The Formation of Gall Stones

The Scientific Basis of Medicine

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Introduced by Dr Ian Gilliland.

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<Opening titles>

<Dr Gilliland to camera>

Dr Hermon Dowling is a lecturer in medicine at the Royal Postgraduate Medical School of Hammersmith where he is also Deputy Director of the Medical Research Council's Intestinal Malabsorption Group. Prior to this, he worked as a research fellow Boston and before that in Belfast. His work has always been concerned with the gastrointestinal problems and, in particular, recently with the formation and dissolution of gallstones[1]. The title of this discourse is the formation of gallstones. Dr Hermon Dowling.

<Dr Dowling to camera and then close-up of gallstones held in Dowling's palm>

[1] Spelt 'gall stones' on title in film. The spelling 'gallstones' has been used for consistency in these transcriptions.

Good day. During these next two programmes, I should like to discuss the problem of the common gallstone. Now, I say a common problem because it has been estimated at a conservative figure that there are something like 10% of the population who suffer from gallstones, or more correctly who have gallstones present in Western civilisations. And given a population in this country of some 50 odd million people, this means that there are at least 5 million people with gallstones. Now, many of these, of course, will be asymptomatic or silent gallstones. But the natural history of the problem suggests that somehow between one third and one half of patients would suffer one of the major complications, namely biliary colic, cholecystitis or obstructive jaundice during a 5 to 10 year period and which, therefore, will necessitate surgery. But quite apart from the economic considerations, I should like to discuss gallstones for another reason: that of the exciting advances which have been made in the physicochemical basis of gallstone formation, which have really been made in the past 5 years. And I should like to start today by discussing first, some of the different types of gallstones which we may see, and then how bile chemistry is disturbed and how this may lead to the formation of gallstones. Because it's by understanding this process that enables us to put the whole business into reverse and to move the material which has precipitated into the stone, put it back in solution in bile and hopefully to dissolve gallstones.

But first then, a word about the different types of stones.

<Dowling refers to large screen display behind him and narrates over slide showing pigment stone>

The first slide shows the picture of a typical pigment stone. Now, I say it's a so-called pigment stone, in fact, because crystallographic analysis of these stones suggests that they are largely the calcium salts: the calcium palmitate, phosphate and carbonate. Although, there are certain amounts of calcium bilirubinate present. These black pigmented stones are soft and crumbly. Because of the calcium salt they are usually radiopaque and they are found commonly in association with haemolytic disorders and also with cirrhosis of the liver. Now, we know very little

about ways of dissolving these gallstones, but the formation of these stones has been now explained and this is shown diagrammatically on this first chart.

<Dowling narrates over diagram illustrating formation of gallstones>

Most of these patients have infection in the gallbladder, mainly with E. coliforms and these bacteria possess the enzyme β-glucuronidase which deconjugates the water-soluble bilirubin diglucuronide to form the water-insoluble unconjugated bilirubin, which then combines with calcium to form these black pigment stones.

Now, as I've said, we have no way of dissolving these stones, but fortunately they're very much in a minority and the vast majority of gallstones in Western civilisations consist predominantly of cholesterol.

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<Dowling to camera, then narrates over slides showing examples of gallstones>

And I should now like to show you a few examples of cholesterol gallstones and we define these as stones containing more than 70% cholesterol by weight. Let's have a look at a few of these stones. Now here is a large solitaire cholesterol stone and, as you can see, the surface is somewhat irregular. It's stained here with trace amounts of a light brown pigment, but remember that it only takes a very small quantity of pigment to produce quite strong discolouration of this stone and, in fact, when this stone was analysed it contained more than 95% cholesterol.

Now, the nature of this stone is obvious on section, both transverse and longitudinal. Next slide please. And here you can see the obvious crystalline nature in a rather radial display. There is, in fact, a small dark nucleus present which may have formed the nidus around which the crystals grew. But perhaps more common than the pure cholesterol gallstone is the mixed stone. <*Next slide*> And here is an example of a whole stone with a faceted surface and a sister stone on the right which shows the

different composition in the interior of the stone. In this centre, you can see that the dark material is again pigment. The body of the stone, however, is largely crystalline arranged in a radial display. And on the surface, there is a laminated structure which, in this case, contains some calcium salts and therefore produced a radiopaque rim. Now, even at this magnification, I think you can probably make out the crystalline content of the stone, but it's easily seen under the dissecting microscope. <*Next slide*> And here you can see the obvious crystalline interior of the stone and the laminated outer surface which contained the calcium salts, but even then this stone consisted predominantly of cholesterol, more than 90% by weight.

Another familiar type of stone is the multiple small faceted stone. And the surgeon often finds that the gallbladder is absolutely crammed full of these small stones to such an extent that their surfaces rub together to produce this apparently smooth faceted exterior.

<Dowling to camera, then narrates in turn over: scanning electron micrograph of cholesterol gallstones; polarising light micrograph of dust on surface of gallstone; micrograph of melting crystals; polarising light micrograph of bile>

Now, I say apparently smooth because these stones, in fact, are far from smooth. And if we take young cholesterol gallstones, the sort we have just seen, and examine them under the scanning electron micrograph, you can see that the surface is a rather jagged alpine profile due to these sheets of sharp crystalline structures, and if we take a few flakes of dust from the surface of this stone and examine it under the polarising microscope, you can see that against the dark background of polarised light, this crystalline material is highly refractile.

Now, a property of all crystals is the fact that they have a very specific melting point and by incorporating a heating element in the microscope stage, linking this to a thermostatically controlled device, one can observe these individual crystals as they melt. And when we did this, these particular crystals melted at exactly at 148.5 degrees centigrade, the melting point of cholesterol. Now, we can also sometimes see cholesterol present in bile in this crystalline form. <*Next slide*> And this shows a

drop of bile from a patient with cholesterol gallstones examined under the polarising microscope and you can see quite clearly the classical rhomboidal configuration of cholesterol crystals.

<Dowling to camera, then over slide showing configuration of cholesterol molecule>

In fact, in our experience, some 50% of patients with gallstones have microcrystals of this sort in their bile. So that we have established that the stones consist predominantly of cholesterol. Now, as you know, cholesterol is a molecule whose configuration is shown here, a steroid molecule with a short side chain. It contains in total 27 carbon atoms, and the steroid ring and side chain is almost exclusively hydrocarbon, as is shown in the centre in this Stuart-Brieglib space-filling molecular model. In fact, at a water-air, or indeed at a water-oil interface, this water-hating or hydrophobic molecule hides itself as far away from the water as possible. In fact, there is only one small water-loving group, shown here, the hydroxyl group at the 3-beta position of the molecule. And this hydrophobic characteristic of cholesterol means that it is almost completely insoluble in water.

<Dowling to camera>

And yet when you think of it, bile is essentially an aqueous medium, which contains quite considerable amounts of cholesterol. And cholesterol in bile is dissolved there because of phospholipids and bile salts. The phospholipid molecule is shown on the next picture.

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<Dowling narrates over diagrammatic slide illustrating a phospholipid molecule>

And this shows the phospholipid molecule which now has a water-loving part and a fat-loving. And here's how it orientates itself at a water-air or, indeed, at a water-oil

interface. The hydrophilic or water-loving part of the molecule is a phosphorylcholine group with positive and negative charges so that over here on the right, you can see that it is electrically neutral. The fat-loving part of the molecule consists of two long-chain paraffin chains or hydrocarbon chains. One of them has a double bond in this position which produces the kink in the space-filling molecular model. Now, although this molecule loves both fat and water and is therefore called an amphipath, it is, in fact, insoluble in water. However, when there is a lot of phospholipid present and water is added to the system, the phospholipid molecules swell and they arrange themselves in a bilayered structure as shown here.

<Dowling narrates over diagram illustrating a bilayered structure of hydrated phospholipid molecules>

This shows the phospholipid molecules, as we have just seen, with the fatty acid tails –the phosphorylcholine group, and these project towards each other; the fatty acid tails project towards each other so that they form bilayered structures. And in between these bilayered structures, water interdigitates itself. And this tends to lubricate these bilayered structures so that in one dimension they move, but because of the constant spacing between the bilayers, they behave in another dimension like crystals. They therefore provide an example of liquid crystals and this is shown over here on this chart.

<Dowling narrates over diagram illustrating lamellar liquid flow in hydrated phospholipid molecules>

Here is the bilayered structure and the constant spacing between these bilayers means that a beam of light or indeed a beam of X-rays will be bent or refracted. So in that dimension, it behaves like a crystal. However, the water acts as a lubricant between these layers so that there is movement which takes place: lamellar liquid flow and these, indeed, are liquid crystals.

Now, a hydrated phospholipid system, such as this, can hold a certain amount of cholesterol in solution. And this is illustrated on this chart.

<Dowling narrates over diagram illustrating retention of cholesterol in a hydrated phospholipid system>

Here the molecules of phospholipid once again and interdigitated between them are the cholesterol molecules. So this is a lecithin cholesterol liquid crystalline structure, and occasionally in bile, we can see liquid crystals of this sort. But by far and away the greatest detergent action in bile is due to the presence of bile salts. And the bile salt molecule is shown on the next slide.

<Dowling narrates over slide showing structure of bile salt molecule>

As you can see from the upper portion here, it is again a steroid ring; this time it has only 24 carbon atoms, the 24th carbon atom having a carboxyl group linked here in peptide linkage with the amino acid glycine or taurine. And in this case we are showing the sulphur containing amino acid, taurine. This particular model that we've chosen is cholic acid which has 3 hydroxyl groups here at 3-, 7- and 12-positions. Now, the conventional structural formula of this sort is very misleading because it gives the impression that these water-loving parts of the molecule, the hydroxyl groups, stick out randomly in space. In fact, they do nothing of the sort. They're orientated here, here and here on one side of the molecule, and here on the other part of the molecule is the polar head group. These form the water-loving part of the molecule, the hydrocarbon back forms the fat-loving part of the molecule.

<Dowling to camera>

So, again, we're dealing here with an amphipathic molecule, but this time the bile salt is freely soluble in water and it's this characteristic of water-soluble amphipathic molecules which enables them to form micelles and to act as detergents. And I'd now like to say a brief word about how micelles form and about the different types of detergent which are present.

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Perhaps the simplest of all detergents is the sodium salt of a fatty acid, the common soap. Let's have a look at this molecule.

<Dowling narrates over diagrams illustrating the molecular structure of the sodium salt of a fatty acid>

Now, here is the sodium salt of a fatty acid. Here's the chemical formula: it's a paraffin chain – a carboxyl group shown in the ionised form in association with the sodium cation. In the middle, we have shown the conventional shorthand way of representing this molecule, rather like a drumstick, although it's often shown as having a wiggly tail. And below is another Stuart-Brieglib molecular model. Now, this also is an amphipathic molecule. It has a fat-loving portion, here, and the polar head group, the carboxyl group with the sodium cation, forms the water-loving part of the molecule.

And let's see now how these simple soaps form micelles.

<Dowling narrates over diagram illustrating formation of micelles by simple soaps>

Now, the old adage that oil and water don't mix is true. And here we've shown an oil-water interface magnified many thousand times. Here is our simple soap, the detergent molecule with its water-loving polar group and its fat-loving paraffin chain. This is how it orientates itself at an interface, the fat-loving group projecting into the fat and the hydrophilic group sticking into the aqueous phase. Now, it's easy to see if the affinity for this group for water is relatively great. We can have individual detergent molecules or monomers and if we increase the detergent concentration then, just by random chance, these molecules are likely to collide and we may end up with dimers which are attracted to each other by van der Waals forces. If we increase the detergent concentration even further, we end up with suddenly a molecular aggregate, a cluster, a roughly spherical structure which is known as a micelle. And this is not a rigid structure because the individual molecules are

monomers hop on and off the micelle and, in fact, it's been estimated that the lifespan of an individual molecule in the micelle is less than a millisecond. And schematically here, we've shown a blob of fat, or triglyceride, solubilised in the middle of the micelle in this aqueous medium.

Now, this is the principle of detergent solubilisation of fat and that's the sort of detergent that the household uses in the kitchen sink when she's dissolving fat from the dinner plate.

<Dowling to camera, then over slide showing molecular structure of sodium salt of cholic acid>

But here we're talking about the biological detergent, the bile salt molecule. Let's have another look at this molecule. The next slide shows another example of a bile salt molecule, in this case not conjugated form but the sodium salt of free or unconjugated cholic acid. And now you can see how the hydroxyl groups, shown here and here, and the polar head group all lie on one side of the molecule. Now, these space-filling molecular models give us a fair idea about the mobility of the individual atoms or groups of atoms on the molecule.

<Dowling briefly to camera, then over slide showing molecular model of sodium salt of cholic acid displayed on the top of a rod>

And this is easy to see if we prop the molecule up on a rod which is shown on the next slide. Here are the hydroxyl groups, here, here and here, and here is the polar head group hanging down as it would do at an oil-water interface. Now, having considered this molecular model, let's now move over to the chart again and see how bile salts form micelles.

<Dowling narrates over diagram illustrating formation of micelles by bile salts>

Here is the bile salt molecule with its 3 hydroxyl water-loving groups and its polar head group. This is how it orientates itself at an oil-water interface. Here is an

individual molecule and here are 2 dimers. This time they are attracted to each other by hydrophobic bonding back to back. Now, if we increase the concentration of the bile salt molecules again, we reach this critical concentration, the so-called critical micellar concentration where the molecules form a cluster, or a molecular aggregate. And they do so in the best way to preserve their integrity in this rather hostile environment of water. The hydroxyl groups are on the outside of the molecule, the polar head group here, and the hydrophobic part of the molecule tucked away from the water. Now this is all very well, that's how a bile salt molecule would form in the test tube, but clearly we don't have simply bile salts alone either in bile or indeed in the intestinal lumen where bile salts are playing their major role in promoting fat absorption. And this is a conception of how we believe a mixed micelle forms and it's based on studies by my former colleague, Donald Small in Boston. And he suggested that bile salts form mixed micelles as a sort of bimolecular disc and this representation shows a cut away version to show the inside of the molecule. The bile salt molecules are plastered around the outside, the hydroxyl groups here and the water-loving head groups here. This shows a phospholipid molecule with its 2 fatty acid tails, its polar head group and here is a cholesterol molecule with its 1 solitary water-loving hydroxyl group so that in bile, we've got 3 different types of lipid. We've bile salts, phospholipids, and cholesterol.

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<Dowling briefly to camera, then sits down to narrate over diagrams illustrating proportions of components of bile salts>

Let's see now how we can represent these various components in bile when we move over to this diagram. Now, if we had only 2 variables such as bile salts and cholesterol, then we could represent any mixture of the 2 by a single point using a conventional graph. But here we've got 3 components: bile salts, phospholipids and cholesterol. And by using this triangular coordinate system, one can still represent any mixture of the 3 as a single point, provided that the sum of the 3 components equals 100. Here on this system, at this apex, is 100% bile salts. This represents 0% bile salts and as we move from this base and parallel to the base towards this apex,

so we have increasing amounts of bile salt present plotted here on the base of the triangle. Similarly, this apex represents 100% cholesterol, this base 0% cholesterol, and as we move towards the apex, so plotted on this axis, there are increasing amounts of cholesterol. And in the same way phospholipid is plotted here on this axis. Now, just to take an example, if we had a mixture containing 65% bile salts, 15% cholesterol and 20% phospholipid, it would be represented by this point, whereas if we had equal amounts of all 3 components, the point would fall in the middle of the triangle.

<Dowling to camera>

Now, this is simply a graphical or mathematical way of expressing our results. And it's a system that was used by Bill Admirand and Donald Small some 5 years ago when they looked at cholesterol solubility in vitro. And to do this, they took literally hundreds of mixtures of bile salts, phospholipid and cholesterol made up to represent gallbladder bile.

<Dowling narrates over chart showing proportions of components of bile salts in liquid and crystal phases>

And when they did this they found that mixtures represented by points falling in this small shaded zone, in the bottom left-hand corner of the triangle, were the only mixtures where cholesterol was completely dissolved as a clear one phase micellar solution. These were mixtures which contained quite a lot of bile salts, not very much cholesterol and variable amounts of phospholipid. And elsewhere in the triangle, there was insufficient bile salt and phospholipid present to hold the cholesterol in solution so that in addition to the clear micellar phase, or liquid phase, there was now a crystalline phase of precipitated cholesterol. This curved line, therefore, represents the absolute limits of cholesterol solubility in bile. And points falling on this line would represent mixtures which are truly saturated solutions.

<Dowling to camera, then over chart>

Now recently there's been considerable controversy about this boundary line and, depending on the physicochemical methods which have been used to define the limits of cholesterol solubility, different investigators have found different boundary zones. In fact, it seems likely that this boundary zone should be somewhat near the base of the triangle, but in general Small's principles hold true and most investigators have found that this indeed does represent a fair way of plotting bile composition.

Now, Small's studies were done in vitro, but they went on to show that these results also apply in vivo because bile taken from normal individuals was represented by points falling in the micellar zone, while bile composition in patients with gallstones either fell on the boundary zone, indicating a saturated solution or in this 2 phase zone here, the triangle. We ourselves have confirmed this result.

<Dowling narrates over chart showing percentage of cholesterol present in gallstones>

And we've done this by analysing bile rich duodenal fluid and this shows the results in control subjects and in patients with cholesterol gallstones. This spidery symbol represents 1 standard deviation about the mean for each of the variables. That, for example, is cholesterol. This is bile salt and that is phospholipid and while there is a considerable overlap between these two populations, in general Small's findings seem to hold true.

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<Dowling to camera, then walks to diagrams on display wall. Narrates over diagram showing passage of bile salts through hepatocyte>

Now, so far, we've been talking about the role of bile salts as detergents, but bile salts also control both the synthesis and secretion of phospholipids and to a certain extent the secretion of cholesterol in bile. Let's go now and look at a diagram to explain this phenomenon. This diagrammatically shows the hepatocyte as bile salts pass through it from the sinusoid to the canaliculus. And here we've shown

diagrammatically presumably how they act on the rough endoplasmic reticulum to influence phospholipid synthesis and phospholipid secretion in bile. Their influence on cholesterol secretion is somewhat less, but it is, nonetheless, important so that coming into the canaliculus we now have a mixture of bile salts, phospholipids and cholesterol which, of course, is a micellar solution of hepatic bile. The relationship between bile salts and phospholipids and between bile salts and cholesterol is therefore of enormous fundamental importance.

<Dowling to camera, then returns to seat and narrates over diagram showing relationship between biliary phospholipid secretion and biliary bile acid secretion>

Let's see now how these factors are now interrelated. This diagram, which although is based on actual results is a hypothetical diagram, shows the relationship between biliary phospholipid secretion and biliary bile acid secretion and, in general, there is probably a curvy linear relationship between these two, although, in fact, one can derive a highly significant correlation coefficient from data of this sort.

<Dowling narrates over diagram showing relationship between biliary cholesterol secretion and biliary bile acid secretion. Interspersed with talk to camera>

For cholesterol secretion the situation is somewhat different. At high bile acid secretion rates, between points B and C, we again have this curvy linear relationship, but at very low bile acid secretion rates, the situation between A and B here is somewhat different because for every mole of bile acid produced, there is a relatively greater amount of cholesterol secreted. And as a result the bile becomes abnormal. This is shown on the relevant corner of the triangle here in the right. Between points A and B, the bile is abnormal or supersaturated with cholesterol, whereas in the normal range of bile acid secretion, between points B and C, we have a normal micellar solution of cholesterol.

Now, this suggests that the abnormal bile in patients with gallstones represents a physiological variant of normal, but it remains possible that the increased cholesterol secretion is indeed an abnormal phenomenon. And some recent data from Phoenix in Arizona suggests that there is an excess secretion of cholesterol in patients with gallstones.

<Dowling to camera, then over diagram of enterohepatic circulation>

Well now, if bile salts regulate the secretion of biliary phospholipids, what controls the concentration of bile salts? Well, as we see over here on this chart, this is regulated by the enterohepatic circulation. And just to remind you briefly: bile salts are made in the liver from cholesterol, they are conjugated with the amino acids glycine and taurine, and they are temporarily stored and concentrated in the gallbladder. And then in response to a meal, the bile salts pass into the upper intestine where they play their very important role in promoting both the digestion and absorption of fat, but the bile salts themselves largely pass down the intestine to the ileum where they are reabsorbed by a special active transport mechanism, returning to the liver in the portal vein and so completing the central hepatic circulation.

Now, it's a very efficient system and there's normally only a small spillover or excretion of bile salts into the faeces. About 2 to 5 % of the total circulating bile salts each day so that to maintain the status quo, the liver has only got to supply enough new bile salts to match the faecal loss.

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<Dowling to camera>

Now, it's been known since about 1965 when Playoust, Lack and Weiner showed in dogs that resection of this part of the intestine causes bile salt malabsorption. The question arises, therefore, when there is disease or resection of the distal small intestine, is this going to deplete the bile of its bile salt content? Or to put it another way, will patients who have Crohn's disease, radiation ileitis, or surgical resection or

bypass suffer an increased incidence of gallstones? Well, the evidence certainly seems to suggest this and we ourselves have looked at this phenomenon and I should now like to describe some studies by my colleague Dr Duncan Bell, where we've looked at bile composition again in bile rich duodenal fluid. And we first demonstrated that this bile composition is very similar to the composition of gall bladder bile obtained at surgery.

<Dowling narrates over diagram showing percentage cholesterol in bile salts of control patients>

Let's look now at the results in control subjects. And here, again, on the relevant corner of the triangle are the findings in 25 control subjects. The males are represented by the closed circles and the females by the open circles. And as you can see the mean value represented by this square symbol lies just inside the limits of cholesterol solubility. This hexagon again represents 1 standard deviation about the mean for each of the variables, but you can see that there is a wide scatter of results and this is particularly true for the women since no less than 5 of the 10 female subjects had an abnormal bile, whereas only 4 of the 15 men had a bile composition represented by points falling outside the micellar zone.

<Dowling narrates over diagram showing percentage cholesterol in bile salts of patients with ileal dysfunction>

In contrast, in a group of patients with ileal dysfunction and a broken enterohepatic circulation, all of them with one exception had an abnormal bile and the difference between this population and the control subjects was highly significant. The difference, of course, is particularly striking for the men since all 5 men here had an abnormal bile.

<Dowling to camera, then over graph showing incidence of gallstones in patients with ileal disease compared that in control populations>

And since this chart was prepared, we have now studied 4 other patients with ileal dysfunction, all of whom have an abnormal or lithogenic bile. These studies, therefore, provide a logical and physicochemical explanation for the clinical observation first made by Heaton and Read in 1969, when they showed that the incidence of gallstones in patients with ileal disease was very much greater than in matched control populations, whether in a necropsy series or patients with nonspecific gastrointestinal symptoms. And Heaton and Read's findings have subsequently been confirmed from this study in the United States by Cohen and his colleagues from Boston.

<Dowling narrates over graph showing prevalence of gallstones in patients with ileal disease compared with that in control populations>

And they found that some 34% of patients with ileal disease had gallstones, a very much higher incidence than their control series which came from the well-known Framingham epidemiological study.

Now, patients with ileal dysfunction have a diminished bile salt pool.

<Dowling narrates over graph comparing bile salt pool in patients who have had ileal resection with those of controls>

And this has been demonstrated many times but these particular results come from Abaurre and his colleagues in Denver. And they showed that patients with ileal resection have a diminished total bile sample pool when compared to control subjects. And in their particular study they found that the primary bile acid, cholic acid pool, was particularly reduced.

<Dowling to camera>

Now, so far, we've been talking about secondary cholelithiasis complicating intestinal disease, but a reduced bile sample pool may well be the fundamental and basic defect that leads to the formation of lithogenic bile in patients with primary

cholelithiasis who are thought to have a normal intestine because a bile salt pool is also reduced in patients with cholesterol gallstones.

<Dowling narrates over graph showing total bile salt pool in patients with gallstones compared with that in control populations>

And this was first shown by Reno Vlahcevic, a Yugoslav working in Richmond in Virginia, who found that the total bile sample pool was reduced in gallstone patients when compared with controls. In fact, it's approximately half of the normal level.

<Dowling narrates over graph comparing the chenodeoxycholate pool in gallstone patients with that in control populations>

Similarly, further studies from the Mayo clinic by Danzig and his colleagues showed that the chenodeoxycholate pool was reduced in gallstone patients when compared with controls

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<Dowling briefly to camera, then over slides summarising primary abnormality for gallstone formation>

So just to summarise, we can say that the primary abnormality must be a metabolic defect with a diminished bile salt pool, and as a result, there is a low bile salt and phospholipid output or secretion in bile. Since there will be a diminished bile acid return to the liver, the negative feedback mechanism regulating the conversion of cholesterol to bile acids through the enzyme 7α-hydroxylase must be deficient and we will discuss this defect in the next programme. It has also been suggested as I've mentioned, from the studies from Phoenix, that there is excess biliary cholesterol.

<Dowling to camera>

Now, without this primary abnormality, we cannot have an abnormal bile, crystals cannot form and stones cannot grow. But of course, there are many important secondary factors. Let's now consider some of these.

<Dowling narrates over series of slides summarising secondary factors in gallstone production. Briefly to camera in between>

Well, first there's a question of stasis. There obviously must be sufficient time for a nidus of crystals to form and for these crystals to grow in a lamellar fashion to form a macroscopic gallstone. What about infection? Well, the presence of bacteria are known to remove a fatty acid from the phospholipid lecithin[2] to form lysolecithin, which may then be absorbed from the gallbladder. Alternatively infection may stimulate the production of mucous from mucopolysaccharide, which may not only provide a nidus or a focus for crystal formation but it also may provide a cement and we know very little about the basic cement structure or matrix of gallstones. Infection through its inflammation on the gallbladder might conceivably cause selective bile acid or, for that matter, phospholipid absorption and while this may be considered as a theoretical possibility, this is yet to be proven in man.

<*Next slide*> Sex hormones may well be important and we know that gallstones occur more often in women than men in a ratio of about 2 or 3 to 1. And indeed gallstone formation may be related to parity. However, we know relatively little about the influence of oestrogens and progesterones on bile composition.

Finally, the question of diet. We know, of course, that gallstones are the product of affluent societies and that gallstone formation may be correlated with calorie intake. Studies in experimental animals by Damm and his colleagues and by Ballint from the United States have shown that essential fatty acid deficiency or high calorie diets may lead to the formation of gallstones. In one particular species, the prairie dog, a high cholesterol diet has produced cholesterol gallstones, but this is a rather unique situation and I don't think it has very much relevance to man.

[2] Spelt 'leicithin' on slide.

<*Next slide*> Finally, the question of where the abnormal bile is formed. Is it a lithogenic liver or, indeed, is it a guilty gallbladder? Let's consider the liver first of all. <*Next slide*> The evidence that the liver may be responsible is suggested by many lines of studies including the fact that hepatic bile, at the time of operation, is more abnormal than gallbladder bile. This was first demonstrated by Small and Rapo in 1970, and in conjunction with Dr Daniel Prandi from the Hôpital Beaujon à Clichy, we ourselves have confirmed this. We've discussed already the insensitivity of the rate-limiting enzyme 7α- hydroxylase in response to a diminished bile acid return to the liver and we have mentioned the studies in North American Indians who have an excess biliary cholesterol excretion? Now what about the gallbladder?

<*Next slide*> Well, last year, Simmons and Bouchier from the Royal Free Hospital showed that while hepatic bile was indeed more abnormal than gallbladder bile at the time of surgery, subsequently hepatic bile became normal. And they suggested that cholecystectomy improved bile composition. Furthermore, Dr Bell and I have shown that in patients with non-functioning gallbladders, that is, gallbladders which failed to opacify after both oral and intravenous cholecystography, have both a normal hepatic and gallbladder bile composition. Now, at first sight, this might suggest that the gallbladder was modifying the composition of the bile and was making it abnormal or lithogenic <*to camera*> but, of course, the sequestration of the bile sample bile in the gallbladder, which is normally functioning or which remains before surgery, itself can physiologically interrupt the enterohepatic circulation. And we know from animal studies in the rhesus monkey that acute interruption of the enterohepatic circulation produces an abnormal bile supersaturated with cholesterol. Indeed, it seems possible that all of us throughout the 24 hour period may from time to time have an abnormal or lithogenic bile. And some studies recently from the Mayo Clinic have suggested that simply gallstone patients have simply more hours of lithogenic bile in the day.

<*Next slide*> And finally, if we can go back to our caption chart, there is the question of the inflamed gallbladder. This we've already discussed.

<Dowling to camera, then narrates over diagram illustrating formation of gallstones>

So just to summarise what we've already discussed today, we can say that there are 3 stages in the formation of gallstones. In stage 1, there is an abnormal or lithogenic bile which is supersaturated with cholesterol. This then proceeds to the second stage where microcrystals of cholesterol are present in bile similar to those which we saw earlier in the programme. In the third stage, these microcrystals have aggregated and grown to form macroscopic gallstones.

In this next programme, I should like to tell how we can put this process into reverse and take the cholesterol from the gallstone and put it back into solution in bile. Thank you.

<End credits>