The Organic acidaemias

The Scientific Basis of Medicine

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<Opening titles>

<Gilliland to camera>

Dr Gompertz is lecturer in biochemistry at the Royal Postgraduate Medical School and Consultant in Biochemical Genetics to Hammersmith Hospital. He previously worked as a lecturer in biochemistry at University College London. He has done much fundamental work in establishing a new generation of inborn errors of metabolism which form the subject of this discourse: the organic acidaemias. Dr David Gompertz.

<Gompertz to camera>

The organic acidaemias have been described rather loosely as a second generation of inborn errors of amino acid metabolism, however, this is a useful definition with which to start today. The first generation of inborn errors of amino acid metabolism

include those amino acidopathies in which there is an accumulation of the affected amino acid in body fluids, blood, neurone. And these amino acids can easily be picked up using amino acid screening techniques, and one laboratory can get up to a hundred thousand samples per year. These diseases obviously include such conditions as phenylketonuria and homocystinuria. However, the organic acidaemias, the second generation, are less easily detected. And this is probably best explained by turning to one of a major metabolic pathways associated with the production of the organic acidaemias. And this is shown in this chart here.

<Gompertz refers, with indicator stick, to charts displayed on wall and narrates over them, interspersed with talk to camera>

These are the degradation pathways of the three branched-chain amino acids: leucine, isoleucine and valine. As you can see there's a line across here, and any inborn error of an enzyme here or here <*indicates stages before coenzyme A*> is associated with accumulation of isoleucine or valine; whereas an inborn error below this line is not associated with a reverse accumulation of the offending amino acid.

Now, this is probably best described in terms of the metabolic reactions of these pathways. The first step is a transamination, producing the equivalent branched-chain α-keto acid. The second stage is an oxidative decarboxylation with a loss of CO_2 to give a short-chain organic acid usually attached to coenzyme A. This step here is irreversible, so if there is a block anywhere below this step one can't get a reverse accumulation back through the pathway to give a raised concentration of amino acids in plasma and urine. However, a block above this step, for instance, at either this level or at this level is associated with a raised level of amino acids.

Now let's look at the organic acidaemias associated with this pathway. The first one I'm going to put up is due to a block at this stage here: at the oxidative decarboxylation of branched-chain keto acids. And this is maple syrup urine disease. It's always been listed as one of the primary amino acidopathies, but perhaps we could consider it as a first organic acidaemia because it has much in common, both

biochemically and clinically, with the other organic acidaemias that have been described since.

The next inborn error to be described in these pathways was isovaleric acidaemia on the block here <*indicates next stage of isovaleryl-CoA action*>. This was first described by Tanaka and Isselbacher. And they described two children who presented with an offensive smell during episodes of intercurrent infection, and they also showed neurological changes. The smell was first identified by two industrial food and flavour chemists as being due to a volatile fatty acid – and this was identified by Tanaka, using gas chromatography, as isovaleric acid and the block being here.

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Now, perhaps, we can explain the episodes of acute acidosis and the remissions between in biochemical terms by moving across to this chart here. Here is an amplified portion of the leucine degradation pathway. Here is isovaleryl-coenzyme A with a block here. In the remission periods and also during the acute attacks, the isovaleryl-CoA is conjugated with glycine to give isovaleryl-glycine. This glycine conjugation mechanism is the same sort of mechanism as used to conjugate salicylates. However, when there is an increased catabolic load due to an intercurrent infection, the throughput of this pathway is too much for this step here <*indicates glycine to isovaleryl-glycine*>. This is overwhelmed and free isovaleric acid spills out into the body fluids to give the abnormal smell, 'the sweaty foot smell', and this gives the unfortunate name to the disease, the sweaty foot syndrome.

Now, the next inborn error of these pathways is best seen back on this chart here, and is of a block here <*indicates next stage of β-methyl-crotonyl-CoA action*>. The first child with this condition was described in Norway by Elgin[?] and his colleagues. And this child was excreting two abnormal metabolites in his urine. The abnormal metabolites are best discussed in relation to this chart here. Once more, we have an amplified part of the leucine degradation pathway. Here was the previous defect we discussed. Here is the metabolite β-methyl-crotonyl-CoA, the normal metabolite. And

here is the impaired enzyme. This metabolite <*indicates β-methyl-crotonyl-CoA*> can have two possible fates. It can either have water added across the double bond to give β-hydroxyisovaleric acid, which appears to be the major metabolite in most of the children who've been seen with this condition, however, the β-methyl-crotonyl-CoA can also be condensed with glycine to give β-methyl-crotonyl glycine. This is similar to condensation of is isovaleryl-CoA with glycine to give isovaleryl glycine and isovaleric acidaemia.

<*Gompertz walks back to first chart*> Now, the next inborn error of these pathways to be described has been described recently from Scriver's laboratory in Montreal, and the block has been tentatively put at this position here <*indicates below α-methyl acetoacetyl-CoA*>. He's described two families in which children excrete α-methyl β-hydroxybutyric acid and α-methyl acetoacetic acid. These are obviously analogues of the two normal ketone bodies. These metabolites were picked up and identified using gas chromatography and gas chromatography mass spectrometry.

As you can see from these pathways, they all end up with three small molecular weight intermediates: acetoacetate, acetyl-CoA and propionyl-CoA. Acetoacetate and acetyl-CoA can enter the Krebs tricarboxylic acid cycle through the acetyl-CoA pool. However, propionic acid in propionyl-CoA is a three-carbon acid and cannot enter the Krebs cycle until it has been converted into a four-carbon acid. This conversion is performed by the propionate methylmalonate pathway, and this is seen in this next board over here. Here is propionyl-CoA which as we saw in the previous chart comes from valine and isoleucine although there are other precursors of propionyl-CoA. Now, propionyl-CoA is converted by an enzyme called propionyl-CoA carboxylase to D-methylmalonyl-CoA. CO_2 is added here and this is a four carbon acid ready for entry into the Krebs tricarboxylic acid cycle. However, this has to be converted to its optical isomer L-methylmalonyl-CoA before it can be converted to a Krebs cycle intermediate. And this is performed by methylmalonyl-CoA racemase. This step here is a step that converts the methylmalonyl-CoA into succinyl-CoA which is a Krebs cycle intermediate, and this is performed by methylmalonyl-CoA mutase.

Inborn errors have been identified of all these three enzymes. The first one to be identified was methylmalonic acidaemia and this was described by Oberholzer and Levin in 1967. They described two children excreting vast amounts of methylmalonic acid in their urine. Now, clinical and experimental evidence have shown that this inborn error of metabolism made be due to a failure of a production of an enzyme, the mutase, or a failure of a production of a coenzyme for this step which is coenzyme B12.

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The next inborn error of metabolism of this pathway that could be described was propionic acidaemia. This is due to a defect of the enzyme at this stage here <*indicates* CO_2>. The first child was described by Hommes and his colleagues in 1968, and the second child presented at the Hammersmith Hospital two years later. These children have devastating metabolic acidoses with a plasma propionate of a thousand time normal – 5 millimolar. And they are untreatable with this form of presentation and die within the first fortnight of life.

In the child that we investigated, after death a portion of his liver was removed, mitochondria were made, and the enzyme here <*indicates* CO_2> was assayed and was shown to be defective, and this identified the enzyme block at this step here. More recently a child has been described with methylmalonic acid urea and it appears that the defect may well be at the racemase stage. So, we have the three inborn errors of this pathway <*indicates* CO_2> giving rise to propionic acidaemia, or these two <*indicates racemase and mutase*> to methylmalonic acidaemia.

Now, after describing these organic acidaemias, let us turn to the presentations.

<Gompertz narrates over slide>

There are really three main types of presentation of the organic acidaemias. The first is an acute neonatal acidosis and ketosis. This has been described in propionic acidaemia, methylmalonic acidaemia and maple syrup urine disease. Within 2 to 4

days of life, the child develops a rapidly increasing metabolic acidosis and ketosis, uncontrollable with alkalis, and the blood pH falls to 6.9 to 7.0 in spite of alkali therapy. And frequently a child dies between 5 and 12 days of life unless a specific treatment can be instituted.

The second presentation is of a child who fails to thrive during the first few months of life, is lethargic, fails to feed normally, vomits continuously and is hypotonic. Laboratory investigation shows a metabolic acidosis. Analysis of blood and urine shows the accumulation of abnormal organic acid. This is a common presentation of methylmalonic acidaemia.

The third type of presentation is of a child, who was previously clinically well, who starts vomiting and shows neurological changes, finally lapsing into coma in response to a minor respiratory tract infection. The children may have had several of these episodes previously and may have been shown to have a metabolic acidosis and ketosis during them. This presentation has been reported in nearly all the organic acidaemias.

<Gompertz to camera and then refers to photographs and narrates over them, interspersed with talk to camera>

I'd like to show you two types of examples of this type of presentation. This little girl over here, Anita, the child who had been physically well, who had normal intelligence, and she was later shown to have a variant of maple syrup urine disease. I'd like to contrast her with another child who was described by Scriver in Montreal with this defect here <*indicates next stage from α-methyl acetoacetyl-CoA*>. This child was excreting α-methyl β-hydroxybutyric acids and α-methyl acetoacetic acids.

Both of these children were well; they were both of normal intelligence and they both lived in agricultural areas. And the initial diagnosis when they were admitted was agricultural poisoning. However, gas chromatographic diagnosis showed them to be excreting the abnormal metabolites characteristic of the two inborn errors of metabolism they have. One of the most important alerting factors in these inborn

errors of metabolism is a child who responds clinically out of all proportion to the minor degree of intercurrent infection that he has. And an example of this is shown by this child over here, Mohammed. This child was admitted moribund with an upper lobe pneumonia. The paediatrician in charge, Dr Jon Scopes, was not satisfied that the pneumonia was sufficient to account for the child's poor clinical and neurological state, and he sent off urine for toxicological examination and also for us to look for organic acid excretion. Now, this child was found on gas chromatography to be excreting two abnormal metabolites in his urine, and these are shown on this chart here.

The child was excreting the metabolite associated with β-methyl-crotonyl-CoA carboxylase deficiency. He was excreting vast amounts of β-hydroxyisovaleric acid and lesser amounts of β-methyl-crotonyl glycine. So, here was a child who presented moribund due to a minor pneumonia but, in fact, was found to have an inborn error of metabolism.

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Now let us summarise again the three possible presentations of these organic acidaemias. There is a severe neonatal ketosis and acidosis. There's a failure to thrive with vomiting, continual metabolic acidosis and perhaps neurological symptoms. And then there is a clinically normal child who suddenly goes down when intercurrent infection shows neurological changes, finally lapsing into coma, and is found later on to have an inborn error of metabolism.

Now let's turn to the treatment of these conditions. Treatment in the first instance is intravenous bicarbonate to control the acidosis. Dextrose is given to maintain an adequate calorie intake, and protein is stopped immediately. One of the treatments that can be used in the acute situation is peritoneal dialysis and this is shown in this chart over here.

<Gompertz refers to charts and narrates over them, interspersed with talk to camera>

This is the chart of a child we have been studying in collaboration with Dr George Russell in Aberdeen. This child had propionic acidaemia. Although the diagnosis was made antenatally, the child was being maintained fairly successfully for first 8 days of life, and then she developed a septicaemia. Her plasma propionate shot up to disastrously high proportion. And although antibiotic treatment had perhaps started to cause a fall in the plasma propionate, peritoneal dialysis caused an immediate fall and brought the child back under biochemical control. The propionate was recovered in the dialysis fluid.

Now, there is another treatment which is both exciting from a theoretical and from a practical point of view. If one looks at this pathway here <*indicates coenzyme B12*>, if one considers children with methylmalonic acidaemia, Rosenberg found that some of these children would respond to massive doses of vitamin B12, a dose of up to 1 milligram of B12 intramuscularly per day. Now, these children probably have a defect in the biosynthesis of coenzyme B12 and this is best explained by looking at this chart over here.

Here is a pathway for the conversion of hydroxy-B12 through to Coenzyme B12, the coenzyme of methylmalonyl-CoA mutase. Hydroxy-B12 is converted enzymically at each of these steps through to coenzyme B12. Here are two reductive steps going from trivalent cobalt in B12 down to monovalent. ATP is added here <*indicates next step from monovalent cobalt*> to give coenzyme B12, the coenzyme of methylmalonyl-CoA mutase, and if it's a failure here in the synthesis of coenzyme B12, the enzyme will not work and the child will get methylmalonic aciduria. However, there are other children, who have been discovered who may well have defects earlier in this pathway of B12 interconversions, defects perhaps here or here <*indicates reductive steps of cobalt*>. These children can't make coenzyme B12; they also cannot make methyl-B12, the other coenzyme form of vitamin B12. Methyl-B12 is used in homocysteine methionine conversions. And these children, with a block at these earlier stages, present with both methylmalonic aciduria and homocystinaemia. So, we have the situation of inborn errors of coenzyme B12 interconversions here.

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The other vitamin-responsive organic acidaemias I want to talk about are the biotin responsive conditions and there are two. And let me point these out on the two major metabolic maps. First of all, biotin is a coenzyme for propionyl-CoA carboxylase as it is for all carboxylases. But, biotin is also the coenzyme for β-methyl-crotonyl-CoA carboxylase. We have demonstrated that both these inborn errors of metabolism can be responsive to massive doses of biotin.

Now, this can be illustrated by looking at two patients that we have treated with biotin. They were both admitted to Great Ormond Street under Dr David Hull. The first one: his progress is illustrated in this chart over here. This child had propionic acidaemia with a plasma propionate of over 60 times normal. He was put on a very low protein diet and was stabilised for several days before he was given an isoleucine load. Isoleucine is a precursor of propionyl-CoA. His plasma propionate rose and was maximal at 12 hours and was back to normal at 24 hours. This child went home for the weekend and came back and was started treatment with biotin at 5 milligrams b.d. This is a massive dose, perhaps a hundred times the amount needed to keep an adult in normal biotin status. He was put back on the low protein diet and he was re-challenged with an isoleucine load. His plasma propionate response to this load was completely modified. It was lower and it was only sustained for perhaps about 4 hours. During this initial load, before biotin therapy was started, the child showed the characteristic long-chain ketonuria of propionic acidaemia. There was no ketonuria during the second loading test.

Now, the other child we tried biotin on was a child with β-methyl-crotonyl-CoA carboxylase deficiency. And this is illustrated over here. This child was admitted at 5 months of age with continual vomiting and an acute metabolic acidosis, the base excess down here of -18. This child was taken off all protein whatsoever, and, as you can see, there was an improvement in his acid-base status. A small amount of protein was added to his diet to try and establish a stable baseline of excretion of these metabolites. Once this protein was added, the child immediately became

acidotic and ketotic again. And it was thought it was inadvisable to wait any longer before trying the effects of biotin therapy. Biotin was started here and there was an immediate correction of a metabolic acidosis with some overshoot. Bicarbonate therapy was stopped and the abnormal metabolite β-methyl-crotonyl glycine disappeared from this child's urine.

So, these are two biotin responsive inborn errors of metabolism. Now, what's the mechanism of biotin responsiveness? It is probably different from the mechanism of B12 responsiveness in methylmalonic acidaemia and it is probably best explained by looking at this chart over here. Here is propionyl-CoA carboxylase. The active form of the enzyme with biotin attached is called holo-propionyl-CoA carboxylase. The inactive enzyme without biotin is called the apoenzyme. Biotin is added to the inactive apoenzyme by another enzyme called apoenzyme-biotin ligase. So, there are two possibilities for biotin responsiveness. They may either be a failure of the enzyme that sticks biotin on to the apoenzyme protein. This enzyme <*indicates apoenzyme-biotin ligase*> may only work at very high biotin concentrations. On the other hand, this enzyme might be normal and there may well be a defect in the binding site on the apoenzyme that can't accept the biotin normally. So, these two conditions would be biochemically and genetically different, but both may well give rise to biotin responsive carboxylase defects.

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Now, having discussed biotin responsiveness, let us turn to the inheritance of these conditions. Most of these conditions are assumed to be autosomal recessive and there's a high degree of consanguinity in these families. And this can be illustrated by a family that we studied with propionic acidaemia. This is shown in this chart here. This is a first child we identified with this condition, the child in whom we established the metabolic block. There was a previous child who had died in the neonatal period, who had had his plasma lipids identified by gas chromatography, and this child had an abnormal lipid present found mainly in propionic acidaemia, allowing us to make the diagnosis in retrospect. This unfortunate lady became pregnant again and had twins, dizygotic – a boy and a girl, and both were affected. As you can see this is a

first cousin marriage between the parents, and here's another first cousin marriage with another neonatal death, although we can't describe this to propionic acidaemia. But, as you can see, this pattern of inheritance is consistent with an autosomal mode, autosomal recessive mode.

Now, after the inheritance pattern, can we make an antenatal diagnosis in any of these diseases? And the answer is probably, yes. It has been reported three times for methylmalonic acidaemia, and recently we have achieved an antenatal diagnosis of a child with propionic academia, the child which I described earlier that we treated with peritoneal dialysis. The inheritance pattern has been investigated in in vitro situations and, in fact, the heterozygote has been identified using fibroblasts in both propionic acidaemia and in lymphocyte preparations in maple syrup urine disease. Although in the other organic acidaemias, the heterozygote has not been identified as yet.

Now, what about the prognosis in these children? There are many of these children known who have varying degrees of brain damage. But, there are some children known who have completely normal intelligence. We do not know if acids such as propionic acid or methylmalonic acid are toxic, per se. It is possible that brain damage is caused during the periods of acute acidosis and ketosis when the children may be nearly moribund. Maybe there is a low cerebral perfusion during these periods of shock. On the other hand, the difference between the children with a normal intelligence and the children with brain damage may be at an entirely different biochemical level. There may well be phenotypic differences in the other enzymes' minor pathways that are involved in trying to get rid of the abnormal metabolites that are accumulating.

And now, Nyhan recently has reported a rather encouraging finding: a child with methylmalonic acidaemia. Treatment was started at the age of 14 months. By the time the child was 4½, there was a doubling in the child's DQ or IQ.

So, if I can summarise – first of all, we have children presenting with three different sorts of presentation: the acute severe neonatal acidosis and ketosis in the first week

or two of life; the failure to thrive in the first few months of life; or the intermittent episode that can present as late as 6 or 8 years of life. Secondly, the diagnosis is impossible to make simply on clinical grounds; it is probably best made using gas chromatography techniques, preferably with mass spectrometry available. Once the diagnosis has been made, there's a possibility of rational therapy. There's a possibility of both dietary modification and, in some cases, treatment with massive doses of a vitamin, giving rise to a coenzyme of the enzyme affected.

<End credits>