Wellcome Film Project

Disorders of the Skeletal System: Mucopolysaccharidoses, Part Two

Uptodate

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University of London Audio-Visual Centre, 1975.
Made for British Postgraduate Medical Federation.

Directed by Trevor A Scott.

Black-and-white
Duration: 00:42:50:17

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<**Opening titles**>

<**Leaback to camera** >

Hello. In the first of these two programmes on the mucopolysaccharidoses we heard an account by Dr Stephens of the classification of this kind of disease and something of the clinical symptoms involved. In the same programme we heard from Dr Sutcliffe, Dr Lake and Dr Whiteman, indications of how radiological, histological and biochemical investigations can help greatly in the diagnoses of these disorders.

We now wish to turn our attention to the consideration to what might be done for known sufferers of these diseases or known carriers. In order to do this, we would like first of all to hear from Dr Dean something of the very interesting recent experiments he's been carrying out in aspects of enzyme therapy. This will be followed by a short account by Dr Stephens of what can be done at present for such cases. Dr Whiteman will tell us of some of the interesting techniques that he has

been using in the investigation of prenatal diagnosis of the mucopolysaccharidoses in foetuses from parents who are known carriers of these diseases. Finally, I shall try to outline what I think are important human, scientific and medical aspects of work in this area and try to indicate where further effort is required.

First of all then may I introduce Dr Michael Dean.

<**Dean to camera**>

Thank you. The catabolism of mucopolysaccharides, or glycosaminoglycans as they are now called, is a complicated procedure during which each one of the sugar residues in turn must be cleaved from the end of the carbohydrate chain. This involves a precise ordered sequence of enzyme reactions and if any one of these enzymes is deficient, we then get an accumulation of partially degraded glycosaminoglycans within the tissues of the patient and it is these partially degraded fragments that are excreted in enormous excess in the patient's urine.

<**Dean narrates over diagram**>

We can illustrate this process by a diagrammatic representation of a portion of a dermatan or a heparan sulphate molecule which as you can see consists of alternating units of amino sugars represented by the rectangles and uronic acids represented by the triangles. The uronic acids are of 2 types – glucuronic acid which is not sulphated and iduronic acid which is invariably always sulphated. Before this terminal iduronic acid can be removed from the chain, it must first be desulphated; a different enzyme being required for this job than that which is necessary to remove the sulphate groups from the amino sugars. When this so-called sulfo-L-iduronate sulphatase enzyme is deficient then all of those saccharides that have a terminal sulphated iduronic acid accumulate giving rise to the defect that we know as Hunter syndrome. Again, if the sulphatase is normal but the iduronidase enzyme necessary to remove the terminal iduronic acid is sufficient, then we have the disease known as Hurler or Scheie syndrome.

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In order, therefore, to alleviate the symptoms of mucopolysaccharidosis we must remove these partially degraded glycosaminoglycan fragments.

<**Dean to camera**>

And the most direct way of doing this is to supplement the enzyme. Some information as to how this might be done by the research work of Dr Elizabeth Neufeld and her group at the NIH […]

< **Dean narrates over diagram**>

[…] who showed that fibroblasts grown from normal skin were able to correct the deficiency in patient cells when grown together in vitro. This correction was induced by lysosomal enzymes produced by normal cells and released into the culture medium where they were taken up by abnormal cells and localised within the lysosomes.

< **Dean to camera** >

Subsequent to this discovery there were a number of different attempts at enzyme replacement therapy. The first of which were those of Dr D Fioranty[?] and his co-workers at the University of Texas. He infused normal human plasma into a group of Hurler and Hunter patients and observed a number of changes taking place subsequent to treatment. These changes included increased excretion and breakdown of urinary glycosaminoglycans coupled with temporary clinical improvements. However, other workers who repeated this plasma infusion therapy were not able to produce the same results. Because of this, we attempted plasma infusion therapy in a group of Sanfilippo and Hunter patients and were able to demonstrate the following beneficial effects.

< **Dean narrates over table** >

First of all we observed a temporarily increased excretion of uronic acid subsequent to treatment, followed by changes in the size distribution of oligosaccharides eluted from Sephadex G-25, changes in sulphate uronic acid ratios of urinary glycosaminoglycans and finally some temporary clinical improvements which included increased mental awareness and a decrease in the patient's palpable liver size.

< **Dean to camera** >

These changes were not observed when we infused an isosmotic dextran solution into a control patient. However, the benefits from this plasma infusion therapy were only temporary and it was obvious that this method of treatment had severe limitations. The main limitations being the difficulties of continued long-term patient management and the possible dangers of immunogenic reactions ensuing from repeated infusion therapy.

Other groups of workers have attempted to prolong the activity of enzymes administered as a method of alleviating similar enzyme deficiency diseases by encapsulating these enzymes either in artificial lipid membranes, known as liposomes, or alternatively in erythrocytes which had been lyced by first subjecting them to hypertonic shock.

In both cases these workers were successful in prolonging enzyme activity but these procedures suffered from the drawbacks that first of all the erythrocytes or liposomes were targeted primarily into the patient's liver; and secondly, before this treatment could be undertaken it was first necessary to purify the enzyme responsible for the genetic deficiency and then to encapsulate it within the liposome or erythrocyte.

Because of these drawbacks we decided to try another way of producing corrective enzyme over a long period of time. And the method we chose was to try and implant normal fibroblasts into the patient. Our reasons for this approach were firstly that it had already been demonstrated by Dr Neufeld's group that normal fibroblasts were

capable of correcting abnormal fibroblasts in vitro, secondly we did not need to purify any enzyme before implantation took place and thirdly one normal fibroblast would contain all the enzymes necessary to correct any one of the different types of mucopolysaccharidosis.

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The patient we selected for our preliminary trials was a boy with Hunter syndrome and with this patient we took a […]

< **Dean narrates over illustration** >

[…] full skin thickness sample of 10cm from the arm of each of his parents and grafted this onto the patient's forearm. Because both donor types of skin were only partially HLA histocompatible, the patient was given steroid immunosuppressant therapy for 7 days before grafting and therapy was continued in decreasing doses throughout the period of observation.

As with the plasma infusion therapy, we observed a number of changes taking place in the urinary glycosaminoglycans subsequent to treatment. The first of these changes was a large increase in the total 24 hour excretion of uronic acid […]

< **Dean narrates over series of quickly changing graphs** >

[…] which reached a peak almost 3 times higher than pre-treatment levels, 35 days after grafting. Again, when the urinary oligosaccharides isolated before treatment were subjected to gel chromatography on Sephadex G25, we separated 3 components. The largest of which was eluted closely behind the void volume followed by an oligosaccharide of lower hydrodynamic size and a smaller tail fraction. Subsequent to grafting the relative proportions of the largest of these oligosaccharides steadily decreased and there was a corresponding increase in the lower molecular rate components, here illustrated by samples taken from various days following grafting.

As we can see here, the largest of these oligosaccharides contained about half of the total uronic acid eluted from the column prior to treatment. By the fifth day after grafting this had decreased to one third of the total and continued to decrease steadily amounting to only 14% of the total 24 hour uronic acid by day 422 following from treatment.

<**Dean to camera**>

We attributed this change in size distribution to increased breakdown of accumulated oligosaccharides induced by the enzyme supplied by the donor cells. Since we know that in Hunter syndrome the defect is a sulfo-L-iduronate sulphatase enzyme we would expect that if our hypothesis were correct, the sulphate content of the accumulated oligosaccharides should decrease as treatment progressed.

<**Dean narrates over graph**>

And this was in fact the case as we can see here by the figures given above. The sulphate uronic acid ratio of samples taken before treatment and immediately after treatment was in the region of 2.1 and this steadily fell to 1.1 some 235 days after treatment and by day 422 was down to a ratio of only 0.5:1.

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<**Dean to camera**>

Having obtained evidence for increased breakdown of glycosaminoglycans subsequent to treatment, we next looked for the appearance of the deficient enzyme in the patient's urine. We did this by preparing enzyme samples from [?] control people and from different samples of patient's urine by ammonium sulphate precipitation and checking these enzyme preparations for their ability to correct the metabolism of radioactive labelled fibroblasts in tissue culture.

<**Dean narrates over graph**>

Here we observed that in normal [?] controls we were able to isolate an average of 94 units of enzyme activity excreted per 24 hours – 1 unit of activity being that amount of enzyme which would induce half maximal correction of glycosaminoglycan metabolism. Whereas the patients' urine sample taken 270 days before treatment and 15 days after had very very little enzyme and the total amount of enzyme gradually began to increase reaching almost 5 units per 24 hours 237 days after treatment.

<**Dean to camera**>

Encouraged by the results of this type of therapy, we decided to take the treatment one step further and implanted 200,000,000 fibroblasts into a second patient with Hunter syndrome. These fibroblasts were donated by a normal sibling and had previously been shown to be HLA histocompatible and to correct sulphate metabolism in the patient's cells in vitro. Again, we observed a similar set of changes taking place subsequent to treatment as with the previous patient.

<**Dean narrates over series of graphs**>

total 24 hour excretion of uronic acid increased noticeably, the average level of excretion after treatment being some 50% higher than the average level achieved before treatment. Again when we looked at the elution profiles of the low molecular weight material, this time fractionated on Bio-Gel P.2, we observed that in samples taken before treatment, indicated by day 7 on the left hand panel, and 1 day after treatment we have a large accumulation of this oligosaccharide component here. 54 days and 58 days after treatment this large component had decreased in total amount and there was a corresponding increase in lower molecular weight material, again being indicative of breakdown occurring subsequent to treatment.

This is illustrated graphically here where we can see that the larger molecular weight component, fraction 1, accounted for between 65 and 70% of the total uronic acid

excreted prior to treatment and that this amount gradually fell and accounted for only 40-45% of the total uronic acid excreted some 2 or 3 months after fibroblasts had been implanted.

<**Dean to camera**>

Furthermore, since we know that the defect in Hunter syndrome is a defect in sulfo-L-iduronate sulphatase, the accumulated oligosaccharides before treatment ought to contain a large proportion of iduronic acid as well as a high sulphate content. And furthermore, the amount of this iduronic acid ought to decrease as treatment progressed. This, in fact, was born out by our analyses.

<**Dean narrates over graphs**>

When we looked at the iduronate content of the oligosaccharides isolated from the patient's urine before implantation, we found that they accounted for some 20-25% of the total uronic acid excreted. As treatment progressed, the relative amounts of iduronic acid in the large molecular weight material, fraction 1, decreased. And there was a corresponding increase in the iduronic content of the lower weight material again indicative of breakdown of oligosaccharides taking place subsequent to treatment.

<**Dean to camera**>

One final piece of evidence that correction had taken place was that in this patient, as in the skin graft patient, we observed a steady increase in sulfo-L-iduronate sulphatase enzyme being excreted in the urine.

So then, if I can summarise the position. Two cases of Hunter syndrome have been treated by implantation of fibroblasts, either in the form of a skin graft or a cell suspension, in an attempt to find a long-term source of corrective enzyme. In both of these patients we have noted a number of changes taking place subsequent to treatment. These changes have included an increased total mobilisation of

glycosaminoglycans, increased breakdown of glycosaminoglycans together with increases in sulphate contents and ratio of iduronic to uronic acids. In addition to this, in both patients we observed an increase in the amount of iduronic sulphatase enzyme excreted in the urine subsequent to treatment.

We therefore feel that fibroblast implantation therapy offers some possibility for long-term replacement therapy in cases of mucopolysaccharidosis. However, the method is still in very experimental stages at the moment, but if it should prove to be effective this one method of treatment should be useful not only for all different types of mucopolysaccharidosis but for all other types of genetic deficiency diseases involving an absence of lysosomal enzymes, provided that it can be demonstrated that the patients' cells are capable of taking up the corrected enzyme released by the host donor cells.

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And now I'd like to introduce you to Dr Rosemary Stephens who's going to talk about her experience of patient management. Dr Stephens.

<**Stephens to camera**>

Thank you. At present there is no effective therapy. Dr Michael Dean has discussed recent research which could be the first step towards real progress in treatment. Meanwhile the management of patients is mainly symptomatic and in trying to prevent the onset of complications.

Frequently treatments for middle ear and respiratory tract infections is required and sometimes adenoids have to be removed. The provision of a hearing aid may provide great improvement in a child's learning ability. Regular dental supervision is important. Most hernia should be removed even though there is a likelihood of recurrence. Mentally normal patients with severe corneal clouding may be helped by a corneal graft providing there is no underlying cause for the blindness such as optic atrophy or retinal degeneration – the results can be good even though the grafted

cornea may recur after 2 or 3 years. Surgical treatment to relieve carpal tunnel compression of the median nerve is sometimes necessary. Orthopaedic procedures to relieve limb deformities should be done with caution as the detrimental effects may outweigh the beneficial ones.

In Morquio disease, posterior fusion of the upper cervical spine can be a life-saving as cord compression due to acute atlanto subluxation is probably the commonest cause of death. A special splint or halo brace, such as that used by Copets[?] in the United States to immobilise the head and neck should be worn before, during and after the operation to avoid the dangers of flexing the head during intubation and of prolonged bed rest afterwards.

All patients tolerate general anaesthetics badly therefore only essential operations should be done and complications should always be anticipated. These are probably caused by mechanical obstruction of the upper airways due to anatomical abnormalities, excessive lymphoid tissue, a large tongue, short neck, profuse nasal secretions and by an unusual sensitivity to certain drugs.

An important part of management consists of giving continuing support and encouragement to the patient and his family. The impact of one of these progressive and crippling diseases and its genetic implications upon a family is great. Fortunately it is now possible to diagnose certain of these diseases early in pregnancy.

Dr Paul Whiteman will now discuss prenatal diagnosis. Dr Whiteman.

00:26:15:13

<**Whiteman to camera**>

Thank you. Prenatal diagnosis is a service that can be offered to some parents of patients with mucopolysaccharidosis. Ideally amniotic fluid should be obtained by amniocentesis at about the 14th week of pregnancy.

<**Whiteman narrates over table**>

Prenatal diagnosis of a mucopolysaccharidosis should include biochemical studies on cultured amniotic fluid cells this should include the demonstration of abnormal sulphate metabolism or the demonstration of a specific enzyme deficiency. Useful information can also be obtained from the analysis of amniotic fluid glycosaminoglycans.

<**Whiteman narrates over diagram**>

Fratantoni[?] and colleagues showed that normal amniotic fluid cells incorporated small amounts of sulpho-35 labelled sulphate when they were incubated in a medium containing this isotope although the rate of accumulation fell considerably after the first day or two. However, cultured amniotic fluid cells from pregnancies affected by Hurler and Hunter diseases continued to increase their intracellular pool of labelled sulphate and did so more rapidly than normal cells.

This has, up to now, been the mostly widely used method for the prenatal diagnosis of the mucopolysaccharidosis and has proved reliable in experienced hands.

<**Whiteman to camera**>

This method will probably be largely superseded by methods involving the demonstration of specific enzyme deficiencies in cultured amniotic fluid cells. However, only a few of the necessary substrates are readily available at the moment and if mistakes are to be avoided it is essential that such a service is carried out in units experienced in this field.

Hurler disease has been diagnosed prenatally by the demonstration of an iduronidase deficiency in cultured amniotic fluid cells using a phenyl iduronide substrate. Also Sanfilippo A disease had been diagnosed prenatally by the demonstration of a sulfamidase deficiency using a labelled heparin substrate.

Unfortunately there are some disadvantages associated with methods based on cell culture. At least 2 weeks and sometimes as long as 4 weeks is required to grow sufficient cells for the appropriate biochemical studies. The patient may therefore be more than 18 weeks pregnant before the results of such tests become available. Even worse, cell lines may fail to grow, become infected or die, necessitating a further amniocentesis and further delays. It is also obvious that special precautions are required for the transportation and storage of specimens involved in cell culture studies.

The glycosaminoglycan content of amniotic fluid has been abnormal in several pregnancies affected by mucopolysaccharidosis but the reliability of this finding for prenatal diagnosis has been seriously questioned particularly during early pregnancy. After the first trimester, some of the glycosaminoglycans in amniotic fluid probably arise from foetal urine.

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Specially devised microtechniques are required for the isolation and analysis of glycosaminoglycans in amniotic fluid and often as little as 2ml is available for analysis. Some of the methods used in the past may have failed to reveal an unusual glycosaminoglycan content when it has existed. It seems clear that the individual glycosaminoglycan components present in amniotic fluid must be measured with reasonable accuracy if this method of prenatal diagnosis is to be properly assessed. The measurement of the total glycosaminoglycan content alone is inadequate since considerable variations occur in unaffected pregnancies. It is important to obtain an answer to this problem since this method of prenatal diagnosis would have several advantages over methods involving cell culture, particularly the early availability of results after amniocentesis.

<**Whiteman narrates over diagrammatic graph**>

The major glycosaminoglycan components in normal amniotic fluid are hyaluronic acid and chondroitin sulphate. The hyaluronic acid content is highest in early pregnancy and gradually falls to become a minor component after the 20th week. At the 14th week, chondroitin sulphate is the minor component but becomes the major component by about the 20th week. The heparin sulphate content is negligible in early pregnancy but trace amounts may be found at later stages. Some workers have also noted the presence of small amounts of dermatan sulphate in normal amniotic fluid. Two methods at least are capable of measuring the abnormal glycosaminoglycan components in amniotic fluid and both have been used to predict the presence of an affected foetus. One involves the measurement of a portion of hyaluronidase-resistant glycosaminoglycans present in amniotic fluid. Dermatan sulphate and heparin sulphate are mostly resistant to digestion by bovine testicular hyaluronidase. Whereas chondroitin sulphate and hyaluronic acid are not.

<**Whiteman narrates over electrophoretogram, uses indication stick**>

Another method involves the separation of individual glycosaminoglycan components by 2-dimensional electrophoresis. After staining these electrophoretograms with alcian blue 8GX it is possible to measure the proportions of the individual glycosaminoglycan components present by eluting the complexes in a suitable reagent. This picture shows the separation of six standard glycosaminoglycan preparations by 2-dimensional electrophoresis. The first run is in a volatile [?] acetic acid buffer at pH 6, and the second run is in a barium acetate solution. Chondroitin sulphate, keratin sulphate, hyaluronic acid, dermatan sulphate, heparan sulphate and heparin <*points to diagram with indication stick*> are all reasonably well separated by this technique. Standard markers are included in the second run to aid identification.

<**Whiteman narrates over further electrophoretogram, uses indication stick**>

The next picture shows a typical separation of amniotic fluid glycosaminoglycans in a pregnancy affected by Hurler disease. An abnormally high content of dermatan sulphate and heparan sulphate is present. Dermatan sulphate and heparan sulphate <*points to diagram with indication stick*> in addition to the normal components of

chondroitin sulphate and hyaluronic acid. <*points to diagram with indication stick*> A control amniotic fluid is shown here for comparison. Chondroitin sulphate component and the hyaluronic acid component. <*points to diagram with indication stick*> Abnormal patterns of this type have been found as early as the 14th week in pregnancies affected by Hurler disease.

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<**Whiteman narrates over further electrophoretogram, uses indication stick**>

The next picture shows the pattern obtained in a pregnancy affected by Sanfilippo A disease and clearly demonstrates an abnormal heparan sulphate component at 25 weeks. This is not seen in the control amniotic fluid which again shows the chondroitin sulphate and hyaluronic acid components. Note that the hyaluronic acid component is in fact a minor component at this stage in both the normal and abnormal amniotic fluids. The chondroitin sulphate component can usefully act as an internal marker and the measurement of a dermatan to chondroitin sulphate ration and a heparan sulphate to chondroitin sulphate ratio is particularly helpful in distinguishing affected pregnancies from unaffected pregnancies.

<**Whiteman to camera**>

Obviously more observations are required to establish the reliability of these techniques for prenatal diagnosis, particularly in early pregnancy. It is advisable that such techniques be used in conjunction with studies on cultured amniotic fluid cells.

And now I'll hand you back to Dr Leaback.

<**Leaback to camera**>

One authority estimates the incidence of the mucopolysaccharidoses to be about 4 cases in 100,000 of the population. While this is probably an underestimate there is

no question of this class of diseases being a very common one. What then are the social, medical and scientific reasons for particular interest in this kind of disorder?

From this foregoing estimate we would expect between 2000 and 3000 individuals to be affected by the mucopolysaccharidoses in Britain at any one time, and since screening of the population for the heterozygotes is unlikely to ever take place on economic grounds, we must assume that fresh cases of these disorders must continue to present themselves.

It will be clear from the presentations of Dr Stephens that these disorders can cause considerable distress not only to the individuals affected but also to relatives. Therefore, approaches of the kind described by Dr Dean which seek to alleviate the distress in these conditions must be encouraged.

Of course, when a case of mucopolysaccharidosis has been diagnosed in a family, then the possibility of counselling is there and with the techniques described by Dr Whiteman for the prenatal diagnosis becoming available, the possibility of averting further incidence of the disease presents itself.

Now what have we learnt on the biochemical and metabolic side of these diseases?

<**Leaback narrates over diagram**>

Dr Dean represented the structures of dermatan and heparan sulphate as shown in this illustration. You will recall that dermatan and heparan sulphates are the materials which are most commonly involved in the mucopolysaccharidoses. These materials are fairly long chain polysaccharides which show considerable heterogeneity along the length of their chains.

<**Leaback to camera**>

I have chosen to represent this heterogeneity as in the next diagram […]

<**Leaback narrates over diagram**>

[…] where we see the repeating sugar sequences of dermatan sulphate. Various linkages in this structure are known to be affected in some of the mucopolysaccharidosis syndromes. For example, this sulphate linkage here is known to be affected in Maroteaux-Lamy syndrome and this uronic acid linkage here is known to be affected in Hurler syndrome.

Heparan sulphate whose structure we now see – these are the repeating sugar sequences in heparan sulphate – is even more complex than dermatan sulphate and a considerable number of syndromes have been related to various linkages in this structure. For example, Sanfilippo A and Sanfilippo B syndromes affect the sulfamido and glucosamino linkages here. The Scheie / Hurler syndromes affect this linkage here and the Hunter syndrome affects this sulfa-iduronide linkage here.

<**Leaback to camera**>

A few years ago we were not even sure whether dermatan sulphate or heparan sulphate were degraded at any significant rate in the tissues. Today we know that these materials must be degraded in a variety of tissues and that they are degraded by the sequential action of a number of specific hydrolases.

Much remains to be done on these mucopolysaccharidosis syndromes. For example, we need much better methods for study of the enzymes concerned. In a number of cases the causes of the various syndromes is still to be discovered. Dr Dean's approach to the enzyme therapy is encouraging but it is too early to say whether his approach will prevent the accumulation of these materials in all the tissues of the body. Nevertheless a start has been made towards alleviating the distress caused by these mucopolysaccharidoses and we have learnt a great deal in the course of this work.

<**End credits**>