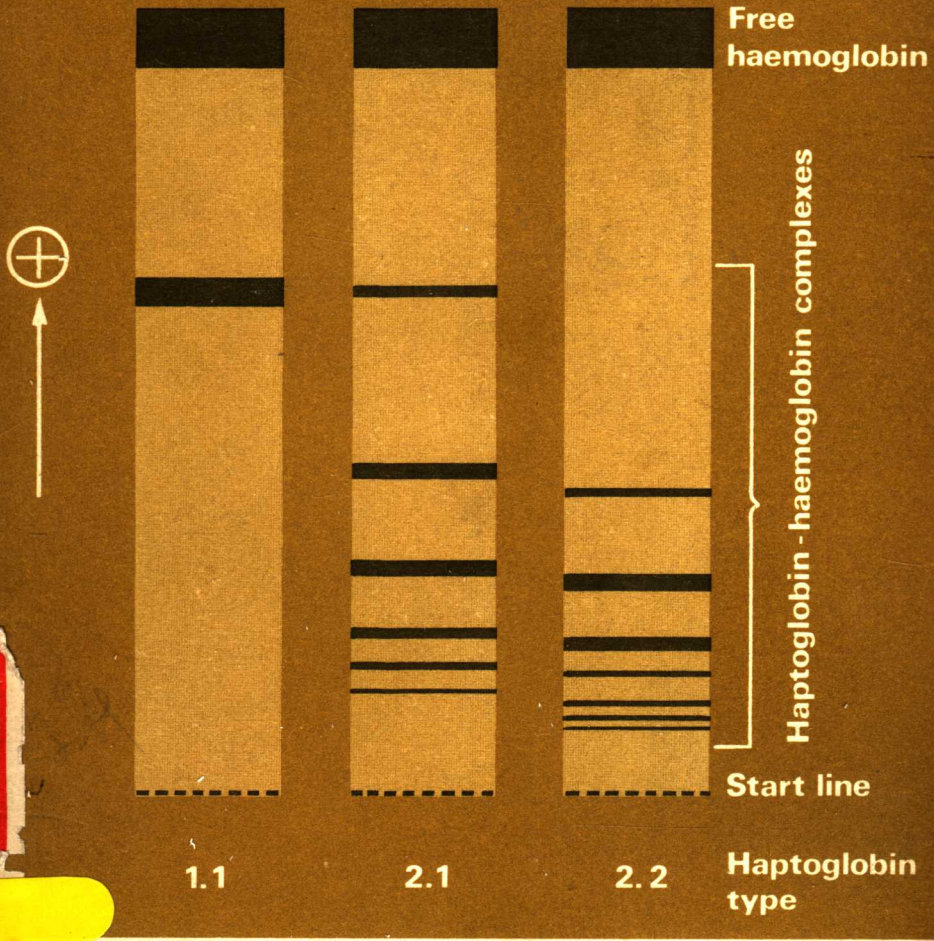
H. HARRIS

HUMAN BIOCHEMICAL GENETICS



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HUMAN BIOCHEMICAL GENETICS

BY

H. HARRIS

WITH A FOREWORD BY

L. S. PENROSE, F.R.S.

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FOREWORD

At the present time the science of human genetics is growing very rapidly. This development was predictable because of the increasing number of workers who are coming into the field and the new techniques which are being applied in the recognition of hereditary characters. In one special region, that concerned with biochemistry, however, advances have exceeded all expectations.

When Garrod produced the second edition of his *Inborn Errors of Metabolism* in 1923, he was able to describe in detail only some half dozen rare anomalies, and only a few discerning people had then realised that a revolution in the study of heredity was in progress. The new outlook was destined to be especially significant in human genetics and it altered eugenic philosophy. No longer could hereditary defects be attributed to the action of mysterious noxious influences, carried by degenerate germ plasm and perhaps engendered by parental vice. Under biochemical scrutiny, hereditary defects were repeatedly found to be quite specific inborn peculiarities. These individual chemical differences seem quantitatively very slight, like the substitution of just one aminoacid residue in an otherwise perfect chain. No stigma can be attached to such impersonal variations. If there are unfavourable effects connected with a chemical variant, these merely present a therapeutic challenge.

In his previous book, *Introduction to Human Biochemical Genetics*, published in 1953, Dr Harris reviewed the position of the subject and described all the well-established advances which had been made since Garrod's time. Now there is, again, much fresh information available and a larger book is required. It is extremely difficult to keep pace with the rate at which discoveries are being made. To present the very latest views in every detail cannot be a practical aim. Dr Harris has again minimised this disadvantage by devoting the book mainly to the description of well-authenticated material, leaving out speculative interpretations of anomalies which are, so far, not well understood. This plan does not involve neglect of dramatic discoveries such as acatalasaemia and analbuminaemia. How do people get on when their blood is deprived of such apparently essential substances as catalase or albumen? The answers are unexpected.

A book of this kind must necessarily include an account of the principles of human population genetics. It is now quite insufficient simply to describe an inherited condition and to demonstrate its mode of transmission in a few pedigrees. The human geneticist must ascertain the prevalence of each defect and must seek to determine why it occurs with the population frequency actually observed. When a trait has been identified in terms of a biochemical peculiarity, accurate incidence figures eventually can be obtained and the situation is favourable for the exploration of problems concerning mutation and natural selection.

In presenting this well-balanced and learned account of the subject, Dr Harris has done a valuable service to medicine, to biochemistry and to genetics. I have much pleasure in drawing the attention of workers in these subjects to this book, for it can open a new world to them.

L. S. PENROSE

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June 1958

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CHAPTER 1

INTRODUCTION

This book is about inborn differences between human beings. More particularly it concerns those differences which can be formulated in biochemical terms. These include differences in structures of macromolecules such as proteins and mucopolysaccharides, differences in the formation of certain enzymes, differences in the composition of body fluids and secretions, and differences in excretory products. Ultimately, however, they must each depend on differences in the biochemical characteristics of the fertilised ova from which all individuals develop. The significant differences here probably lie in the structural organisation of the desoxyribose nucleic acid present in the cell nucleus.

The inborn errors of metabolism and biochemical individuality

The scientific study of inherited differences in human biochemistry began with the work of Garrod at the turn of the century. In 1902 he published a paper in the *Lancet* called 'The incidence of alkaptonuria, a study in chemical individuality'(1), and in it he first drew attention to the biological significance of differences of this kind.

Alkaptonuria is a rare condition which is characterised by the excretion of large quantities of homogentisic acid (Fig. 1). Several grams of this substance may be passed daily in the urine, and its excretion is continuous and goes on throughout life. It is a very striking peculiarity because the urine goes black on standing, and the disorder is often recognised in early infancy by the characteristic staining of the napkins. The affected individuals are in other respects quite healthy, though as they get older they are rather more prone than other people to develop osteoarthritis.

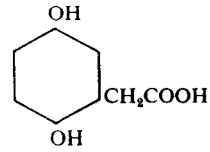

Fig. 1. Homogentisic acid.

Garrod made some fundamental points about the condition. He observed that a person is either frankly alkaptonuric or conforms to the normal type, that is, he either excretes several grams of

homogentisic acid a day, or none at all. Garrod pointed out that the homogentisic acid must be largely derived from the aminoacid tyrosine, and that the essential peculiarity of such people was an inability to break down the benzene ring of this aminoacid in the normal way. Instead they passed it intact in the urine. It seemed more appropriate, he suggested, to regard the peculiarity as an aspect of the person's inborn individuality rather than a disease process in the ordinary sense.

The other striking feature of the condition to which he drew attention was its familial distribution. It was often found among several members of the same family. Frequently two or more of a group of brothers and sisters would be affected, the parents being quite normal as were other more distant relatives. Furthermore, the parents of alkaptonurics were often blood relatives, usually first or second cousins. The familial distribution of the condition showed a highly characteristic pattern and Garrod had little hesitation in concluding that it implied a hereditary or genetical basis for the disorder. It was possible to take this conclusion further. Garrod consulted Bateson, one of the earliest of British geneticists, who pointed out that the situation might be readily explained in terms of the then recently rediscovered laws of Mendel. The frequent occurrence of the disorder among the brothers and sisters of an alkaptonuric, its rarity among their antecedents or descendants, and the high incidence of consanguinous marriage among the parents who were themselves unaffected, was precisely the type of familial distribution to be expected if alkaptonuria was inherited as a Mendelian recessive character. This was in fact the first example of recessive inheritance to be recognised as such in man.

Garrod therefore interpreted alkaptonuria as an inborn metabolic variant inherited as a recessive Mendelian character. The metabolic anomaly apparently lay in a peculiar inability to break down the benzene ring of the aminoacid tyrosine, and he suggested that this kind of genetically determined biochemical variation was not a unique phenomenon, but was probably of general occurrence. He instanced a number of other metabolic peculiarities which he thought might be regarded as further examples of such 'chemical individuality'.

Concerning the biological significance of such conditions he made the following remarks:

> If it be, indeed, the case that in alkaptonuria and the other conditions mentioned we are dealing with individualities in metabolism and not with

the results of morbid processes, the thought naturally presents itself that these are merely extreme examples of variations of chemical behaviour which are probably everywhere present in minor degrees and that just as no two individuals of a species are ever absolutely identical in bodily structure neither are their chemical processes carried out on exactly the same lines. Such chemical differences will be obviously far more subtle than those of form, for whereas the latter are evident to any careful observer the former will only be revealed by elaborate chemical methods.

This passage is remarkably modern in outlook. Alkaptonuria and the other metabolic peculiarities of which Garrod was then aware were all extremely rare. At that time and indeed until very recently such conditions have been regarded in medicine as little more than curiosities of no general importance. At a time when both the study of genetics and of human biochemistry were still in their infancy, Garrod showed singular insight in perceiving the biological interest of these disorders. He was probably alone among the physicians of his day in appreciating that the detailed study of these rare and peculiar conditions was likely to throw considerable light on the general nature of human variability.

Garrod developed the argument in his Croonian Lectures in 1908 (2) and in later works (3), with a detailed examination of a number of other analogous disorders such as cystinuria, porphyria, and pentosuria. He called them 'inborn errors of metabolism'. Since then many further examples of the same kind of thing have been discovered. These include phenylketonuria, galactosaemia, fructosuria, the glycogen storage diseases, and the various forms of goitrous cretinism. Although they represent a very diverse series of biochemical peculiarities and differ widely from one another in their clinical significance, they nevertheless have in common certain characteristic features which make it profitable to study them together.

They each occur much more frequently among the close relatives of affected individuals than in the general population. Munro (4), for example, found among 179 brothers and sisters of phenylketonuric patients thirty-eight further examples of the condition. In contrast he estimated that the incidence of the disorder in the general population from which they were drawn was something of the order of 1 in 40,000. This high familial concentration cannot in general be accounted for in terms of differences between environments in which different family groups live, because in each case the underlying biochemical peculiarity appears to be little influenced by ordinary

environmental variations. The simplest explanation in fact is that the conditions are each genetically determined. The occurrence in some cases of an increased frequency of parental consanguinity, and the existence of highly characteristic types of pedigree configurations afford further evidence that the peculiarities are inborn and hereditary.

Characteristically, each of these peculiarities serves to divide human beings more or less sharply into separate groups differing metabolically from each other in some particular respect. The metabolic anomaly is in each instance highly specific. A fructosuric, for example, is unable to metabolise fructose completely, but his ability to deal with glucose and other sugars is not impaired. His brothers and sisters are more likely than an unrelated person to exhibit fructosuria. They are, however, no more likely than anybody else to manifest other quite different metabolic peculiarities. Even closely related disorders such as phenylketonuria and alkaptonuria, both of which represent abnormalities in the oxidation of the aromatic aminoacids, occur independently and are inherited quite specifically.

The effects on the viability of the individual and on his biological fitness, that is his capacity to reproduce, are very diverse. Fructosuria and pentosuria, for example, seem to confer no disadvantage at all on the affected individuals. They remain quite healthy and their reproductive ability is not curtailed; such conditions can only be regarded as normal variants. In other cases serious consequences are regularly associated with the metabolic abnormality. Galactosaemia, for example, if left untreated, usually results in severe liver damage, cataract formation, mental impairment, and retardation of growth. It is often fatal in early life. Phenylketonuria, while not usually leading to early death, is nevertheless always associated with some degree of mental defect. This usually amounts to idiocy or imbecility, and necessarily involves a gross curtailment of biological fitness. In practice it is difficult in these various conditions to draw any sharp line between what may be regarded as normal variations and what must be considered as pathological. Every degree of intermediacy may be encountered.

Thus the 'inborn errors of metabolism' can be regarded as genetically determined biochemical variations, which sharply characterise human beings. They are highly specific and represent many diverse metabolic phenomena, which result in very varied effects on the viability and fitness of the individual.

Garrod developed a simple hypothesis which he thought might explain in a general way the common features of the various 'inborn errors of metabolism'. He suggested that in each condition the body was unable to perform some particular step in the normal course of metabolism. This was presumably due to the congenital absence of the enzyme usually concerned in catalysing the step in question, and as a result a block occurred at this point in the metabolic processes. The unusual concentrations of metabolites in the body fluids and excretions, and the various clinical signs and symptoms with which they might be associated, could all ultimately be traced back to the inability to perform this single step in the metabolic sequence. Thus, if we consider a series of reactions which can be written

$$\begin{array}{ccccccccc} A & \rightarrow & B & \rightarrow & C & \rightarrow & D & \rightarrow & E \\ & \alpha & & \beta & & \gamma & & \delta & \end{array}$$

the congenital absence of the enzyme γ would be expected to result in a failure to form the metabolite D from its precursor C, and in consequence C would tend to accumulate and perhaps be excreted in unusual amount. The formation of C in excessive quantities or the failure to form D might result in diverse consequences for the organism. Their effect on its viability and fitness would depend on the character of the particular metabolites involved, and on the relative importance of the disturbed sequence of reactions in the overall biochemical economy of the body.

There is little doubt that this concept of an inborn metabolic block provides an accurate and useful way of understanding the biochemical disturbances to be found in many inherited abnormalities. For example, the sequence of reactions which are believed to take place during the normal oxidation of phenylalanine and tyrosine are shown in Fig. 2. The peculiar composition of the body fluids and urine in phenylketonuria can largely be accounted for in terms of a metabolic block at the point A, and it has now been demonstrated directly that the enzyme system necessary to carry out this step is indeed deficient in phenylketonuria. Similarly a block at point B would be expected to result in the kind of changes which are observed in alkaptonuria. Here again a specific deficiency of the relevant enzyme has been demonstrated directly.

However, it should be pointed out that not all the conditions which Garrod originally regarded as examples of metabolic errors can be explained in terms of abnormalities of intermediary metabolism. The

excessive excretion of cystine and certain other aminoacids in classical cystinuria, for example, is now thought to be due not to a defect in the intermediary metabolism of cystine, but to a failure in renal tubular reabsorption from the glomerular filtrate. The abnormality is 'renal' rather than 'metabolic'. From the theoretical standpoint the discrepancy is probably only a superficial one. Transport of metabolites across the renal tubule cells, as across other membranes in the body, is in general an active and highly specific process. Although little is known about the detailed mechanisms involved, they are probably enzymically controlled. An inborn defect in the formation of a specific enzyme in the renal tubule cells concerned in the transport of cystine might well be the explanation of classical

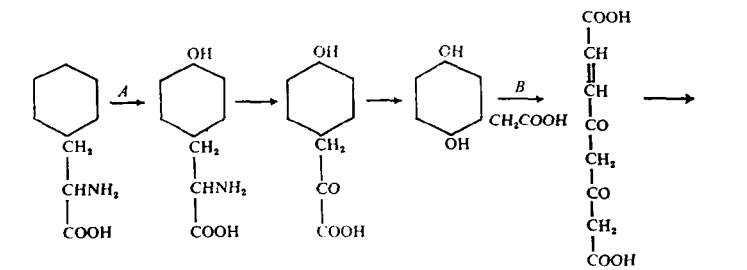

Fig. 2. Steps in the oxidation of phenylalanine and tyrosine.

cystinuria, and such an explanation would in principle be much the same as that advanced to account for the inborn abnormalities in intermediary metabolism.

The important implication of this general theory of the inborn errors of metabolism was that genetical factors could play a specific role in the formation of enzymes. The idea was later to be considerably extended and elaborated. In the form of the one gene–one enzyme hypothesis it became a key working hypothesis in experimental genetics. This was largely the result of the experimental production in large numbers of 'biochemical' mutants in certain organisms such as the bread mould, *Neurospora*. In essence these mutants exhibited specific blocks in metabolism of the kind originally envisaged by Garrod to explain the 'inborn errors of metabolism'.

Although all enzymes are probably proteins, not all proteins behave as enzymes. If it is true that inherited differences can lead to differences in enzyme formation, then it might be anticipated that

inborn differences in the formation of proteins with other kinds of functional properties also occur. This, indeed, turns out to be the case. In some instances the inherited peculiarity results in a complete or almost complete failure to synthesise the particular protein or group of proteins. This is so, for example, in afibrinogenaemia, agammaglobulinaemia, and analbuminaemia. Perhaps of more fundamental significance, however, are those inherited differences which result not in the failure to form a particular protein, but in the formation of proteins similar in most respects to those usually encountered but differing from them in the finer details of their structural organisation and their physico-chemical properties. The most important example of this kind of phenomenon is provided by the series of different types of haemoglobin which have now been identified in human beings. The character of the haemoglobin or haemoglobins which an individual possesses appears to depend more or less directly on certain features of his genetical constitution.

Another example of inherited variation in protein synthesis is the differences which have been encountered between people in the properties of certain of their plasma proteins, the haptoglobins. Individuals may be divided quite sharply into three classes according to the type of haptoglobin they possess. In European populations these three classes of individual occur with a frequency of about 16 per cent, 48 per cent and 36 per cent respectively. In this situation it is clearly impossible to regard one type of haptoglobin as 'normal' and another as 'abnormal'. Each type represents a different version of 'normality', and in any one individual it reflects one particular facet of his biochemical individuality and genetical constitution.

In fact, the first demonstration that subtle chemical differences between human beings may be a common phenomenon was provided by Landsteiner's fundamental work on the blood groups, the first results of which were published at about the same time as were Garrod's. It was found that people could be classified into four groups according to whether they possessed one (A), another (B), both (AB), or neither (O) of two different antigenic substances on the surfaces of their red blood cells. These substances are now thought to be mucopolysaccharides. The differences in their immunological specificity imply differences in their chemical structures and hence in their biosynthesis. These differences are genetically determined.

Subsequently a number of other genetically determined systems of blood-group antigens such as the MN system and the Rh system were

discovered. In general, each system is determined independently of the others, so that any single individual has a complex of antigenic substances present on the surface of his red cells which includes components belonging to each of these different systems. By using all the antisera which are available to characterise these different antigens, it is now possible to define more than a million different classes of people according to whether they possess in their red cells one or another combination of these antigenic substances. Many of these possible combinations are extremely rare, but the discriminative power of the technique is illustrated by the findings of Race and his colleagues (5) who, using seventeen different antisera, were able to classify 475 Londoners into 296 distinct types. Of these, 211 antigenic combinations occurred only once, and no more than ten individuals had the same combination. This affords some measure of the individual differentiation occurring in respect of what is only one relatively minor feature of the body's biochemical architecture.

The uniqueness of the biochemical make-up of the individual is further illustrated by the phenomena which occur when attempts are made to transplant skin or other tissues from one person to another. In general such transplants fail to take unless the individuals concerned are uniovular twins and therefore have the same genetical constitution, or unless the recipient of the graft has some defect in his capacity to form antibodies, as for example occurs in agammaglobulinaemia. The failure of a tissue transplant to take successfully can be attributed to an immunological reaction which develops in the recipient against foreign substances which are introduced. These foreign substances which induce the reaction must be macromolecules, and one can conclude that the transplanted tissue contains macromolecules different in structure from the equivalent ones present in the recipient's own tissues.

There is thus ample indication of inborn diversity in the biochemical make-up of human beings. This may be reflected in differences in the patterns of metabolic processes, or in differences in the structures of macromolecules. A particular kind of variant may be rare or common. It may result in pathological consequences for the individual or it may lead to no obvious effect on viability or biological fitness. It is probable that, with the exception of uniovular twins, no two human beings are exactly alike in their inborn biochemical potentialities. The analysis of this biochemical individuality forms the subject-matter of human biochemical genetics.

Chromosomes and genes

Modern genetics views heredity as an atomistic process. The genetical constitution of an individual is regarded as being composed of a large number of specific functional units which are directly inherited from his parents. Inborn differences between people are thought of as being due to specific differences in the character of these units, and differences in the combinations in which they occur.

This view is based on a vast amount of experimental evidence in animals, plants, and micro-organisms. The principles that have emerged appear to have a remarkable degree of generality, and although direct evidence for certain of them is not available in the human species, nevertheless there seems little doubt that they apply. An account of general genetics is outside the scope of this book. However, in order to facilitate the subsequent discussion some of the key concepts will be outlined somewhat dogmatically.

An individual arises from the fusion of two cells or gametes derived from his parents: the ovum from the mother, and the sperm from the father. The hereditary potentialities of the new individual or zygote formed by the fertilisation of the ovum by the sperm are derived from the characteristics of these two component cells. Most of the genetical character of these cells is determined by the particular properties of a series of thread-like structures present in their nuclei and known as chromosomes. Chromosomes are differentiated longitudinally into more or less discrete regions with genetically distinct properties. These chromosomal subdivisions are called genes, and they represent the specific units involved in Mendelian heredity. Each chromosome contains a large number of genes, possibly hundreds or even thousands. They are arranged in a characteristic order along its length, so that each gene can be said to have its own special position in any one particular chromosome. This is called its locus.

The nucleus of each human gamete is now thought to contain twenty-three chromosomes (6, 7). Each of them is different from the others in its genetical properties. When the ovum and sperm come together their nuclei fuse, so that the fertilised egg thus formed has a nucleus containing forty-six chromosomes, made up of twenty-three pairs. In general, each member of such a pair of homologous chromosomes is similar in its genetical properties to its fellow. Each member of the pair contains the same number of genes, and the gene loci are arranged in exactly the same order. There is, however, one

pair of chromosomes that is exceptional in this respect. They are called the sex chromosomes. In males, the pair of sex chromosomes is made up of one long one (the X chromosome) and one short one (the Y chromosome). The X chromosome certainly contains genes not represented on the Y chromosome, and it is possible that the Y chromosome contains genes not represented on the X. The X chromosome is derived from the mother, and the Y from the father. In females there are two X chromosomes and they resemble one another in the same way as do the members of the other twenty-two pairs of so-called 'autosomal' chromosomes.

In the development of the individual from the fertilised egg a successive series of cell divisions takes place. Prior to each cell division the chromosomes are duplicated in such a way that each of the two daughter cells comes to contain a set of chromosomes exactly like the other and like those of the parent cells. Hence they possess the same complement of genes. Thus with a few exceptions every cell in the body is thought to possess the same content of genetical determinants in its nucleus as every other cell. The main exception occurs in the formation of the sex cells or gametes. Here a special type of cell division occurs (meiosis), the result of which is that only one member of each pair of chromosomes appears in each gamete. Thus the gametes contain twenty-three chromosomes each.

A gene is an extremely stable entity and its replication at each cell division is very exact. Occasionally, however, it may undergo a sudden change, called a mutation. The new form of the gene so produced is then duplicated at each cell division in the same way as the old one, so that the mutant form persists. As a result of past mutations a number of alternative forms of the same gene may occur at a particular chromosomal locus. These are called alleles. The same chromosomal locus will in general be represented twice in a fertilised ovum, once on each of a pair of homologous chromosomes. Thus only two alleles of the same gene can be present, one derived from the father and one from the mother. If these two alleles are the same the individual is said to be homozygous with respect to the genes present at that locus. If they are different he is said to be heterozygous. A heterozygous person will, with the rare exceptions resulting from further mutations, come to possess replicates of the same two alleles in the nucleus of each of his somatic cells. Since the sex cells or gametes only contain one member of each pair of chromosomes, half of them will contain one allele, and half the other.

An individual is likely to be heterozygous for genes at many different loci on many different chromosomes. Because only one gene from each pair of alleles is transmitted to any one of his offspring, the alleles at the various chromosomal loci are continually being reassorted into new combinations. The degree of possible reassortment is greatly extended by the phenomenon known as crossing over. This involves the exchange of material between two homologous chromosomes during meiosis. In general, the closer together two genes are on the same chromosome the less likely are they to be separated by crossing over. Gene loci on the same chromosome are said to be linked, and the analysis of the relative frequency with which different genes on the same chromosome are separated by crossing over allows an assessment to be made of the relative positions of the different loci. In this way it is possible to construct detailed 'maps' of the different chromosomes which indicate the order of the gene loci present. So far, however, only a very few examples of 'linked' genes have been identified in human beings.

Each gene can be regarded as having a specific functional role in the biochemistry of the cell, and hence in the biochemical economy of the body as a whole. A mutation presumably alters the structure of a gene in some way and this may be expected to be reflected in its functional behaviour. In practice we can only know anything at all about a particular gene if as a result of past mutations it occurs in more than one allelic form, and if the different possible combinations of allelic genes result in detectable differences between the individuals who carry them. The simplest situation which can be analysed occurs where in any population of individuals there exist at a particular chromosomal locus one or other of two allelic genes. If these are called **A** and **a**, then with respect to this particular chromosomal locus three genetically distinct types of individual will occur, and they can be designated **AA**, **Aa**, **aa**. Each of these three types of individual will be in some respect biochemically different from the others. If our techniques of investigation are adequate it may, in fact, be possible to distinguish each of these three types clearly from the others. Often, however, only two classes of individual may be recognised; one which we can designate $\bar{a}$, corresponding to the genotype **aa**, and the other designated as $\bar{A}$, corresponding to the genotypes **AA** and **Aa**, which are indistinguishable. In such circumstances it may be said that the gene **A** is dominant to the gene **a**, and that **a** is recessive to **A**. The class $\bar{A}$ is called the phenotype corresponding to the genotypes **AA** and **Aa**.