The Dissolution of Gall Stones

The Scientific Basis of Medicine

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<Opening titles>

<Dr Dowling to camera>

Hello again. You may remember that in the last programme we talked about the formation of gallstones[1] with particular emphasis on the physicochemical changes which occur in bile composition in patients who develop cholesterol gallstones; and in this first chart, you'll remember how we plotted out the bile salt, phospholipid and cholesterol content of bile on this triangular coordinate diagram.

<Dowling narrates over series of diagrams plotting bile salt composition>

The small shaded area in this bottom left-hand corner is the only area where cholesterol is completely soluble in bile. Points falling below this curved boundary line, therefore, indicate a bile which is less than fully saturated with cholesterol.

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

Points falling above the line indicate a bile which is carrying more cholesterol than can be carried in a stable equilibrium. You will also remember that <*next diagram*> based on Admirand and Small's original observation, we confirmed that patients with cholesterol gallstones have, by and large, a more abnormal bile than control subjects whose bile composition falls within the micellar zone. Now, these results were based on analysis of bile aspirated from the duodenum of the fasting patient after cholecystokinin. The question arises: what is this abnormality due to? These samples were analysed fresh before filtration or centrifugation. And the first possibility to explain this abnormality is that there may be microcrystals present in the bile.

<Dowling refers to large screen display behind him and narrates over slides of microcrystals in bile, before and after filtration>

And if look at this first slide, it illustrates the presence of microcrystals seen under the polarising microscope in a drop of bile from a patient with cholesterol gallstones. In fact, this slide illustrates something else. If we just focus on this area of the slide, you can see that there is a dark, roughly spherical structure with the appearance of a Maltese Cross. And this is a liquid crystal. You may remember that we discussed, in the last programme, how liquid crystals may solubilise a certain amount of cholesterol in bile. Now, when a sample of bile, such as this, is passed through a Millipore filter – and we've used a 0.22 micron filter – and we re-examine the filtrate under the polarising microscope, you see that the crystals have completely disappeared. And were it not for these two little bits of dust which are diffracting the polarised light, we would be looking at a completely plain surface.

<Dowling narrates over slide of photomicrograph showing bile residue taken from Millipore filter and then over charts showing bile salt composition>

The next slide shows the scraping taken from the Millipore filter. And you can see that the microcrystals here have been retained by the filter. Now, Millipore filtration changes the bile lipid composition. And if we turn to the chart again, we can see just what happens: this shows the results of bile lipid composition before (the closed circles) and after (these open circles) Millipore filtration. And these bile samples

contained no microcrystals. It seems, therefore, that simple Millipore filtration itself non-specifically affects the amount of cholesterol present in the lipid composition of bile.

<Dowling to camera>

However, microcrystals are probably not the major factor accounting for the abnormal bile composition in patients with cholesterol gallstones. In fact, in our experience, they're present in some 50% of patients.

<Dowling narrates over slide showing test tube containing bile from patient after cholecystokinin administration>

Now, there's another explanation for this abnormality. If we look at this slide, it shows you the appearance of a test tube containing bile aspirated from a patient after cholecystokinin and it's a dark rich concentrated example. When this sample has been stored in the deep freeze at minus 20 degrees centigrade and subsequently thawed and centrifuged, we almost invariably find a precipitate, suggesting that the process of freezing had disturbed the equilibrium of the supersaturated solution. This is explained diagrammatically on this chart.

<Dowling narrates over chart illustrating precipitate formation in bile after freezing and thawing>

At the time of aspiration, the bile looked clear and yet its composition was represented by a point falling outside the micellar zone and here is our hypothetical micelle shown overloaded with cholesterol molecules. After freezing, thawing and centrifugation, a precipitate occurs which now forms a true stable equilibrium with the supernatant. And if we reanalyse the supernatant, it now is a truly saturated solution. Instead, the micelle forms a stable configuration and we see the microcrystals of cholesterol in the bottom of the tube. Now, this is the theory.

Let's now and go and look at the actual results when we analyse bile before and after freezing, thawing and centrifugation.

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<Dowling sits down and narrates over chart showing composition of bile salts after centrifugation>

And the results are shown on this chart. Again the solid circles represent the results before, and the open circles the results after this freezing, thawing, centrifugation. And again you can see this markedly influences bile lipid composition. Not only is there a reduction in the proportion of cholesterol present but there is also a reduction in the proportion of phospholipid.

<Dowling to camera>

It seems therefore that freezing, thawing and centrifugation disturbs the equilibrium of bile and precipitates the combination of cholesterol and phospholipid.

Well, I'd now like to turn to the other major point which we made last week, that is, the reduction in bile salt pool size in patients with cholesterol gallstones.

<Dowling walks to display wall and narrates over chart showing bile salt pool size in patients with gallstones and in control subjects>

To my mind one of the most important and fundamental problems is why the patient with cholesterol gallstones has a diminished bile salt pool? Now, if we consider this diagram which shows the bile salt pool size in control subjects, 2.5 grams, and in gallstone patients, about 1.25 grams. It's obvious that synthesis normally matches faecal loss and we know from several studies that faecal bile salt loss is the same in control subjects and in patients with cholesterol gallstones. Now again, in the steady state, input matches output so that the reduced bile salt pool size is independent of synthesis and excretion. What sets the homeostatic mechanism and tells the body

that the pool should be this reduced size, as opposed to the normal size, is completely unknown. And this is one of the fundamental problems which is yet to be solved.

Now, if we consider this in a theoretical way and turn to the next chart: we've shown here diagrammatically the enterohepatic circulation, first in normal individuals and in patients with gallstones.

<Dowling narrates over diagram illustrating enterohepatic circulation>

As we've just seen, faecal excretion is the same in both groups of patients and therefore in a steady state. Synthesis in the liver will be the same in normal and gallstone patients. Now, if we make the assumption that the number of enterohepatic cycles remains constant in patients with gallstones, the total bile salt output must be drastically reduced in the patient with gallstones. Pool size times circulation frequency gives us the total output or secretion rate of bile salts, which in the gallstone patients is about half that of the normal individual. Now, the proportion of bile salts escaping absorption from this output is clearly greater than from this output. And this implies that the patient with gallstones has a relative absorptive defect in the intestine. In fact, the absorption efficiency drops from something like over 98% to just under 97%. But on accumulative basis, this results in a marked reduction in bile salt return to the liver: 14.75 grams per day in the normal individual compared to 7.25 per day in the patient with gallstones.

Now, normally the liver is extremely sensitive to very slight reductions in the amounts of bile salts returning. And in the normal case, one would have expected that bile salt synthesis through this enzyme 7α-hydroxylase would markedly increase, but this is not the case. And we can only assume, therefore, that there are two defects in the patient with cholesterol gallstones: one, a relative intestinal malabsorption, and secondly, a defect in the hepatic synthesis. Now, an alternative explanation might be that in the face of a reduced bile salt pool, the number of enterohepatic cycles would increase, perhaps to 12 times per day, to maintain the total output unchanged. Which

of these theories is correct awaits the proof from experimental data and so far this is not yet available.

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<Dowling to camera, then over series of charts and diagrams illustrating results of experiments on gallstone dissolution, interspersed with talk to camera>

Well, we've talked about the formation of gallstones and about the enterohepatic circulation and in the rest of today's talk, I should like to concentrate on the main topic for today: gallstone dissolution.

<*Next chart*> Now, here in this chart are the results of some in vitro studies carried out by Donald Small on gallstone dissolution in vitro. Human cholesterol gallstones placed in water as a control showed no change as a percent of the original stone weight when plotted on this expanded scale against time. However, when the human gallstone is placed in a bile salt solution containing 6% bile salts, there is initially rapid dissolution and then a plateau is reached. When phospholipid is added to the bile salt, and this phospholipid of course is a principal phospholipid found in human bile, there is much more rapid rate of dissolution. Now, these in vitro studies provide us with the theoretical background to some studies which were carried out in San Francisco last year.

<*Next chart*> And this is a study of the dissolution of retained common bile duct stones after gallbladder surgery. When the patient has been left with a T tube in the common bile duct, Admirand and Way found that by perfusing a high concentration, 100 millimolar sodium cholate at a rate of 30 ml per hour, into the common bile duct, they could achieve a high detergent concentration locally. Now, obviously this bile salt spills into the intestine and because of the cathartic action of bile salts, this would normally produce quite profound diarrhoea. But by feeding cholestyramine in this relatively high dose of 2 grams every hour, they were able to prevent to a certain extent the diarrhoea suffered by these patients. They found, in fact, that out of 8 patients who had single gallstones, 6 dissolved, and 9 patients who had multiple

stones, 5 dissolved in this relatively short period of time. So far, we have been unable in our own laboratory to repeat this experience, but if these experiments are proved and confirmed, this will be a valuable adjunct to our main form of treatment for retained common duct stones.

But I'd now like to return to the main stream of our talk and to consider human gallstone dissolution in vivo. <*Next chart*> And this slide, for which I accept absolutely no responsibility, showing these rather exotic beasties, demonstrates that human gallstones placed in the dog gallbladder dissolve extremely rapidly. In fact, this has been known since about 1892 when it was shown by the German physiologist, Naunyn. Human gallstones placed in – I think this is a rhesus monkey but again I apologise for any similarity between that and our own experiments, in any case, human gallstones placed in the monkey gallbladder also dissolve, but at a very much slower rate.

Now, why should this be so? Well, it's explained on the basis of bile composition. And this is shown on this chart. <*Next chart*> Dog bile contains virtually no cholesterol, somewhat less than 1 mole percent; it is therefore an extremely high cholesterol solubilising capacity. In contrast, rhesus monkey bile and human bile are almost completely saturated with cholesterol. And it's about stone dissolution in three different species that I should like to discuss this afternoon.

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Many of our studies, carried out in conjunction with my colleague Dr Duncan Bell, have been carried out in the rhesus monkey. And we chose this animal because of the similarity of its bile composition to that of man. Now, we train these animals to sit in a special restraining chair for some 2 weeks before operation.

<Dowling narrates over diagram illustrating experiment set up to investigate bile composition in rhesus monkeys>

And then at surgery, we tied off the common bile duct here and inserted a T tube, where possible below the entry of the cystic duct so that our cannula drained both gall bladder and hepatic bile. The drops of bile passed between the light source and photoelectric cell of this electronic stream splitter which we could adjust to divert, by means of these moving funnels, every 20th drop of bile into a sampling tube. The remaining 95% passed into this small cup, balanced in such a way that only a few drops were required to close the switch, activate a pump and return the bile to the antrum region of the stomach. In so doing, we maintain a virtually intact enterohepatic circulation since 95% of the bile is being returned to the intestine, but this 5% which we divert to our sampling tube provides us with a measure of the volume of bile produced during any given period of time, the concentration of the substances in the bile and, of course, an accurate measure therefore of the secretion rate during any given period of time.

Now, this model was modified to include a small glass chamber in which we placed a human cholesterol gallstone and the detail of this is shown better on the next slide.

<Dowling narrates over slide showing glass chamber in which gallstone is placed>

And here you see the glass chamber below and the completed assembly above so that knowing the stone composition, its surface area and the composition of bile flowing over the stone, we can see which of these factors is responsible for promoting gallstone dissolution.

<Dowling narrates over chart illustrating rates of dissolution of gallstones of varying cholesterol content>

Now, let's look at the data when we consider first the cholesterol content of the stone. And on this scale, this expanded scale, we've plotted the percent weight loss of the stone against days. As a control, an almost pure cholesterol stone, containing 95% cholesterol, was analysed chemically, was placed in water and removed at regular intervals for weighing. And as you might expect, there was no change in its

weight. This shows the results for 3 different stones containing 79, 85 and 90% cholesterol. And as you can see, the greater the amount of cholesterol, the more rapid the rate of dissolution. Now, in these studies, the content of the stone varied but the content of the bile was the same.

This next chart shows what happens when we keep the content of the stone constant, 90% cholesterol, but vary the degree of saturation of the bile.

<Dowling narrates over chart illustrating rates of dissolution of gallstones in varying bile saturations>

And again, plot weight loss against time. This is a bile which is almost completely saturated with cholesterol, whereas this bile is 83% saturated. And this relatively small difference in the degree of saturation almost doubled the rate of gallstone dissolution.

<Dowling to camera and then over diagram illustrating experiment set up to study effect of surface area of gallstone on its dissolution>

Now, I'd like to consider the question of stone surface area. And in these studies, we took a large solitaire gallstone and divided it up as follows: we took a single section and 12 small cubic sections, each weighing about the same in a region of 800 milligrams, but having quite different surface areas. And these were exposed to monkey bile. As a control for these studies, similar segments were placed in water. Let's see now the results.

<Dowling narrates over chart showing rates of dissolution of gallstones of varying surface area>

Both control stones, shown by this single line, showed no change in weight with time. Here's the result for the single stone and here for the 12 small segments and, as you might expect, with a greater surface area to weight ratio, the small segments dissolved more rapidly. But f course, these studies will depend on relatively crude

planimetry. And, if you remember from last week, the surface of the gallstones, seen under the scanning electron micrograph, is far from smooth.

<Dowling narrates over photomicrographs of gallstones>

In fact, shown here, you can see these jagged crystals so that we can't take into account the microclimate on the surface of the stone. And furthermore, if we look at the stone under the dissecting microscope, you can see this whitish material which is due to cholesterol in a mixed stone, but a predominantly cholesterol stone, before exposure to bile. And if we look at the same stone after exposure to bile, you can see it's developed this rather granular pitted surface with cracks and crevices so that dissolution is not simply a surface phenomenon.

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<Dowling to camera>

I'd now like to turn to the question of a calcified stone. And this is a common problem in clinical practice. In other words, we're simply asking: if a patient has a radiopaque gallstone, is he likely to prove unsuitable for medical therapy? Well, to answer this problem, we have again taken dog bile and we harvested this from a large number of animals. We've chosen this to study this in vitro simply because the dog bile dissolves gallstones extremely rapidly. And if we look at the slide, here you see a single stone and a split stone taken from the same patient and above are the X-rays of these stones.

<Dowling refers to slide showing gallstones and their X-rays>

The calcified rim is quite clearly seen, and perhaps better seen in the split stone. As a control for these studies, a segment of an almost pure cholesterol stone was taken. And as a result, it is virtually radiolucent. Now, let's have a look at these results.

<Dowling narrates over chart illustrating rates of dissolution of gallstones with calcified rim>

Over here we've plotted, again, the percent weight loss against time for the whole stone, the half stone and the control containing almost nothing but cholesterol. And as you might expect, the pure cholesterol stone dissolves rapidly, but the half protected split stone does dissolve, although the wholly protected stone with the calcified rim also dissolves, it does so considerably more rapidly.

So just to summarise the results to date.

<Dowling narrates over slide summarising factors affecting gallstone dissolution>

We've shown that the rate of gallstone dissolution depends first on the cholesterol content of the gallstone; secondly, on bile composition with reference to the proportion of cholesterol present; thirdly, on the surface area of the stone; and fourthly, on the presence or absence of a calcified rim.

<Dowling to camera and then over chart comparing bile salt pool size in patients with gallstone with that in control patients>

I'd now like to go on to discuss the question of gallstone dissolution in men and if we could go back to our chart where we've shown pool size. If, by feeding bile acids, it's possible to expand this depleted pool and return it to normal, then we should be able to first improve bile composition and secondly promote gallstone dissolution. And based on this theory, in 1971, Thistle and Schoenfield showed, for the first time, that feeding the primary bile acid, chenodeoxycholic acid improved bile composition in 4 young Chihuahua Indian women.

<Dowling narrates over chart showing bile composition before and after administration of chenodeoxycholic acid. To camera in between>

And this shows the results of the bile composition before (the closed circles) and after (the open circles) chenodeoxycholic acid. Now the Chihuahua are one of seven North American tribes who have an extremely high prevalence of gallstones. Bile composition in these Chihuahua Indian women first becomes abnormal about the time of puberty. And by the time they're 25, some 70% of these women have gallstones. Now, Thistle and Shoenfield's studies, shown here, were carried out on women who had a lithogenic bile or an abnormal bile but who had not yet developed gallstones. And the same group from the Mayo Clinic went on to show that feeding this bile acid chenodeoxycholic acid promoted gall stone dissolution in 4 out of 7 patients. And these studies were first reported about a year ago.

I'd now like to tell you about the result of some studies which my colleagues, Dr Duncan Bell and Brian Whitney from Hammersmith Hospital, have been carrying out on gallstone dissolution. And first, the results of bile composition.

<Dowling narrates over charts showing results of further experiments investigating bile samples>

This chart shows the results before and after treatment with chenodeoxycholic acid and in virtually every case, there is an improvement in cholesterol solubility in bile. In fact, this chart probably underestimates the degree of improvement and this is for various technical reasons in the way we process the bile samples.

Now, another way of looking at this data is to consider the so-called cholesterol solubilising capacity which is shown over here *<next chart>.* This is quite simply the ratio of bile salts plus phospholipids, the solubilisers, over cholesterol, the material to be dissolved. And here we show the ratio before and after treatment with chenodeoxycholic acid in 15 patients with a normally functioning gallbladder. And in virtually every case, we've shown an improvement in bile composition. Although in some patients there was little change and in 1 patient, there was actually a deterioration. But taking the mean value and the standard error of the mean, shown by this E symbol, there was an overall improvement in bile composition which was

significant. Now, the results indicated by these arrows were the results in a young woman who had multiple small radiolucent gallstones.

<Dowling narrates over slide of X-ray of radiolucent gallstones>

And this is shown by her X-ray in December of 1970. She declined surgery which was advised at that time and 1 year later, the appearance of her X-ray was essentially unchanged. We began treatment at this time and she was given chenodeoxycholic acid in a dose of 1 gram per day.

<Dowling narrates over chart showing results of bile composition in a patient with gallstones before and after treatment, then over X-ray after treatment>

And this chart shows the results of her bile composition before, 1 month and 3 months after treatment, showing a progressive improvement in cholesterol solubility. More important – 6 months after starting treatment, her X-ray shows that the gallstones had completely disappeared.

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<Dowling narrates over series of X-rays showing results of treatment of gallstones>

I'd now like to show you a few more X-rays illustrating the results of treatment. And in this next film, you see the X-ray of a woman taken in March of this year, of 1972, showing 3 small radiolucent stones. She was again given chenodeoxycholic acid in a dose of a gram per day and 6 months later, the stones had completely disappeared. These then are examples of gallstone dissolution.

I'd now like to show you an X-ray illustrating a reduction in gallstone size. On the left is the cholecystogram in a woman who had 5 medium-sized radiolucent stones. 6 months after starting treatment, the stones have appreciably reduced in size. And although the gallbladder is shown in the contracted position after a fatty meal, by

comparing the size of the vertebra in the 2 films, we can say that there was no change in the magnification between these films. And we believe that there was a significant reduction in gallstone size.

And finally an X-ray showing the appearance of calcification: a patient, whose X-rays were taken in December of 1971, showing a fairly dense calcification around the periphery of this stone. After 1 year's treatment, there has as yet been no reduction in gallstone size in this patient.

<Dowling to camera, then over slides summarising results of study of patients receiving treatment for gallstones>

So just to summarise the overall results: we have now studied some 18 patients who have been treated for a period of 6 months and re-X-rayed after this period of time. 12 patients had small radiolucent gallstones. Of those who had small stones, in 3 patients the stones have completely disappeared. With medium-sized stones, 4 patients have shown a significant reduction in gallstone size, while the 5 patients who had large gallstones have, as yet, shown no change in gallstone size, although it must be stressed that this is the earliest that one could possibly hope to find changes, given the rate of gallstone dissolution which we have seen from our experimental animal studies.

We've also looked – next slide please – at 3 patients who had calcified stones. One of these had small faintly calcified stones, which after 6 months of treatment had completely disappeared. Although in this patient's case, there was an attack of biliary colic immediately before the X-rays were taken and we believe that the reduction in gallstone size produced by treatment had enabled the stones to be small enough to squeeze into the biliary tree and hence produce colic, which heralded the disappearance of the stones. 2 patients had medium or large gallstones which again have shown no change in size. And with 3 patients who had a non-functioning gallbladder, defined by failure to opacify the gallbladder after either oral or intravenous cholecystography, there has been, as yet, no return of function.

<Dowling briefly to camera, then over slides listing complications of gallstones>

It seems, therefore, that we can indeed dissolve gallstones, but there are many questions which remain. What about the complications for example? Well, we can consider these, I think, under two headings: complications due to any patient who has untreated gallstones; and complications specifically due to bile acid treatment with chenodeoxycholic acid. Let's take the complications of the stones themselves. 1 patient had cholecystitis and was referred to surgery. This was a patient who had a non-functioning gallbladder, and at operation, a stone was found blocking the cystic duct, behind which the gallbladder was distended with sterile pus. 1 patient developed obstructive jaundice and this was a patient who had common bile duct stones which were present before treatment began, and she too was referred to surgery. 3 of our patients had developed biliary colic, but with the exception of 1 patient, who required an overnight admission to hospital, this has been relatively mild and the patients have been treated conservatively.

What about the dyspeptic symptoms that many gallstone patients complain of. Well, our subjective impression is that the symptoms, if anything, have been improved by treatment. We certainly would not like to lay claims to any significant pattern of results with this form of treatment.

00:30:23:11

<Dowling to camera, then over slides listing complications of bile acid treatment>

And now the complications of bile acid treatment itself. Chenodeoxycholic acid, or indeed any bile acid, might be expected to produce gastric irritation, but none of our patients complained of dyspeptic symptoms. Because of spillover of bile salts which have not been absorbed into the colon, the cathartic effect of producing diarrhoea is to be expected and about 50% of our patients complained of somewhat loose stools during the first 2 or 3 weeks of treatment, but almost invariably this settles

spontaneously with time. Theoretically, bile acids may be hepatotoxic. Chenodeoxycholic acid could affect hepatic function either directly as a microsomal toxin or indirectly through conversion to its bacterial metabolite lithocholic acid, which is known to be hepatotoxic and cholestatic. We have carefully monitored the changes in liver cell enzymes at monthly intervals and we have found a slight, but of doubtful clinical significance, rise in several hepatocellular enzymes. We arbitrarily decided that we will carry out liver biopsy only if there was increase BSP retention. And somewhat to our surprise, the 45 minute BSP retention figures were low rather than high. We believe this indicates increased clearance or an increase in the maximum excretory rate of BSP that has been produced by bile acids, an observation originally made by O'Maille, Richards and Short from Liverpool in 1966.

Finally, what about cholesterol accumulation? The relationship between bile acid and cholesterol metabolism is indeed complex, but theoretically at least, we might have expected cholesterol accumulation to have occurred in our patients. We therefore monitored not only the serum cholesterol levels, but the fasting serum triglyceride levels in our patients during treatment. These studies were carried out at monthly intervals and I've simply selected the results at 1 time interval, 2 months after treatment to illustrate the overall pattern of results.

<Dowling narrates over chart showing serum cholesterol levels and serum triglyceride levels before and 2 months after bile acid treatment>

Here we have plotted the fasting serum cholesterol levels and serum triglyceride levels before and 2 months after treatment. There is no change in serum cholesterol levels, but, somewhat to our surprise, the serum triglyceride levels consistently were markedly reduced and this pattern was repeated at every time interval and became more significant as time went on.

<Dowling to camera, then over slide listing questions>

Well, what are the problems which remain? I think we've got to admit that we're really only at the start of this very exciting and promising era. The questions include: what

happens to bile composition after treatment stops? Will it revert to becoming abnormal or lithogenic? And if so, will the gallstones reform? And if they are going to re-form, how soon after cessation of treatment? We also don't know which bile acid is going to be most effective, although preliminary results suggest that chenodeoxycholic acid is a more suitable form of treatment than cholic acid, the other primary bile acid. We've yet to determine what is the most efficacious dose? Although our own studies in experimental animals and indeed our clinical studies, which I've just described, indicate that a very much smaller dose than that previously used should be effective in promoting gallstone dissolution.

And finally, the question for the physical chemist of the future: will synthetic detergents ever replace the naturally occurring bile acids in their capacity to promote cholesterol dissolution in vivo and hence lead to gallstone dissolution?

<Dowling to camera>

I think in these two talks, I've illustrated a very personal view of how gallstones form and how they may be dissolved. I hope I've also indicated that there are many problems which remain to be answered. Thank you.

<End credits>