Papers and Originals

Genetical Theory and the "Inborn Errors of Metabolism" *

HARRY HARRIS,† M.D., F.R.S.

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In recent years considerable advances have been made in our understanding of how the genetic constitution of an individual determines the enzymes and proteins that he makes; and how abnormal genes which have arisen from specific mutations in earlier generations may, because they result in the abnormal synthesis of a specific enzyme or protein, lead to the occurrence of a characteristic form of disease. The central idea is that the primary sequence of amino-acids in each of the distinctive polypeptide chains that may occur in an enzyme or protein is coded in the sequence of purine and pyramidine bases in the deoxyribonucleic acid (D.N.A.) of a corresponding gene. It can be conveniently expressed by the simple formula: one gene—one polypeptide chain; and it implies that there are very many so-called "structural" gene loci in the genetic constitution of each individual, and that the genes that are present determine in a very precise manner the structure and hence the properties and functional activities of the thousands of enzymes and proteins that he makes. Furthermore, a gene mutation can now be thought of as resulting in some kind of alteration of the coded sequence of bases in the D.N.A. of the particular gene, and this will in many cases be reflected by a corresponding change in the structure of the protein which the gene defines.

Classical Work of A. E. Garrod

It is in the context of this general theory that I would like to consider certain aspects of the group of rare diseases usually referred to as "inborn errors of metabolism." This term was first introduced into medicine by A. E. Garrod (1909) in his now classical Croonian lectures given to the Royal College of Physicians in 1908. Garrod's concept of the pathogenesis of these disorders was largely based on his studies of alkaptonuria, a rare condition characterized by the continuous and life-long excretion of homogentisic acid in the urine in large amounts. Arguing from the results of a variety of metabolic experiments on such patients, he suggested that homogentisic acid was in fact an intermediate in the normal metabolism of two aromatic amino-acids present in dietary and tissue proteins, phenylalanine and tyrosine, and that its excessive formation in alkaptonuria was most simply explained by a metabolic block due to a congenital deficiency of the specific enzyme normally concerned in its further degradation. This was a novel idea at the time, and difficult to pursue experimentally because enzymology was still in its infancy. Indeed, Garrod's hypothesis was not eventually confirmed until some 50 years later, when it became possible to assay the activities of the series of enzymes normally involved in the degradation of phenylalanine and tyrosine in liver from alkaptonuric subjects, and it was shown that there was indeed a specific deficiency of the critical enzyme, homogentisic acid oxidase (La Du *et al.*, 1958).

The other point that Garrod noted about alkaptonuria was its characteristic familial distribution. Two or more of a set of brothers and sisters might show the abnormality, but it was rarely seen in the parents, children, or other relatives of affected patients. Furthermore, there was clearly an increased incidence of cousin marriage among the parents of alkaptonurics. Garrod consulted Bateson, one of the earliest geneticists, who pointed out that the situation could be readily explained in terms of the then recently rediscovered laws of Mendel. The pedigrees were those to be expected if alkaptonuria was determined by a rare recessive Mendelian factor, or, as we should now say, gene. This was indeed the first example of so-called recessive inheritance to be recognized as such in man.

Thus Garrod interpreted alkaptonuria as being caused by the congenital deficiency of a specific enzyme due to the presence of an abnormal Mendelian factor, or gene. This interpretation carried an important implication for genetical theory. This was that the normal allele of the gene determining alkaptonuria must itself in some way be necessary for the formation of the enzyme in the normal organism. It was in fact the first clue to the now well-established generalization that genes exert their effects in the organism by directing the synthesis of enzymes and other proteins.

Garrod predicted that in due course other inherited diseases would be found to have a similar underlying basis, and he also suggested that other kinds of abnormalities, such as drug idiosyncrasies, might occur as a result of analogous enzyme defects. His predictions have been amply fulfilled, and a wide variety of different conditions have now been recognized in which the characteristic biochemical, pathological, and clinical disturbances can be confidently attributed to a primary genetically determined defect in the synthesis of a specific enzyme. In fact, more than 60 different conditions are now known in which the specific enzyme defect has been demonstrated directly by in-vitro assay. Furthermore, judging from the rate at which new examples are currently being reported in the literature, one cannot doubt that many more remain to be identified and characterized.

Enzyme-deficiency Disorders

The enzymes which have been found to be deficient in these different disorders are very diverse, and may affect almost any aspect of normal metabolism. Not surprisingly, the

* Leonard Parsons memorial lecture delivered at the University of Birmingham on 6 November 1969.

† Galton Professor of Human Genetics and Honorary Director M.R.C. Human Biochemical Genetics Unit, Galton Laboratory, University College, Wolfson House, Stephenson Way, London N.W.1.

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character and the severity of the clinical conditions which result also vary widely, both in their detailed characteristics and in their severity. They may for one reason or another turn up in virtually every branch and specialty in medicine, though, of course, they are of particular importance in paediatrics.

A point of some interest which has emerged from the progressive analysis and characterization of these disorders is that often what at first may have appeared to be a single disease entity, albeit with somewhat variable clinical manifestations, has proved, when the enzyme defect has been examined in different cases, to represent a number of distinct conditions, each due to a deficiency of a different enzyme. That this should be so is perhaps not very surprising. Loss of function of one or another of a series of enzymes which are involved in a sequence of reactions in a metabolic pathway, or which are associated together in a complex of physiological relationships, could well lead to the same or very similar end-results at the clinical level. The point is illustrated by the series of clinically not dissimilar haemolytic anaemias now known to be due to specific deficiencies of different enzymes normally concerned with catalysing successive steps in red cell glycolysis. The several forms of glycogen-storage diseases due to deficiencies of one or another of the enzymes normally concerned with the breakdown of glycogen illustrate the same general phenomenon. So also does the work currently being reported from several laboratories, on the differentiation and characterization of various types of mucopolysaccharidoses and gangliosidoses in terms of specific deficiencies of different lysosomal enzymes.

But I would like to suggest that the genetic heterogeneity of the "inborn errors of metabolism" is likely to prove much more extensive than even these examples would imply. In particular it seems probable that very often different cases of a particular metabolic disorder with a deficiency of the same specific enzyme may be attributable to different abnormal genes, each causing the enzyme defect in its own characteristic manner. Sometimes the several conditions may closely resemble each other clinically or even be indistinguishable, because the degree of enzyme deficiency present is much the same in each of them, though it is brought about in different ways. In other cases variation in clinical severity between patients from different families may be the consequence of differences in the degree of enzyme deficiency brought about by the presence of distinct abnormal genes.

Gene Mutations and Protein Structure

The abnormal genes that give rise to "inborn errors of metabolism" must be presumed to have arisen by spontaneous mutations which have occurred in individuals in earlier generations, and to have been subsequently transmitted from generation to generation, so that they are now somewhat haphazardly distributed among living members of the species. It is convenient therefore to start by considering the manner in which it is thought that different mutations of a single gene may affect the synthesis of a corresponding enzyme protein.

The fundamental molecular relationships between the structure of the D.N.A. which occurs in a particular gene and the structure of the protein which it determines—the so-called genetic code—are now well known. D.N.A. is composed of polynucleotide chains which contain four different sorts of purine and pyrimidine bases arranged in linear sequence. Proteins are composed of one or more polypeptide chains, each of which is made up of a long string of amino-acids which may be of 20 different kinds, and which are also arranged in a definite linear sequence. The essential point is that the precise sequence of bases in the D.N.A. defines the sequence of amino-acids in a corresponding polypeptide chain. Each amino-acid is specified in the D.N.A. sequence by a set of three bases. The two sequences are co-linear; that is to say, a base triplet or codon in the D.N.A. sequence specifying one amino-acid in the polypeptide chain is immediately followed by a base triplet specifying the next, and so on. The four bases in D.N.A. may occur in 64 different triplet sequences, and of these, 61 each specify 1 out of 20 amino-acids. Thus most amino-acids are coded by two or more different base triplets. The remaining three triplets—the so-called "nonsense" triplets—do not code for any amino-acid, but are apparently concerned in designating chain termination, that is the point in the synthesis of the polypeptide at which the end of the chain is reached.

There is now a very extensive body of evidence which indicates that many, and perhaps the majority, of all the spontaneous gene mutations which occur in nature involve no more than the change of one base for another at some point in the sequence of the several hundred or thousand bases contained in the D.N.A. of the particular gene. Furthermore, these may apparently occur more or less at random anywhere along the sequence. So it follows that many different alternative genes or alleles may in principle be generated by different mutations in a given gene. Thus from a typical gene with, say, 900 bases coding for a polypeptide chain 300 amino-acids long, as many as 2,700 different alleles might arise from different mutations causing the replacement of a single base, since each of the 900 bases may be altered to one of three others by different mutational events.

Single Base Changes

The effects of such single base changes in the D.N.A. of the gene on the synthesis of the corresponding protein may be very diverse.

In some cases, because the base change simply alters a triplet coding for one particular amino-acid to another triplet coding for the same amino-acid, no alteration in the structure of the protein will occur at all. From what is known about the nature of the genetic code and the amino-acid composition of proteins, it seems likely that some 20 to 25% of all possible single base changes are of this type. Probably most such mutations are without significant consequences. It has been suggested, however, that occasionally they may affect the rate of synthesis of the protein concerned, and so alter the quantity that is formed. There is as yet very little direct evidence on this point.

In about 70 to 75% of cases a single base change may be expected to result in a change of a triplet coding for one amino-acid to a triplet coding for 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 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 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 at which the substitution has occurred. Some mutations of this sort may be expected in the case of enzyme proteins to so modify the molecular structure as to lead to a gross reduction, or even complete loss, of the specific enzyme activity. This, of course, is precisely the situation characteristically found in "inborn errors of metabolism." There are, however, likely to be many other amino-acid substitutions which do not alter the properties of the

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enzyme protein in such a critical way, and consequently cause little or no change in its functional activity.

In addition, one may expect that some 2 to 4% of all the possible mutations involving single base changes will have quite different effects on protein structure. These are the cases where the base change results in the alteration of a triplet coding for a particular amino-acid at some point in the sequence to one coding for chain termination. Such a mutation may be expected to result in the synthesis of a shortened polypeptide 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 is unlikely to result in the appearance of a viable and functional form of the protein, and obviously such mutations represent yet another possible cause of "inborn errors of metabolism."

Besides gene mutations that involve only the change of a single base in the D.N.A. sequence, other kinds of mutations which have a more drastic effect on D.N.A. structure are also known to occur. They may involve deletions, duplications, or other kinds of rearrangement of part of the base sequence and they may be expected to result in corresponding alterations in the polypeptide structure which is coded. Again, it is unlikely where these changes are at all extensive that a viable and functional protein will be produced. So this represents yet a further class of mutants which may give rise to "inborn errors of metabolism."

Thus in theory a very great variety of different mutant alleles may be generated by mutational events within any given gene, and each will in general be expected to have its own specific effects on the synthesis and structure of the corresponding enzyme. Furthermore, there is an increasing body of evidence which suggests that quite a large number of these possible mutant alleles may at any one time be present in different members of the species, even though individually most of them are extremely rare.

Haemoglobin Variants

The most direct evidence on this point is provided by the multiplicity of different variant forms of haemoglobin A which have now been identified, and which can be attributed to different mutant alleles of one or other of the genes that determine the alpha and beta polypeptide chains of this protein (Lehmann and Carrell, 1969). Nearly a hundred such variants have been characterized. The great majority differ from normal haemoglobin A by only a single amino-acid substitution, and each of these can be accounted for by a single base change in the D.N.A. of the corresponding gene. There are also a few haemoglobin variants in which an absence of one or several amino-acids from one of the chains occurs, and here a small deletion of a sequence of bases in the D.N.A. may be postulated.

Some of the haemoglobin variants were discovered as a result of the investigation of patients with particular kinds of haematological disease, which turned out to be a consequence of the specific haemoglobin abnormality. Others were found in the course of screening large numbers of randomly selected healthy individuals in different populations. Though the studies which have led to the discovery of these many haemoglobin variants have been very extensive, they clearly can have covered only a small fraction of all patients with diseases due to abnormalities in this protein, and the random populations surveyed can represent only a minute fraction of the total world population. Furthermore, the electrophoretic methods mainly used for the detection of variants in these studies are known to be capable of detecting only a proportion of all the possible variants that might occur. Nevertheless, many different variants have already been discovered, and judging from the current literature the rate of discovery of new examples is not yet slowing down. So it is likely that the different variants so far identified are only a small proportion of the total number that actually occur among living members of the species.

The effects of these different mutant alleles vary very widely, and depend on the specific alteration in the properties of the haemoglobin molecule which they produce (Perutz and Lehmann, 1968). Many appear to be relatively harmless, but others result in one way or another in clinical abnormality, and it is of interest to see how several quite distinct disease entities can result from different mutations in a single gene. Thus in the case of the gene which codes for the beta chain of haemoglobin, different alleles are the cause of sickle-cell disease, or of various other kinds of chronic haemolytic disorders, or of the syndrome usually referred to as congenital methaemoglobinaemia. Furthermore, one may note that what can appear clinically as a single kind of disorder may be very heterogeneous in its genetical basis. Thus in the last few years at least eight different mutations at the beta chain locus have been identified, each of which results in the synthesis of a haemoglobin molecule which is excessively unstable, and so undergoes denaturation in the red cell much more readily than the normal form (Lehmann and Carrell, 1969). This leads to a type of chronic haemolytic disease with characteristic red cell inclusion bodies (Heinz bodies). Though there is variation in severity of the condition from case to case, the syndrome might well be regarded by ordinary clinical and pathological criteria as a single disease entity.

There is no reason to think that the occurrence of the large array of different mutant alleles which affect haemoglobin is a phenomenon peculiar to this protein, and one may anticipate that essentially the same kind of thing occurs with other proteins, including enzyme proteins. Indeed, in recent years electrophoretic studies carried out on a variety of different enzymes in randomly selected individuals from different popu-
many inherited variants which are essentially analogous to the
several inherited variants which are essentially analogous to the numerous variants of haemoglobin discovered by the same method. From the extensive body of data which has accumulated one may reasonably infer that at virtually any gene locus coding for the structure of a particular enzyme a large number of different alleles generated by separate mutations probably exist in human populations, though most of these are very rare. Furthermore, though many of the variant molecular forms produced by these alleles are similar to the usual form of the enzyme in their functional properties, others may differ quite markedly.

Causes of Enzyme Deficiencies

Now we have said that the essential feature of the so-called "inborn errors of metabolism" is a specific deficiency of a particular enzyme activity, and in many cases it has been possible to demonstrate this deficiency by direct in-vitro assay. In general, this has involved measurement of the level of activity of the enzyme and related enzymes in tissue or blood samples, or in some cases in cells grown in tissue cultures.

But the level of activity of an enzyme as it is usually determined is clearly a complex function. In the first place it depends on the specific catalytic properties of the enzyme protein, which are determined by its three-dimensional molecular structure, and which in turn depends on the amino-acid sequence of its constituent polypeptide chains. Secondly, it depends on the quantity of the enzyme protein which is actually present. This is the resultant of the rates of two different

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and distinct processes: the rate at which it is being synthesized in the cells at the particular time, and the rate at which it is being broken down and denatured. Finally, the activity observed may also depend on a variety of other factors, such as the presence of particular activators, inhibitors, repressors, coenzymes, and so on.

Main Possibilities

Thus it is evident that a particular gene mutation may result in a reduction in the level of a particular type of enzyme activity in a variety of ways, and it is perhaps helpful to consider briefly some of the main possibilities.

In some cases the mutational change may lead to the synthesis of a structurally altered protein with defective or modified catalytic properties. A single amino-acid substitution, for example, if it affected the active centre of the protein, might, by altering the facility with which the substrate or coenzyme is bound to the protein during catalysis, result in a pronounced modification in the kinetics of the catalytic process and hence in loss of activity. In several "inborn errors" such an alteration in the kinetics of the defective enzyme has indeed been demonstrated. Thus in one form of citrullinaemia, Tedesco and Mellman (1967) have shown that the Michaelis constant of arginino-succinate synthetase, whose deficient activity is the immediate cause of the metabolic disturbance, is grossly raised. This effect, which is presumably a consequence of a specific structural alteration in the enzyme protein, is apparently sufficient to account for the metabolic disturbances seen in the condition. A catalytically abnormal enzyme has also been demonstrated in some but not in other cases of pyruvic kinase deficiency causing haemolytic anaemia (Boivin and Galand, 1967; Paglia *et al.*, 1968). Several other examples of the same type of phenomenon have also been found in other metabolic disorders.

In other cases 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 in vivo, 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. Indeed, it might be so low as to be undetectable.

A particularly clear example of this kind of effect is provided by the mutants of the gene determining glucose-6-phosphate dehydrogenase, which give rise to two of the well-known forms of G-6-PD deficiency (Yoshida *et al.*, 1967; Piomelli *et al.*, 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 the acute haemolytic disease favism, which develops when individuals with this form of enzyme deficiency eat fava beans. The half-life of normal G-6-PD 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 13 days, and that of the Mediterranean form is even more curtailed (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 case of 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.

These "unstable" molecular variants of G-6-PD can be regarded as analogous to the different "unstable" haemoglobins mentioned earlier. In general, in view of the many different ways such molecular instability may be brought about, it would not be surprising if it represents a common cause of different enzyme deficiencies seen in inborn errors of metabolism.

Further Possibility

A further possibility is that the mutation may result in a complete failure to synthesize the specific enzyme protein at all, or in a severe reduction in the rate of its synthesis so that very little is actually present at any one time. Complete failure to form the enzyme might result, for example, from deletion of parts of the D.N.A. sequence of the gene, or it could arise from a single base change causing premature chain termination. A gross reduction in rate of synthesis may also possibly occur from other kinds of mutation in the gene (or genes) which define the amino-acid sequence of the enzyme protein. But it could also perhaps be the consequence of mutations of other genes, so-called regulator and operator genes, which may normally be involved in controlling the rate of synthesis of the enzyme.

Unfortunately, not much is yet known about the manner in which genetic control of rates of protein synthesis is exercised in higher organisms, largely because of the lack of satisfactory methods for studying this kind of phenomenon. Nevertheless, it remains quite possible that at least some of the enzyme deficiencies found in the inborn errors of metabolism may be due to mutations in genes normally concerned in some way in regulating rates of synthesis of specific enzymes.

Genetical Heterogeneity

Thus, many different mutant alleles can in different ways result in a complete loss or gross reduction of an enzyme's activity. But, of course, however the deficiency of a particular enzyme activity comes about, provided it is of a sufficient degree, its effect in terms of the metabolic disturbance that ensues and the clinical consequences observed may be much the same in different cases. Thus one can expect that many of the diseases which have been shown to be due to a complete or nearly complete deficiency of a specific enzyme, and which are usually attributed to the defects of a single abnormal gene, are likely in reality to be genetically heterogenous.

It is quite possible, for example, that the loss of activity of the enzyme phenylalanine hydroxylase in phenylketonuria could be the consequence of a number of quite different mutations in the gene that codes for the amino-acid sequence of a polypeptide chain in this enzyme protein. If so, patients with phenylketonuria may include not only individuals homozygous for one or other of these genes but also individuals heterozygous for two of them.

By the same token one may expect that these various mutations will not necessarily all have the same degree of effect on the activity of the enzyme. Some may result in a complete loss of activity, while in others the deficiency may be incomplete and the amount of residual activity remaining may vary considerably from one mutant to another. Such differences in residual activity can be expected to influence the degree of metabolic upset which is produced, and hence the clinical findings. Some evidence for this has indeed been obtained in phenylketonuria (Auerbach *et al.*, 1967; Justice *et al.*, 1967; Woolf *et al.*, 1967). But the matter is difficult to elucidate fully because it requires direct assay and characterization of the enzyme deficiency in an extensive series of cases, and this can be carried out only with tissue obtained from liver biopsy.

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The point is well illustrated, however, by some recent work on the enzyme hypoxanthine-guanine phosphoribosyl transferase, which in the normal individual is concerned with the regulation of uric acid formation. This enzyme can be readily studied because it happens to occur in red cells. It is also particularly convenient to study because it turns out to be determined by a gene located on the X chromosome, and since males having only one X chromosome carry only one of the possible alleles, examination of the effects of different alleles can be made separately.

Lesch and Nyhan (1964) described a complex and very severe neurological syndrome in children which is characterized by mental retardation, spastic cerebral palsy, choreoathetosis, and a curious behavioural disorder manifested by self-destructive biting. This was found to be associated with hyperuricaemia due to overproduction of uric acid, and in some cases uric acid renal calculi are present with signs of gout. The condition turned out to arise from what appeared to be an apparently complete and quite specific deficiency of hypoxanthine-guanine phosphoribosyl transferase (Seegmiller *et al.*, 1967).

Because of its relationship to uric acid formation, the enzyme was then examined in a number of adult patients with typical clinical histories of acute gouty arthritis or uric acid nephrolithiasis and with hyperuricaemia shown to be due to uric acid overproduction. Among this series of patients some were found to have quite pronounced deficiencies of the enzyme, though not as severe as in the Lesch–Nyhan syndrome (Kelley *et al.*, 1967; Kelley, 1968). The results suggest that several quite distinct types of defect may occur and give rise to hyperuricaemia, but in any one family the same specific abnormality is present among the affected individuals. Thus in one family the affected individuals showed levels of activity as measured in red cells of only about 1% of the normal when either hypoxanthine or guanine was used as substrate. Furthermore, the enzyme protein was found to be significantly more thermolabile than the normal enzyme. In another family the affected individuals also showed reduced enzyme activity, but the reduction was much more pronounced with guanine as substrate than with hypoxanthine, a finding which indicates an altered pattern of substrate specificity. Furthermore, here the enzyme protein appeared to be less thermolabile than the normal one.

Distinct Abnormal Genes

One may infer that several distinct abnormal genes, each producing a structurally altered form of this enzyme protein with abnormal properties, separately occur in the various families. They each evidently lead to pronounced enzyme deficiency resulting in overproduction of uric acid with hyperuricaemia, and the clinical consequences (gout and nephrolithiasis) are very similar in the different cases. But the clinical picture contrasts very strikingly with that of the Lesch–Nyhan syndrome, which is apparently the consequence of a complete or almost complete deficiency of the enzyme. Here a very severe neurological disorder manifesting in childhood occurs.

Variations of this sort in the degree of enzyme deficiency resulting from different mutations at a single gene locus are likely to be a very general phenomenon. They may well be the reason for the variation in clinical manifestation and in severity that is often observed among those patients who have a particular sort of metabolic abnormality.

The possible occurrence of genetic heterogeneity is of obvious interest if one is trying to understand the fundamental nature of a particular disease. But awareness of this possibility may also, at least in certain cases, be of considerable therapeutic importance. This point is illustrated by some recent work on a rare disorder known as methylmalonic aciduria (Oberholzer *et al.*, 1967; Rosenberg *et al.*, 1968; Morrow *et al.*, 1969). The condition is characterized by the excretion of large amounts of methylmalonic acid in the urine. The affected children fail to thrive and show pronounced ketoacidosis. They may be severely retarded, and many die in early life. The abnormality has been shown in several cases to be due to a specific deficiency of the enzyme methylmalonyl-Co A carbonyl mutase, which catalyses one step in the reaction sequence by which propionate is converted to succinate. The enzyme requires for its normal activity the cobamide coenzyme form of vitamin B_{12}.

A virtually complete deficiency of the enzyme activity was found in liver samples from a series of such patients, and in several of the cases no increase in activity was obtained when a large excess of the cobamide coenzyme was added to the reaction mixture. But there was also another type of case in which the addition of the coenzyme in excess led to the appearance of the enzyme activity in essentially normal amounts (Morrow *et al.*, 1969). Furthermore, in the latter type of case a remarkable therapeutic response can be obtained by the administration of very large doses of vitamin B_{12} to the patient, whereas no clinical improvement results in the others.

One possibility is that in the vitamin-B_{12}-responsive cases some structural defect in the enzyme makes it very inefficient in binding the cobamide coenzyme, so that to obtain adequate functional activity a very high concentration of the coenzyme is required. It is, however, also possible that the primary defect might lie in the formation or destruction of the coenzyme itself, though it should be noted that no other signs of vitamin-B_{12} deficiency are evident. The patients who are quite unresponsive to vitamin-B_{12} evidently have some other defect of the enzyme which is presumably the consequence of a quite different mutation.

Thus one cannot conclude that because certain patients with a particular sort of inherited metabolic disorder have proved unresponsive to a specific line of treatment this will necessarily be so in other cases. One sometimes finds in the literature apparently contradictory reports about a given disorder. An example is homocystinuria, in which some workers—for example, Barber and Spaeth (1967)—reported remarkable improvement of the biochemical disturbance following the administration of vitamin B_6, whereas others have found no such response (Perry, 1967). This might well arise if the different patients carried different abnormal alleles which result in a deficiency of the same enzyme but in different ways.

Random Genetic Drift

There is one other aspect of the genetical heterogeneity of these disorders to which I would like to draw attention. This concerns the distribution and relative incidence in different human populations of the abnormal genes that cause them.

Most of the inborn errors of metabolism appear to be inherited as autosomal recessive characteristics. This implies that though the diseases may each in themselves be very rare, the abnormal genes that cause them must be much more widely distributed in the population, since the great majority will in fact occur in healthy heterozygous carriers who have received an abnormal gene from one parent and a functionally normal allele from the other. Thus, for a disease which occurs in say 1 in 100,000 births, one may estimate that as many as 1 in 160 individuals in the population are likely to be heterozygous carriers.

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Now the abnormal genes present in individual members of the population today must have originated by spontaneous mutations in ancestors in the more or less remote past. But the chance that any particular abnormal gene arising from a fresh mutation will persist in a population and eventually achieve any appreciable incidence is small. The new gene will on average be transmitted to only half the children of the individual who first receives it. So there is a distinct chance that it will not be transmitted to the next generation, and the chances of it being lost are compounded in successive generations. Thus it has been shown (Fisher, 1930) that in a stable population where each pair of parents is on average replaced by two children who become parents of the next generation, the probability because of chance effects alone that a new mutant will be present after say 15 generations is only about 1 in 9. The odds in favour of the mutant persisting are greater if the population happens to be increasing in numbers when it appears, and less if the population is declining. But in general the majority of new mutant genes produced by fresh mutations are likely to be eliminated in the course of the next 10 to 20 generations in a more or less random manner.

Though the majority of new mutants will be lost very quickly some will persist for many generations, and occasional ones purely by chance will tend to spread. Thus in any given population there are always likely to be quite a number of abnormal genes whose incidence and distribution reflects the result over a long period of time of the continuous generation of new genes by mutation, and the accidental and haphazard loss of most of them by chance.

Furthermore, since, as we have seen, a great variety of alleles, each capable of producing an enzyme defect in different ways may be produced by separate mutational events at a single gene locus, a number of different ones may well be present in any large interbreeding population, and the actual ones that occur, and their relative incidence, are likely to vary considerably from one population to another according to their different ancestral histories.

Natural Selection

Superimposed on these essentially random phenomena are, of course, the effects of natural selection, which tends in general to limit the overall incidence of deleterious mutants, because individuals carrying the abnormal genes in particular combinations tend on average to contribute fewer offspring than other people to the next generation, or perhaps none at all. Many of the patients with inborn errors of metabolism, for example, die in early life, and others, because of the severe degree of physical or mental handicap which the disease imposes, are less likely than other people to survive to adult life or to become parents of the next generation. Thus natural selection tends to prevent perpetuation of the particular genes they carry. But, of course, most of the abnormal genes that cause these diseases occur in the population, in healthy heterozygous carriers. They are consequently largely protected from the operation of natural selection, and there is plenty of scope for chance effects, or what is often referred to as random genetic drift, to influence their incidence and distribution.

The general theory, which I have outlined only very roughly, suggests not only that more than one different abnormal allele producing a specific enzyme deficiency may occur among the members of any given population, but that there are likely to be considerable differences both in the actual alleles that are present and in their relative frequencies from population to population with different ancestries. So the incidence of particular inborn errors of metabolism, and the detailed character of the specific enzyme deficiencies which cause them, may be expected to vary from one human ethnic group to another, and within ethnic groups from one population or community to another.

"Founder Effect"

One somewhat special illustration of random genetic drift, which is of interest because of the rather dramatic results it may produce, is often referred to as the "founder effect." This term is used to refer to situations where a relatively small number of individuals from one population have migrated elsewhere to found a new community, which has subsequently increased considerably in numbers, more or less in isolation. If by chance one of the founder members happened to be a heterozygous carrier of a particular rare mutant gene, then this might quite fortuitously come to have an extremely high frequency among the descendants.

A particularly striking example is the remarkable incidence of the disease known as hereditary tyrosinaemia found in an isolated French-Canadian population living in Northern Quebec (Laberge, 1969). The disease, which is exceedingly rare in other parts of the world, is due to a specific deficiency of the enzyme *p*-hydroxyphenylpyruvic acid oxidase, and results in a severe form of infantile cirrhosis of the liver, often fatal in early life. As many as 1 in every 25 members of this population are heterozygous carriers of the particular abnormal gene, and pedigree studies suggest that they are all descendants of one of the founder members of the population who immigrated from France in the seventeenth century and who presumably was himself heterozygous for the gene.

The unusual incidence of a gene which determines Tay–Sachs disease among Jewish patients whose ancestors mainly came from Southern Lithuania or North-East Poland is probably to be accounted for in the same way (Livingstone, 1969), and a variety of other examples involving different communities and different abnormalities have been reported. They give us some insight into the fortuitous and haphazard way particular deleterious genes may be perpetuated and spread, and their closer study may help us to understand more clearly the irregular distribution of other inborn errors in much larger populations where there are, or have been in the past, limitations in various degrees on interbreeding between different communities within the total population.

Conclusion

At the beginning I suggested that Garrod's classical work on the inborn errors of metabolism was a landmark in medicine because it delineated a new class of diseases with a characteristic type of pathogenesis; and that it was a landmark in genetics because it provided the first clue to the manner by which genetical determinants exert their effects in the individual organism. This was a quite remarkable outcome from the study of a very few extremely rare diseases.

Since Garrod's time many more "inborn errors" have been identified, and a great deal has been discovered about them. But in considering the position today it is perhaps important to emphasize how ignorant we still remain about many aspects of these disorders. In particular we know very little, in the great majority of cases, about the precise nature of the specific enzyme deficiencies in different patients, and at best we have only a very rough idea about the incidence and distribution in different human populations of the abnormal genes that cause them.

So the "inborn errors of metabolism" are likely to remain a very active field of research for many years to come. And perhaps the main justification for the kind of theoretical discussion in which I have indulged is that it may be useful in directing attention to some of the questions that need to be answered in specific cases. Also it may perhaps serve to emphasize, once again, how the precise and detailed study of even exceedingly rare diseases may still provide information of very wide and general relevance to human biology and medicine.

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Speculation on Pathogenesis in Death from Respiratory Syncytial Virus Infection

P. S. GARDNER,* M.D., DIP.BACT. ; J. McQUILLIN,† B.SC., F.I.M.L.T. ; S. D. M. COURT,‡ M.D., F.R.C.P.

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Summary: The lungs of three infants, two with bronchiolitis and one with pneumonia, were examined by fluorescent antibody techniques for the distribution of respiratory syncytial (R.S.) virus, and also for the presence of human globulin. In bronchiolitis the lungs contained little virus, whereas in pneumonia virus was abundant and widespread; and, paradoxically, while in bronchiolitis human globulin had the same scanty distribution as virus it was absent in pneumonia. It is suggested that the essential process in bronchiolitis is a widespread type 1 allergic reaction dependent on a second encounter with R.S. virus antigen, whereas in R.S. virus pneumonia the mucosal necrosis and alveolar and interstitial inflammation are the result of direct virus damage to the lungs. The alternative explanation put forward is that the process may be a type 3 allergic reaction.

Introduction

For the past 10 years we have been studying the role of respiratory syncytial (R.S.) virus in acute respiratory infections of children (Gardner *et al.*, 1960; Andrews and Gardner, 1963; Elderkin *et al.*, 1965; Holdaway *et al.*, 1967; Gardner, 1968), and a recent study of 22 deaths, in which four cases of pneumonia and five of bronchiolitis could be attributed to this virus, has emphasized our uncertainty regarding the precise processes involved (Aherne *et al.*, 1970).

Three problems particularly concern us. (1) Why does 80% of acute bronchiolitis, almost entirely due to R.S. virus, occur in the first six months of life when maternal antibody is still present? (Newcastle upon Tyne Respiratory Survey 1969). (2) Why, in infants vaccinated against R.S. virus, did subsequent infection with naturally occurring virus produce illnesses of greater severity than in those not vaccinated? (Chin *et al.*, 1969; Fulginiti *et al.*, 1969; Kapikian *et al.*, 1969; Kim *et al.*, 1969a).(3) Is the pathogenesis in fatal bronchiolitis and fatal pneumonia due to R.S. virus the same or different? If it differs, what criteria of differentiation can be accepted?

We are attempting to answer these questions by bringing forward additional, though still incomplete, evidence of the cell-virus relationship in bronchiolitis and pneumonia caused by R.S. virus, derived from the immunofluorescent study of the lungs of three children who died.

Materials and Methods

Virus Techniques

Since the validity of our observations and deductions depends on the methods employed, these are described in some detail.

(*a*) *Preparation of Inoculum for Virus Isolation.*—Lung material from each case was processed by methods already described (Gardner *et al.*, 1967; Aherne *et al.*, 1970).

(*b*) *Preparation of Impression Smears.*—On arrival in the laboratory the post-mortem lung material was promptly stored at 4° C. until ready for processing. Within 30 minutes of reception two small pieces of tissue, about 2 mm. sq. by 1 mm. thick were removed from the lung and placed about 1in. (2·5 cm.) apart on a glass slide. A series of impression smears were made from the two pieces of tissue by pressing a succession of glass slides on top of them. Six to eight slides could be prepared in this way before the tissue was completely used. The smears were then allowed to dry in air and fixed in acetone at 4° C. for 10 minutes. They were stained by the technique already described, and controls for specificity and efficiency of techniques were included (Gardner and McQuillin, 1968; McQuillin and Gardner, 1968). For obvious reasons negative controls could not be tested, but since many lungs which were negative on culture and also lungs infected with viruses other than R.S. virus were examined in this way and gave no fluorescence with R.S. virus antiserum, specificity of the R.S. virus antiserum for R.S. virus antigen in lung tissue could be accepted.

(*c*) *Preparation of Frozen Sections.*—At the same time as the pieces for impression smears were cut from the lung two pieces about 3-4 mm. sq. and 1-2 mm. thick were cut and placed in bijou bottles. These pieces were snap-frozen on to the side of the bottle in a dry ice and alcohol mixture for storage at −70° C. until required. Cryostat sections were cut and a series of single sections were placed on clean glass slides; the sections were fixed in acetone at 4° C. for 20 minutes. The staining procedures have been described elsewhere, two sections being treated with R.S. virus

* Consultant Virologist and Honorary Reader.
† Senior Scientific Officer, Department of Virology.
‡ Professor of Child Health, Royal Victoria Infirmary and University of Newcastle-upon-Tyne, Newcastle-upon-Tyne 1.

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