Changes in biliary lipid concentrations in bile duct obstruction: an experimental study

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Dr Xu was a British Council Scholar during the period of this study

Summary

Changes in biliary concentrations of bile acids, phospholipids and cholesterol and biliary pressures were measured in dogs. These parameters were studied during 7-day periods of partial biliary obstruction, of varying degrees, and after 24-hour and 48-hour periods of complete obstruction. The samples were obtained via an exteriorized but intact enterohepatic circulation allowing the introduction of varying degrees of obstruction and bile sampling.

Biliary obstruction reduced the concentration of all biliary lipids especially when the obstruction produced pressures in excess of 75% of the maximum biliary secretion pressure. Only immediately after the release of a 48-hour period of complete obstruction did the risk of cholesterol supersaturation of bile occur. However, at that time there was a greatly reduced concentration of lipids in the bile and the amount of cholesterol that could potentially have precipitated was very small. It is suggested that this supersaturation would not play a significant role in the formation of gallstones.

Introduction

A correlation between the formation of gallstones and stenosis in the common bile duct has been recognized clinically[1-3]. It has also been shown that supersaturation of bile with cholesterol is a principal factor of the formation of cholesterol gallstones, the supersaturation being determined by the relative proportions of the three major biliary lipids, i.e. bile acids, phospholipids and cholesterol[4]. In animal studies it has been reported that partial, but not complete, biliary obstruction in dogs produced gallstones in the bile duct[2,5], and that biliary obstruction altered the composition of biliary lipids and might favour the precipitation of cholesterol in bile[6,7]. Redinger *et al.*[7] postulated from their preliminary results in monkeys that intermittent biliary tract obstruction may play a predisposing role in gallstone formation by decreasing cholesterol solubility in bile. The formation of gallstones in the bile duct is always associated with chronic biliary obstruction. The effects of such obstruction are thus of particular interest.

The present study was therefore designed to investigate the alterations in composition of biliary lipids caused by various kinds of biliary obstruction during a chronic course and whether these alterations might play a role in the formation of cholesterol gallstones. Thus it was necessary to develop an animal model that enabled repeated sampling and pressure and flow measurements. Various methods for the production of biliary obstruction have been reported[2,8-12], but were difficult to control or were unsuitable for long-term studies.

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Materials and methods

Animal model

The animal model used incorporated an exteriorized biliary tubing system. Seven dogs, 2 female and 5 male, weighing between 12 and 30 kg, were used. The abdominal wall was opened under general anaesthesia via a right subcostal incision and a cholecystectomy performed.

The common bile duct was mobilized about 1–1.5 cm superior to the duodenum and cannulated via a transverse incision. The proximal end of the duct was dilated to accommodate a silicone arteriovenous shunt (SAF-T shunt ST-714 Extracorporeal Medical Specialities Inc) of internal diameter 2.5 mm and external diameter 5.0 mm, which was fixed in place by two 2/0 (3 metric) silk sutures placed in the grooves on either side of the flange near the tip of the catheter. The distal end of the bile duct was similarly cannulated but with a slightly different cannula (ST-704). After cannulation the posterior wall of the duct was transected to prevent recanalization. The abdominal muscle layer was divided between the transversalis and oblique abdominal muscles via the original abdominal incision to the right flank, and two tracheostomy tubes (Blue line, FG 24, Portex Ltd) were buried within the muscular layer. One end of the tracheostomy tube protruded through the skin and the other through the transversalis muscle into the peritoneal cavity. The tubes were fixed with nylon sutures. The cannulae were brought out through these tubes and interconnected with a three-way tap (Figure 1). The abdominal wall was closed with continuous nylon and the skin with interrupted silk sutures. Postoperatively the dogs wore an 'Elizabethan' collar and a dressing covering from neck to thigh. Bile sampling and biliary pressure measurements were carried out via the three-way tap. A complete biliary obstruction was produced by shutting the proximal channel of the three-way tap. To introduce a partial biliary obstruction, a stenosis made from a hypodermic needle was fixed within the exteriorized biliary tubing and three-way taps were placed on either side (Figure 2). The distal three-way tap was used for bile sampling and the proximal one for biliary pressure measurements.

Throughout the studies the dogs were fed 750 g of tinned food and 300 g biscuits each morning after all measurements had been taken for the day. The dogs were trained to lie in left lateral recumbency during the period of measurement.

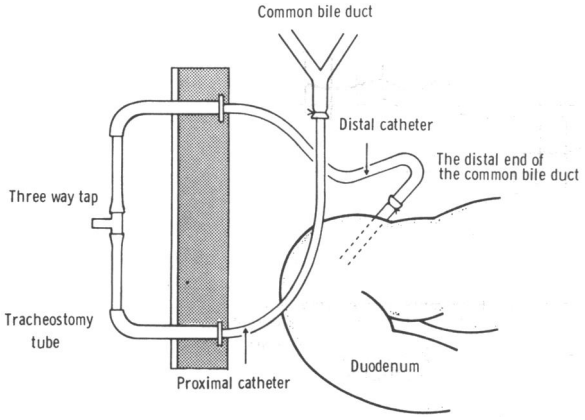

Figure 1. Diagram of biliary tubing system during control phase

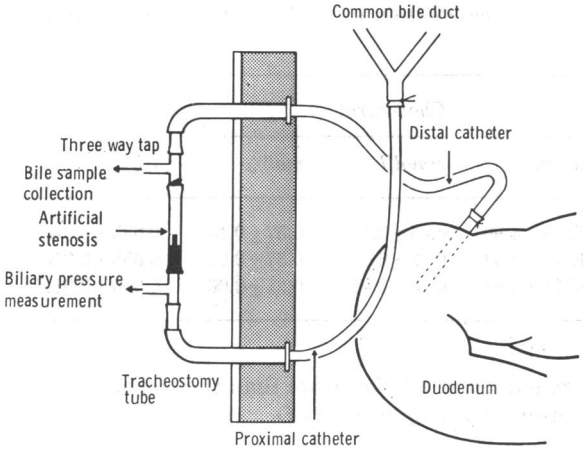

Figure 2. Diagram of biliary tubing system with partial stenosis

Measurement techniques

Bile flow rate and bile collection: The distal end of the three-way tap was shut off after a 10 cm manometer tube was attached to the free end of the tap. The free end of the manometer tube was adjusted so that it was 2 cm above the animal's vertebral column. The vertebral column is taken as being at the same level as the biliary tract when the animal is in left lateral recumbency. The additional 2 cm was designed to prevent a siphon effect and to imitate the resistance of the distal part of the duct. The time taken for the collection of a 3 ml bile sample was recorded.

Biliary pressure: The three-way tap was connected via a saline-filled manometer tube to a pressure transducer (Kulite) attached to the recording system (M19 Ormed Engineering). The transducer was kept at the level of the vertebral column. The tap was adjusted so that three channels were in communication. After the animal had become relaxed and the biliary pressure had stabilized, readings were taken for at least 15 minutes.

The maximum biliary secretion pressure (MBSP), defined as the pressure at which hepatic bile secretion ceases, was measured similarly except that the distal end of the three-way tap was completely blocked. The biliary pressure then increased gradually until, within about 20 minutes, it reached a maximum. When this level had remained constant for 10 minutes the level was recorded and regarded as the MBSP. The MBSP was measured in the control phase of the experiment.

Chemical methods: Bile acids and cholesterol in bile were estimated by gas chromatography using the method described by Evrard and Janssen[13] as modified by Sian[14]. A 1.5 m QF-1 (3%) column was used at 220°C for cholesterol and a similar column of 0.9 m at 230°C for bile acids. The phospholipid concentrations in bile were estimated by the method described by Morrison[15] and Anderson and David[16].

The molar ratio of bile acids to total biliary lipids (mol%BA) was calculated as:

$$\text{mol\%BA} = \frac{\text{Bile acids (mmol/l)}}{\text{Total biliary lipids (mmol/l)}} \times 100$$

where the total biliary lipids is the sum of the concentrations of bile acids, phospholipids and cholesterol. The molar ratios of phospholipids (mol%PL) and cholesterol (mol%Ch) were similarly calculated.

The cholesterol lithogenic index (CLI) was calculated from the tables recommended by Carey[17]: it is defined as the ratio of the mol%Ch present in the sample to the maximum amount that would be soluble at the phospholipid/bile salt ratio of the sample[18].

Stages of experiment

This study consisted of three stages: control, partial biliary obstruction (PBO), and intermittent complete biliary obstruction (ICBO).

The control stage, which lasted 7 days, began at least one month postoperatively to allow the animal to recover. There was no stenosis within the tubing system during that time. Bile sampling and biliary pressure measurements were carried out every morning. This time schedule was repeated in the partial biliary obstruction stage.

The PBO stage consisted of periods of moderate and severe obstruction. Moderate PBO was produced by introducing a 21 gauge needle (0.6 mm internal diameter) into the tubing system and severe PBO by a 23 gauge needle (0.4 mm internal diameter). Each obstruction lasted 7 days followed by 2 days without obstruction.

The ICBO stage included 24-hour and 48-hour periods of obstruction. During 24-hour ICBO, the proximal channel in the three-way tap was blocked for 24 hours then released for 24 hours. This was repeated twice more. During 48-hour ICBO, the tubing was blocked for 48 hours and then released for 24 hours. This was also repeated twice more. The bile samples were collected in the morning 24 hours after release of the obstruction, before the animal was fed. In addition, bile samples were also collected immediately, 30 minutes and 6 hours after release of the first episode of 48 hours ICBO.

Results

Results are expressed throughout as the mean ± standard deviation, and probabilities for comparisons with controls were calculated by paired *t* tests.

Biliary pressure and flow rate

The biliary pressure during the control stage was 4.8 ± 1.5 mmHg. The maximum biliary secretory pressure (MBSP) was 20.57 ± 1.27 mmHg. The biliary pressure increased to 11.86 ± 2.6 mmHg (approximately 50–65% MBSP) during moderate PBO and 17.0 ± 1.2 mmHg (77–88% MBSP) during severe PBO. The biliary pressures 24 hours after introduction of a complete obstruction were 20.1 ± 2.0 mmHg, and after 48 hours of obstruction were 19.0 ± 1.1 mmHg. Figure

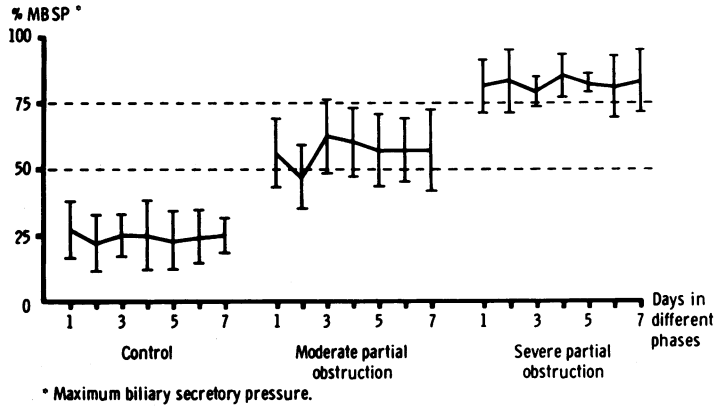

Figure 3. Mean biliary pressures in seven dogs during control and partial obstruction phases as a percentage of the maximum biliary secretory pressure (MBSP)

Table 1. Biliary lipids during moderate and severe partial biliary obstruction (PBO) (mean ± s.d.). (CLI = cholesterol lithogenic index)

	Bile acids		*Phospholipids*		*Cholesterol*		
	mmol/l	*mol%*	*mmol/l*	*mol%*	*mmol/l*	*mol%*	*CLI*
Control	54.65 ± 12.17	67.00 ± 4.98	25.29 ± 4.48	32.19 ± 4.98	0.46 ± 0.15	0.57 ± 0.14	0.085 ± 0.023
Moderate PBO	49.12 ± 9.11	69.50 ± 3.50	20.20 ± 3.19	30.12 ± 3.53	0.26 ± 0.07	0.37 ± 0.07	0.058 ± 0.010
Severe PBO	18.49 ± 3.00	56.19 ± 5.67	13.03 ± 1.68	43.51 ± 5.63	0.10 ± 0.01	0.31 ± 0.06	0.066 ± 0.012

3 shows the biliary pressures obtained in each phase as a percentage of MBSP. During the control phase the bile flow rate was 0.32 ± 0.05 ml/min.

Bile composition: PBO (Table 1)
The bile acid concentration decreased after introducing a moderate PBO ($P<0.02$) and dropped sharply with a severe PBO ($P<0.001$). The mol%BA did not significantly change during moderate PBO ($P>0.5$) but became lower than the control ($P<0.01$) during severe PBO. Both concentration and mol%BA were within the control range 24 hours after release of either moderate or severe PBO.

The phospholipid concentration decreased during moderate PBO ($P<0.01$) and further dropped in severe PBO ($P<0.001$). The mol%PL did not significantly change during moderate PBO but it increased ($P<0.01$) after introducing a severe PBO. Both concentration and mol%PL returned to the control range within 24–48 hours after release of either moderate or severe PBO.

The concentration of cholesterol decreased during moderate PBO ($P<0.01$) and fell further during severe PBO ($P<0.001$). The mol%Ch decreased after moderate PBO ($P<0.01$). Although mol%Ch during severe PBO was lower than the control ($P<0.01$), there was no significant difference between the mol%Ch in severe and moderate PBO. Both concentrations and mol%Ch returned to the control range within 24–48 hours after release of moderate PBO but were still lower than the control level in 48 hours after release of severe PBO. The cholesterol lithogenic index (CLI) decreased during moderate PBO ($P<0.02$). Although CLI was lower in severe PBO (0.066 ± 0.012) than that in the control (0.085 ± 0.023), there was no significant difference between these two groups ($P>0.05$). CLI returned to the control range 24–48 hours after release of moderate PBO, whereas it remained at a lower level than the control 48 hours after release of severe PBO.

Bile composition: ICBO
Immediately after release (Table 2): The bile composition immediately after release of complete biliary obstruction was only measured after the first episode of 48-hour obstruction.

The bile acid concentration was very low (3.32 ± 2.37 mmol/l), only about 5% of the control value (54.65 ± 12.17). There was no significant difference in bile acid concentration between the immediate sample and the sample collected 30 minutes later, but it increased after 6 hours compared with that in the immediate samples ($P<0.001$). The mol%BA in the bile collected immediately and 30 minutes after release was lower than that in the control ($P<0.01$). The mol%BA almost recovered to the control level in 6 hours after release.

The concentration of phospholipids in the immediate bile samples was 5.39 ± 0.86 mmol/l, only about 20% of the control (25.29 ± 4.48), and little changed in 30 minutes. Although the concentration of phospholipid was significantly increased after 6 hours ($P<0.01$), it only reached approximately 50% of the control level. The mol%PL was higher than the control in the bile collected either immediately or in 30 minutes ($P<0.01$). The mol%PL had returned to the control range in 6 hours.

The cholesterol concentration in the bile collected immediately after release was 0.06 ± 0.02 mmol/l, only about 13% of the control value (0.46 ± 0.15) and changed little in the following 6 hours. The mol%Ch in the immediate and 30 minutes bile was not significantly higher than the control and became lower than that in the control in 6 hours ($P<0.01$). However, those bile samples collected immediately and 30 minutes after release were supersaturated with

Table 2. Biliary lipids after release of first 48-hour period of complete obstruction (mean ± s.d.)

	Bile acids		*Phospholipids*		*Cholesterol*	
	mmol/l	*mol%*	*mmol/l*	*mol%*	*mmol/l*	*mol%*
Control	54.65 ± 12.17	67.00 ± 4.98	25.29 ± 4.48	32.19 ± 4.98	0.46 ± 0.15	0.57 ± 0.14
0 ●	3.32 ± 2.37	34.32 ± 12.78	5.39 ± 0.86	64.98 ± 12.71	0.06 ± 0.02	0.69 ± 0.12
30 min	2.23 ± 2.8	24.75 ± 17.59	4.74 ± 1.53	74.56 ± 17.57	0.04 ± 0.03	0.68 ± 0.52
6 hr	16.13 ± 2.67	60.86 ± 8.25	10.36 ± 2.67	38.81 ± 8.31	0.07 ± 0.02	0.27 ± 0.07

● Time from release

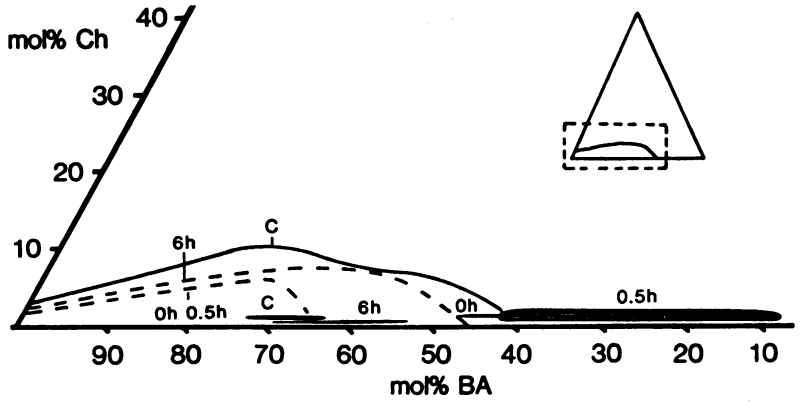

Figure 4. The composition of biliary lipids in the early period following release of 48-hour complete obstruction during chronic biliary obstruction. (Admirand and Small's[4] triangle). The dashed lines represent maximum solubility of cholesterol as determined by total concentration of biliary lipids in the bile collected immediately (Oh), 30 minutes (0.5 h), and 6 hours (6 h) after release of obstruction. (C = control, Ch = cholesterol, BA = bile acids)

Table 3. Biliary lipids during intermittent complete biliary obstruction (ICBO) phases (mean ± s.d.). Results from bile obtained 24 hours after release of obstruction. (CLI = cholesterol lithogenic index)

	Bile acids		*Phospholipids*		*Cholesterol*		
	mmol/l	*mol%*	*mmol/l*	*mol%*	*mmol/l*	*mol%*	*CLI*
Control	54.65 ± 12.17	67.00 ± 4.98	25.29 ± 4.48	32.19 ± 4.98	0.46 ± 0.15	0.57 ± 0.14	0.085 ± 0.023
24-hr ICBO	43.64 ± 6.64	70.45 ± 1.86	17.57 ± 2.94	31.98 ± 8.10	0.18 ± 0.03	0.29 ± 0.05	0.046 ± 0.011
48-hr ICBO	38.67 ± 6.36	68.41 ± 5.03	16.88 ± 1.79	32.41 ± 7.83	0.15 ± 0.03	0.28 ± 0.05	0.044 ± 0.008

cholesterol (Figure 4), their composition falling outside the micellar zone described by Admirand and Small[4] as subsequently modified[19,20]. The CLI in bile had almost returned to the control range in 6 hours.

24 hours after release (Table 3): The bile acid concentration was still lower than the control level 24 hours after release of 24-hour ($P<0.01$) and 48-hour ($P<0.02$) complete obstruction, though it had recovered considerably. The mol%BA at that time had returned to the control level after both 24- and 48-hour obstructions. There was no significant difference in concentration or mol%BA 24 hours after release of either 24- or 48-hour periods of complete obstruction.

The phospholipid concentration was still lower than the control in the bile 24 hours after release during 24- and 48-hour ICBO ($P<0.01$). The mol%PL at that time remained in the control range.

The cholesterol concentration remained lower than that in the control ($P<0.01$) 24 hours after release during ICBO, as did the mol%Ch ($P<0.01$) and the CLI ($P<0.001$ for 24-hour and $P<0.01$ for 48-hour ICBO).

Discussion

It is arguable that the dog is not an ideal experimental animal because the concentration of cholesterol in the bile is very low relative to phospholipid[21]. However, in this study it was not intended to design an animal model which would produce cholesterol gallstones but to examine the effect of biliary obstruction on the composition of bile. Smaller animals with suitable biliary physiology, for example the rat and guinea pig, could not be used. The guinea pig is not capable of surviving the necessary operative procedure and in both species the bile ducts are too fine to allow progressive stenoses to be artificially produced. The pig, on the other hand, has ideal biliary anatomy and recovers well from the operation; however it proved impossible to take the necessary measurements as the animal was not

capable of being trained to the level of cooperation required. Primates would be ideal physiologically and anatomically, but the provision and keeping of such animals present great difficulties. The dog was therefore chosen as it can be easily trained, the biliary anatomy is similar to that in humans[22] and the biochemical and physiological background of biliary secretion in dogs is known.

The results suggest that this relatively simple system is capable of producing consistent controlled degrees of biliary obstruction. The biliary pressures obtained in the control phase were similar to those reported by Parry *et al.*[23]. Flow rates were within the range reported by Wheeler and Ramos[24]. It has been suggested[25] that replacement of a segment of bile duct with a widely patent, inert tube would produce an obstruction in the proximal biliary tree with dilation of the proximal bile duct. The biliary pressure was measured in the 7 dogs at intervals throughout the first postoperative month. Three of these dogs initially had abnormally high pressures, but these were considered not to be due to the inert tubing since (*a*) the readings were taken distal to the junction of the proximal common bile duct with the tubing; (*b*) the high levels returned to normal within 2 to 3 weeks; and (*c*) 4 out of 7 animals had normal pressures throughout. Two possible explanations of the raised biliary pressures are, first, that the trauma of the procedure could produce oedema and inflammation of the epithelium of the duct or spasm of the sphincter of Oddi. Secondly, removal of the gallbladder has been reported to be followed by raised pressures and dilated CBD[26,27]. For these reasons, one month postoperative recuperation was allowed before the control phase was commenced. Cholecystectomy was carried out, as the presence of a gallbladder tends to dampen the effect of obstruction[28].

This study shows that biliary obstruction reduces the concentration of all biliary lipids. The concentrations of biliary lipids were altered when a stenosis was introduced that produced a biliary pressure of about 50–65% of the MBSP. However, it is not clear whether the biliary lipids are decreased as soon as the biliary pressure is more than 50% of the MBSP. It seems that the concentrations of biliary lipids just begin to decrease under these conditions because the extent of decrease in bile acids and phospholipids is limited. On the other hand, there is a marked decrease in concentration in all these lipids during severe PBO. This suggests that the major alteration occurs when biliary pressure increases to more than 75% of the MBSP.

In this study, biliary obstruction usually resulted in a decrease of concentration and molar percentage of cholesterol in bile. The effect of depressing the secretion of cholesterol lasts some time after the release of chronic biliary obstruction. It seems that cholesterol secretion is more sensitive to biliary obstruction than bile acids and phospholipids. During moderate PBO, cholesterol concentration and mol%Ch markedly decreased, whereas mol%BA and mol%PL did not significantly change and their concentrations decreased proportionally less than that of cholesterol. The recovery of cholesterol in bile was always slower than that of bile acids and phospholipids. It has been reported that cholecystectomy is followed by a reduced cholesterol proportion in biliary lipids[29,30]. On the other hand, several authors were unable to obtain any evidence of improvement of cholesterol solubility[31–33]. Nevertheless, it is unlikely that in this study the reduced mol%Ch was due to the effect of cholecystectomy, which was performed during the operation to construct the biliary tubing system, because (1) there was no gradual decreasing tendency in mol%Ch during the control phase; (2) the reduced mol%Ch recovered to the control range within 24 hours after release of moderate PBO; and (3) the mol%Ch decreased sharply as soon as a severe stenosis had been introduced. For the same reasons it is also unlikely that, of itself, the biliary tubing system caused the reduction of mol%Ch seen in this study. Prolonged starvation may result in a reduced mol%Ch in bile[34], but starvation initially results in an increased cholesterol saturation[35]. The decrease of mol%Ch actually commenced as soon as PGO had been produced, yet mol%Ch was reduced while the dogs ate normally during moderate PBO. Thus, the alteractions of the composition of bile are unlikely to have resulted from reduced food intake.

The risk of cholesterol supersaturation of bile only emerged immediately following release of 48-hour ICBO, though mol%Ch at that moment was not significantly higher than the control. This supersaturation was due to a relatively increased proportion of phospholipid in bile and greatly decreased total biliary lipids which reduced the micellar zone[36]. It must be emphasized that biliary obstruction does not stimulate the liver to secrete more cholesterol into the bile duct; on the contrary, it depresses the secretion of cholesterol.

In their investigations on monkeys, Redinger *et al.*[7] suggested that intermittent biliary tract obstruction might decrease cholesterol solubility (increase mol%Ch) in bile in patients with or without cholelithiasis, thus predisposing to enhanced gallstone formation. They reported that the composition of biliary lipids in the 'immediate' bile was normal, but the increase of mol%Ch occurred in two hours and lasted for 36 hours. However, in the present study mol%Ch in bile collected during partial biliary obstruction or 24 hours after release of partial and complete obstructions was reduced, not increased. Only in bile collected immediately following release of a 48-hour period of complete obstruction did cholesterol supersaturation occur. At that time there were markedly reduced concentrations of all biliary lipids. This supersaturation had disappeared by 6 hours after release of the obstruction.

The differences between the results reported by Redinger *et al.*[7] and those from this study are possibly due to the species difference. The concentration of cholesterol in dog bile is very low[21], but is higher in monkey bile[37]. Differences of cholesterol metabolism may exist in these two species and thus they may react differently to biliary obstruction. On the other hand, the apparent discrepancy might not be simply due to the species difference: Redinger *et al.*[7] studied acute complete obstruction, but in the present study the complete obstruction commenced after a period of partial obstruction. It is not known if the results obtained in this study after 48 hours of complete obstruction were affected by previous partial obstruction.

In considering whether biliary obstruction favours the formation of cholesterol gallstones, it is of note that there was only a trace of cholesterol in the bile immediately following the release of a 48-hour

obstruction. Should precipitation of cholesterol occur, it would be very little. The composition of the biliary lipids at that time gave a point located in the area of Admirand and Small's triangle[4], usually associated with liquid crystal plus liquid. There were no flat parallelogram-shaped cholesterol crystals in the sediment of the centrifuged bile. Following release of the obstruction, the cholesterol saturation returned to normal in 6 hours and bile contained proportionately even less cholesterol in 24 hours. Thus, this kind of supersaturation with cholesterol would not play a significant role in the formation of cholesterol gallstones.

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