The Mediterranean Anaemias: Thalassaemia

The Scientific Basis of Medicine

Presented by Professor David Weatherall.

Introduced by Dr Ian Gilliland.

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<Opening titles>

<Dr Gilliland to camera>

Professor Weatherall has been appointed Nuffield Professor of Clinical Medicine in the University of Oxford. He has previously been Professor of Haematology in the University of Liverpool and Consultant Physician to the United Liverpool Hospitals. His research work on haematology has been outstanding and he has published many important scientific papers on the fundamental aspect of haematology, in particular in the Mediterranean anaemias which form the subject of this discourse: thalassaemia. Professor Weatherall.

<Professor Weatherall to camera, standing in front of a diagram of a haemoglobin molecule>

In 1925, a rather obscure Detroit paediatrician called Thomas Cooley described a group of young babies who were anaemic from the first few months of life and as

they grow older developed enlargement of the spleen and liver, became pigmented and died within the first year of life. Further patients of this type were described in the United States in the subsequent years and because of the Mediterranean background of these patients, the condition was called thalassaemia from the Greek *thalassa* – the sea. It's subsequently been realised that this disease occurs in all racial groups and is not localised to that region, but the condition is still called thalassaemia, or Mediterranean anaemia, or sometimes Cooley's anaemia.

There's been a tremendous interest in this disease in the last few years for several reasons. First of all, it's been realised that the thalassaemia disorders are by far the commonest genetic disorders of the blood and, in fact, are the commonest single gene disorders to cause a massive world health problem. Secondly, of course, in this country, we're seeing these disorders with increasing frequency, particularly in the immigrant population, but we also realise that the diseases do occur in the endogenous British population, people of so-called pure British stock. And finally, and really the subject of my talk today, we are realising that all the thalassaemia disorders result from a defective rate of production of the globin part of the haemoglobin molecule. And therefore, we are dealing with disorders due to a defective rate of protein synthesis and therefore the disorders do offer us a model for understanding all genetic disorders associated with a deficient rate of globin production. And I'm going to try and concentrate today on recent work on the molecular defect in the thalassaemia disorders.

Now, to understand these disorders, I think it really is essential to have a very simple understanding of the basic physiology or anatomy and physiology of haemoglobin and the way haemoglobin is genetically determined. So let's first look at a haemoglobin molecule and decide what we're asking of a genetic system which is going to produce it.

<Weatherall refers to diagram, on display board, showing structure of haemoglobin molecule and narrates over it>

This is the molecule as it appears to the x-ray crystallographers, particularly Dr Perutz and his colleagues in Cambridge. It's a spherical molecule and it consists of a protein background, the globin chains, which are shown as these wavy line here, and then the oxygen-carrying part of the molecule, the haem groups, which are shown as these discs which are pushed into deep clefts in the surface of the globin. Now, you'll see from the shading that there are separate pairs of globin chains. There are two alpha chains, shown in the light shading here, and two beta chains, shown in the dark shading here. And you can see that there is a central cavity and we're looking down on top of the molecule.

<Weatherall to camera>

Now, this complex 3-dimensional structure is absolutely essential if haemoglobin is going to act as an oxygen carrier because there are subtle changes which occur in the shape of the molecule during the giving up and taking up of oxygen and these are entirely dependent on this complicated 3-dimensional structure. And this in turn, of course, is entirely dependent on the order of amino acids in those globin chains. So the question we must ask then is how are these amino acids determined and how is this molecule put together?

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<Weatherall refers to diagram illustrating genetic control of amino acid chains in haemoglobin. Interspersed with talk to camera>

Now, let's look at the genetic control in another model. Here is our adult haemoglobin with two alpha chains and two beta chains, haemoglobin A, and this makes up about 98% of the adult haemoglobin in health. In all adults, there is a minor component with two alpha chains and two distinct chains called delta chains, which is called haemoglobin A2, and this makes up about 2% of haemoglobin in health. And it's important in thalassaemia because the relative amount does alter in some forms of thalassaemia.

Now, in foetal life the main haemoglobin is haemoglobin F and this has alpha chains, in this case combined with gamma chains. So this is represented $\alpha_2\gamma_2$. Back in the early embryonic life there is a separate haemoglobin, Gower haemoglobin, or the embryonic haemoglobin, but this is only important clinically and we're going to stick to these three haemoglobins for the purposes of the thalassaemia talk.

Now, what happens during development? Well, in uterine life, the alpha chain genes, controlling the alpha chain, and the gamma chain genes, controlling the gamma chains, are active. Alpha chains are produced, gamma chains are produced and haemoglobin F is produced.

At the period round about birth, a remarkable change occurs in the haemoglobin pattern. Alpha chains continue to be produced but now we stop making gamma chains and we start making beta chains. And this beautifully controlled switch occurs from foetal to adult haemoglobin production. At the same time we start making delta chains and haemoglobin A2.

Now, the importance of this model clinically is this: if you've got a defect at the alpha chain gene, then you can't make foetal or adult haemoglobin properly. If you've got a defect at the beta chain gene, then you can make foetal haemoglobin all right but you can't make adult haemoglobin. And this really is the basis of the thalassaemia disorders. So let's take this model and let's transfer this to the clinical problem of thalassaemia.

<Weatherall refers to chart showing classification of thalassaemias and narrates over it. Interspersed with talk to camera>

Now, here we have a model of the different types of thalassaemia, a simple classification based on the system that we've just been looking at. To remind you again, here's haemoglobin A and here's foetal haemoglobin. Now, there are a group of genetic disorders of haemoglobin synthesis, the beta-thalassaemias in which there's an inability to make beta chains. Most of these patients, if they're severely affected, go on synthesising alpha chains after the neonatal period and so the beta

thalassaemias are characterised by persistent beta chain production. You notice I keep saying beta thalassaemias because there's heterogeneity and there are probably about ten different types of beta thalassaemia, but we're just going to stick to the general principle of defective beta chain synthesis as the basis for all of them.

Now, if you can't make alpha chains, well, you're in trouble because you can't make foetal or adult haemoglobin. Now, in intrauterine life, deficiency of alpha chains leads to an excess of gamma chain production and these come together to form this curious haemoglobin, gamma 4 or haemoglobin Bart's. If these patients survive, they go on making excess beta chains in adult life and these form a curious tetra-haemoglobin, consisting entirely of beta chains, haemoglobin H. People get confused over this nomenclature and the reason it's arisen is very simple. In the 1950s as new haemoglobins were discovered, they were given a letter of the alphabet. And when haemoglobin H was discovered, H was the next available letter. When Bart's was discovered, there were no letters of the alphabet left and therefore, with the customary modesty of the London teaching hospitals, they named it after their own teaching hospital, haemoglobin Bart's. So, we can define alpha thalassaemia, then, as a condition characterised by the presence of haemoglobin Bart's in infancy and haemoglobin H in adult life.

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<Weatherall to camera and then narrates over photograph of child with beta t**halassaemia>**

Now, let's now look at the clinical picture of beta thalassaemia and if we look at this child, we see that he is a typical beta thalassaemic. He's stunted in growth and he has a rather large bossed skull, a splenectomy scar and pigmentation of the skin. He was well until the sixth month of life when he started not to thrive well. He was noted to have an enlarged liver and spleen and he required regular blood transfusions throughout infancy.

Now, if we look a little closer at this child's head, we will see that he has quite striking bossing of the skull. He's got overgrowth of the upper jaw and he's got an inability for his teeth to meet properly, a malocclusion. This type of jaw associated with bony overgrowth is called a gnathopathy, a ghastly term which has recently come into the medical literature, presumably a genetically termed inability to gnash the teeth. And all this is due to an overgrowth of the maxilla due to excessive expansion of the bone marrow cavity.

<Weatherall to camera and then narrates over photomicrographs in turn of red blood cells and bone marrow from child with beta thalassaemia>

Now, if we look at this child's blood picture, it's obvious even to a non-haematologist that all is not well with the red cells. These cells are excessively pale, they vary in shape and size and some of them are no more than little broken-off pieces. And quite clearly, there is a severe defect in haemoglobin synthesis in these patients. If we look at the bone marrow, we see that there's tremendous erythroid activity, spreading down the long bones. And it's has been known for many years that the marrow of these individuals has suffered from a basic ineffective erythropoiesis. In other words, tremendous activity with a very poor output. And this usually means that there are being cells destroyed within the bone marrow before they get into the peripheral blood. Now, if we look closely at this marrow, we see that there are some black-staining areas within the red cell precursors and it's now known that these black inclusions are, in fact, lumps of denatured alpha chain which have been produced during the development of the red cell precursor.

<Weatherall to camera>

Now, in the last few years it's been possible, by taking reticulocytes or bone marrow from these patients and incubating them with radioactive amino acids, to actually measure the rate of individual globin chain synthesis in these individuals. And what has come up in all cases of thalassaemia is a basic imbalance of globin chain production. Now, knowing the clinical details that we've just gone over and knowing

about imbalanced chain synthesis, we can really make a nice pathophysiological model to explain the whole clinical picture in these children.

<Weatherall refers to diagram illustrating defective pathway in red blood cell production in beta thalassaemia, and narrates over it>

Now, here's the model. We have basically an inability to make beta chains effectively. We make our alpha chains fine and we make some gamma chains but not enough to bind all the excess alpha chains, so some cells have got quite a lot of foetal haemoglobin in them, others don't. Now, the excess of alpha chains undergo really two fates. If there's a large excess, they produce a large amount of junk in the red cell precursor and this is precipitated and one produces a solid red cell which just can't get out of the bone marrow into the peripheral blood. Therefore death of cells in the bone marrow, ineffective erythropoiesis. Those cells which do make it have precipitated material which is removed in the spleen and reticular endothelial system with a shortening of the red cell survival and therefore one ends up with a severe degree of anaemia, which is secondary both to ineffective erythropoiesis and to haemolysis.

Now, the cells which do get out with a lot of foetal haemoglobin tend to have a high oxygen affinity and so the severe anaemia and the high oxygen affinity produce a tremendous drive to produce erythropoietin, tremendous stimulation of bone marrow and then, of course, the striking skeletal changes which occur in these children. So one can really take all the clinical features and relate them back to this basic defect in beta chain synthesis.

<Weatherall to camera and then narrates over photograph of stillborn baby>

Now, let's very briefly now consider alpha thalassaemia and let's look at this baby. Now, this is a stillborn infant at about 36 weeks, who was born in one of the hospitals in Singapore, and if it was seen in this country, you would say that this baby's got hydrops foetalis. It's an absolutely identical clinical picture: a baby who's oedematous and pale with massive hepatosplenomegaly, but in this case, if you look at the blood

picture, it shows changes of severs thalassaemia, and if you look at the haemoglobin pattern, it consists entirely of Bart's. These babies make no alpha chains at all; they just make Bart's haemoglobin and they die in utero and this is a very common cause of death in Southeast Asia: a genetically determined inability to make alpha chains, severe alpha thalassaemia.

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<Weatherall narrates over diagram outlining defective pathway in haemoglobin H disease >

Now, there's a milder form of alpha thalassaemia which is called haemoglobin H disease, which actually is compatible with survival into adult life. And we've shown here how if you get two alpha thalassaemia genes, a severe one and a mild one, you get a moderate decrease in alpha chain synthesis, excessive beta chain production, beta 4 molecules. Now, these are relatively stable; they make it into the peripheral blood, but then as the red cell ages, they precipitate and form these big large inclusion bodies and these, of course, are picked out of the red cells in the spleen and RE system and there's a shortened red cell survival due to red cell membrane damage.

<Weatherall to camera>

That disease is extremely common all over Southeast Asia and has been described now in pretty well every racial group including, again, the British.

So, you'll see that we have in all the thalassaemia disorders a defect in protein synthesis, characterised by deficient alpha or beta chain production, either total or partial. Now, how can we explain this in terms of the basic molecular abnormality? Well, to do this, we've obviously got to look very briefly at current ideas about protein synthesis and then relate this to the particular problem, the particular clinical problem that we have.

<Weatherall refers to and narrates over diagram illustrating genetic control of protein production>

Now, I've got here a very simplified model of the genetic control of protein production. Obviously, the order of amino acids in each globin chain is directed by information from the cell nucleus, held in the DNA of the particular gene for that chain, and this information has to be transported into the cell's cytoplasm where haemoglobin synthesis occurs. Now, the genetic information is held by virtue of the triplet genetic code, the series of bases, each triplet of which, of course, directs the insertion of one particular amino acid in the globin chain. Now, this information has got into the cell cytoplasm by means of a messenger called messenger RNA. Now, when messenger RNA is going to be synthesised on a DNA template, the DNA unwinds, one strand is copied and the messenger, the bases here are put in a position exactly complementary to those in the DNA. So the DNA message is faithfully copied to the RNA message which then moves out into the cytoplasm where it acts as a kind of template or workbench for protein synthesis.

Now, you've got to get your amino acids to this template or workbench and this is done on specific transfer RNAs which have a series of bases called the anticodon, which are complementary to those of the messenger RNA, so they'll only put the amino acids into the right position on the messenger template. Now, one particular triplet says START, here, and once this has started, the first transfer RNA is put in position and then the next transfer RNA comes along and puts the next amino acid in position. Now, the growing chain is held together or in position by a jig or pulley, little bodies here, shown here as the small and large circles called ribosomes. And the ribosomes move along the message carrying the growing globin chain, each new amino acid being put in position until they reach another code word which says STOP. And then the ribosomes drop apart and the chain is completed. It combines with another chain to produce a haemoglobin molecule.

<Weatherall to camera and then over diagram illustrating outcomes of different defects in protein synthesis pathway>

Now, based on that very crude and over-simplified account of protein synthesis, we can then look at the possibilities in the thalassaemia disorders. And I think an easy way of looking at them is shown here. What we've done, we've taken three messages with their ribosomes moving across. And if you look at them rather as a three lane highway; I thought about this very much sitting in the three lanes of the Mersey Tunnel in a traffic jam every morning, in which the cars are all going in one direction, and ask yourself the question: how could you reduce the number of cars getting through to the end of the tunnel on those three lanes? Well obviously, you could easily do this by shutting two lanes down and just having one lane open. And in molecular terms that would mean reduce the amount of messenger RNA in the cell. You could have a rather inefficient character at the pay booth at the opening end of the tunnel such that he only lets a few cars through at a time. And therefore, at any one time, you'd have fewer cars moving through. In molecular terms, there would be a defect in chain initiation.

You could also have a breakdown on the three lanes simultaneously, and abnormality of the lanes themselves, the defect actually in the road. And in molecular terms, this would be a defect in the rate at which the message was translated. The message might be abnormal, there might be a pile up of ribosomes and a deficient chain production.

Or finally, you might have a rather stupid policeman at the other end of the tunnel, maybe just measuring the tyre pressure on every car that came out of the tunnel and therefore causing a tremendous pile up right back down the tunnel, very few cars getting through; in other words, in molecular terms, a defect in chain termination.

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<Weatherall to camera>

Now, in the last few years, it's been possible to take each of these processes and take them apart in the test tube using reticulocytes and bone marrow from thalassaemic patients, and everybody is now agreed that in most forms of

thalassaemia, it's number 2 here, a deficiency of messenger RNA, which seems to be the most likely explanation. We don't know why the message is deficient. In some cases there may be no message at all and maybe a deletion of the gene. In others, there may be a defect in the normal processes of control of message production about which we know very little.

<Weatherall refers to previous diagram outlining defective pathway in haemoglobin H disease, and then over slide listing structure of abnormal haemoglobin>

Now, in the last year or two, we've re-looked at some patients with haemoglobin H disease. And if you remember, we said that haemoglobin H disease was a common form of thalassaemia which resulted from the inheritance of two types of thalassaemia gene. When we looked at some of these patients very closely, we found that some of them had a very small amount of an abnormal haemoglobin. When we looked at the structure of this haemoglobin, it was very unusual. What it shows, in fact, is that it has a normal alpha chain but the normal alpha chain ends at position 141 as tyrosine. This alpha chain, however, in addition to this has an extra 31 amino acids tacked on the end as shown here. We call this haemoglobin variant, haemoglobin Constant Spring, or haemoglobin CS, because it was found in a family from a suburb of Kingston, Jamaica, called Constant Spring. Now, as you see, haemoglobin CS ends in tyrosine, arginine. And the next amino acid along here is glutamine. Now, how can a haemoglobin molecule end up with 31 extra amino acids tacked on the end? In other words, it's a molecule with a tail, which presumably it will wag since this tail of extra material will be lying around on the surface of the molecule somewhere. Well, there are a variety of ways in which this could happen, but perhaps the simplest explanation, and what we now think is almost certainly so, is the following.

<Weatherall narrates over diagram illustrating globin chain synthesis>

Now, we've already said that protein synthesis starts with a specific 'start' word and then the ribosomes move along the message until a specific 'stop' word is reached

and then they drop and globin chain synthesis is complete. Now, we do know that at least one of the mammalian stop words is UAA. And what we could propose is that, like any other mutation, a single base has changed and U has changed to C. Well, we now have a situation where we've got CAA here, which happens to be the code word for glutamine, so instead of stopping making the chain, glutamine is put in here and then we must presume that this extra message material which is not normally translated, which in this particular case is translated until another stop word is reached. So we now end up with a globin chain, the Constant Spring chain with 141 amino acids and then glutamine and then 30 extra amino acids tacked on the end. This chain is produced at a reduced rate and if you inherit this gene with an alpha-thalassaemia gene, then you end up with a fairly severe form of haemoglobin H disease.

And we now know that this Constant Spring gene is extremely common all over Southeast Asia. In fact, it accounts for about half of all the cases of haemoglobin H disease in that part of the world. And it's now turning up in other populations, particularly in Greece.

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<Weatherall to camera and then over diagram showing mRNA to cDNA pathway>

So that's another molecular defect altogether as the basis of a thalassaemia disorder. Now, perhaps the last and most recent development in the thalassaemia field has been the attempt to make DNA based on messenger RNA from mammalian and, recently, human cells. As you know the central dogma of molecular biology is that DNA leads to RNA, leads to protein, but there are certain enzymes produced by organisms which will reverse this process. In other words, using messenger RNA as a template with a reverse transcriptase, an enzyme which will do this, it's possible to synthesise DNA.

Now, if you take this message and isolate it from mammalian cells and then use radioactive bases, you can make a form of DNA called cDNA, or complementary DNA, which in fact exactly mirrors the messenger RNA which you've used as a template. In other words, you can make a gene. Now, when you've got hot or radioactive DNA of this type, you've got a very sensitive probe for analysing the amount of RNA that you've got in any test solution because you can use the DNA, you can add your test material and then you can see how much DNA will hybridise with RNA and, therefore, determine how much RNA is present in the test sample. And using this type of probe, it's been possible to show that in certain forms of beta thalassaemia and haemoglobin H disease, there is a deficiency of either alpha or beta message.

<Weatherall to camera>

This work is in its very early stages at the moment, but if confirmed, we'll be further evidence that many of these disorders are due to a primary defect in messenger RNA production.

So let's just summarise what we've said about the thalassaemia disorders. We've said first of all that they are the commonest genetic disorders and cause a vast world health problem. They're heterogeneous. Within the general heading of alpha and beta thalassaemia, there are many genetic disorders, probably each with a different basis. They cause, in the homozygous states, severe chronic anaemia, and in the heterozygous state, a mild iron refractory anaemia. It's been possible using in vitro biosynthetic techniques to provide a very clear model of the pathophysiology of these disorders and we're now getting to a stage where we're starting to unravel some of the molecular bases for these conditions.

We know that many beta thalassaemias and some alpha thalassaemias result from a deficiency of messenger RNA for the affected chain and we also know that some of them result from the synthesis, or the inefficient synthesis, of haemoglobin molecules which are structurally abnormal. Where this is going to take us in the future in terms of management one doesn't know. One of the exciting prospects, of course, is that

one would be able to make alpha and beta genes using a reverse transcriptase, and the possibility of getting them into the cells is an exciting area in the general area of genetic surgery. From the other management point of view, we do know that these patients do much better if they produce large amounts of foetal haemoglobin, and a lot of work now is going into the elucidation of the mechanism of the switch from foetal to adult haemoglobin production. And so I think we have a very exciting area here where molecular biology and clinical medicine are meeting very closely and some of the work of the molecular biologist is starting to have some spillover into something useful in clinical medicine.

<End credits>