REVIEW

The Formation of Gallstones

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Abstract. There are two types of gallstones; cholesterol and pigment or bilirubinate. Cholesterol stones are formed in the gallbladder as a consequence of altered hepatocellular and gallbladder function. Overproduction of cholesterol by the liver is the major metabolic precedent of cholesterol gallstones and this may occur because of obesity, drugs, or other factors. Gallbladder factors which promote stone formation include hypomotility and the secretion of nucleating factors such as mucus glycoprotein. It is possible that both of these two factors are mediated by an increase in the prostaglandin production by the gallbladder mucosa. Pigment stones are either brown or black. Brown stones are formed of calcium bilirubinate and are usually associated with biliary infection. They occur in both the gallbladder and the bile ducts. Black pigment stones are extremely hard bilirubin polymers and are found mainly in the gallbladder. Biliary sludge is a necessary precedent of gallstones. It comprises cholesterol monohydrate crystals, glycoproteins and granules of calcium bilirubinate. (Keio J Med 41 (1): 1-5, March 1992)

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There are two types of gallstones—cholesterol and pigment or bilirubin stones. Cholesterol gallstones are more common in the Western world where they comprise about 75% of all stones. Pigment gallstones are more common in the East and are particularly associated with biliary tract infection. This review will deal firstly with cholesterol stones and subsequently with the formation of bilirubin stones.

Epidemiology

Gallstone disease increases with age and is commoner in females than males. Our data from studies in Scotland show that at the age of 30 years about 5% of females have gallstones, whereas by 60 years nearly 30% of females have formed gallstones. The data for males shows that the stones are 50% less frequent. This data is comparable with figures from the rest of the world and the evidence from postmortem studies matches very closely the newer data based on ultrasonographic evaluation of populations.

Environmental factors leading to cholesterol gallstones include female sex, social class (I<V), reduced physical activity, a diet high in calories and low in legumes, a low intake of vegetables and possibly a high polyunsaturated fatty acid diet. The mechanism whereby these factors lead to gallstone formation is as yet poorly understood. There is evidence that a moderate alcohol intake is negatively associated with cholesterol stone formation.1,2

There are clear racial differences in the prevalence of gallstones. The highest prevalence is found in Pima Indians where it amounts to 80% of mature females. This is followed by Chilean Indians, Germans and Italians. The prevalence in African tribes is around 14% and studies in Japan indicate a prevalence of 10%. The lowest recorded prevalence is apparently China where it is 5%. Genetic factors also play a role although the precise genetic influence has still to be determined. Gallstones are five times commoner in gallstone families, and twice as common amongst first degree relatives. There is no increase in spouses. The suggestion has been made that there is possibly a polygenic inheritance.

While gallstones are recognised as common in females, the precise mechanism is still not entirely clear.3 The frequency of stones increases with an early menarche and with multiple pregnancies. Whether or not oral contraceptives are associated with a greater frequency of gallstones remains controversial. Most studies suggest that there is an increase in gallbladder disease in patients taking oral contraceptives and this is either because of chronic cholecystitis or an increased tendency to gall-

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stimulation of the hepatic lipoprotein receptors with stones.4–6 When oral contraceptives are used, there is an increased input of cholesterol into the hepatic pool. It is probable that there is also an inhibition of the regulatory oxysteroid with an increased cholesterol input which is not associated with reduced cholesterol synthesis. There is inhibition of the metabolism of cholesterol to bile acids. The overall effect of these various metabolic changes is an increased biliary lithogenicity.3,7

There is, however, little doubt that pregnancy is associated with increased tendency to form biliary sludge or cholesterol stones and at the time of delivery about 50% of women have sludge, 15% have gallstones. After one year about 4% of patients have gallstones. It is likely that the major factor determining gallstones in pregnancy is gallbladder hypomotility.8,9

Physical–chemical State of Supersaturated Bile

Cholesterol is carried in bile in four different ways. A small quantity is associated with bile salts to form simple micelles containing cholesterol and bile salts but no lecithin. The major quantity of cholesterol in bile is associated with mixed micelles containing a core of lecithin and cholesterol with a perimeter of bile salt molecules which act as a water solubilising surface. The third carrier system is unilamellar vesicles which are bilayers of cholesterol and phospholipid (C/P) molecules. These vesicles are liquid crystalline fragments which are considerably larger than micelles in size. Finally a small quantity of cholesterol is associated with a biliary lipoprotein. By far the most important interactions determining gallstone formation are micellar cholesterol and cholesterol in the vesicles.10,11

Bile salts are secreted from the hepatocyte as monomers and in the course of their secretion stimulate lecithin and cholesterol cosecretion from the liver cell membrane in the form of small unilamellar vesicles. Once within the bile canaliculus the small cholesterol/lecithin vesicles coalesce with bile salts to form mixed micelles. However in the dilute hepatic bile this is an incomplete process and about 90% of the cholesterol in hepatic duct bile is carried in the form of C/P vesicles. As the bile is concentrated in the gallbladder, with absorption of water the bile salts and lecithin can solubilise proportionately more cholesterol so that the cholesterol saturation index falls as the bile becomes more concentrated. In gallbladder bile proportionately more of the cholesterol is carried in the form of mixed micelles.

The bile of patients forming cholesterol gallstones has an excess of cholesterol. This can be recorded by using the cholesterol saturation index. When the cholesterol saturation index is increased above one it indicates that there is excess cholesterol being secreted into the bile. But this is a relatively poor index of gallstone-forming capacity. The excess cholesterol is also carried in vesicles and the cholesterol content of the C/P vesicles in gallstone-forming bile is higher than in non stone forming bile. In gallstone-prone subjects the cholesterol rich C/P vesicles coalesce to form larger cholesterol rich micellar vesicles which eventually form cholesterol monohydrate crystals.11,12 In non stone forming bile the process of crystallisation may take many days, whereas the characteristic feature of stone forming bile is that it can form cholesterol crystals within a matter of hours. The secretion of excess cholesterol by the liver is essential for the formation of cholesterol stones. Cholesterol secretion is increased during the fasting state, with age,13,14 in obesity,15–17 as a consequence of certain drugs such as Clofibrate, and possibly as a result of dietary factors. There is no evidence that patients in the Western world who form cholesterol stones have an abnormality in the activities of either 3-hydroxy-methyl-gluteryl CoA-reductase (the rate limiting enzyme for cholesterol synthesis) or cholesterol 7 alpha-hydroxylase (the rate limiting enzyme for bile salt synthesis). Haptic hyposecretion of bile salts is not thought to be important in the pathogenesis of cholesterol gallstones.

Nucleation

While cholesterol saturation of bile is essential for stone formation, not all saturated biles form cholesterol stones. By far the most important factor distinguishing stone forming from non stone-forming bile is the capacity to nucleate cholesterol out of solution in the form of cholesterol monohydrate crystals.18,19 The nature of the nucleating factors remain to be determined with precision but they include mucus glycoprotein (mucin), non mucus glycoproteins, calcium ions, immunoglobulins and possibly free fatty acids.20,21 In addition it is recognised that there are antinucleating factors which include apolipoproteins, lecithin and ursodeoxycholic acid. The precise balance of activity of these different factors, and how they influence stone formation remains to be determined. It is important to distinguish the nucleation of cholesterol crystals i.e. the initial formation of a crystal, from crystal growth and stone formation. It is probable that different factors determine these two processes.

Gallbladder Factors Involved in Gallstone Formation

Cholesterol gallstones form only in the gallbladder and factors which promote stone formation include stasis, the concentration of bile,20 the stratification of bile, the addition of nucleating factors, and possibly alterations in bilirubin and phospholipid metabolism by the gallbladder wall. The gallbladder mucosa influences bile composition by altering the acyl groups of that small percent of chol-
esterol that is esterified in bile.\textsuperscript{22} Gallbladder absorption of cholesterol will influence cholesterol saturation in bile favourably.\textsuperscript{23,24} It is also known that gallbladder absorption of water increases during early gallstone formation.\textsuperscript{25}

**Mucus**

It has been known for some time that mucus glycoprotein is important in gallstone formation. The glycoprotein is necessary for the development of biliary sludge, for nucleation of bile, and for the growth of the gallstone. Mucus interferes with gallbladder emptying, causes poor equilibration of the bile salt pool, binds biliary lipids and bilirubin, and enhances the nucleation of cholesterol monohydrate crystals.\textsuperscript{26,27}

**Calcium in Bile**

Calcium is important in the formation of both cholesterol and pigment stones. Calcium bilirubinate forms the nidus of cholesterol stones and precipitation of calcium carbonate can be found as surface calcification in cholesterol stones. Calcium also aids the nucleation of cholesterol monohydrate crystals from cholesterol/phospholipid vesicles. Normally calcium is prevented from precipitating in bile because of the low pH which results in a reduction in the calcium solubility products so that no calcium carbonate crystals are formed. When the gallbladder fails to acidify bile precipitation of calcium carbonate and phosphate can take place.

**Phospholipids in Bile**

Ninety-five per cent of phospholipids in bile are in the form of lecithin (phosphatidyl choline) which contains one saturated and one unsaturated fatty acid. Patients who form cholesterol stones have an inherent excess of arachidonic acid phospholipids and these can be metabolised in the gallbladder mucosa to prostaglandins which promote mucus production and inhibit gallbladder motility. It is thought that this mechanism operates in patients who have a tendency to form cholesterol gallstones.\textsuperscript{28}

**Gallbladder Motor Function**

There is gallbladder hypomotility in patients forming gallstones and recently observations in patients whose gallstones have been dissolved by bile acids or using extracorporeal shock wave lithotripsy demonstrate that the gallbladder motor abnormality precedes gallstone formation. It is not a consequence of the gallstones.\textsuperscript{29–31} Hypomotility enhances the formation of crystals and, of course, prevents biliary sludge from being evacuated by the gallbladder. Hypomotility is known to be a factor in pregnancy, in post operative states and other stasis syndromes including somatostatinoma and therapy with octreotide, and spinal cord injury. Gallstone forming gallbladders have reduced isometric tension of the muscles strips in response to CCK stimulation. There is increased cystic duct resistance. The degree of hypomotility correlates with an increase in the cholesterol saturation index.

Human studies and studies on experimental gallstones suggest that these gallbladder changes are secondary to the secretion of bile which is saturated with cholesterol. Hypersecretion of mucus by the mucosa and the gallbladder hypomotility both precede the presence of gallstones. The sequence of events would therefore appear to be the production of cholesterol-enriched bile by the liver which is accompanied by, or which induces gallbladder hypomotility and mucus hypersecretion; this in turn, promotes the nucleation of cholesterol crystals and the retention of cholesterol monohydrate in a "sludge" of mucus, cholesterol crystals, and calcium bilirubinate crystals. Once nucleation has taken place and the gallstone has formed it will grow through the further deposition of cholesterol and calcium crystals. Human gallstones are either single or multiple; the multiple stones are usually of one size suggesting that when nucleation does occur it does so at one particular time. Any further precipitation of cholesterol forms larger stones, not more stones. It is unlikely that waves of nucleation with new stone formation occur.

**Pigment Stones**

There are two types of pigment stones—black or brown. Black stones are hard, found mainly in the gallbladder, are formed of bilirubin polymers, and do not contain either bacteria or cholesterol. Brown pigment stones tend to be soft and are found both in the gallbladder and the bile duct. They are radiolucent and are formed of crystals of calcium bilirubinate. These stones are usually associated with infected bile and contain bacilli, and cholesterol crystals. Calcium palmitate produced by the hydrolysis of lecithin is a very characteristic component of brown pigment stones.

It is probable that bilirubinate stones are formed in bile which is supersaturated with calcium bilirubinate. This may occur because of an increase in the free, ionised calcium in bile or because of an increase in unbound, unconjugated bilirubin ion. The latter may come about from an increase in unconjugated bilirubin being secreted by the liver for example in haemolysis, or because of increased ionisation of unconjugated bilirubin due to a raise in pH. There may also be a decrease in the binding of bilirubinate ions for example when there is a drop in bile salt concentration or increase in lecithin concentration,\textsuperscript{32} thus making them more available to
precipitate as calcium salts.

**Brown Pigment Stones**

Brown pigment stones are formed primarily from a combination of stasis and infection. Stasis can occur because of a biliary stricture, or a disorder of the sphincter of Oddi or due to foreign bodies in the ducts. Infection is primarily due to E. coli and results in hydrolysis of bilirubin to unconjugated bilirubin, the formation of lysolecithin which will promote the formation of cholesterol precipitates, and the formation of free bile acids which may also form calcium salts. Bacteria also produce glycoprotein and possibly stimulates glycoprotein from the gallbladder and this aids in the nucleation of bilirubinate and cholesterol crystals and development of biliary sludge. We have found that the pH of the bile of patients forming pigment stones is lower than that of patients forming cholesterol stones. This is contrary to what has been reported before, namely that when the gallbladder fails to acidify bile precipitation of inorganic and organic calcium salts occurs which is essential for the formation of pigment gallstones. Our observations, however, suggest that the presence of acidic bile is more common in pigment forming bile than in bile containing cholesterol stones. It is possible in these acidic bile that there is more calcium ion available to form insoluble salts preferentially with bilirubin than with phosphates or carbonate and this determines the formation of bilirubinate stones.

**Black Pigment Stones**

The mechanism of formation of black pigment stones remains unclear. Factors which play a role include an altered pH, an increase in ionised calcium, and an increase in unconjugated bilirubin. Because there is no evidence that infection plays a role it is possible that abnormal glucuronidase activity by the gallbladder mucosa may be important. Haemolysis is known to be an aetiological factor.

As with cholesterol stones, biliary sludge is important in the formation of pigment stones. A combination of concentrated bile which is viscid and rich in crystals precedes the formation of pigment stones. It is likely that biliary sludge is not a permanent event but is transient. Thus sludge may form in the gallbladder intermittently under conditions when there is gallbladder hypomotility or when factors results in an increased tendency for biliary pigment to precipitate. If normal gallbladder motility exists, the sludge would be ejected from the gallbladder; on the other hand failure of the gallbladder to empty itself will result in retention of sludge and ultimately by the formation of gallstones.33

**Conclusions**

A great deal is now understood about the formation of gallstones. Factors causing the secretion of either cholesterol or bilirubin rich bile on the one hand and a gallbladder which promotes the formation of cholesterol and bilirubin crystals together with stone growth on the other lead to gallstone formation. Insight into bile physiology and gallstone formation has resulted in the introduction of agents which are capable of dissolving cholesterol stones, and these have now been introduced into clinical practice. The capacity to dissolve bilirubin stones is, however, limited and this is an area of pharmacology which is waiting to be developed.

**References**

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