

原 著

Initiating Factors of Cholesterol Gallstones and Pancreatitis

by

HIROSHI TANIMURA, RYUZO SHIODA, HIROHISA MUROYA,
MASAFUMI TAKENAKA and MASAO NAGASE

The 2nd Surgical Department, Kyoto University School of Medicine
(Director: Prof. Dr. YORINORI HIKASA)
Received for Publication 2 Oct., 1975

I. Introduction

Recently cholesterol cholelithiasis is seen increasingly in Japanese people, especially in urban people, although pigment stones are seen still not infrequently in rural people. Table 1 summarizes the cases of cholelithiasis operated on at a hospital situated in a small city which has both urban and rural people, and the cases operated on at Kyoto University Hospital treating mainly urban people.

The high incidence of gallstones in modern western society is believed to be dietary in origin and mediated through abnormal metabolism of cholesterol and bile salts.

The harmful respects of the western diet are as follows:

- 1) It contains too much refined or highly available carbohydrate such as sugar, and contains too little vegetable fibres.
- 2) It is too rich in fats, especially in saturated animal fats such as butter fat, and contains too little essential fatty acids.

The mechanism by which such a diet initiates the formation of cholesterol gallstones has been studied in our laboratory¹⁾²⁾³⁾. Moreover, some experimental studies have been done to elucidate the mechanism on syntropy of acute pancreatitis with

Table 1

Cases of cholelithiasis operated on during the period of 1954 to 1974 at Yamato-Takada City Hospital

Years	number or cases	cholesterol stones	pigment stones
54-56	19	0	19
57-59	28	7	21
60-62	39	10	29
63-65	54	25	29
66-68	72	38	34
69-71	102	63	39
72-74	178	108	70
Total	492	251	241
70-74	101	83	18

(at Kyoto University Hospital)

Key Words : Metabolism of Cholesterol and Bile Acids, Essential Fatty Acids, Lithogenic Diet and Bile

Present address : The 2nd Department of Surgery Kyoto University, Medical School, Sakyo-ku, Kyoto, Japan. 〒606

cholesterol gallstones.

II. Biophysics of bile

Organic solid components of bile are bile salts, lecithin, cholesterol and others. Cholesterol itself is insoluble in water and the capacity of bile to dissolve cholesterol resides within the bile salts and lecithin fractions which combine to disperse cholesterol in mixed micelles as shown in Fig. 1⁴⁾.

The composition of any bile can be plotted as a single point in a phase diagram

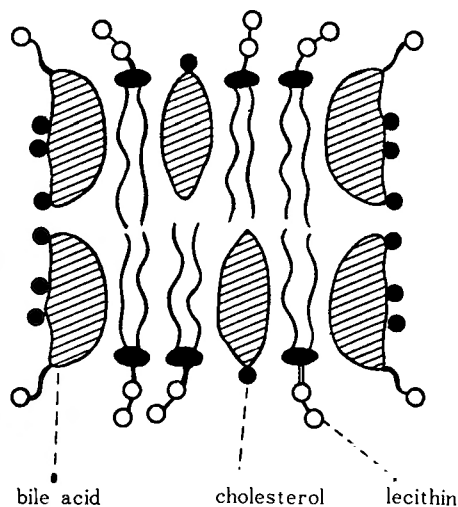


Fig. 1 ○ charged polar group
● less polar hydroxyl or ester group
cited from Small¹⁾

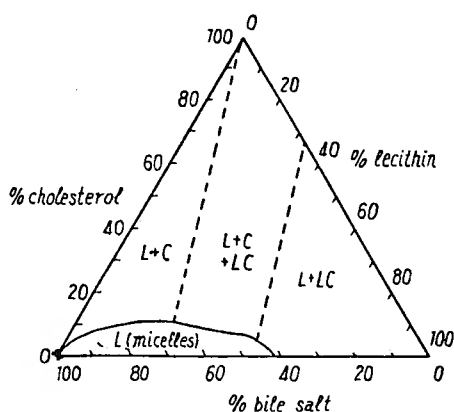


Fig. 2 Phase diagram showing the phase equilibria of bile salt-lecithin-cholesterol mixtures at 90 per cent water content and 37°C.
L=liquid, C=crystals, LC=liquid crystals.
cited from Admirand & Small⁵⁾

showing the phase equilibria of bile salt-lecithin-cholesterol mixture, and only mixtures in the micellar zone (the lower left hand of the triangle) are fully soluble (Fig. 2)⁵⁾. Outside the micellar zone, mixtures are supersaturated with cholesterol, and cholesterol begins to precipitate. This is the first step of formation of cholesterol gallstones.

Recently, Holzbach et al. have reported that the micellar zone is significantly smaller than that previously reported and that normal human bile, in contrast to bile from other mammalian species, is commonly supersaturated with cholesterol. According to them, bile from patients with cholesterol gallstones has a micellar zone similar to normals but differs compositionally in that there is a greater excess of cholesterol above saturation. From these facts, they conclude that cholesterol supersaturation may be a necessary but not solely sufficient cause for gallstone formation⁶⁾.

In anyway, the mechanism by which bile is supersaturated with cholesterol should be elucidated.

Since lecithin secretion is largely dependent on bile salt secretion, the metabolism of the latter should be discussed chiefly.

III. Metabolism of Bile Acids

Bile acids (cholic acid and chenodeoxycholic acid) which are produced from chol-

esterol in the liver are secreted in bile and into the intestine, where almost all of them (about 97%) are reabsorbed either in their original forms or in reduced forms (deoxycholic acid and lithocholic acid), and then again secreted into the intestine (enterohepatic circulation).

The whole amount of the circulating bile acids is called bile acid pool, which is continually depleted by fecal excretion and replenished by hepatic synthesis (Fig. 3 and 4)⁷⁾. The normal bile salt pool is about 2-4 g in size, circulating twice each meal,

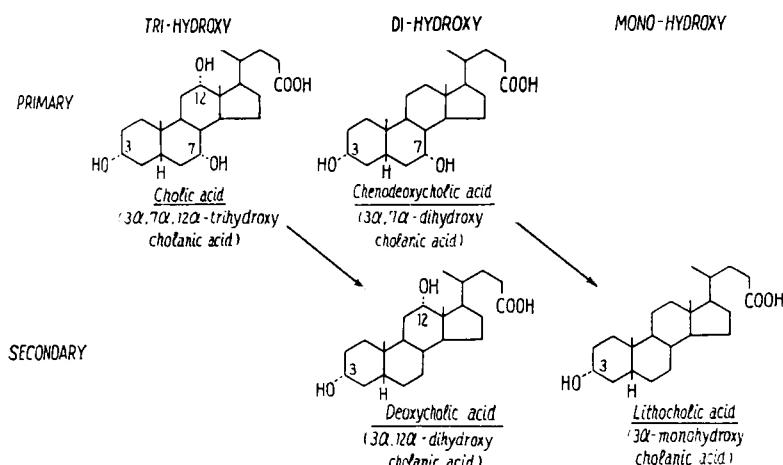


Fig. 3 Human bile acids acids.

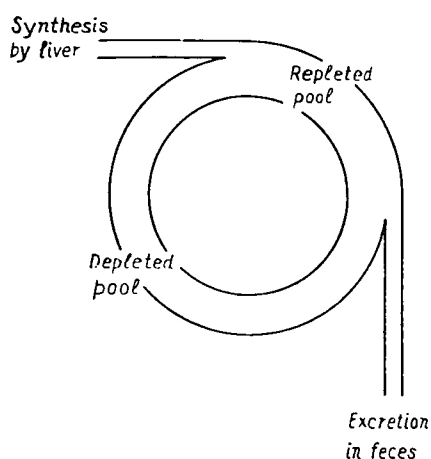


Fig. 4 A simple model of the enterohepatic circulation.

and each day it loses and is replenished with 500-700mg.

According to a recent study of Vlahcevic et al⁸⁾, the total bile acid pool is reduced to 1.3 g, or little more than half normal, in man with gallstones present in radiologically functioning gallbladders.

Reduced pool sizes have also been reported in U.S.A. Indian women with a high probability (70%) of developing cholelithiasis but with as yet normal gallbladders⁹⁾.

Therefore it is evident that reduced bile acid pool is not the result but the cause of gallstones.

The significance of reduced bile acid pool is illustrated by Heaton⁷⁾ as follows (The figures used are based on the data of Bell et al.¹⁰⁾).

A normal subject, with a 2 g bile salt pool circulating eight times daily, perfuses

his liver with a total of 16 g bile salt each day. The 16 g of bile salts returning to the liver keeps daily synthesis by feedback inhibition down to the normal 400mg, this being all that is necessary to replace fecal losses. If the daily return of bile salts is reduced, that is, if the enterohepatic circulation is interrupted, then liver synthesis increases. From monkey experiments, one would expect a 20 per cent interruption to cause maximal synthesis.¹¹⁾ A 20 per cent interruption is equivalent to a return of 12.8 g bile salts daily. Therefore, a normal subject should synthesize maximally (approximately 3.3 g/day) if his bile salt is reduced to 12.8 g/day¹²⁾.

A man with lithogenic bile has on average a bile salt pool of 1.1 g. Assuming he recycles his pool the normal eight times per day, he perfuses his liver with 8.8 g bile salts daily. In a normal person this would constitute a supra-maximal stimulus to liver synthesis. Nevertheless, the secretor of lithogenic bile synthesizes only the normal 400mg/day. His liver is insensitive or inhibited on synthesis of the bile acids. Expressed differently, the lithogenic liver is a liver in which cholesterol catabolism is pathologically suppressed.

IV. Experimental and Clinical Studies in Our Laboratory

A) Experimental formation of cholesterol gallstones in golden hamsters

i) The golden hamsters maintained on a fat free diet containing only sugar or glucose as carbohydrate (an experimental lithogenic diet of which composition is shown in Table 2) for 2 to 3 weeks formed pure cholesterol gallstones in their gallbladders (Fig. 5)^{13),14)}.

ii) Additions of fats rich in essential fatty acids (abbreviated as EFA in the followings) to the lithogenic diet suppressed the incidence of stones, and this suppression was enhanced by administration of active vitamin B₆ (PALP) (Fig. 6).

Various kinds of fats which differed in their fatty acid composition were added to the lithogenic diet, and it was found that the higher was the ratio of EFA to oleic acid plus saturated fatty acids, the lower was the incidence of stones, and that the fats with the lower ratio enhanced the stone formation (Fig. 7)¹³⁾.

iii) Although when sugar or glucose of the lithogenic diet was replaced by α or β starch the animals did not form stones, they did form stones frequently when they were fed with a diet containing α starch and some animal fats (such as butter fat) which were rich in short or medium chain

Table 2

	Lithogenic diet		Rat chow diet
Carbohydrate	Glucose	70%	wheat & corn
	or	60%	56.0%
Protein	Casein	20%	crude casein
			24.0%
Fat	none		vegetable oil
	(or butter fat	10%)	3.5%
Salts	U.S.P.XII	5%	
Vitamines		1%	6.0%
Fibres	Carboxyl-me		
	thyl-cellulose	3.5%	cellulose 4.5%
Choline		0.5%	
chloride			

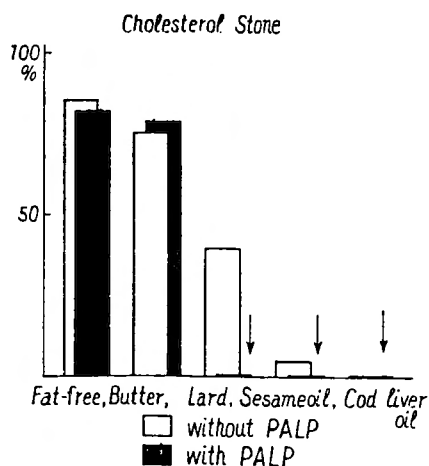


Fig. 6

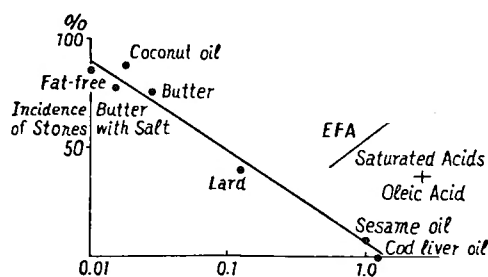


Fig. 7

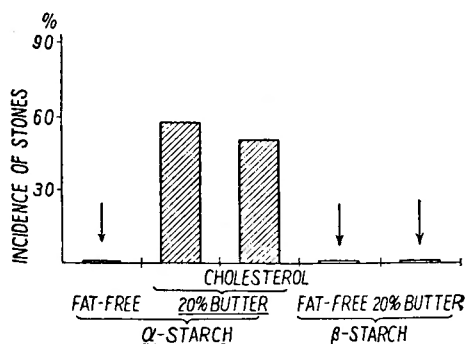


Fig. 8

and saturated fatty acids and were poor in EFA (Fig. 8)¹⁵⁾.

These results were of practical importance from the viewpoint of our dietary habits eating the starch almost in α form.

The same results were obtained when medium chain triglycerides (MCT) consisting of 8 to 10 carbon atoms were given in place of butter fat.

iv) The formation of gallstones in the animals fed with the lithogenic diet was suppressed when a large amount of carboxyl-methyl-cellulose (CMC) or agar was added to the diet, and also when straws were floored in the animal cages¹⁶⁾.

B) Studies on the mechanisms inducing the abnormal metabolism of cholesterol and bile acids.

i) The liver of the patients of cholesterol cholelithiasis showed an increased content of total and esterified cholesterol, and a decrease of total bile acids was seen in their bile. Moreover, their content of C-18 and C-20 fatty acids showed an abnormal pattern which was typical in EFA-deficiency, that is a decrease in tetraene having four double bonds (C-20:4) (Fig. 9)¹⁷⁾

EFA cannot be synthesized in animal bodies and should be taken by human being in about 6 g each day.

EFA are contained in the liver in large quantities (Fig. 10), and are supposed to play a very important role in liver functions.

In a collaboration with the late Prof. Dr. Kinsell, it was demonstrated in human being that administration of trilinolein composed of EFA in place of coconut oil composed of saturated fatty acids increased the secretion of total bile acids, especially that of cholic and chenodeoxycholic acids in bile (Fig. 11)¹⁸⁾⁻²¹⁾.

These facts show that EFA play a very important role in the catabolism of cholesterol to bile acids, and nowadays it is theoretically believed that about 1g

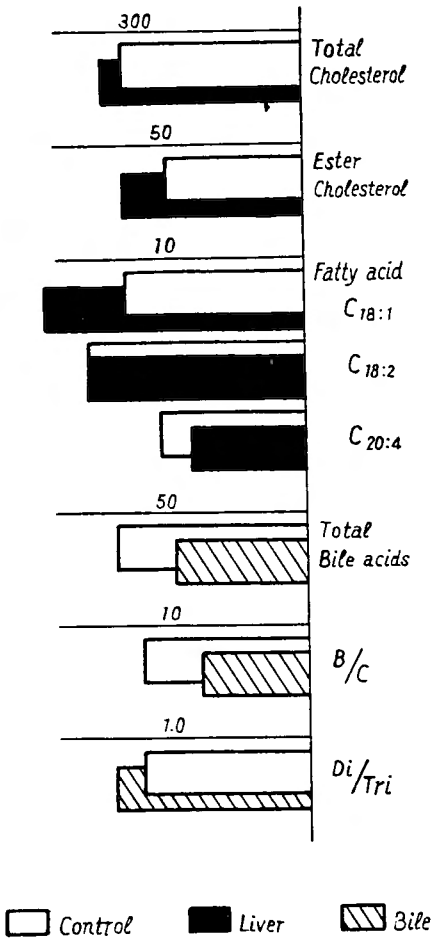


Fig. 9

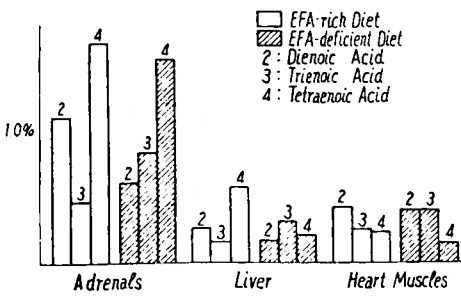


Fig. 10

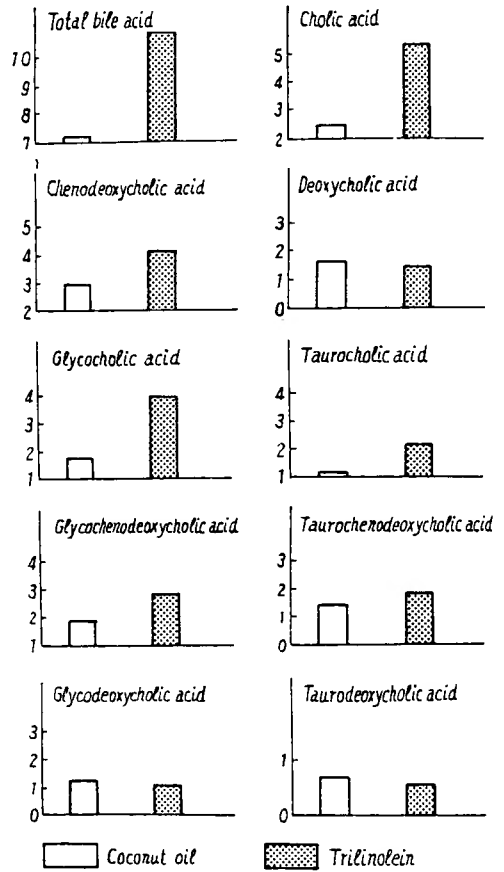


Fig. 11 Bile acid secretion in 24 hours

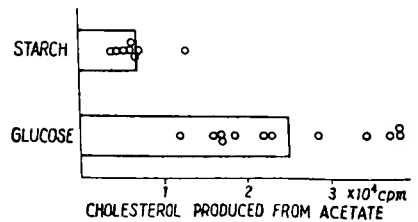


Fig. 12

linoleic acid is required for the daily catabolism of about 1 to 2 g cholesterol.

Thus, it has been elucidated that the catabolism of cholesterol is inhibited in the body which either is deficient in EFA or has an abnormal metabolism of EFA.

ii) In a collaboration with Prof. Dr. Hayaishi of the Department of Biochemistry of Kyoto University School of Medicine, biosynthesis of cholesterol from acetate in the liver was studied on the animals fed either with the experimental lithogenic diet or with the diet added with β starch.

As shown in Fig. 12 and 13, the feeding of the lithogenic diet accelerated rather promptly the biosynthesis of cholesterol in the liver^{(22), (23)}.

These facts indicate that acceleration of cholesterol synthesis in the liver is one of the necessary factors for cholesterol gallstone formation.

The blood sugar levels of the patients of cholesterol cholelithiasis are frequently higher than normal (Fig 14), and the patients are in conditions which should be called possible diabetic and oxyhyperglycemia in glucose tolerance tests (Fig. 15)^{(24), (25)}. The animals fed with the lithogenic diet showed also an abnormal metabolism of glucose in glucose tolerance tests (Fig. 16).

The abnormal acceleration of cholesterol synthesis in the liver which was found in these bodies is believed to be caused by an abnormal metabolism of glucose.

iii) The characteristics of the highly refined carbohydrates such as sugar are that they are absorbed very rapidly and induce a transient hyperglycemia, and that they contain no fibres at all.

As previously mentioned, gallstone for-

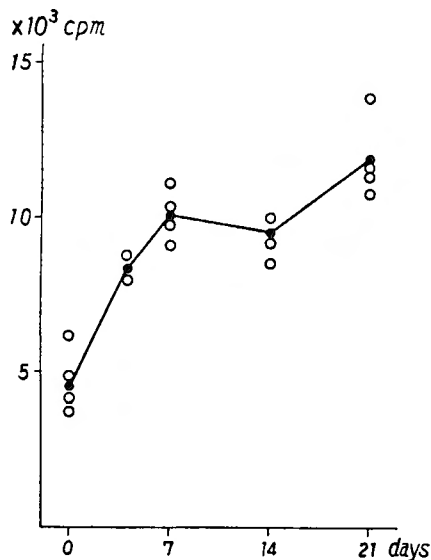


Fig. 13

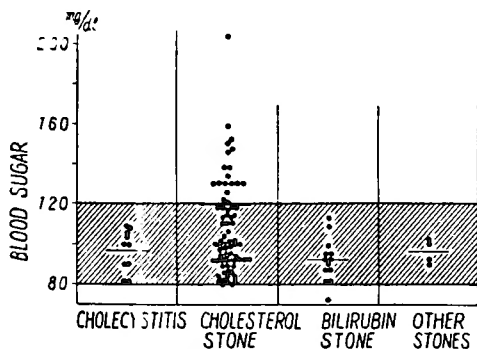


Fig. 14

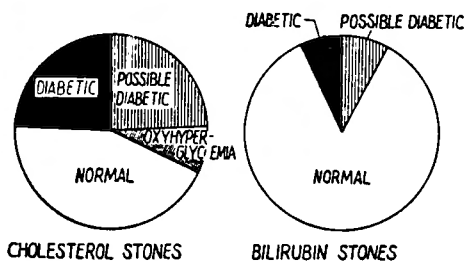


Fig. 15

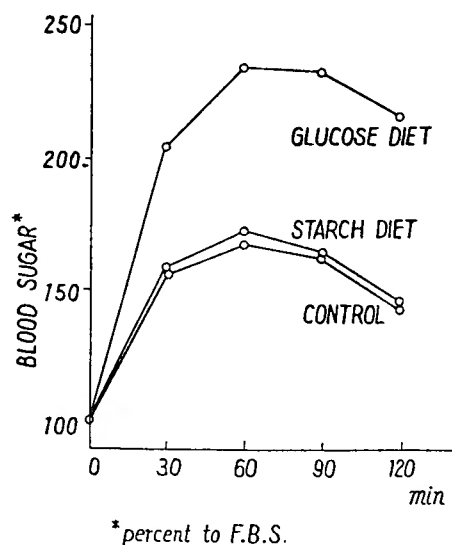


Fig. 16

mation was suppressed when fibres were added to the lithogenic diet. The vegetable fibres have hypocholesterolemic properties, increase the secretion of bile acids and therefore promote the catabolism of cholesterol.

These effects of the fibres are supposedly attributed to their following two functions.

- 1) They adsorb the bile acids.
- 2) They promote the bowel movements and inhibit the absorption of the bile acids.

Bile acids vary in their tendency to be bound by fibre in vitro, the degree of binding being greatest with lithocholic acid.²⁶⁾ This may be important in view of the ability of lithocholate to impair hepatic cholesterol metabolism⁷⁾

V. The Role of the Gallbladder in Stone Formation

Rats having no gallbladder did not form cholesterol gallstones even if they were fed with the lithogenic diet, although their bile became lithogenic.

The golden hamsters fed with the lithogenic diet did not form stones when their gallbladders had previously been removed.

Therefore it is certain that the gallbladder is the site of cholesterol gallstone formation. Expressed differently, cholesterol precipitates from the bile supersaturated with cholesterol and aggregates and grows to stones in the gallbladder.

It has still not been elucidated whether the function of the gallbladder and the sphincter of Oddi has any influences on the composition of the hepatic bile.

A clinical study of Shaffer et al. is very interesting in that the hepatic bile composition of the patients with cholesterol gallstones was restored to a normal pattern after cholecystectomy²⁷⁾.

VI. Studies on the Pathogenesis of Acute Pancreatitis

As a causative factor of acute pancreatitis, gallstones have been incriminated in modern western society. According to Schmieden, Ivy, Dragstedt and others, about 50 to 70 per cent of patients of acute pancreatitis were accompanied with gallstones (28)~(31).

In Japan their concurrences were rather infrequent findings ranging about 6 per cent in the past. In recent years, however, acute pancreatitis is seen increasingly in patients with cholesterol gallstones.

The fact that acute pancreatitis occurs much more frequently with cholesterol gallstones than with pigment stones cannot be explained by current concepts on the pathogenesis of acute pancreatitis.

Following studies have been done in our laboratory to elucidate the mechanism on syntropy of acute pancreatitis with cholesterol gallstones.

i) A mixture of taurocholate and trypsin was infused into the pancreatic duct through the common bile duct of hamsters (Fig. 17).

In the animals fed with the lithogenic diet, the infusion provoked typical hemorrhagic pancreatitis (Fig. 18) and a marked rise of amylase levels in the serum, urine and ascites (Fig. 19).

In control animals, there was only seen a slight edema in the head of the pancreas (Fig. 20).

The inclination caused by feeding of the lithogenic diet to develop acute pancreatitis could be easily cured by feeding of control diets³²⁾.

ii) Pancreatic acinar cells of the experimental animals were suspended in Hank's solution, and the resistance of the cell membrane against various toxic agents was evaluated.

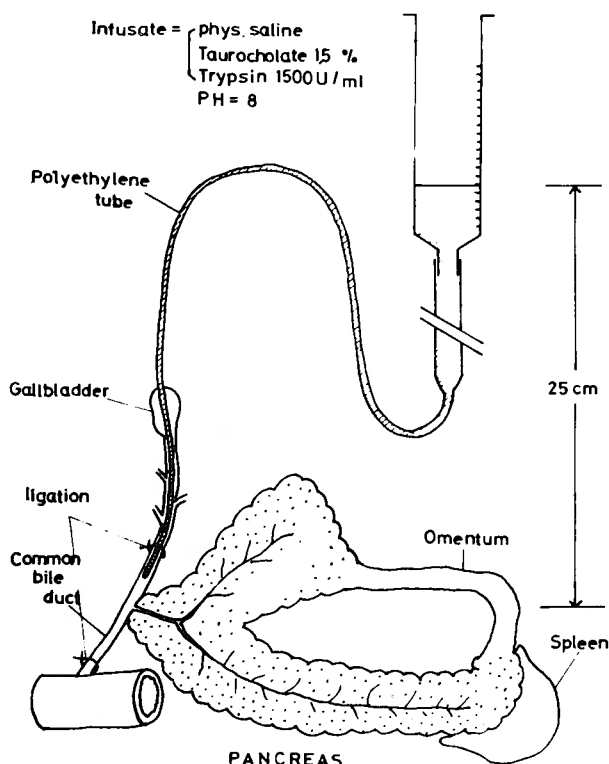


Fig. 17

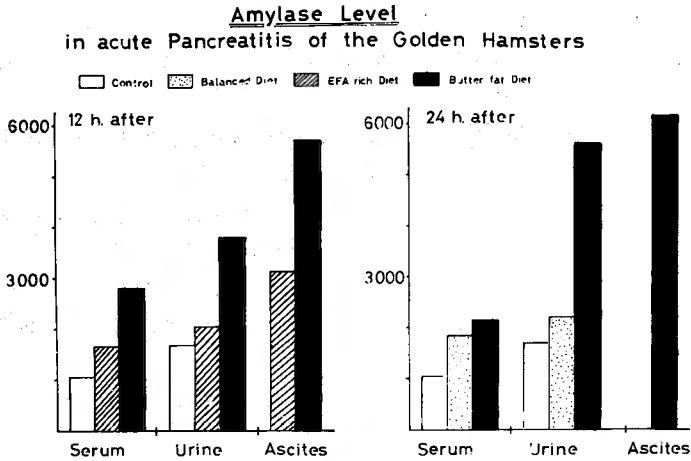


Fig. 19

The pancreatic acinar cell membrane of the animals fed with the lithogenic diet showed a markedly decreased resistance not only against a mixture of their own bile and trypsin but also against their own bile alone (Figs. 21, 22 and 23)³³⁾.

iii) Pancreatic acinar cells obtained from the patients with cholesterol gallstones showed same decreased resistance as those of the animals fed with the lithogenic diet (Fig. 24)³⁴⁾.

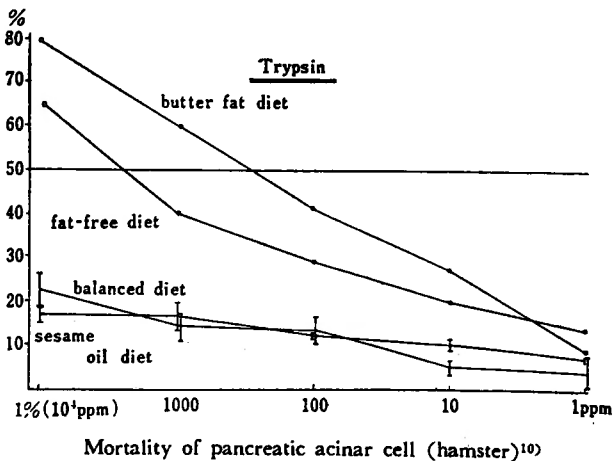


Fig. 21

These results show that acute pancreatitis can be induced by reflux of bile into the pancreatic duct both in the animals fed with the lithogenic diet and in the cholesterol gallstone patients.

As pointed out by Kune, it is well known that reflux of bile into the pancreatic duct is almost certainly not harmful in itself³⁵⁾. In the patients with cholesterol gallstones, however, refluxing bile may destroy the acinar cells having a reduced

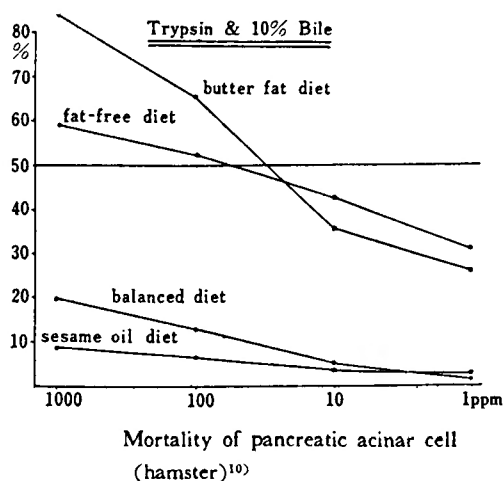


Fig. 22

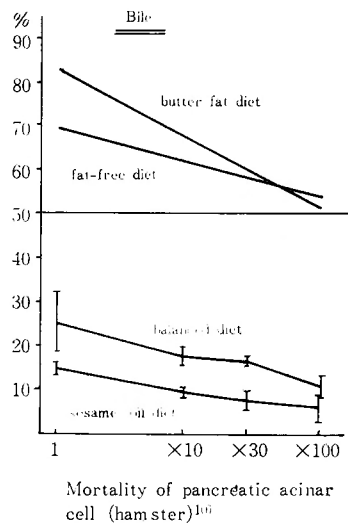


Fig. 23

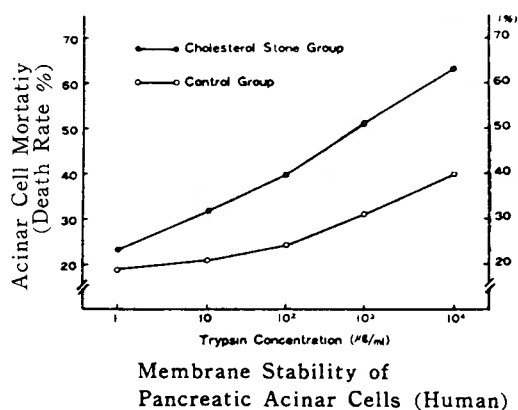


Fig. 24

membrane resistance and liberate cytokinase from the cells.

The liberated cytokinase activates trypsinogen to trypsin, and the latter converts phospholipase A, which is secreted by the acinar cells, to phospholipase A³⁶⁾.

VII. Summary

The initiating factors of cholesterol gallstones were discussed and a large intake of highly refined carbohydrate such as sugar and of animal fats such as butter fat containing a large amount of short or medium chain and saturated fatty acids, and a deficient intake of vegetable fibres were incriminated.

In addition, the mechanism on the syntropy of acute pancreatitis with cholesterol gallstones were elucidated.

References

- 1) Hikasa, Y., S. Matsuda, M. Nagase et al.: Initiating factors of gallstones, especially cholesterol stones (III). Arch. Jap. Chir., 38 : 107, 1969.
- 2) Tanimura, H. et al.: Cholelithiasis-especially on the initiating factors of cholesterol gallstones. (in Japanese) Nihon-Rinshō, 31 : 2085, 1973.

- 3) Nagase M. et al. : Westernization of diet and gallstone formation. (in Japanese) *Sôgô-Rinshô*, **23** : 394, 1974.
- 4) Small, D. M. : The formation of gallstones. *Adv. internal Med.*, **16** : 243, 1970.
- 5) Admirand, W. H. and D. M. Small : The physicochemical basis of cholesterol gallstone formation in man. *J. Clin. Invest.*, **47** : 1043, 1968.
- 6) Holzbach, R. T. et al. : Cholesterol solubility in bile. *J. Clin. Invest.* **52** : 1467, 1973.
- 7) Heaton, K. W. : Bile salts in health and disease. Churchill Livingstone, 1972.
- 8) Vlahcevic Z. R. et al. : Diminished bile acid pool size in patients with gallstones. *Gastroenterology*, **59** : 165, 1970
- 9) Vlahcevic, Z. R. et al. : Relationship of bile acid pool size to the formation of lithogenic bile in female Indians of the Southwest. *Gastroenterology*, **62** : 73, 1972.
- 10) Bell, C. C. et al. : Relationship of bile acid pool size to the formation of lithogenous bile in male Indians of the Southwest. *S. G. O.*, **134** : 473, 1972.
- 11) Dowling, R. H. et al. : Effects of controlled interruption of the enterohepatic circulation of bile salts by biliary diversion and by ileal resection on bile salt secretion, synthesis and pool size in the Rhesus monkey. *J. Clin. Invest.*, **49** : 232, 1970.
- 12) Carey, J. B. & G. Williams : Maximum primary bile salt synthesis rates in man. *Gastroenterology*, **56**, A. 121 (abstract), 1969.
- 13) Tanimura, H. : Experimental studies on the etiology of cholelithiasis. *Arch. Jap. Chir.*, **34** : 1160, 1965.
- 14) Shioda, R. : Experimental studies on gallstone formation. *Arch. Jap. Chir.*, **34** : 571, 1965.
- 15) Hashimoto, K. : Experimental studies on gallstones in hamsters. *Arch. Jap. Chir.*, **35** : 981, 1966.
- 16) Togo, M. : Cholesterol gallstone formation in hamsters correlated with histological findings in livers and gallbladders. *Arch. Jap. Chir.*, **38** : 565, 1969.
- 17) Hirano, Y. : Role of essential fatty acids in liver on the conversion of cholesterol to bile acid, especially significance of essential fatty acids concerning the formation of cholesterol stones. *Arch. Jap. Chir.*, **34** : 939, 1965.
- 18) Shioda, R., H. Weizel, P. Wood and L. Kinsell : Dietary influences on daily hepatic output in bile of lipids and steroids. *Clinical Research*, **16** : 160, 1968.
- 19) Shioda, R., P. D. S. Wood and L. W. Kinsell : Human biliary output of lipids in relation to diet. 51st Annual Meeting Federation of American Societies for Experimental Biology, 0268, 1967.
- 20) Shioda, R., P. Wood and L. Kinsell : Dietary effects on human bile composition. *Clinical Research*, **14** : 138, 1967.
- 21) Shioda, R., P. D. S. Wood and L. W. Kinsell : Determination of individual conjugated bile acids in human bile. *J. Lipid Research*, **10** : 546, 1969.
- 22) Muroya, H., R. Suzue and Y. Hikasa : Stimulation of hepatic cholesterol biosynthesis by dietary glucose, and its relation to gallstone formation in the hamster. *Arch. Biochem. Biophys.*, **124** : 12, 1968.
- 23) Muroya, H. : Stimulative effect of dietary glucose on hepatic cholesterol biosynthesis and formation of cholesterol gallstones in hamsters. *Arch. Jap. Chir.*, **37** : 76, 1968.
- 24) Tobe, T. et al. : Clinical observation of cholelithiasis. (I) Especially on preoperative and postoperative glucose metabolism. The 100th Kinki Surgical Meeting (Kyoto) in 1966.
- 25) Tobe, T. et al. : Glucose tolerance test at the pre-and post-cholecystectomy. The 10th Jap. Diabet. Ass. in 1967.
- 26) Eastwood, M. A. and D. Hamilton : Studies on the adsorption of bile salts to non-absorbed components of diets. *Biochem. Biophys. Acta*, **152** : 165, 1968.
- 27) Shaffer, E. A., J. W. Braasch and D. M. Small : The influence of cholecystectomy on bile composition in cholesterol gallstone patients. *Gastroenterology*, **62** : 809, 1972.
- 28) Schmieden, V. and Sebenig, W. : Chirurgie des Pankreas. Langenbecks Arch. Klin. Chir., **148** : 139, 1927.
- 29) Ivy, A.C. and G. E. Gibbs : Pancreatitis. A review. *Surg.*, **31** : 614, 1952.
- 30) Dragstedt, L. R., H. E. Haymond and J. C. Ellis : Pathogenesis of acute pancreatitis.

- Arch.Surg., 28 : 232, 1934.
- 31) Scholze, H. : Die Pankreatitis, Das klinische Bild, Die chirurgische Behandlung. Ferdinand Ercke Verlag, Stuttgart, 1972.
 - 32) Takenaka, M. : To be published.
 - 33) Yamazaki, H. : Experimental studies on initiating factors of pancreatitis associated with gallstones, especially cholesterol stones. Arch. Jap. Chir., 42 : 169, 1973.
 - 34) Koyama, T. : Clinical and experimental studies on the etiology of pancreatitis with cholelithiasis. Arch. Jap. Chir., 43 : 146, 1974.
 - 35) Kune, G. A. : Current Practice of Biliary Surgery. Little, Brown and Company, 1972.
 - 36) Creutzfeldt, W. and H. Schmidt : Aetiology and pathogenesis of pancreatitis (current concepts). Scand. J. Gastroent., 6 : 47, 1970.

和文抄録

コレステロール系胆石及び急性膵炎の発生機序について

京都大学医学部外科学教室第2講座（主任：日笠頼則教授）

谷村弘，塩田隆三，室家大久，竹中正文，長瀬正夫

先進欧米諸国と同様に，最近わが国でも胆石症，特にコレステロール系胆石が急増している．その主な原因は食餌の欧米化であるが，われわれは永年にわたる実験的並びに臨床的研究の結果，砂糖のように高度に精製された糖質及び低級～中級飽和脂肪酸含量の大きい

バターのような動物性脂質の大量摂取，ならびに植物性繊維の摂取不足がコレステロール系胆石形成の原因であることを明らかにした．

同時にコレステロール系胆石症に急性膵炎がしばしば合併する機序を明らかにし得た．

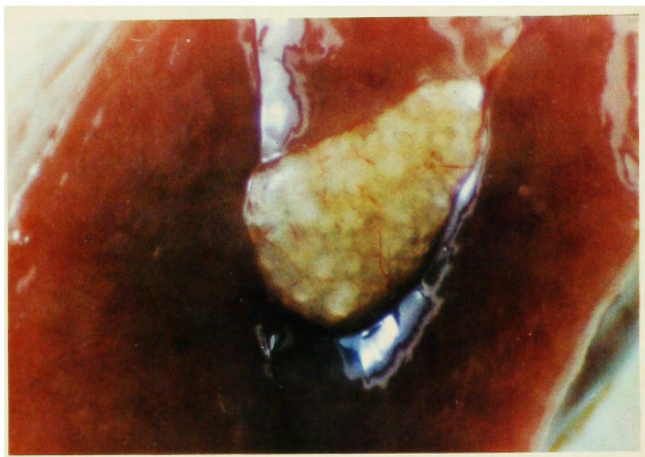


Fig. 5

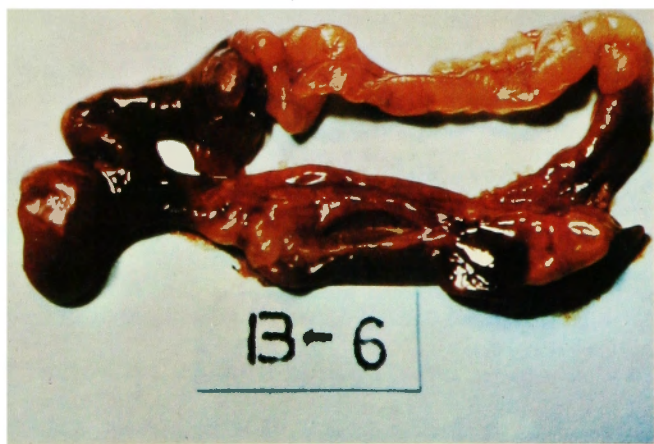


Fig. 18



Fig. 20