The genetics of metabolic disorders*

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THE first work on the genetics of metabolic disorders in Man was of course carried out by that great pioneer Sir Archibald Garrod at the turn of the present century (Garrod, 1902). From a study of the hereditary background of a small series of patients with a rare disorder, alcaptonuria, he discovered the first example of recessive Mendelian inheritance in Man and with remarkable foresight he suggested that this unusual condition was an example of 'chemical individuality'—an inborn alteration in the metabolism of tyrosine. He also suggested that other conditions such as albinism and cystinuria could be manifestations of inborn changes or defects in metabolism. He called them 'freaks' or 'sports' of metabolism. This was as long ago as 1902—almost 70 years ago.

A few years later in the Croonian lectures, Garrod (1908) developed the subject further and formulated a theory to account for the metabolic freaks or 'inborn errors of metabolism' as he now called them. His idea was that in each of these conditions there was a specific congenital enzyme defect at some point in the normal metabolic chain. This deficiency could lead either to an accumulation of intermediary metabolites which might be toxic or to the failure of adequate synthesis of some essential final product and thus in various ways result in pathological changes.

Arguing from the pedigree data he put forward the view that the congenital deficiency of a specific enzyme was due to the presence of an abnormal Mendelian factor or what we would now call a gene. Implicit in his argument was the idea that the normal gene directed the synthesis of the enzyme in the healthy individual. Thus, well before his time, Garrod formulated the now well recognized concept that genes exert their effects in an organism by directing the synthesis of enzymes and other proteins. Garrod also predicted that in due course other inherited metabolic disorders would be found and with a similar underlying basis.

Since that time, ample evidence has accumulated to support this view and numerous fresh examples of inborn errors have been described and the specific enzyme deficiencies recognized. In a recent publica-

tion (Harris, 1970) more than sixty such disorders were listed and further examples are regularly being reported.

It is interesting to note that there is no one particular aspect of metabolism which seems peculiarly susceptible to genetic variation of this kind. Nor is there any indication that enzymes catalysing particular types of reaction or utilizing specialized cofactors are more prone. On the one hand we have disorders like acatalasia in which there is a deficiency of catalase, the enzyme responsible for the simple reaction splitting hydrogen peroxide to water and oxygen, and on the other hand we have the glycogen diseases in which there are deficiencies of one or other of the enzymes involved in the synthesis or degradation of the rather complex molecule glycogen.

There is a great variation too in the clinical manifestations of these metabolic disorders ranging from the severe and incapacitating mental retardation which may be a feature of phenylketonuria, to the benign and symptomless passage of fructose in the urine in benign fructosuria. In other cases, like glucose-6-phosphate dehydrogenase deficiency, the metabolic disorder may come to light in the presence of specific unusual environmental factors such as particular foodstuffs in the diet or perhaps drugs given for therapeutic purposes. Such people may lead completely normal lives and be untroubled and unaware of their metabolic 'abnormality'. And the existence of this type of metabolic disorder is exactly in accord with an earlier prediction of Garrod that genetically determined differences in enzyme activity may occur without obvious symptoms and be detected only by specialized techniques.

Genetic heterogeneity with the 'inborn errors of metabolism'

A point of considerable interest which is beginning to emerge from the progressive analysis and detailed characterization of the inborn errors of metabolism is the remarkable degree of genetic heterogeneity which appears to exist. Quite often it has been found that what appeared to be a single disease entity, with perhaps rather variable clinical manifestations, is in reality a number of distinct conditions, each due to the deficiency of a different enzyme. This is

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true for example in the group of disorders affecting glycogen metabolism where there are several different forms of glycogen disease due to deficiencies in one or another of the enzymes involved in the synthesis or degradation of the macromolecule glycogen.

The point is also illustrated by the series of congenital haemolytic anaemias, which are clinically fairly homogeneous, but are now known to be due to specific deficiencies of different enzymes normally concerned with catalysing successive steps in red cell glycolysis (Valentine, 1968, Brain, 1971).

This is perhaps hardly surprising if one looks at the principal pathways of metabolism in red cells (Fig. 1). Glucose is the main source of energy for the erythrocytes and this energy is generated by glycolysis and also in the hexose monophosphate shunt pathway. There are no citric acid cycle enzymes present in the red cell or other alternative means of ATP synthesis on the required scale. Similarly there are no other effective ways of generating the reduced coenzymes NADH or NADPH. Thus, a deficiency of virtually any one of the enzymes in the glycolytic sequence or the hexose monophosphate shunt pathway might be expected to have the same effects—depletion of ATP and depletion of reduced coenzyme concentrations and hence premature lysis of the red cells. In other words, the clinical signs and symptoms of haemolytic anaemia would be expected and do indeed occur from deficiencies of red cell hexokinase, phosphohexose isomerase, glucose-6-phosphate dehydrogenase, pyruvate kinase and several other enzymes, due to the occurrence of mutant alleles at the various structural loci which determine these particular enzymes.

In addition to this kind of genetic heterogeneity within the inborn errors of metabolism, that is variation at separate gene loci leading to the same or similar clinical syndromes, another type of heterogeneity exists. This is allelic variation at the same genetic locus and this kind of heterogeneity may well prove to be the rule rather than exception for the genetically determined metabolic disorders. It seems that very often different cases of a particular metabolic disorder may be attributable to different abnormal alleles at the same gene locus, each allele causing the enzyme defect in its own characteristic manner. Sometimes the different patients may resemble each other clinically or even be indistinguishable, because the degree of enzyme deficiency is virtually the same in each of them, though it is produced in different ways. In other cases, variation in the clinical manifestation among patients in different families may occur and be found to be due to differences in the degree of enzyme deficiency brought about by the presence of different abnormal alleles.

A very striking example of this is provided by the

dozens of different variant forms of glucose-6-phosphate dehydrogenase which have now been reported (Motulsky & Yoshida, 1969; Kirkman, 1971). Each variant may be attributed to a different mutant allele at the same structural gene locus and appears to be a structurally different form of the enzyme protein with its own characteristic properties. In some cases the variants result in chronic haemolytic anaemia, which may be mild or very severe depending on the degree of enzyme deficiency. In other cases the variant may appear to be quite harmless until the affected individual comes into contact with particular drugs or foodstuffs, in which event haemolysis may occur. In yet other cases the variants seem to be quite innocuous.

For various reasons glucose-6-phosphate dehydrogenase has been studied more extensively than any other human enzyme and it may be supposed that the many variants which have been described are somewhat exceptional. However, this does not seem to be true. Many similar variants of other enzymes have been discovered and glucose-6-phosphate dehydrogenase does not appear to be a special case. If we look again (Fig. 1) at the main metabolic pathway of the red cell for example we can easily find several other good examples of multiple variants of particular enzymes. For instance, several different kinds of red cell hexokinase deficiency have been encountered in patients with congenital non-spherocytic haemolytic anaemia (Necheles, Rai & Cameron, 1970), and similar heterogeneity due to multiple allelism has been encountered in phosphohexose isomerase deficiency (Baughan *et al.*, 1968; Paglia *et al.*, 1969; Detter *et al.*, 1968) and also in pyruvate kinase deficiency (Valentine, 1968).

Practical considerations

The occurrence of several different mutant alleles at a given locus, each resulting in marked deficiency of the specific enzyme coded, means that certain ideas about the inborn errors of metabolism have to be altered. For example in the past it was generally assumed that individuals with such disorders showing the well known familial pattern of autosomal recessive inheritance were invariably homozygous for a single mutant allele. It now seems likely that very often such individuals may be heterozygous for two different mutant alleles, rather than homozygous for just one such allele. Indeed heterozygotes of this kind have already been identified in phosphohexose isomerase deficiency anaemia (Baughan *et al.*, 1968; Paglia *et al.*, 1969; Detter *et al.*, 1968), methaemoglobinemia due to diaphorase deficiency (Kaplan & Beutler, 1967; West *et al.*, 1967; Bloom & Zarkowsky, 1969; Hsieh & Jaffe, 1970), pyruvate kinase deficiency anaemia (Valentine, 1968) and

FIG. 1. Enzymes concerned in the metabolism of glucose in red cells (from Harris, 1970).

there are probably examples in phenylketonuria (Hsia, 1970). It seems reasonable to suggest that each new case of a particular metabolic disorder should be regarded as a distinct entity with its own charac-

teristic kind of enzyme defect, until it has been compared with previous cases.

This concept is important at the practical level when considering the type of treatment which should

be given to the patient. Each new patient should be considered individually and judgement about therapy should not be influenced by previous reports. This was recently highlighted in the description of a new case of maple syrup urine disease (Scriver *et al.*, 1971). Previous patients with this particular metabolic disorder have failed to respond to therapy with thiamine and treatment has been limited to dietary restriction of protein and certain amino acids. In the new case, however, thiamine was found to be very successful in the treatment of the disorder. This suggests that in this particular case of maple syrup urine disease the mutant allele or alleles present led to enzyme deficiency by altering the coenzyme binding properties of the enzyme protein, whereas in previous cases the mutant alleles presumably have led to different structural alterations in the enzyme protein which have not altered the coenzyme binding.

Normal variation

So far, I have mentioned only genetically determined enzyme deficiencies: that is enzyme deficiencies which were identified in the first instance because of some striking clinical syndrome or disturbance of metabolism—enzyme deficiencies which in many cases result in frank pathological effects. In other words, I have been discussing a highly selected group of what are usually quite rare mutant genes. But mutations probably occur more or less at random, and many of the mutations which occur are likely to produce only moderate or minor changes in enzyme function and the majority may have no effect at all. This raises the very fundamental question as to whether there are a large number of mutant genes in the general population which determine structurally different forms of particular enzyme proteins but which have no obvious pathological consequences. If so, can these genes, or rather, can the variant forms of the enzymes which they determine be demonstrated in the general population? If they can be demonstrated how commonly do they occur?

Now the approach to the study of enzymes in normal healthy individuals is of course quite different from that used in the investigation of the inborn errors. In the case of the inborn errors, the approach is essentially clinical, and attention is largely confined to the affected patients and their families; and quite sophisticated biochemical analyses can be carried out and quite a large range of different tissues can be studied in each case.

In assessing the general population the methods which can be used and the tissues which can be studied are much more limited. The general aim is to examine large numbers of individuals. Thus, the enzymes which are studied must be in readily

available tissues such as blood and the screening methods must be quick and easy. Then, if any variants are found family studies have to be done and also more detailed biochemical investigation can be carried out.

So in the first instance one wants to examine fairly large numbers of individuals and the screening method which has been most extensively used for this is electrophoresis. This method has the advantage of being a quick and reliable technique and also it is particularly powerful in detecting molecular charge differences such as those which are produced by point mutations involving charged basic or acidic amino acid residues. However, it is not very good for picking up point mutations which do not alter the molecular charge. Nevertheless, despite this limitation this method has led to the recognition of a great deal of enzyme variation among normal healthy individuals.

A good example is provided by some work on a particular acid phosphatase which occurs in human red cells. Using an electrophoretic method it was found that there are person-to-person differences in red cell acid phosphatase isozyme pattern in the general population (Hopkinson, Spencer & Harris, 1963; Hopkinson & Harris, 1969). Five types of pattern occur quite commonly in the English population. They are designated types A, BA, B, CA and CB and occur with frequencies of about 13, 43, 6, 3 and 5% respectively. A sixth type C also occurs but this is rather rare and is found only in about one in 600 individuals. These different types of acid phosphatase pattern are constant individual characteristics and family studies have shown that they are genetically determined. Thus, types A, B and C represent homozygotes and types BA, CA and CB represent heterozygotes for three alleles which occur with appreciable frequency in the English population.

This particular example of enzyme variation in normal healthy individuals appears to be fairly typical. Red cell acid phosphatase happened to be one of the first enzymes which we studied in our electrophoretic survey but since then several other examples of common enzyme variation, often referred to as polymorphism, have been encountered. For example we have examined about 30 different human enzymes in varying degrees of detail for genetic variation (Harris, 1969). About two-thirds of these enzymes exhibit multiple alleles—some of these variant alleles are relatively rare but others are, like the acid phosphatase alleles, relatively common and give rise to genetic polymorphism.

The variation of each enzyme appears to occur independently of all the others, thus, a very large number of different combinations of enzyme types are possible in the general population. Indeed it seems no exaggeration to say that each individual

will eventually be found to have his own unique enzyme constitution.

Conclusion

It is important I think to try and place the genetically determined metabolic disorders, that is the inborn errors of metabolism, in context against this background of 'normal' variation when it can then be seen that they are simply extreme examples of a kind of variation which is widespread throughout the species and indeed one of its fundamental characteristics.

Finally what can we say about the precise biological significance of this variability? Just how and when did the polymorphisms and the rare variants arise? How many fresh mutations occur in each generation? Are the polymorphisms balanced or transient? What effects do they have on our biological fitness? What selective factors in our environment are involved? Are some of the polymorphisms the legacy of previous evolutionary activity?

The answers to most of these questions are at the moment unknown. In certain cases, like the polymorphism of glucose-6-phosphate dehydrogenase, selective effects have been identified, but they are by no means clear-cut and the only thing one can really say at the present time is that we are what our enzymes make us, and that the genetically determined diversity in enzyme constitution which has been revealed in the last few years provides us with the biochemical basis of an explanation for the inherited differences that we can easily observe between different members of our own species: differences in physical and physiological characteristics, differences in mental capabilities, and of course differences in susceptibilities to various diseases, but the edges are rather blurred and the phenomenon of enzyme variation raises many interesting questions and problems for the future.

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