,3}.

The administration of such lithogenic diet mixed with 1% cholesterol to animals resulted in the reduction of the number of animals with gallstones as compared with the animals fed with the lithogenic diet alone and permitted observation of adequate supply of phospholipids in bile of these animals that was brought forth by their plentiful synthesis in the liver⁴.

On the other hand, however, dietary cholesterol is considered to play an important role for the recently increasing incidence of gallstone diseases. In addition, a variety of environmental factors such as sex, drugs^{5,6}, heredity, alcoholic drinks and other favorites are also considered influential on the biliary cholesterol excretion.

An experiment was performed to evaluate the effect of exogenous cholesterol on the biliary and serum lipids in animals fed with the normal diet supplemented with 1% cholesterol and thereby to analyze the above discrepancy in the role of dietary cholesterol for the lithogenicity of bile between the animal experiment and the statistical observation in the human beings.

METHODS

The methods employed in the present experiment were basically identical to what had been adopted previously with an exception of the diet contents^{1,4}.

Sixty-four female golden hamsters were fed with a 1% cholesterol diet (standard rat chaw supplemented with 1% cholesterol, Oriental Food Co., Japan).

Specimens of bile, serum and liver were obtained prior to (i.e., maintained under a normal diet) and 10, 20 and 30 days after feeding the

cholesterol diet.

Biliary lipids (cholesterol and bile acids) were analyzed by gas-liquid chromatography according to the methods described by Kawamoto et al.^{5,6}.

Biliary lecithin (phospholipids⁷), serum cholesterol⁸, triglycerides⁹ and phospholipids⁷ were determined by the enzyme methods.

The lithogenic index was calculated according to the formula described by Thomas and Hofmann¹⁰ based on the Holzbach's index¹¹.

Fatty acids of biliary phospholipids were analyzed by gas-liquid chromatography packed with 25% DEGS¹².

The serum lipoprotein analysis was performed with agarose gel lipoproteinelectrophoresis (Nippon Shoji Co., Japan).

Fresh liver slices were incubated with (1-¹⁴C) palmitate in Krebs-Ringer phosphate buffer (pH 7.4) for three hours in order to determine the synthesis rates of triglycerides and phospholipids¹³. Lipids after extraction were separated on silica Gel G thin layer chromatography. Phospholipid and triglyceride fractions were scraped and transferred into scintillation vials. The radioactivity of lipids was counted with an Aloca LSC liquid scintillation spectrometer.

RESULTS

I. Hourly output of biliary lipids and gallstone formation:

The output of biliary cholesterol showed a slight but significant increase 20 and 30 days after feeding the cholesterol diet. ($p < 0.01$)

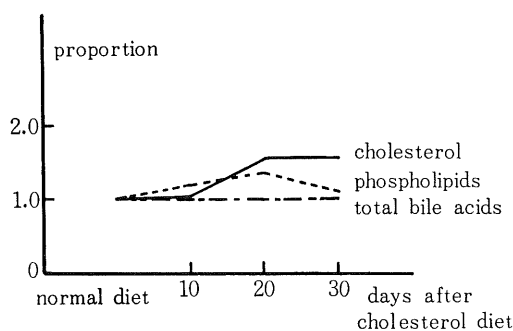
The output of phospholipids also seemed to be larger in animals fed with the cholesterol diet after 10 (N.S.) and 20 days ($p < 0.05$) as compared with those fed with the normal diet. Contrary to the above two lipids, the biliary total bile acid output after feeding the cholesterol diet stayed constant.

The gallstone was not detected at all in the gallbladder at any time during the period of cholesterol diet feeding. (Table 1)

Figure 1 shows the proportion of the increase in the biliary lipid outputs. The proportion is expressed for three biliary lipids in the ratio to the output by the normal diet. The cholesterol output reached the plateau 20 days after the cholesterol diet feeding, and the ratio of the increase in phospholipids tended to ascend upto

Table 1. Hourly output of biliary lipids and number of animals with gallstone

		(μ mole/hour)			
		Cholesterol	Phospholipids	Total bile acids	Number of animals with gallstone(%)
Normal diet	(6)	21.04 \pm 5.16	122.92 \pm 22.27	1567.50 \pm 181.95	0
10 days after cholesterol diet	(5)	22.28 \pm 6.00	148.35 \pm 29.31	1589.85 \pm 385.27	0
20 days after cholesterol diet	(5)	32.48 \pm 3.81**	168.37 \pm 29.86*	1624.20 \pm 208.95	0
30 days after cholesterol diet	(6)	32.63 \pm 4.49**	130.5 \pm 15.90	1593.0 \pm 342.7	0

* $p < 0.05$ ** $p < 0.01$ **Fig. 1.** Proportions of the increase in bile lipid outputs

20 days after the cholesterol diet feeding but descended thereafter.

The individual bile acid outputs are listed in Table 2. Both the primary and secondary bile acid outputs were not influenced by feeding the cholesterol diet.

II. Fatty acid composition of biliary phospholipids:

Figure 2 indicates the fatty acid composition in percentage of biliary phospholipids, classified into saturated, mono-unsaturated and poly-unsaturated fatty acids. Percentage saturated fatty acids showed a slight decrease 30 days after and mono-unsaturated fatty acids, successive increase 10, 20 and 30 days after feeding the cholesterol diet. The percentage of poly-unsaturated fatty acids was lower 10 and 20 days after feeding but returned almost to the level of the normal diet 30 days after feeding the cholesterol diet.

III. Lithogenic index (Holzbach):

The lithogenic index tended to increase slightly after feeding the cholesterol diet but was within the unsaturated range (index less than 1.0) throughout the period of cholesterol diet feeding.

IV. Serum lipids:

Serum total cholesterol, phospholipids and triglycerides increased remarkably and significantly so shortly as 10 days after feeding the

Table 2. Individual bile acid outputs (μ mole/hour)

		Primary bile acids		Secondary bile acids	
		Chenodeoxycholic acid	Cholic acid	Lithocholic acid	Deoxycholic acid
Normal diet	(6)	358 \pm 43	781 \pm 140	165 \pm 49	428 \pm 57
10 days after cholesterol diet	(5)	465 \pm 107	676 \pm 172	257 \pm 109	489 \pm 151
20 days after cholesterol diet	(5)	556 \pm 246	723 \pm 124	180 \pm 34	387 \pm 744
30 days after cholesterol diet	(6)	436 \pm 172	693 \pm 102	230 \pm 112	463 \pm 101

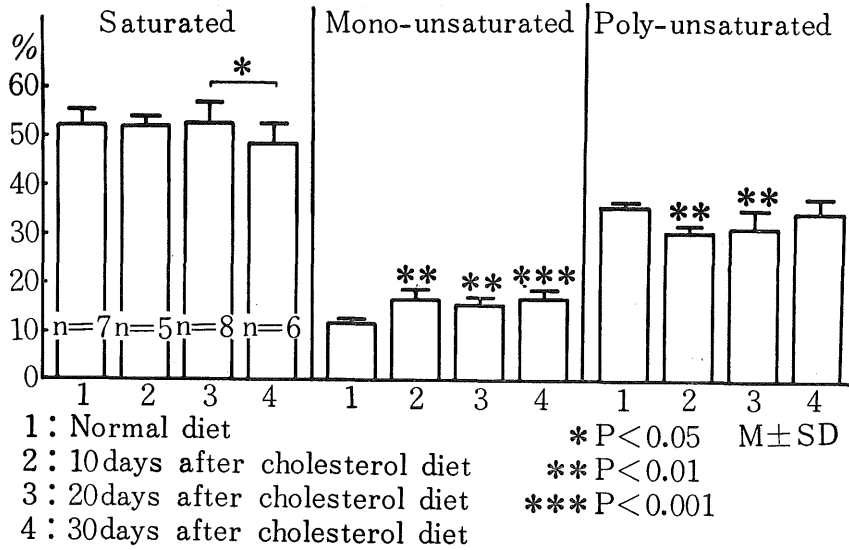


Fig. 2. Fatty composition of biliary phospholipids

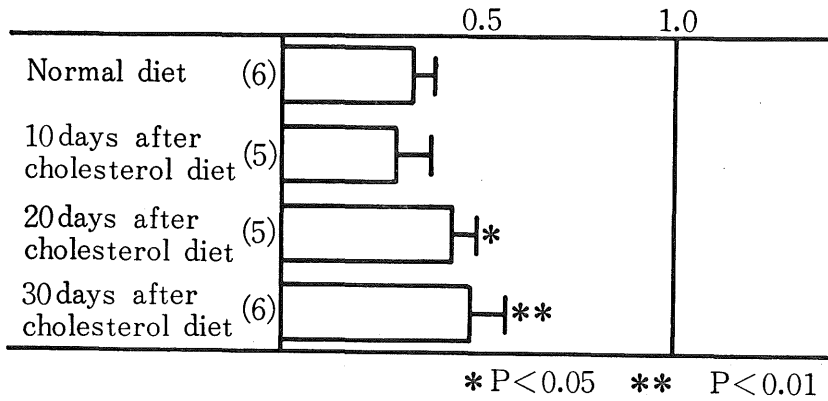


Fig. 3. Lithogenic index (Holzbach)

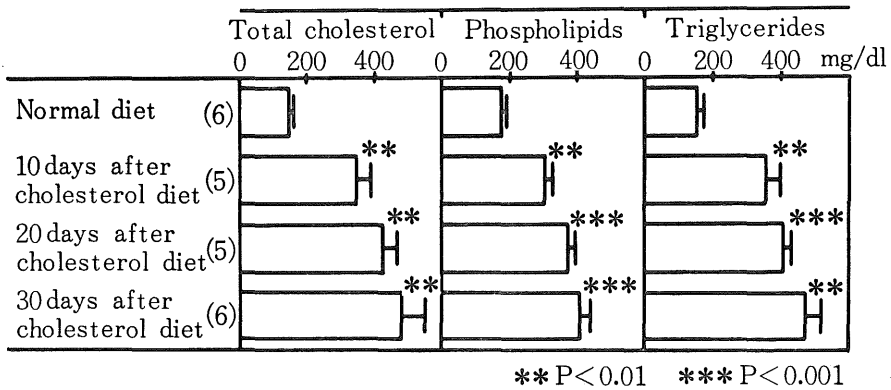
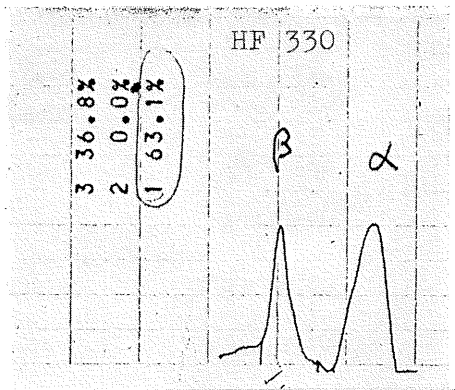
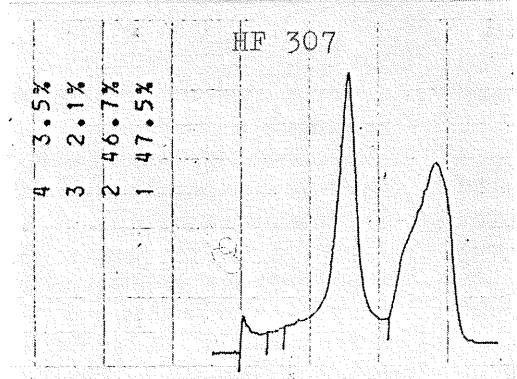


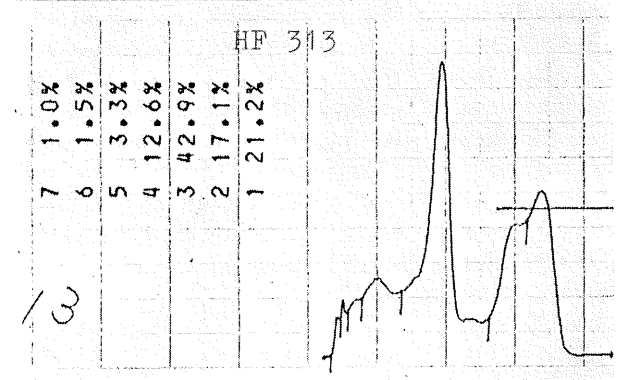
Fig. 4. Serum lipid concentrations



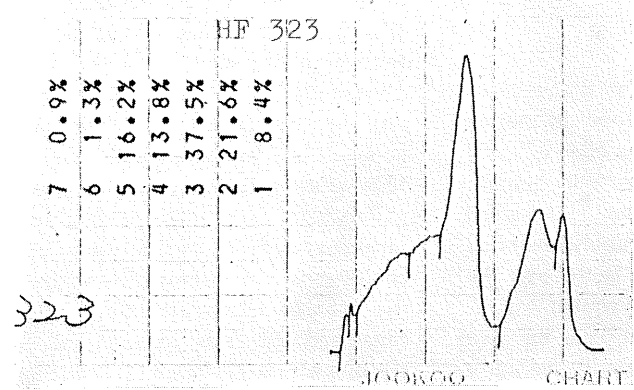
Normal diet



10 days after
cholesterol diet



20 days after
cholesterol diet



30 days after
cholesterol diet

Table 3. (1-¹⁴C) palmitate incorporated into phospholipids and triglycerides

		DPM in phospho- lipid fraction	DPM in trigly- ceride fraction	Triglycerides Phospholipids
Normal diet	(4)	9178±1202	10153± 736	1.116±0.080
10 days after cholesterol diet	(6)	12095±977**	11702±1315*	1.028±0.118
20 days after cholesterol diet	(6)	12180±626**	12947±1678**	1.057±0.161
30 days after cholesterol diet	(4)	12414±767**	12882±1644*	1.035±0.089

* p<0.05 ** p<0.01

cholesterol diet and continued increasing until 30 days after the diet feeding.

V. Serum lipoprotein electrophoresis:

Figure 5 shows the representative examples of serum lipoprotein electrophoretic patterns. The cholesterol diet feeding to animals caused the characteristic change in the form of the most remarkable elevation of β -lipoprotein peak.

VI. Synthesis of phospholipids and triglycerides in the liver: ((1-¹⁴C) palmitate incorporated into phospholipids and triglycerides)

More (1-¹⁴C) palmitate was incorporated into phospholipids and triglycerides in the liver slices of animals fed with the cholesterol diet than in those with normal diet as shown in Table 3.

The ratio of radioactive triglycerides to phospholipids was slightly lower in the liver slices of animals fed with the cholesterol diet than those with the normal diet, although there was entirely no statistically significant difference between these two groups of animals.

DISCUSSION

Dietary cholesterol had proved in the previous experiment to inhibit the gallstone formation to be caused by the lithogenic diet (72.3% glucose) to some extent for the time being⁴⁾. It had then been concluded that synthesized phospholipids induced by dietary cholesterol had lowered the lithogenesis of bile, decreasing the gallstone formation in that experimental condition⁴⁾. On the other hand, it can be also speculated that synthesized cholesterol, the main source of cholesterol gallstone in animals fed with lithogenic diet, was reduced in the liver

by dietary cholesterol through the action of the feedback mechanism.

Dam et al. also reported earlier that dietary cholesterol did not contribute to the formation of gallstone in hamsters¹⁹⁾.

These experiments suggest in a way that dietary cholesterol has influence neither on the biliary cholesterol content nor the deterioration of lithogenesis of bile and further that dietary cholesterol improves the lithogenesis by reducing the cholesterol content in bile.

However, many investigators warned that there is a possibility of dietary cholesterol to enhance the gallstone formation and disturb the dissolution of gallstone intended by CDCA administration^{14,15)}. In addition, dietary cholesterol causes a plenty of gallstone in mice when fed with a cholesterol diet combined with cholic acid to accelerate intestinal cholesterol absorption¹⁶⁾.

The present experiment of the authors produced a slight but significant increase in the biliary output of cholesterol in hamsters 20 and 30 days after feeding the cholesterol diet with the temporary increase in the output of biliary phospholipids. There was, however, neither any increase in the total acid output nor any change in the individual bile acid outputs. Moreover, gallstone was not detected in the gallbladders of animals throughout the experimental period, in spite of the increase in biliary cholesterol. In conformity with these results, the average lithogenic indices of hamster bile calculated by the formula of Thomas and Hofmann¹⁰⁾ were below 1.0 during the experimental period.

On the other hand, the total serum choles-

terol level rose prominently and significantly 10 days after feeding, followed by additional increase 20 and 30 days after feeding the cholesterol diet.

The above results proved that although adequate dietary cholesterol is absorbed through the animal intestine, a large portion of dietary cholesterol is easily excreted into serum but merely a very little amount is directly eliminated into bile in this animal.

It was, however, proven by these results that dietary cholesterol apparently increases the biliary cholesterol excretion, although its magnitude does not exceed that of lithogenic diet¹⁾, bile is not supersaturated and gallstone is not formed in the animal gallbladder.

It has been well established that dietary cholesterol inhibits the cholesterol synthesis in the liver (synthesized cholesterol is regarded as the main source of gallstone) by the action of feedback mechanism. Therefore, the reduction of gallstone formation in hamsters fed with the cholesterol-lithogenic diet is considered due to the aid of the feedback mechanism of cholesterol synthesis in the livers of the animals⁴⁾. At the same time, when the animals were fed with dietary cholesterol in combination with the lithogenic diet, phospholipids were synthesized dramatically in the liver with the increase in biliary phospholipids proportionally to the increase in biliary cholesterol to maintain the cholesterol solubility in bile⁴⁾.

The present experiment revealed the significant elevation of serum phospholipid level after feeding the cholesterol diet but it was not so noticeable in bile except for the temporary increase after feeding for 20 days.

However, the significant increase in incorporation of (1-¹⁴C) palmitate into phospholipids by the cholesterol diet feeding indicates the acceleration of phospholipids in the animal liver.

The change in the fatty acid composition of biliary phospholipids may be interpreted to indicate the influence of elevated phospholipid synthesis on biliary phospholipids permitting the augmented cholesterol solubility in bile of the animal.

It can be concluded from the foregoing results that dietary cholesterol increases the biliary cholesterol excretion but its solubility is maintained with the support of the increment of phospholipids synthesized to some extent in the

liver. However, in the event of the phospholipid synthesis being inhibited or disturbed in the liver, dietary cholesterol alone increases in bile resulting in the elevation of lithogenesis of bile. These changes in biliary lipids here brings forth no change in bile acid at all.

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