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Levels of Serum and Biliary Lipids and Their Composition in Patients with Pure Cholesterol and Mixed Gallstones*)

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ABSTRACT

Serum and biliary lipids were analyzed in 20 normal controls and 37 patients with pure cholesterol and mixed gallstones. Incidence of hypertriglyceridemia was significantly higher in patients with pure cholesterol stone than in those with mixed stones.

The patients with both types of gallstones showed an increase in average molar percent cholesterol and decrease in molar percent total bile acids resulting in high elevation of the lithogenic index in many instances. The pure cholesterol stone patients are accompanied with supersaturated lithogenic bile in most cases but, on the other hand, only half of the patients with mixed stones had supersaturated bile.

The pure cholesterol gallstone had significantly increased molar percent deoxycholic acid and decreased percent chenodeoxycholic acid, and the mixed stone had almost the same percent composition of bile acids as normal controls did.

The patients with pure cholesterol gallstone are accompanied by the disorder of lipid metabolism which caused hypertriglyceridemia in many cases and have cholesterol-supersaturated bille in most instances. Therefore, the disorder not only of biliary but also of serum lipid metabolism seemed to be associated with the formation of this type of gallstone.

On the contrary, for the mixed stone formation lithogenic bile may be helpful but not essential and other factor(s) including inflammatory reaction also participate in the initiation and development of this type of gallstones.

INTRODUCTION

The growth of cholesterol gallstones (or cholesterol-rich gallstones) has been believed to be initiated by cholesterol precipitating in the gallbladder due to its supersaturation caused by excess excretion of cholesterol into bile from the liver or by the relative decrease in bile acids. However, cholesterol gallstones are basically classified into two common groups, one is a pure cholesterol gallstone (solitary in most cases) and the other is mixed stones (multiple in most cases)[21].

In 1909 Aschoff[9] et al. already described in "Die Cholelithiasis" that the pure cholesterol stone was considered a product of general disorder of lipid metabolism (Allgemine Stoffwechselstörung) and they proposed "the non-inflammatory congestion theory". On the other hand, the mixed stone (cholesterol-bilirubin-calcium stone) which contains about 90% cholesterol is often produced in the gallbladder affected by inflammatory disease. The earlier Naunyn's[15] theory is fundamentally baced on the congestion of bile and inflammation of biliary tract as the origin of mixed stone formation.

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In the present study the levels and chemical composition of serum and biliary lipids were compared between patients with pure cholesterol and mixed gallstones to investigate the characteristic difference in lipid metabolism between both patients with different type of gallstone.

SUBJECTS AND METHODS

Thirty-seven patients (11 males and 26 females) with gallstones which were confirmed to be the cholesterol rich gallstones by subsequent surgical operation were admitted for preoperative examination at the time of the investigation. The average age of the patients was 44.7.

The extracted stones were divided into two groups, pure cholesterol and mixed gallstones mainly by a stereoscopic microscope and Shimadzu IR-450 infrared spectroscope.

Normal controls (mean age=48.1) were selected from healthy volunteers (medical staffs) and patients who had neither hepatobiliary and pancreactic diseases nor diseases which affect the lipid and bile acid metabolism.

Serum cholesterol and triglyderide levels were determined by the enzyme methods[9,26] respectively. HDL-cholesterol was isolated with sodium phosphotangstate and $MgCl_2$ and determined by the enzyme method mentioned above[4].

LDL-cholesterol was drawn from serum total cholesterol, HDL-cholesterol and serum triglycerides according to the formula proposed by Friedewald et al.[10] (LDL-cholesterol (mg)=total cholesterol—HDL-choresterol—1/5 triglycerides (mg) when serum triglyceride level<300 mg/dl).

B Bile was collected after a regular diet (average total calorie 2460 cal., protein 78 g, carbohydrate 347 g, fat 50 g, vitamines 2.72 g and minerals 552.4 mg) which was maintained at least for one week.

Determination of cholesterol and bile acids was achieved fundamentally according to the method reported by Kawamoto et al.[13] After adding 10% NaOH to 0.5 ml bile, hydrolysis of bile acids was carried out in an autoclave at 18 p. s. i. for three hours. Extracted bile acids in an acidified solution were methylated and propionated as described by Shino et al.[20] Vitamine E caprylate was adopted as a standard for gaschromatographic analysis. Biliary cholesterol was extracted in an alkaline solution and α-cholestane was added as a standard in the gaschromatographic procedure.

A Shimadzu GC-6A gas-liquid chromatograph was equipped with FID which was connected to a Shimadzu-chromatopac E-1-A data analyzer.

Separation was carried out by 3.4 mm×1.5 m (cholesterol) and 3.4 mm×2.0 m (bile acids) glass column filled with 60-80 mesh chromosorb W coated with 2.5% OV-1. The injection port and detector were maintained at 305°C and the column oven at 295°C.

Biliary lecithin was determined by the Takayama's enzyme method[22].

Triangular coordinates were employed for presenting three major components of bile (cholesterol, lecithin and bile acids) by the method decribed by Admirand et al.[1] Each component was expressed as percentage molar ratio of cholesterol, lecithin and total bile acids. Saturation lines were drawn according to the reports described by Admirand et al.[1] (A-B-C) and Holzbach[12] (D-E).

The lithogenic index in each case was calculated from the equation developed by Thomas and Hofmann[23] for the cholesterol saturation limits described by Admirand-Small[1].

RESULTS

1) *Serum lipid concentration in patients with gallstones:*

As shown in Figures 1—3 and Table 1, average total, LDL- and HDL-cholesterol were within the normal range (150—250 mg/dl for serum total cholesterol, 80—170 mg/dl for LDL-cholesterol and 40—70 mg/dl for HDL-cholesterol) and no significant difference existed in these three lepeds between the pure cholesterol and mixed stones.

Figure 4 shows the incidence of abnormally high serum total cholesterol and LDL-cholesterol and abnormally low HDL-cholesterol in the two types of gallstone. Incidence of abnormally high total and LDL-cholesterol tended to be higher in patients with pure cholesterol stone than in those with mixed stones. Lowered HDL-cholesterol was observed in none of the patients with pure cholesterol stone and in a very few (3 out of 21) patients with mixed stones.

The average serum triglyceride level was also almost within the normal range (50—130 mg/dl) and there was no significant difference in this

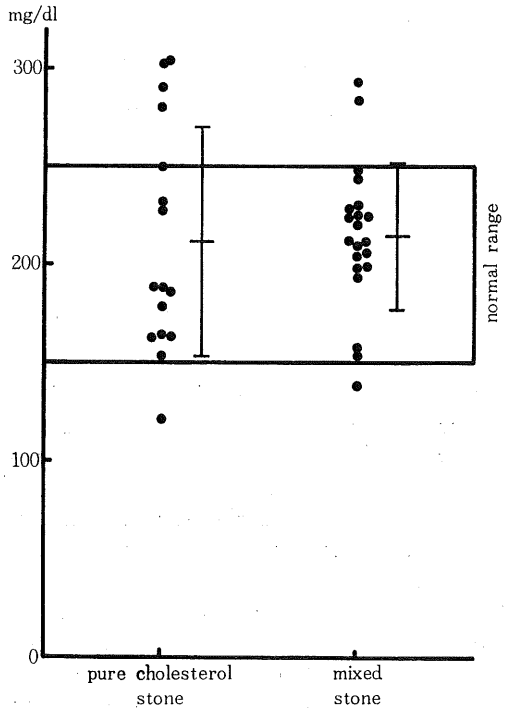

Fig. 1. Serum total cholesterol concentration in patients with pure cholesterol and mixed gallstones

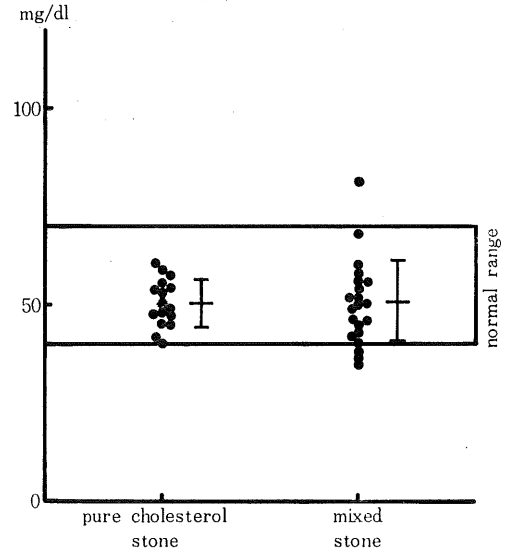

Fig. 3. Serum HDL-cholesterol concentration in patients with pure cholesterol and mixed gallstones

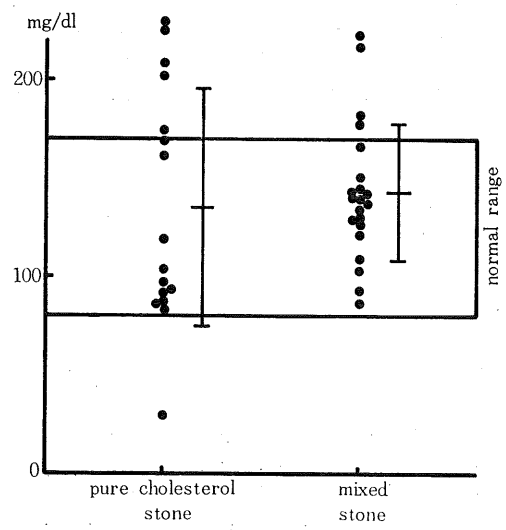

Fig. 2. Serum LDL-cholesterol concentration in patients with pure cholesterol and mixed gallstones

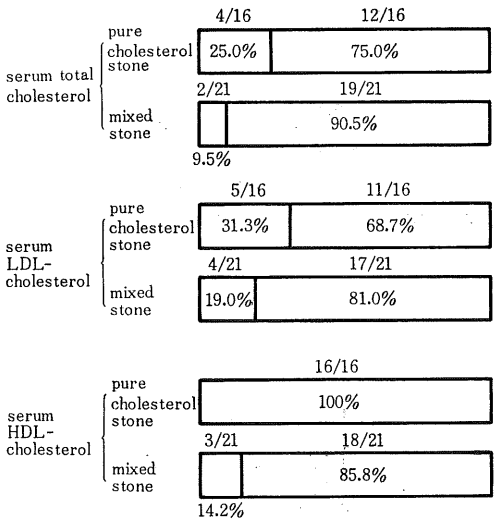

Fig. 4. Incidence of hypercholesterolemia and hyper-LDL-nemia and hypo-HDL-nemia in patients with pure cholesterol and mixed gallstones

Table 1. Average serum total, LDL- and HDL-cholesterol and serum triglycerides concentrations in patients with pure cholesterol and mixed gallstones

(mg/dl)

	No.	Total cholesterol	LDL-cholesterol	HDL-cholesterol	Triglycerides
Normal range		150−250	80−170	40−70	50−130
Pure cholesterol stone	16	211.5±58.2	135.1±60.6	50.3± 6.0	130.5±53.4
Mixed stone	21	214.0±37.2	143.0±35.0	50.8±10.5	113.6±54.3

(Mean±SD)

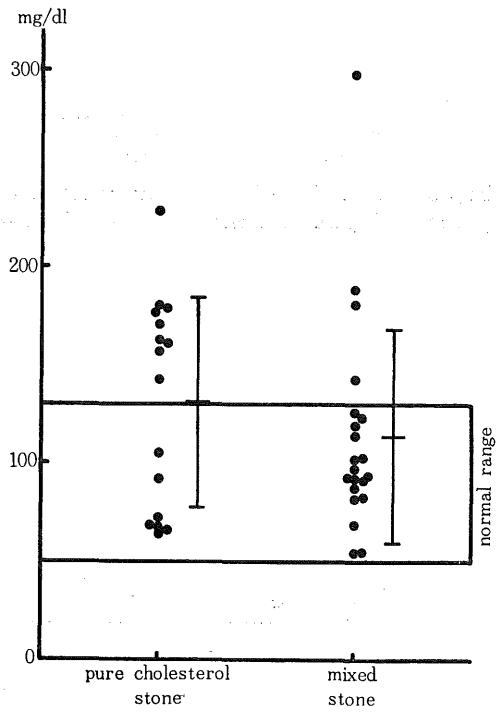

Fig. 5. Serum triglycerides concentration in patients with pure cholesterol and mixed gallstones

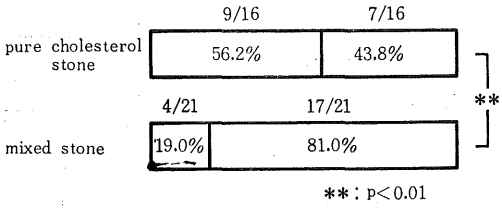

Fig. 6. Incidence of hypertriglyceridemia in patients with pure cholesterol and mixed gallstones

lipid between the two types of gallstones. However, the incidence of hypertriglyceridemia was significantly higher in patients with pure cholesterol stone than those with mixed stones.

The degree of hypertriglyceridemia was slight to moderate in patients with both the types of gallstones.

2) *Bile lipid composition of bile in patients with pure cholesterol and mixed gallstones:*

Average percent biliary cholesterol significantly rose but, on the contrary, percent total bile acids decreased in patients with both pure cholesterol and mixed gallstones by the same degree. Percent lecithin, however, showed no statistical change among the normal controls and two types of gallstone patients as shown in the middle columns in Figure 7.

Figure 8 shows the three major components of bile on triangular coordinates in normal controls, patients with pure cholesterol and mixed gallstones. While the normal controls were positioned under the saturation lines (A-

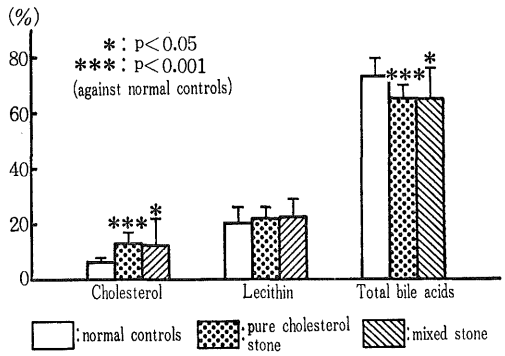

Fig. 7. Three components of bile (cholesterol, lecithin and total bile acids) in normal controls and patients with pure cholesterol and mixed gallstones

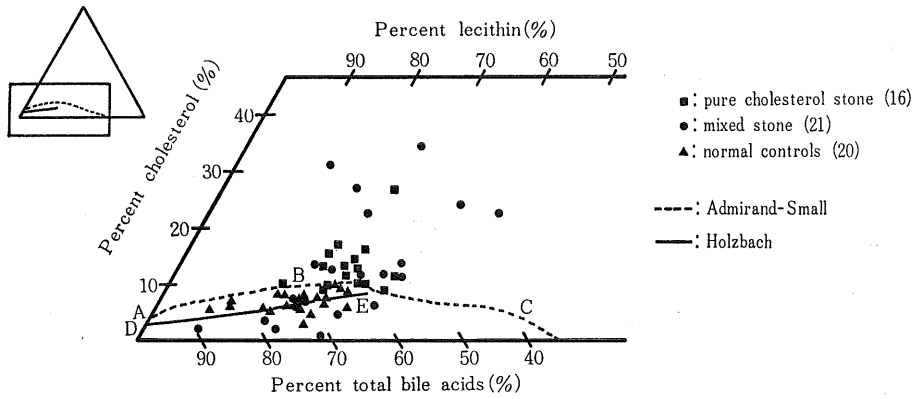

Fig. 8. Three major components of bile (cholesterol, lecithin and total bile acids) on triangular coordinates in patients with pure cholesterol and mixed stones. (The figure represents left lower part of the triangular coordinates)

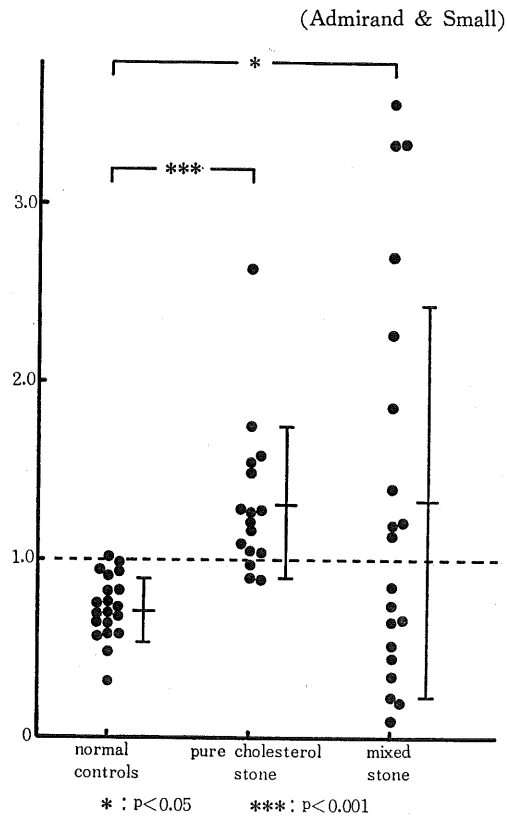

Fig. 9. Lithogenic index of bile in normal controls and patients with pure cholesterol and mixed gallstones

B–C and D–E), patients with gallstones were frequently positioned much higher above the saturation lines.

Among the patients, the mixed stones had more widely spread points as compared with the pure cholesterol stone on the triangular coordinates. Some of the points of mixed stones held very high positions (generally the right upper area of coordinates) and others held the area near to or even lower than those of the normal controls on the triangular coordinates.

The lithogenic indices of bile of the normal controls were below 1.0 almost entirely and those of pure cholesterol stone were above 1.0 in almost all the cases as indicated in Figure 9. Therefore, this created a significant difference in the average indices between the normal controls and pure cholesterol stone. The mixed stones had also higher average index than the normal controls. However, the indices were widely spread individually and about half of them remained below 1.0 (saturation points), that is, unsaturated bile.

3) *Bile acid composition of bile in normal controls and patients with pure cholesterol and mixed gallstones:*

The statistically significant increase in molar percent deoxycholic acid and decrease in molar percent chenodeoxycholic acid were observed in patients with pure cholesterol stone. On the other hand, no change in molar percent bile acids occurred in patients with mixed stones.

An obvious change in percent lithocholic acid

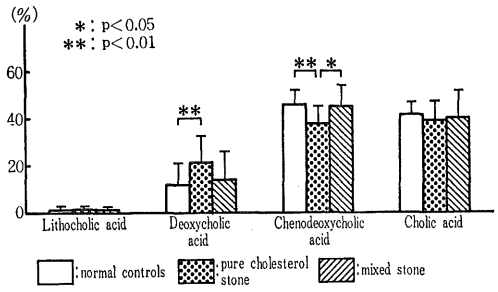

Fig. 10. Bile acid composition of bile in normal controls and patients with pure cholesterol and mixed gallstones

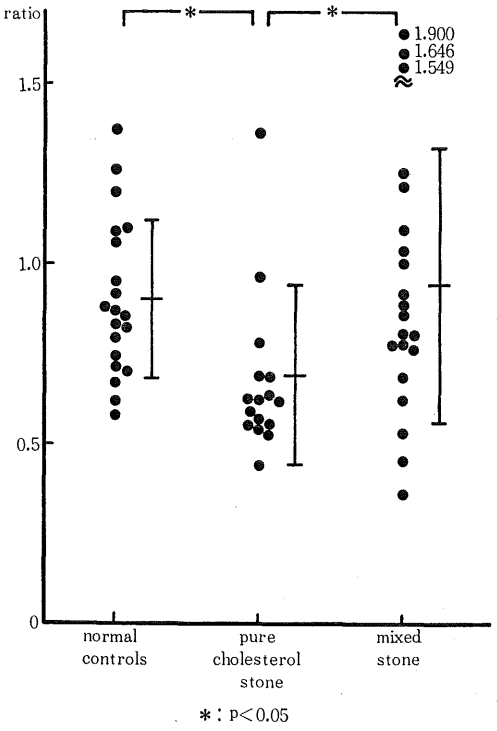

Fig. 11. Chenodeoxycholic and lithocholic acids: cholic and deoxycholic acids ratio in normal controls and patients with pure cholesterol and mixed stone

was not seen among the normal controls and patients with pure cholesterol and mixed stones. Therefore, chenodeoxycholic acid and its secondary bile acid (lithocholic acid): cholic acid and its secondary bile acid (deoxycholic acid) ratio was significantly lower in patients with pure cholesterol stone than normal controls and patients with mixed stones.

DISCUSSION

Because most of the gallstones contain cholesterol, particularly among the Europeans, and because cholesterol was first discovered in gallstone, a search for a link between gallstone formation and disturbance of lipid metabolism seemed a very natural object of research for many investigators.

In the early literatures of the twenties and thirties there are a number of studies on the serum cholesterol levels in patients with gallstones. However, there are three answers already given to the question of the level; too high, normal and too low. Hypercholesterolemia was observed by Wilensky[26] and normal values were found by Campbell[6] and Epstein[8]. On the other hand, finally Robinson et al.[16] reported that these patients had often low values.

Also, according to the recent report by Savage et al.[18] the serum cholecterol level is significantly lower in American Pima Indians (70% of the girls having gallstone by the age of 27[17]) as compared with Caucasian populations and Van der Linden reported that serum cholesterol is not increased at all and may even be reduced in Swedish female patients[24]. Thus the general trend of the recent reports shows that gallstone patients have rather low serum cholesterol levels.

In the present examination average total and LDL-cholesterol concentrations were within the normal range in patients with both pure cholesterol and mixed stones. However, the incidence of hypercholesterolemia and LDL-nemia was higher in patients with pure cholesterol stone than those with mixed atones, although there was no statistical significance betweem them.

The increased serum total and LDL-cholesterol (hypercholesterolemia) levels seem generally to be caured by the disturbed lipid metabolism in the liver which also regulates cholesterol secretion into bile, and the disturbance of lipid metabolism in the liver seems to cause excess secretion of cholesterol into bile. However, the present results indicate the absence of such strong relation between the serum cholesterol levels and gallstone formation as expected, although the incidence of hypercholesterolemia

tended to be higher in patients with pure cholesterol stone (which has been thought to be deeply related to general disorder of lipid metabolism) than those with mixed stones (which has been thought to be related to other factor(s)-e. g., inflammation-than lipid metabolism).

It has been suggested by several investigators[11,14] that HDL transports peripheral cholesterol to the liver. According to the report of Schwarz et al.[19] cholesterol in bile is more derived from HDL than from the newly synthesized cholesterol pool. However, average HDL-cholesterol was also within the normal range in both patients with pure cholesterol and mixed stones.

Hypertriglyceridemia also frequently occurs as a result of disturbed lipid metabolism which is highly related to obesity.

It is obvious that gallstone patients tend to be obese and suffer from other manifestations of overnutrition. Einarsson et al.[7] reported that type IV hyperliproteinemia (hypertriglyceridemia) had a statistically significant high incidence of gallstone as compared with other hyperlipoproteinemia. It was also found that type IV had an increased HMG CoA reducatse activity which regulates cholesterol synthesis in men[2]. (limiting enzyme of cholesterol synthesis).

The present examination elucidates that many gallstone patients had slight to moderate hypertriglyceridemia, although their average levels were almost within the normal range. And, moreover, the patients with pure cholesterol stone had a significantly higher incidence of hypertriglyceridemia than those with mixed stones.

These results may indicate that the general disorder of lipid metabolism in patients with pure cholesterol stone caused not only gallstone formation but also an increase in the serum triglyceride level and that smaller influence of general disorder of lipid metabolism in combination with larger other factor(s) induced a less incidence of hypertriglyceridemia as compared with pure cholesterol gallstone and formation of different type of gallstones in patients with mixed stones.

The patients with gallstones, either pure cholesterol or mixed stones, had relatively increased biliary cholesterol and decreased total bile acids with statistical significance. Therefore, three major components of bile (cholesterol, lecithin and total bile acids) of these patients showed supersaturated bile on the triangular coordinates and their lithogenic indices rose above 1.0 in many cases. However, when these two types of gallstones were compared with each other, the mixed stones showed wider speading of indices than pure cholesterol stone. In addition, the majority of pure cholesterol stone had supersaturated bile and on the other hand half of the mixed stones revealed supersaturated bile and another half remained with unsaturated bile.

Thus, it seems likely that cholesterol supersaturated bile is almost always necessary for the pure cholesterol formation and that it is helpful but not essential for the mixed stone formation.

In addition, some difference in the colloidal and chemical environment of the gallbladder content undoubtedly exists between these two types of stones to account for their different structures. In contrast with pure cholesterol stone, as mentioned above, the mixed stones are thought to be of infectious origin since it is more commonly found in association with chronic inflammatory changes in the gallbladder than is pure cholesterol stone. In many cases, however, the inflammatory reaction may be the result rather than the cause of cholelithiasis, as these smaller calculi undoubtedly produce temporary obstruction to the cystic duct more often than does the larger pure cholesterol stone. The fact that most of the stones occurring after pregnancy are the mixed stone without indication that infection is in a way associated with their formation suggests that there may be factor(s) other than inflammatory reaction for the formation of this stone.

Bile acid analysis of these patients revealed a significant increase in percent deoxycholic acid and decrease in percent chenodeoxycholic acid resulting in a decreased ratio of chenodeoxycholic acid and its secondary bile acid (lithocholic acid): cholic acid and its secondary bile acid (deoxycholic acid) in patients with pure cholesterol stone. This change in the bile acid composition may also be caused by the general disorder of lipid metabolism.

In an earlier study, Cahlin et al.[5] reported that the concentration of chenodeoxycholic acid was very low in pre-β hyperlipoproteinemia gallstone patients (hypertriglyceridemia) and

they speculated that the comparatively high prevalence of gallstone disease in patients with pre-β hyperlipoproteinemia is dependent on a lipid metabolic defect in the liver.

Van der Linden and Bargman[25] also found that there was a significant correlation between chenodeoxycholic acid and the ratio between the two other main bile acids (cholic/deoxycholic acid), that high lithogenesity was associated with low chenodeoxycholic acid and high deoxycholic acid values and finally that serum triglycerides were negatively correlated with the bile acid molar fraction and with the absolute concentration of bile acid.

The results of the present study are almost in accord with the above reports only in patients with pure cholesterol stone but not in those with mixed stones, although approximately 90% of the composition of mixed stone is cholesterol.

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