

The Preventive Effect of Vitamin E on Gallstone Formation

(1) A Study of Biliary Cholesterol and Bile Acids in Vitamin E-Deficient Hamsters

TOHRU SAITO

The 2nd Department of Surgery, Faculty of Medicine, Kyoto University

(Director: Prof. Dr. KAZUE OZAWA)

Received for Publication, Feb. 27, 1987.

Summary

Biliary cholesterol and bile acid concentration in vitamin E-deficient hamsters were measured and the effects of α -tocopherol administration on biliary lipids were evaluated in vitamin E-deficient and chow-fed hamsters. No gallstones were found in any hamsters fed a vitamin E-deficient diet or chow diet during four weeks. In the vitamin E-deficient hamsters, GCA and TCA markedly decreased. Intraperitoneal administration of dl- α -tocopheryl acetate caused a decrease of cholesterol and an increase of GCDCA in the bile. The biliary cholesterol level in vitamin E-deficient hamsters was higher than in chow-fed hamsters. More than four days were required for bile acid metabolism to return to normal levels, when vitamin E-deficient animals were replenished with α -tocopherol.

Biliary cholesterol levels in older hamsters were higher than in young hamsters, but α -tocopherol reduced the cholesterol level, even in older hamsters, and caused an increase in GCDCA and a corresponding increase in the total bile acid/cholesterol ratio of the bile. It was confirmed that vitamin E deficiency produced an increase in biliary cholesterol and a decrease in GCA and TCA, whereas α -tocopherol administration reduced biliary cholesterol and raised GCDCA in the bile.

Introduction

Because the pathogenesis of cholesterol gallstones has been ascribed to an abnormal hepatic cholesterol metabolism^{16,33)}, the ideal therapy may be not only to dissolve gallstones, but also to normalize the impaired cholesterol and bile acid metabolism. However, the efficacy of gallstone dissolution therapy using chenodeoxycholic acid (CDCA) or ursodeoxycholic acid (UDCA) is still less than 40%^{28,47)}. Although CDCA is much more efficacious than this at a high dosage of 750 mg per day⁴²⁾, it produces side effects such as diarrhea, and hepatocellular damage frequently occurs²³⁾.

Key words: α -Tocopherol, Vitamin E-deficiency, Gallstone, Bile acid, Hamster.

索引語: α -tocopherol, ビタミンE欠乏, 胆石, 胆汁酸濃度, ハムスター

Present address: The 2nd Department of Surgery, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

DAM et al⁷⁾ first produced cholesterol gallstones in hamsters in 1952, but later they found that a fat free diet was the real cause. The findings that the activity of anilin enzyme in the enzyme system of cholesterol 7 α -hydroxylase decreases when the amounts of vitamin C and E are low in the liver¹¹⁾, and that vitamin C accelerates the activity of cholesterol 7 α -hydroxylase¹⁸⁾, suggest that vitamin E might improve the cholesterol solubilizing capacity in bile through the effects of α -tocopherol on the biliary lipids. In this paper, the effects of vitamin E deficiency on bile acid were investigated.

Materials and methods

Microdetermination of bile acids

Because the volume of bile obtained from the gallbladder of hamsters is extremely small, microdetermination by high pressure liquid chromatographic enzymatic assay¹⁴⁾ was indispensable.

1. Reagents and standards of bile acids

The standard bile acids of CA, CDCA, DCA, LCA, UDCA and their glycine and taurine conjugates, were purchased from Sigma Chemical A Co. (USA). An immobilized 3 α -hydroxysteroid dehydrogenase (3 α -HSD) column was purchased from Shimazu Co. (Japan), and SEP-PAK C₁₈ cartridge was obtained from Waters Co. (USA). β -NAD was purchased from Oriental Yeast Co. (Japan), and used as a reagent after dissolving it into a Tris-HCl buffer (100 mmol/ml, pH 8.0) containing 0.1% EDTA 2Na. Ethanol, methanol, KH₂PO₄, and K₂HPO₄ were purchased from Wako Co. (Japan).

2. Preparation of samples

Each bile sample was diluted with saline and passed through a SEP-PAK C₁₈ cartridge. Bile acids were then eluted with ethanol. After evaporation of the solvent under flow with N₂ gas, the residue was resolved in 90% ethanol, and a solution was applied to a piperidino-hydroxypropyl 20 Gel (PHP-Gel). Ion-exchange chromatography was undertaken following the method of NAMBARA and GOTO¹²⁾. The PHP-Gel for ion-exchange chromatography was purchased from Shimazu Co. The eluents for ion-exchange chromatography were 0.1 M acetic acid in 90% ethanol, 0.2 M formic acid in 90% ethanol, and 0.3 M potassium acetate solution in 90% ethanol.

3. Apparatus and measuring accuracy

Figure 1 shows a HPLC system. 100 mM potassium phosphate/ethanol/methanol (51/37/12, pH 7.5) was used as a mobile phase. The wave length of the fluorometric detector was 350 nm at excitation, and 460 nm at emission.

The recovery rates of bile acids were 97.8~100.7% in unconjugated bile acids, 89.3~99.7% in glycine-conjugated bile acids except for GCDCA (85.0%), and 96.4~100.7% in taurine-conjugated bile acids, respectively. A calibration curve was constructed by plotting the ratio of peak area to each standard bile acid. Detection with a differential fluorometer showed a linear response ($r=0.999$) to each bile acid in the range of 0~40 μ g.

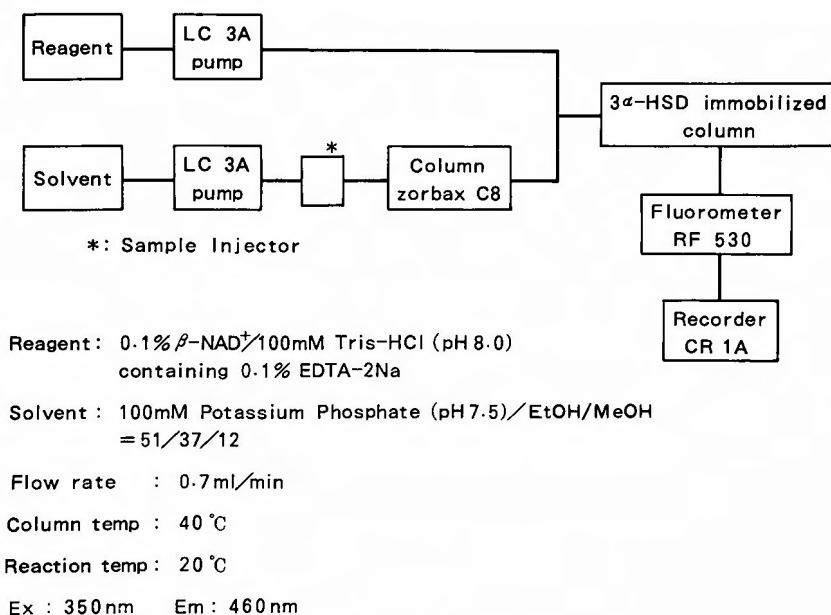


Fig. 1. HPLC system

Experiment I

Fourteen male golden hamsters (four weeks old, weighing 51 to 60 g, purchased from colonies of the Shizuoka Laboratory Animal Center) were kept on the chow diet for one week as an adaptation period, and were then divided into two groups: Group I (chow diet, n=6), and Group II (vitamin E-deficient diet, n=8). All animals were maintained in a 26°C air-conditioned room,

Table 1. Experimental vitamin E-deficient diet

	g/100g diet
Corn starch	36
α -Wheat starch	10
Sucrose	5
Casein (vitamin free)	25
Stripped corn oil*	8
Salt mixture**	6
Vitamin mixture***	2
Purified fiber	8

* Produced by Eastman Kodak Chemistry.

Group 3, corn oil was not added.

** K 692, P 597, Ca 411, Na 270, Mg 86, Fe 41, Zn 0.4, Mn 1.3, Cu 0.08, I 7.7 (mg/100g diet)

*** Vitamin A 1000IU, D₂ 200IU, B₁ 2.4mg, B₂ 8.0mg, B₆ 1.6mg, B₁₂ 0.001mg, C 60.0mg, K₃ 10.4mg, Biotin 0.04mg, Folic acid 0.4mg, Ca-Panthonate 10mg, P-aminobenzoic acid 10mg, Niacin 12.0mg, Inositol 12.0mg, Cholin-CI 4000mg (per 100g diet)

and in individual wire-mesh cages to prevent coprophagia. The vitamin E-deficient diet (Oriental Yeast Co.) contained corn starch (36 g), α -wheat starch (10g), sucrose (5 g), casein (vitamin free 25 g), stripped corn oil (8 g), a salt mixture (6 g), a vitamin mixture (2 g), and purified fiber (8 g per 100 g) (Table 1). The composition of stripped corn oil produced by Eastman Kodak Chemistry was palmitic acid 10.5%, stearic acid 2.1%, oleic acid 26.0%, linoleic acid 60.1%, and linolenic acid 1.3%. The α -tocopherol content confirmed by HPLC analysis was less than 0.1 mg per 100 g diet in the vitamin E-deficient diet, and 1.2 mg in the chow diet. Vitamin C content was 46 mg and 52 mg per 100 g, respectively, in each diet. The food was replaced daily in the cages. The gallbladder bile was aspirated by an insulin injector "Myjector".

Experiment II

Twenty-eight hamsters (four weeks old, weighing 48 to 61 g) were divided into four groups: Group III (a chow diet), Group IV (a chow diet with α -tocopherol administration), Group V (a vitamin E-deficient diet), Group VI (a vitamin E-deficient diet with α -tocopherol administration) (Fig. 2). The α -tocopherol which contained 100 mg of dl- α -tocopheryl acetate, 200 mg of hydrogenetic castor oil 60 and 140 mg of polyethylene glycol 400. Dl- α -tocopheryl acetate was administered intraperitoneally at a dosage of 20 mg/kg body weight for four consecutive days in the fifth week. Blood samples for determination of cholesterol were taken through a 0.25 μ m Milipore filter, and measured by an enzyme assay (the FROMM method)¹⁰. Serum α -tocopherol level was measured by the ABE method¹¹

Experiment III

In 8 of 16 old hamsters fed a chow diet (29~31 weeks old), α -tocopherol was administered intraperitoneally at a dosage of 20 mg/kg body weight for four consecutive days (Group VII). Three days later they were laparotomied. α -Tocopherol concentrations in serum and the liver, serum and biliary cholesterol levels, and bile acids concentrations in the gallbladder bile were evaluated. All the results are shown as "Mean $\pm$ SEM", and the STUDENT'S t-test was utilized to make statistical comparisons.

Experimental design

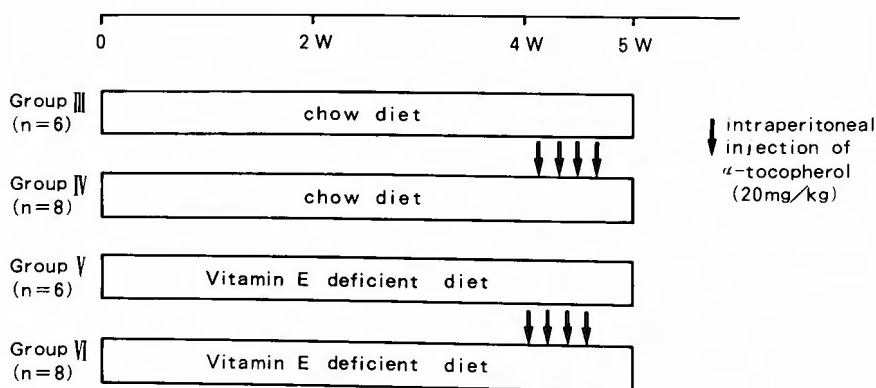


Fig. 2. Protocol of Experiment II

Results

(1) Body weight and liver weight

All animals appeared healthy at the time of sacrifice. The mean body weight and liver weight were not different among all groups.

(2) Serum and liver tocopherol concentrations

Serum α -tocopherol concentration was extremely low in Group V, reflecting the vitamin E deficiency. On the contrary, α -tocopherol administration to vitamin E-deficient hamsters caused a marked increase to $20.75 \pm 0.42 \mu\text{g/ml}$, nearly equal to that in Group IV (Table 2). α -Tocopherol administration also caused a 1.8-fold increase in the liver and serum α -tocopherol concentrations in Group VII (Table 3).

(3) Biliary cholesterol concentration

α -Tocopherol administration caused a moderate decrease in biliary cholesterol concentration, but vitamin E deficiency raised it (Fig. 3). An increase in biliary cholesterol level in older hamsters of Experiment III, compared to the young hamsters of Experiment II, was observed.

Table 2. Serum tocopherol level in hamsters of Experiment II

Group	Diet	Animals	Serum tocopherol level ($\mu\text{g/ml}$)		
			α	β	γ
III	Chow diet	(n=6)	10.36 ± 0.39	0.57 ± 0.04	0.50 ± 0.05
IV	Chow diet with α -tocopherol	(n=6)	22.72 ± 1.24	< 0.02	< 0.02
V	Vitamin E deficient diet	(n=6)	1.33 ± 0.05	0.07 ± 0.01	0.14 ± 0.01
VI	Vitamin E deficient diet with α -tocopherol	(n=6)	20.75 ± 0.42	< 0.02	< 0.02

Table 3. Serum and liver tocopherol concentrations in hamsters of Experiment III

Group	Tocopherol	Serum ($\mu\text{g/ml} \pm \text{SEM}$)			Liver ($\mu\text{g/g} \pm \text{SEM}$)		
		α	β	γ	α	β	γ
VII (n=7)		10.33 ± 1.66	0.51 ± 0.04	0.51 ± 0.04	63.49 ± 11.59	3.56 ± 0.50	4.68 ± 0.66
VIII (n=6)		19.28 ± 1.18	0.20 ± 0.03	0.21 ± 0.02	135.55 ± 28.35	1.40 ± 0.13	1.67 ± 0.19

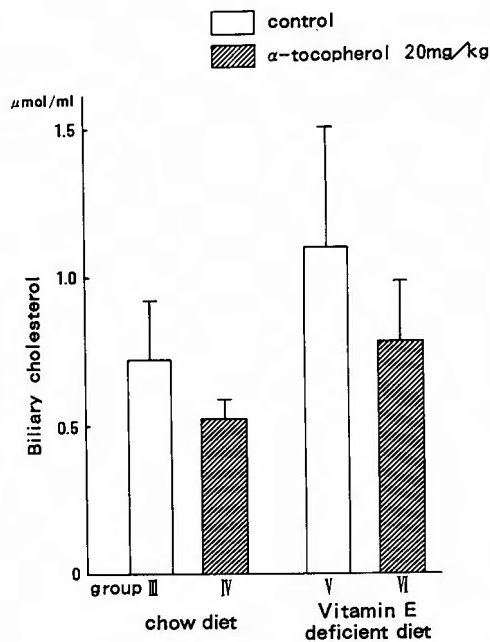


Fig. 3. Biliary cholesterol level in young hamsters (Experiment II)

(4) The composition and concentration of bile acids in the gallbladder bile

The biliary bile acid composition in hamsters fed the chow diet indicated that CA was the major bile acid (60.5%), and that CDCA accounted for 27.9% (Table 4). In animals fed the vitamin E-deficient diet, there was a marked decrease in total bile acids (TBA), glycine-conjugated and taurine-conjugated bile acid concentrations, especially GCA and TCA compared to the chow-fed group (Fig. 4). However, G/T ratio and C/CDC ratio were not different between Groups I and II (Table 4).

In Experiment II, increase in TBA and glycine-conjugated bile acid concentration were observed in Groups IV and VI (Table 5). The animals receiving α-tocopherol (Groups IV and VI) had an increased GCDCA percentage of total bile acids compared with each chow-fed control group (Fig. 5). Thus, α-tocopherol reduced the C/CDC ratio, and raised the TBA to cholesterol (TBA/CH) ratio in the bile of hamsters in Groups IV and VI (Fig. 6).

Table 4. Composition and concentration of bile acids

Group	Total* bile acid	Bile acids (μmol/ml±SEM)		
		Glycine* conjugated	Taurine* conjugated	Unconjugated
I (n=6)	87.0±3.0	53.6±2.4 (61.6±0.9%)	27.8±1.9 (32.0±0.5%)	5.6±1.2 (6.4±0.6%)
II (n=8)	68.2±3.5	40.8±3.9 (59.9±1.8%)	22.0±4.0 (32.2±1.9%)	5.4±2.3 (7.9±1.2%)

*: P<0.001, ★: P<0.01, ‡: P<0.05

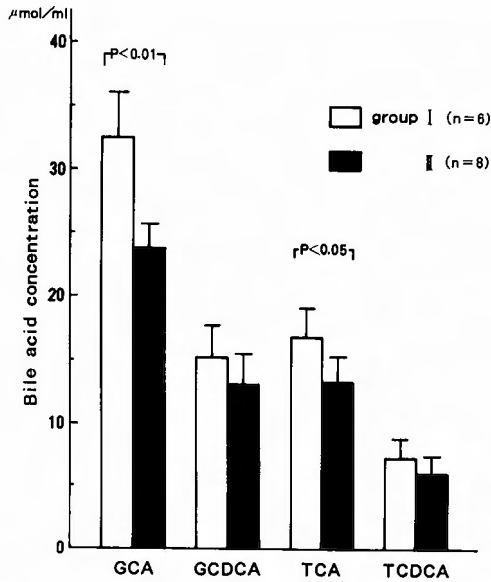


Fig. 4. Conjugated primary bile acid concentration in hamsters fed the vitamin E-deficient diet

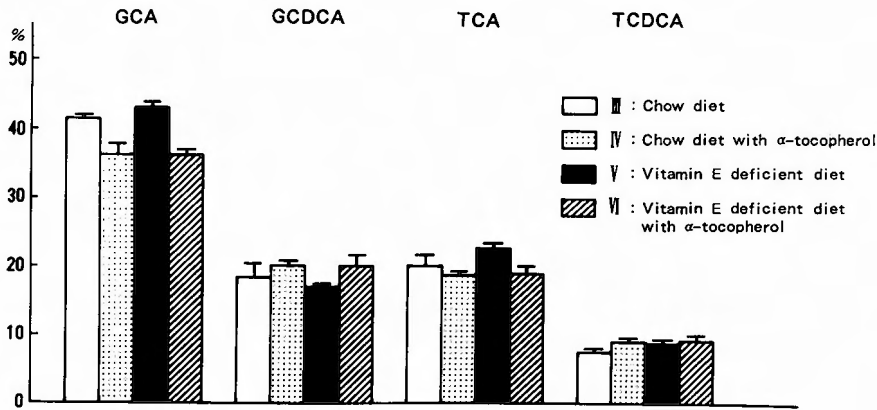


Fig. 5. Conjugated primary bile acid concentration in each group of Experiment II

in hamsters fed the vitamin E-deficient diet

CA#	CDCA	DCA	LCA	Molar ratio	
				G/T	C/CDC
52.7±5.3 (60.5±2.3%)	24.3±3.8 (27.9±1.7%)	5.1±1.2 (5.9±0.6%)	1.2±0.5 (1.4±0.3%)	1.9±0.1	2.3±0.6
40.9±2.2 (60.1±1.0%)	19.9±3.0 (29.2±1.5%)	4.3±2.3 (6.3±1.1%)	1.4±0.6 (2.1±0.3%)	1.9±0.5	2.1±0.4

Table 5. Composition and concentration of bile acids in each group of Experiment II

Group	Bile acids ($\mu\text{mol}/\text{ml} \pm \text{SEM}$)				Molar ratio	
	Total bile acid	Glycine conjugated	Taurine conjugated	Unconjugated	G/T	C/CDC
III (n=2)	77.3 $\pm$ 2.3	51.5 $\pm$ 1.9 (66.7 $\pm$ 0.6%)	23.8 $\pm$ 0.8 (30.9 $\pm$ 2.0%)	1.9 $\pm$ 1.1 (2.5 $\pm$ 1.4%)	2.2 $\pm$ 0.2	2.5 $\pm$ 0.2
IV (n=3)	104.2 $\pm$ 15.9	67.8 $\pm$ 10.9 (64.8 $\pm$ 0.7%)	34.1 $\pm$ 5.4 (32.6 $\pm$ 0.2%)	2.4 $\pm$ 0.4 (2.5 $\pm$ 0.9%)	2.0 $\pm$ 0.1	2.0 $\pm$ 0.1
V (n=2)	65.6 $\pm$ 2.7	41.2 $\pm$ 2.2 (62.7 $\pm$ 0.7%)	21.7 $\pm$ 0.5 (33.1 $\pm$ 0.7%)	2.8 $\pm$ 0.1 (4.2 $\pm$ 0.0%)	1.9 $\pm$ 0.1	2.6 $\pm$ 0.1
VI (n=4)	70.6 $\pm$ 10.9	46.6 $\pm$ 7.9 (65.5 $\pm$ 1.2%)	21.9 $\pm$ 3.0 (31.2 $\pm$ 0.8%)	2.2 $\pm$ 0.5 (3.3 $\pm$ 1.0%)	2.1 $\pm$ 0.1	2.0 $\pm$ 0.2

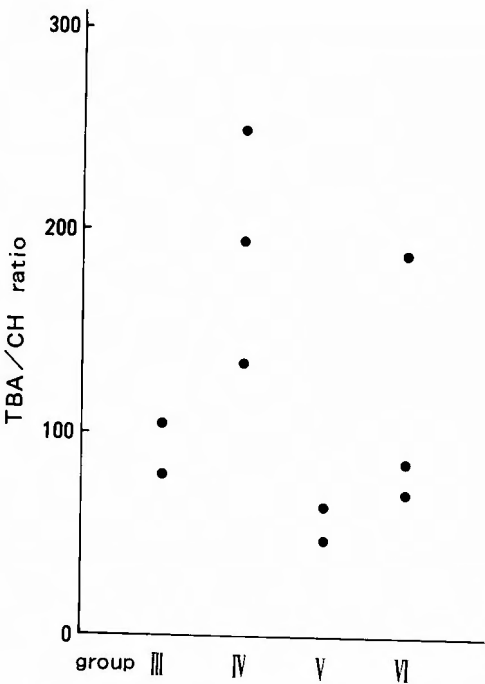
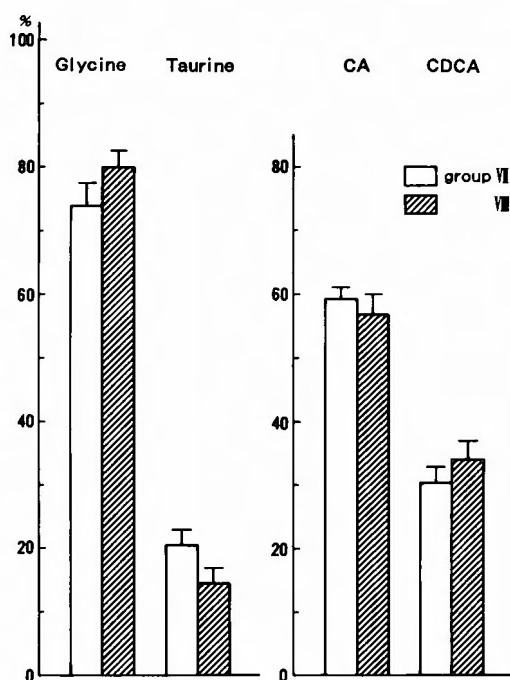


Fig. 6. TBA/CH ratio in each group of Experiment II

A characteristic of the bile acid composition in older hamsters was that glycine-conjugated and unconjugated bile acids accounted for a greater percent of the total bile acids (Table 6). α -Tocopherol administration increased TBA and glycine-conjugated bile acid concentration compared to the control group (Group VIII), whereas taurine-conjugated bile acid or unconjugated bile acid did not change significantly. These animals had a greater percentage of CDCA

Table 6. Composition and concentration of bile acids in older hamsters

Group	Bile acids ($\mu\text{mol/ml} \pm \text{SEM}$)				Molar ratio	
	Total bile acid	Glycine conjugated	Taurine conjugated	Unconjugated	G/T	C/CDC
VI (n=5)	41.6 ± 5.6	31.5 ± 5.2 (74.0 $\pm$ 3.6%)	7.9 ± 0.5 (20.3 $\pm$ 2.6%)	2.2 ± 0.4 (5.7 $\pm$ 1.4%)	4.0 ± 0.6	2.0 ± 0.2
VII (n=6)	53.3 ± 4.5	43.1 ± 4.9 (79.9 $\pm$ 2.8%)	7.5 ± 0.9 (14.6 $\pm$ 2.4%)	2.8 ± 0.5 (5.6 $\pm$ 1.2%)	6.2 ± 0.9	1.8 ± 0.2

**Fig. 7.** Bile acid concentration in older hamsters

and a lower percentage of CA compared with the control group, and α -tocopherol caused an increase of the TBA/CH ratio in the bile (Fig. 7 and Fig. 8).

Discussion

The relationships between vitamin E deficiency and cholesterol metabolism have been reported for several animal species. In rats, there have been reports of both an increase^{3, 6, 13, 24)} and no change³⁷⁾ in serum cholesterol, increases in total lipids³⁰⁾ and cholesterol⁴⁹⁾ in the liver and/or a decreased cholesterol synthesis in the liver³⁹⁾, associated with vitamin E deficiency. In rabbits, an increase in serum cholesterol⁴³⁾, an increased cholesterol synthesis from mevalonic acid, and a decreased conversion of cholesterol into bile acid⁹⁾, were reported. In guinea pigs,

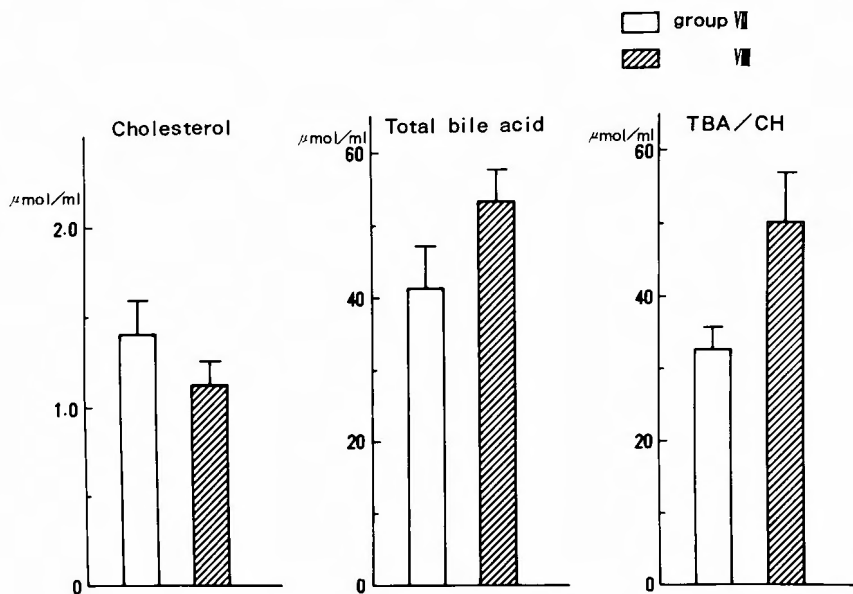


Fig. 8. Biliary lipids in older hamsters

both an increase³⁴⁾ and decrease⁶⁾ in serum cholesterol were reported. These results suggest that vitamin E deficiency may cause an increased cholesterol synthesis in the liver. Unfortunately, cholesterol and bile acid metabolisms in rats, rabbits and guinea pigs are so different from that in humans⁴⁶⁾, that the findings from these animals can not be applied clinically to human study.

However, in a number of ways, the sterol metabolism of hamsters differs from that of these experimental animals, and more closely approximates that seen clinically in humans^{44, 45)}, similarities have been noted in their pancreas and gallbladder^{2, 19)}, and the composition of bile acids^{5, 31)}. Furthermore, in both species hepatic and biliary cholesterol increase in proportion to the amount of dietary cholesterol³⁾, and hamsters produce lithogenic bile and cholesterol gallstones in response to a variety of alimentary manipulations^{8, 36, 40)}. Although KATO established an experimental vitamin E-deficient model in hamsters¹⁹⁾, cholesterol metabolism and bile acid metabolism were not evaluated in his study.

Although there are numerous studies concerning the relationship between dietary α -tocopherol supplementation and serum α -tocopherol concentration, parenteral administration of α -tocopherol has been examined in only a few. YASUNAGA⁵⁰⁾ reported that in mice, serum α -tocopherol level increased to twice that of the control group at dl- α -tocopherol dosages of 10 or 20 IU/kg by intraperitoneal administration, that immunoreactions such as PHA or LPS were suppressed at dosages of more than 80 IU/kg, and that all animals died within three days at a dosages of 400 IU/kg. From his results, the optimal dosage for α -tocopherol to affect biliary lipids was thought to be 20 mg/kg/day. A schedule of α -tocopherol administration for four consecutive days was designed, with reference to the various studies showing that α -tocopherol is mainly deposited in the liver or fatty tissue, and that excessive α -tocopherol is not excreted

rapidly. ZANNONI⁵²⁾ found that the oral administration of α -tocopherol in hamsters caused an elevation of drug metabolism activity within 12 hours, and that α -tocopherol concentration in the liver increased gradually from 6 to 36 hours after oral administration⁵¹⁾. Even if α -tocopherol was given intraperitoneally every day for a four-week period, serum α -tocopherol levels were not different⁵¹⁾.

The present study was the first to evaluate biliary cholesterol and bile acids in vitamin E-deficient hamsters, or animals administered α -tocopherol. It was found that the biliary cholesterol concentration in vitamin E-deficient animals was higher than in chow-fed hamsters, and that GCA and TCA significantly decreased in vitamin E-deficient hamsters as a biological effect of α -tocopherol. On the other hand, intraperitoneal α -tocopherol administration caused a decrease of biliary cholesterol, and an increase of GCDCA as a pharmacological effect of α -tocopherol. Thus, it was suggested that vitamin E deprivation might suppress the conversion of cholesterol into bile acid, as observed in rabbits⁸⁾, or might suppress the activity of cholesterol 12 α -hydroxylase in the liver. It was confirmed that α -tocopherol showed some inhibitory effect on biliary cholesterol output, and that it required more than four days for bile acid metabolism to return to the chow-fed control level, when vitamin E-deficient animals were replenished with α -tocopherol, even though the serum α -tocopherol level reached twice the normal value at a dosage of 20 mg/kg per day.

In the older hamsters, cholesterol, glycine-conjugated and unconjugated bile acids characteristically accounted for a greater percent of total biliary lipids than with the young animals. However, α -tocopherol administration reduced the cholesterol level in bile and caused an increase of GCDCA, as observed in young hamsters. Judging from the value of the TBA/CH ratio in older hamsters, it was concluded that aging is a major factor affecting cholesterol gallstone formation. This result agreed with the report by MATSUSHIRO²⁷⁾ that even if gallstones were not found in the elderly the total bile acids concentration in bile decreased and their lithogenicity increased.

A vitamin E-deficient diet for four weeks in our study did not induce gallstone formation in hamsters and the administration of a vitamin E-deficient diet for six month has not induced gallstones in four surviving hamsters³²⁾. Why were cholesterol gallstones not formed in vitamin E-deficient hamsters in spite of the increased cholesterol and the decreased total bile acids in the bile?

The cholesterol solubilizing capacity of bile can be estimated from the TBA/CH ratio, because that is proportional to the TBA+Phospholipid/CH ratio. α -Tocopherol raised this ratio in both chow-fed and vitamin E-deficient hamsters. Its value was smallest in vitamin E-deficient hamsters, but remained comparatively high ranging between 48.7 and 62.7. Many reports show that the TBA/CH ratio usually ranges from 48 to 101^{8, 20, 29)} in chow-fed hamsters, and there is no evidence of gallstone formation when this value is more than 42³⁸⁾. On the contrary, in hamsters with gallstones, resulting from being fed a lithogenic diet^{15, 29, 41, 48)} or lithogenic regimen³⁹⁾ the TBA/CH ratio is less than 23. The present study confirmed the reason why cholesterol gallstones were not formed in vitamin E-deficient hamsters in spite of the increase in

biliary cholesterol and the decrease in TBA.

From many studies on bile acid composition in bile, CA constitutes 56~70%, CDCA accounts for 18~33%, DCA accounts for 3.2~10.3%, and LCA accounts for 0.6~1.6% in hamsters fed on a chow diet^{21,22,35}. On the other hand, in hamsters with cholesterol gallstones, bile acid composition was 53% CA, 21~31% CDCA, 10~13% DCA, and 3.5~15% LCA when a lithogenic diet was given^{26,41}. A decrease in CA and increases in DCA or LCA were observed in hamsters with cholesterol gallstones. The bile acid composition in hamsters administered α -tocopherol was different from that of hamsters with cholesterol gallstones, although the percent of CA decreased.

The findings that α -tocopherol caused a decrease in biliary cholesterol, and an increase in GCDCA suggesting that α -tocopherol promoted the conversion from cholesterol into primary bile acid in the liver, by acceleration of the enzyme system of cholesterol 7 α -hydroxylase. Explanatory hypotheses for these phenomena were considered. Firstly, α -tocopherol might prevent essential fatty acids from producing peroxide, and as the peroxide itself reduces this enzyme activity⁴, the prevention of fatty acid formation might increase the cholesterol 7 α -hydroxylase activity^{17,25}. Secondly, because the activity of the aniline enzyme in the cholesterol 7 α -hydroxylase enzyme system decreases when both vitamin C and vitamin E are in low concentration in the liver¹¹, and because vitamin C itself accelerates the activity of cholesterol 7 α -hydroxylase, α -tocopherol may improve cholesterol saturation or lithogenicity. More definitive experiments are required to show whether α -tocopherol can improve lithogenicity in hamsters with cholesterol gallstones, and whether it can prevent gallstone formation or dissolve gallstones.

Acknowledgments

The author wishes to express their sincere gratitude to Professor KAZUE OZAWA, Assistant Professor HIROSHI TANIMURA, and Professor YORINORI HIKASA, Second Department of Surgery, Kyoto University, for their overall instruction in the course of this work. This work was in part supported by Scientific Grant-in-Aid No. 59570571 for Scientific Research from the Japan Ministry of Education, Science and Culture. A part of the present study was presented at the XII th International Congress of Gastroenterology, Lisbon, 1984, at the 70th General Meeting of the Japanese Society of Gastroenterology, 1983, and at the 36th General Meeting of the Vitamin Society of Japan, 1984.

References

- 1) Abe K, Yuguchi Y, Katsui G: Quantitative determination of tocopherols by high-speed liquid chromatography. *J Nutr Sci Vitaminol* **21**: 183-188, 1975.
- 2) Becker V: *Spezielle pathologische Anatomie*, Band 6, Springer-Verlag **48**: 1973.
- 3) Behr WT, Filus AM, Rao B, et al: A comparative study of bile acid metabolism in the rat, mouse, and gerbil. *PSEBM* **130**: 1067-1074, 1969.
- 4) Bjorkheim I, Blomstrand R, Svensson L: Effect of different dietary triglycerides on 7 α -hydroxylation of cholesterol and other mixed function oxidations. *J Lipid Res* **19**: 359, 1978.
- 5) Bonorris GG, Coyne MJ, Chung A, et al: Mechanism of estrogen induced saturated bile in the hamster. *J Lab Clin Med* **90**: 963-970, 1977.
- 6) Chen LH, Liao S, Packett LV: Interaction of dietary vitamin E and protein level or lipid source with serum cholesterol level in rats. *J Nutr* **102**: 729-732, 1972.

- 7) Dam H, Christensen F: Alimentary production of gallstones in hamsters I. *Acta Path Microbiol Scand* **30**: 236-242, 1952.
- 8) Dam H: Determinations of cholesterol cholelithiasis in man and animals. *Am J Med* **51**: 596-613, 1971.
- 9) Eskelson CD, Jacobi HP, Fitch DM: Some effects of vitamin E deficiency on in vitro cholesterol metabolism. *Physiol Chem Physics* **5**: 319-329, 1973.
- 10) Fromm H, Amin P, Klein H, et al: Use of a simple enzymatic assay for cholesterol analysis in human bile. *J Lipid Res* **21**: 259-261, 1980.
- 11) Ginter E, Kosinova A, Hudecova A, et al: Synergism between vitamin C and E: Effect on microsomal hydroxylation in guinea pig liver. *Internat J Vit Nutr Res* **52**: 55-59, 1982.
- 12) Goto J, Hasegawa M, Kato H, et al: A new method for simultaneous determination of bile acids in human bile without hydrolysis. *Clin Chim Acta* **87**: 141-147, 1978.
- 13) Harrill I, Minarik G, Gifford ED: Effect of vitamin A and E on lipids in selected rat tissues. *J Nutr* **87**: 424-428, 1965.
- 14) Hayashi M, Ishida Y, Arisue K, et al: A new high pressure liquid chromatography for bile acids determination. Abstract of 24th LC meeting **22**: 91, 1981 (Jpn).
- 15) Hermann WJ, Ward K, Faucett J, et al: The effect of tocopherol on high density lipoprotein cholesterol. *Am J Clin Pathol* **72**: 848-852, 1979.
- 16) Hikasa Y, Kuyama T, Maruyama I, et al: Initiating factors of gallstones, especially cholesterol gallstones (II). *Acta Jpn Chir* **34**: 1430-1461, 1965.
- 17) Hirano M: Role of essential fatty acids in liver on the conversion of cholesterol to bile acids, especially significance of essential fatty acids concerning the formation of cholesterol stones. *Arch Jpn Chir* **34**: 939-973, 1966.
- 18) Holloway DE, Rivers JM: Influence of chronic ascorbic acid deficiency and excessive ascorbic acid intake on bile acid metabolism and bile composition in the guinea pig. *J Nutr* **111**: 412-424, 1980.
- 19) Kato H: An experimental study of vitamin E on the etiology of pancreatitis. *Arch Jpn Chir* **51**: 481-494, 1982 (Jpn).
- 20) Kobayashi N: Experimental studies on gallstone formation after partial ileal bypass operation (II). Effects of partial ileal bypass operation on biliary lipids and enterohepatic circulation of bile acids in hamsters. *Arch Jpn Chir* **49**: 85-99, 1980 (Jpn).
- 21) Kuroki S, Hoshita T: Effect of bile acid feeding on hepatic steroid 12 α -hydroxylase activity in hamsters. *Lipids* **18**: 789-794, 1983.
- 22) Kuroki S, Muramoto S, Kuramoto T, et al: Sex differences in gallbladder bile acid composition and hepatic steroid 12 α -hydroxylase activity in hamsters. *J Lipid Res* **24**: 1543-1549, 1983.
- 23) Leuschner U: Current problem of chemical gallstone dissolution. *Tokai J Exp Clin Med* **8**: 223-233, 1983.
- 24) Levander OA, Morris VC, Higgs DJ, et al: Nutritional interrelationships among vitamin E, selenium, antioxidants, and ethylalcohol in the rat. *J Nutr* **103**: 536-542, 1973.
- 25) Maruyama I: Effect of essential fatty acids and pyridoxine on the formation of gallstones, especially cholesterol gallstones. *Arch Jpn Chir* **34**: 19-34, 1965.
- 26) Matsumoto K, Kameda H: The influence of bile acid on gallstone formation. *Igaku no Ayumi* **89**: 701-702, 1974 (Jpn).
- 27) Matsushiro T, Nagashima H, Yamamoto K, et al: Study on bile composition in the elderly in contrast with those of the young or cholesterol gallstone cases. *Jpn J Gastroenterol* **79** (Suppl): 635, 1982 (Jpn).
- 28) Nakagawa S, Makino I, Ishizaki T: Dissolution of cholesterol gallstones by ursodeoxycholic acid. *Lancet* **2**: 367-369, 1977.
- 29) Oohara H, Shiofiri H, Ono H, et al: The effect of chenodeoxycholic acid on experimental gallstones in hamsters. *Clin Report* **12**: 39-48, 1978 (Jpn).
- 30) Pappu AS, Fatterpaker P, Sreenivasan A: Alterations in lipid composition of subcellular membranes of rat liver in vitamin E deficiency. *Ind J Biochem Biophys* **16**: 143-147, 1979.
- 31) Pearlman BJ, Bonorris GG, Phillips J, et al: Cholesterol gallstone formation and prevention by chenodeoxycholic and ursodeoxycholic acids. *Gastroenterology* **77**: 634-641, 1979.
- 32) Saito T: A study on the composition of bile acids in bile in hamsters fed on a vitamin E-deficient diet for six months. (Unpublished data)
- 33) Sarles H, Chabert C, Pommeau Y, et al: Diet and cholesterol gallstones. A study of 101 patients with cholelithiasis compared to 101 matched controls. *Am J Digest Disease* **14**:(8): 531-537, 1969.

- 34) Shull RL, Ershoff BH, Alfin-Slater RB: Effect of antioxidants on muscle and plasma lipid of vitamin E deficient guinea pigs. *PSEBM* **98**: 364-366, 1958.
- 35) Singhal AK, Finver-Sadowsky J, McSherry CK, et al: Effects of cholesterol and bile acids on the regulation of cholesterol metabolism in hamsters. *Biochim Biophys Acta* **752**: 236-244, 1983.
- 36) Shioda R, Tanimura H, Muroya D: Experimental gallstones- especially the cause of cholesterol gallstones. *Tokyo Tanabe Quarterly* **19**: 26-37, 1973 (Jpn).
- 37) Shirai K, Matsuoka N, Mizobuchi M: The effect of tocopherol deficiency on lipid metabolism of arterial wall in hypercholesterolemic rat. *Arteriosclerosis* **7**: 249-255, 1979.
- 38) Sue SO, Bonorris GG, Marks JW, et al: Dissolution of cholesterol gallstones by bile acids in hamsters. *Med Sci* **284**: 18-23, 1982.
- 39) Takeuchi N, Matsumiya K, Takahashi Y, et al: Thiobarbituric acid reactive substances (TBARS) and lipid metabolism in α -tocopherol deficient rats. *Exp Geront* **12**: 63-68, 1977.
- 40) Tanimura H: Experimental gallstones, Tan to Sui. **2**: 663-669, 1981 (Jpn).
- 41) Tanimura H, Takenaka M: Experimental studies on dissolution of cholesterol gallstones in hamsters with chenodeoxycholic acid. *Arch Jpn Chir* **44**: 3-20, 1975 (Jpn).
- 42) Thistle JL, Hofmann AF: Efficacy and specificity of chenodeoxycholic acid therapy for dissolving gallstones. *N Engl J Med* **289**: 655-659, 1973.
- 43) Tore-Leonhardt E: Effects of vitamin E on serum cholesterol and triglycerides in hyperlipidemic patients treated with diet and clofibrate. *Am J Clin Nutr* **31**: 100-105, 1978.
- 44) Turley SD, Dietschy JM: Cholesterol metabolism and excretion. The liver, biology and pathobiology. Arias I, Popper H et al Ed., pp. 467-492. Raven Press, New York. 1982.
- 45) Turley SD, Dietschy JM: Effects of clofibrate, cholestyramine, zanchol, probucol, and AOMA feeding on hepatic and intestinal cholesterol metabolism and on biliary lipid secretion in the rat. *J Cardiovasc Pharm* **2**: 281-297, 1980.
- 46) Uchida K, Kadowaki M: Comparative biochemistry of bile and bile acids. *Metabolism* **14**: 303-314, 1977 (Jpn).
- 47) Weis HJ, Holtermuller KH, Stiehl A, et al: Clinical experience in bile composition in patients taking ursodeoxycholic acid for gallstone dissolution, In *Biological Effects of Bile Acid*. G. Paumgartner Ed. Lancaster. MTP Press, England. pp. 90-102, 1979.
- 48) Wheeler HO: Liver physiology and disease. Biliary excretion of bile acids, lecithin, and cholesterol in hamsters with gallstones. *Gastroenterology* **65**: 92-103, 1973.
- 49) Yasuda M, Fujita T, Mizunoya Y: Liver and plasma lipids in vitamin E deficient rat. *Chem Pharm Bull* **27**: 447-451, 1979.
- 50) Yasunaga T, Kato H, Ohgaki K, et al: Effect of vitamin E as an immunopotential agent for mice at optimal dosage and its toxicity at high dosage. *J Nutr* **112**: 1075-1084, 1982.
- 51) Yoshikawa T, Yokoe N, Takemura S, et al: Lipid peroxidation and lysosomal enzymes in D-galactosamine hepatitis and its protection by vitamin E. *Gastroenterol Jpn* **14**: 31-39, 1979.
- 52) Zannoni VG, Sato PH: The effect of certain vitamin deficiencies on hepatic drug metabolism. *Fed Proc* **35**: 2464-2469, 1976.

和文抄録

ビタミンEの胆石形成予防効果に関する研究

(1) ビタミンE欠乏ハムスターにおける胆汁中コレステロール濃度と胆汁酸濃度について

京都大学医学部第2外科学教室（指導：小澤和恵教授）

齋 藤 徹

ビタミンE欠乏食で飼育したハムスターに α -tocopherol を投与した際の胆汁中コレステロール濃度および胆汁酸組成とその濃度測定から薬理学的効果を検討し、以下の成績を得た。

1. ビタミンE欠乏ハムスターでは、①胆汁中コレステロール濃度の上昇と、②胆汁酸としてGCAおよびTCA濃度の減少を認め、③胆汁中TBA/CH比が低下したが、④その低下の程度は軽度であり、胆嚢内に胆石を形成しなかった。

2. 高齢ハムスターでは、①胆汁中コレステロール濃度の上昇と、②胆汁中総胆汁酸の濃度の低下を認めたが、③とくにビタミンE欠乏動物よりもTBA/CH比の低下が顕著であり、加齢因子がビタミンE欠乏状

態よりも胆石形成に強く影響することが判明した。

3. α -tocopherol の投与は、ビタミンE欠乏動物および高齢動物のいずれにおいても①胆汁中コレステロール濃度を低下させ、②胆汁中胆汁酸のうちGCDCAおよびGCA濃度を増加させ、③その結果、TBA/CH比が上昇し、胆石形成を阻止する方向に改善した。

以上の実験成績から、 α -tocopherol の投与は胆汁中コレステロール濃度を低下させ、1次胆汁酸であるGCAとGCDCA濃度を上昇させることが確認された。その作用機序としてコレステロールから胆汁酸への異化酵素である肝臓の cholesterol 7 α -hydroxylase の活性を直接的または間接的に亢進している可能性が推測された。