Genetics and clinical enzymology

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A large number of inherited disorders—the so-called inborn errors of metabolism—in which the characteristic metabolic and clinical abnormalities can be attributed to specific genetically determined deficiencies of particular enzymes, have now been identified; and new examples are currently appearing in the literature at a remarkable rate. Furthermore studies *in vitro* of the enzyme defect, as it occurs in erythrocytes, leucocytes, biopsy material from other tissues, or in fibroblasts grown in tissue culture, is already a critical diagnostic procedure for certain conditions. We may expect that this kind of investigation is likely to become of increasing importance in the future.

A point of some interest which is beginning to emerge from such studies is the remarkable degree of genetic heterogeneity which may be uncovered when sensitive analytical procedures are applied to appropriate material from patients who have a deficiency of the same specific enzyme, but come from different families or different populations and are therefore not closely related genetically. For example, even in a condition as rare as methaemoglobinemia due to a deficiency of methaemoglobin reductase several different sorts of enzyme defect, each apparently determined by a different abnormality of the gene, have now been identified in patients from different families (Kaplan and Beutler, 1967; West, Gompertz, Heuhns, Kessel, and Ashby, 1967; Bloom and Zarkowsky, 1969; Hsieh and Jaffe, 1970); a similar heterogeneity has been demonstrated among patients with various other enzyme deficiencies. Indeed it now seems very likely that such genetic heterogeneity will prove to be the rule rather than the exception.

It is perhaps useful therefore to consider the general nature of this phenomenon and some of its implications in chemical pathology.

Gene Mutations

We can presume that the abnormal genes which determine these various enzyme defects originally arose by spontaneous mutation in single individuals in previous generations, some perhaps relatively

recently others in the more remote past. The mutant alleles were subsequently transmitted from generation to generation so that they are now distributed apparently rather irregularly among living members of the species today. They are mostly very rare, but their relative incidence varies widely, and there appear to be quite marked differences in their detailed distribution from one human population to another.

Mutation of a gene must involve some alteration in the structure of its DNA. It has been shown that many, and perhaps the majority, of all spontaneous mutations involve no more than the change of one base for another in the DNA sequence of the several hundred or thousand bases which gives the gene its characteristic specificity. This implies that a very considerable number of different mutant alleles may in principle be generated by separate mutations in any given gene. Thus from a typical gene with a DNA sequence of 900 bases long, and coding for an enzyme protein containing a polypeptide sequence of 300 amino acids, as many as 2,700 different mutant alleles may be generated by separate mutations each causing the replacement of a single base, since each of the 900 bases may be altered to one of three others in different mutations.

The effects of such single base changes in the DNA of the gene on the synthesis of the corresponding enzyme may be very diverse.

From what is known about the nature of the genetic code and about the amino acid composition of proteins, it seems probable that in perhaps 20 to 25% of cases such a single base alteration will not result in any change at all in the structure of the corresponding enzyme. This is because of the 'degeneracy' of the code, which means that many such mutations will simply alter a codon specifying a particular amino acid to another specifying the same amino acid. Probably most, so-called synonymous mutations of this sort are without significant consequences. However it is possible that in some cases they may affect the rate of synthesis of the enzyme.

In about 2 to 6% of cases, a single base change will result in the alteration of a codon

specifying a particular amino acid at some point in the sequence to a so-called 'nonsense' codon which will specify chain termination. Such mutations may be expected to result in the synthesis of a shortened polypeptide chain which lacks the sequence of amino acids normally coded by the base sequence in the gene beyond the point where the mutation has occurred. In most cases such an abbreviated polypeptide chain is unlikely to constitute a viable and functional form of the enzyme protein, so the mutant allele will effectively result in loss of the specific enzyme activity.

In about 70 to 75% of cases, a single base change may be expected to result in a change of a codon specifying one amino acid to a codon specifying another, and thus lead to the synthesis of an altered protein which differs from the original by a substitution of one amino acid for another at a specific point in the amino-acid sequence. Since a typical polypeptide chain may contain a sequence of several hundred amino acids, and any one of these may be replaced by one of several others by this sort of mutation, it is apparent that a considerable variety of structurally altered forms of the enzyme protein may arise in this way. Furthermore, they may be expected to differ in their properties one from another according to the particular amino acid which has been substituted and the particular site in the protein in which the substitution has occurred. Some mutations of this sort may be expected to modify the molecular structure so as to lead to a gross reduction, or even complete loss of the specific enzyme activity. Many other amino acid substitutions however may not alter the properties of the enzyme protein in such a critical way, and consequently may cause only minor changes in its functional activity.

Finally, besides gene mutations that involve only the change of a single base in the DNA sequence, an indeterminate number of other kinds of mutation which have a more drastic effect on DNA structure are known to occur. They may involve deletion or duplication of part of the base sequence, and they may be expected to result in a corresponding alteration in the polypeptide structure which is coded. Again it is unlikely that in the majority of such cases a viable and functional protein will be produced, so this represents yet a further class of mutants which can result in loss of the specific enzyme activity.

Thus a great variety of different mutations can occur within the confines of a single gene, and a proportion of these will result in the appearance of mutant alleles which in different ways lead to a specific deficiency of the corresponding enzyme activity.

Enzyme Deficiencies

In the past it was often assumed that in the 'inborn errors of metabolism' the specific enzyme at fault was completely absent. However with the advent of more sensitive and precise methods for assaying the enzyme activity in different cases, it has become clear that this is often not the case. In an increasing number of examples it has been found that although the specific enzyme activity may be much reduced, some residual activity can still be detected, though it is evidently insufficient to allow normal metabolic function since the defect gives rise to clinical abnormality. This finding in a particular condition opens up the possibility of investigating the nature of the enzyme defect in more detail, for example, by kinetic or stability studies. Such studies have already proved to be very informative in a number of cases and are likely to be greatly extended in the future.

Broadly speaking one may distinguish between three different ways in which a particular mutant allele may result in a specific enzyme deficiency.

First, it may result in the synthesis of a structurally altered form of the enzyme protein with defective or modified catalytic properties. For example, a single amino acid substitution, if it affected the active centre of the protein, might alter the facility with which the substrate or coenzyme is bound to the protein during catalysis as to result in a marked modification of the kinetics of the catalytic process and hence in a loss of activity. In several inherited disorders such an alteration in the kinetics of the defective enzyme has indeed been demonstrated. Thus in one form of citrullinaemia Tedesco and Mellman (1967) showed that the Michaelis constant of argininosuccinate synthetase (EC 6.3.4.5), was more than 25 times greater than in normal controls. This effect appeared to be sufficient to account for the gross metabolic disturbance which was present. Markedly altered kinetics have also been demonstrated in some but not all cases of pyruvate kinase (EC 2.7.1.40) deficiency (Boivin and Galand, 1967; Paglia, Valentine, Baughan, Miller, Reed, and McIntyre, 1968), and in the so-called 'atypical' form of serum cholinesterase (EC 3.1.1.8) which results in excessive sensitivity to suxamethonium (Davies, Marton, and Kalow, 1960).

Secondly, the mutant gene may lead to the synthesis of a structurally altered protein whose catalytic properties are not necessarily altered, but whose inherent stability is much reduced because of the modification of its three-dimensional conformation. Such an enzyme protein would tend to be more rapidly broken down intracellularly, so that its half-life would be much shortened. This would mean that the actual quantity of the enzyme protein

present at any one time, and hence the level of activity observed, would be reduced. A particularly clear example of this kind of effect is provided by the mutants of the gene determining glucose 6-phosphate dehydrogenase (EC 1.1.1.49), which give rise to two of the well-known forms of deficiency of this enzyme (Yoshida, Stamatoyanopoulos, and Motulsky, 1967; Piomelli, Corash, Davenport, Miraglia, and Ambrosi, 1968). One of these is common in negro populations and is the cause of excessive sensitivity to various drugs such as primaquine and sulphonamides which produce acute haemolytic upsets in affected subjects. The other occurs commonly in people living in the Mediterranean area and the middle East, and is known particularly as the cause of favism, an acute haemolytic disease which develops when individuals with this form of enzyme deficiency eat fava beans. The half-life of normal glucose 6-phosphate dehydrogenase in the red cell has been shown to be about 62 days. The defective enzyme in the negro form of the abnormality is, however, only about 12 days, and that of the Mediterranean form is even more curtailed and is probably less than a day (Piomelli *et al.*, 1968). The effects of the rapid decay of this enzyme *in vivo* are of course reflected in the level of activity observed in the red cells. In the negro form this is usually about 15% of that normally found, and in the Mediterranean form it is usually only about 2 to 3% of the normal level.

In a number of other cases it also appears likely that molecular instability is the main reason for the enzyme deficiency. In general, in view of the many different single amino acid substitutions which might be expected to give rise in one way or another to a distortion of the normal three-dimensional conformation of an enzyme protein, it seems very probable that molecular instability will prove to be a common cause of enzyme deficiencies.

Finally, the gene mutation may result in a severe reduction in the actual rate of synthesis of the enzyme protein and thus give rise to the observed enzyme deficiency. So far no certain example of this has been demonstrated for any human enzyme, but by analogy with the defective synthesis of haemoglobin seen in the thalassaemia syndromes it may be expected to occur in some cases. At present convenient techniques for measuring the rates of synthesis of specific enzymes in cells obtained from single individuals are not available; no doubt they will become so in due course, and thus make possible this line of investigation.

It should be noted that these various ways in which an enzyme deficiency may be brought about by a single gene mutation are not necessarily mutually exclusive. A single amino acid substitution may, for example, modify the kinetics of the enzyme, and also

perhaps change its molecular stability or be associated with an altered rate of synthesis.

Multiple Alleles

We have seen that in theory a very large number of different mutant alleles varying widely in their functional effects may be generated by separate mutations of any given gene. An important feature of recent work is the increasing evidence which suggests that quite a large number of these possible mutants may at any one time occur among different members of the species, even though individually the great majority of them are extremely rare.

Perhaps the most striking example is provided by the 60 or more different variant forms of glucose 6-phosphate dehydrogenase which have now been reported (Kirkman, 1971). Each appears to represent a structurally distinct form of the enzyme protein with its own characteristic properties, and each is evidently attributable to a different mutant allele which has arisen at the same gene locus. These variants differ widely one from another in their clinical consequences. Some result in chronic haemolytic anaemia of varying degrees of severity, others appear to result in no ill effects under ordinary circumstances but render the affected individual exceptionally susceptible to haemolysis from particular drugs or other substances, and still others appear to be harmless.

The discovery of this great variety of mutant forms of this particular enzyme has been the consequence of the development of simple and sensitive screening procedures which can be widely applied both in studies on patients with haemolytic anaemia and in random population surveys, and also the working out of a variety of sensitive enzymological methods for discriminating between different variants using quite small amounts of material. Consequently the search for different variants of glucose 6-phosphate dehydrogenase has been carried out very much more extensively than for any other human enzyme. But many similar variants of other enzymes have been discovered in the course of more restricted surveys and there is no reason to think that the multiplicity of glucose 6-phosphate dehydrogenase variants that have been found is an unusual phenomenon.

Thus for any particular gene determining a specific enzyme there probably exist in human populations quite a number of different mutant alleles which may result in a marked deficiency of the enzyme and hence in a clinical disorder. Some of these alleles may each result in complete or virtually complete deficiency of the enzyme, and if so the secondary

metabolic and clinical consequences which ensue will probably be much the same in different patients who are homozygous for one or another of these alleles, or heterozygous for two of them. However, where such mutant alleles differ in the degree of enzyme deficiency which they produce, this may be reflected by variation from patient to patient in the character of the metabolic disorder and in its clinical severity.

The point is well illustrated by recent studies on deficiencies of the enzyme hypoxanthine phosphoribosyltransferase (EC 2.4.2.8) which result in overproduction of uric acid (Seegmiller, Rosenbloom and Kelley 1967; Kelley, Rosenbloom, Henderson, and Seegmiller, 1967; Kelley, Greene, Rosenbloom, Henderson, and Seegmiller, 1969). A virtually complete deficiency of this enzyme has been shown to be the cause of a complex and very severe neurological syndrome in children, usually referred to as the Lesch-Nyhan syndrome. It is characterized by mental retardation, spastic cerebral palsy, choreo-athetosis, and a serious behavioural disorder manifested by self-destructive biting. There is also marked hyperuricaemia with signs of gout. Subsequently it was found that certain adult patients with overproduction of uric acid and histories of acute gout or uric acid calculi but no obvious neurological abnormalities also showed a deficiency of the same enzyme. But in these cases, although the deficiency was marked, it was evidently incomplete since some residual activity could be demonstrated. Furthermore the level of activity in these patients, although it was much the same in affected individuals from the same family, varied significantly from family to family. In general the findings suggest that a number of distinct mutant alleles causing deficiencies of the enzyme of different severity occur. In those patients where the allele results in a complete or virtually complete deficiency, hyperuricaemia associated with severe neurological disorder develops. But where the allele results in a marked but incomplete deficiency, the resulting disease is much less severe and more variable.

Enzyme Variation in Normal Individuals

Besides the variants of a particular enzyme which result in a sufficiently severe degree of functional deficiency as to result in overt clinical consequences, it appears that many others occur which, while not having direct pathological effects, nevertheless cause an alteration in the enzyme activity in individuals who carry them. Sometimes such variants are relatively common in a population, and are a significant cause of enzyme variation among healthy individuals. This may become of interest in clinical

enzymology, if for no other reason than that in investigating the nature of some rare defect it is in general necessary to compare it with suitable normal controls.

A simple illustration of the kind of enzyme variation which may occur among apparently healthy individuals is provided by some work on a particular acid phosphatase (EC 3.1.3.2) which appears to be peculiar to the red cells, though its metabolic role is not as yet understood. It was found by electrophoretic studies that normal individuals may be classified into a series of distinct and common types according to the characteristics of the acid phosphatase in their red cells (Hopkinson, Spencer, and Harris, 1963). These differences turned out to be determined by three common alleles, which in European populations occur with frequencies of about 0.36, 0.60, and 0.04 respectively. Furthermore it was found that red cells from individuals of the different red cell acid phosphatase types differed quite significantly in the average level of activity of this enzyme (Spencer, Hopkinson, and Harris, 1964). Thus type B individuals who are homozygous for one of the alleles and constitute some 36% of the population, show on average about 50% greater activity than type A individuals who are homozygous for another allele and who constitute some 13% of the population. Heterozygous type BA individuals who constitute 43% of the population show intermediate levels of activity.

If the level of red cell acid phosphatase activity is determined in a randomly selected series of healthy individuals, a continuous unimodal distribution is obtained which is not unlike that obtained when many other enzymes are studied quantitatively in different people. However, in this case it is apparent that the overall distribution represents a series of separate but overlapping distributions corresponding to each of the qualitatively different types, which differ from one another in the characteristics of the enzyme proteins present.

Conclusion

Enough has perhaps been said to indicate some of the main implications for clinical enzymology of recent developments in genetics. It is worth noting that these developments are beginning to result in some interesting changes in some long-held basic concepts in this field. For example, in the past it was generally assumed that individuals with disorders showing the well known familial pattern of autosomal recessive inheritance were invariably homozygous for a single mutant allele. It now appears likely that very often they may be heterozygous for two different mutant alleles each producing its own

characteristic kind of enzyme defect (Harris, 1970).

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