Current Practice

GROWING POINTS

Genetics and Medicine—II*

G. R. FRASER,† M.D., PH.D.

Mechanisms of Heredity

Mendel was concerned with the phenotype—visible characters which he could clearly observe and score. For him the gene (a term not introduced till much later) was an entirely tenuous concept. Today's biologists, on the other hand, have considerable knowledge of the actual chemical structure of the genetical material in the chromosomes. Probably the effective determinants of heredity are nucleic acids, which are polymerized structures made up of nucleotides. The chromosomes contain deoxyribose nucleic acid (D.N.A.). This is composed of a pentose, deoxyribose, linked to a purine or pyrimidine base and to a phosphate residue. In its turn the phosphate residue is linked to another pentose, thus ensuring polymerization, which may affect several thousand nucleotide units. The resulting D.N.A. molecule consists of two intertwining helical chains. Most forms of D.N.A., including the human variety, contain four bases—the purines adenine and guanine, and the pyrimidines cytosine and thymine.

The modern theory of D.N.A. structure postulates that the double helices are held together by hydrogen bonding between *base-pairs*—that is, between adenine and thymine and between cytosine and guanine. Such a molecule can reproduce itself (or *replicate*) by splitting into its constituent chains, each of which can act as a pattern to form a new complementary chain and thus reconstitute a double helix.

The specificity of the D.N.A. molecule, and hence its genetical function, is thought to be determined by the sequences of the four bases it contains. Thus a particular sequence, or *triplet*, of three bases will give rise eventually to the formation of a particular amino-acid. This is known as *coding*. Since only 20 amino-acids are commonly found in nature, and since there are 64 possible triplet arrangements of four bases, the code is said to be "degenerate." Possibly the code for any amino-acid may be represented by more than one triplet, and some triplets may code for such features, or "punctuations," as the beginning or the end of the synthesis of a protein molecule.

Control of Protein Synthesis

The method of the control of protein synthesis seems to be as follows. Initially at the surface of the chromosome the D.N.A. code is used as a pattern, or *template*, for the formation of the so-called *messenger* ribonucleic acid (R.N.A.). The chemical composition of R.N.A. is similar to that of deoxyribonucleic acid (D.N.A.) except that the pentose units are replaced by ribose and that thymine is replaced by uracil. Messenger R.N.A. then acts as a template for protein synthesis itself. This takes place on the surface of small intracellular particles called *ribosomes*. The amino-acids corresponding to

the nucleotide triplets of the messenger R.N.A. are carried to the surface of the ribosomes by another type of R.N.A. called *transfer* or *soluble* R.N.A.

In this way each gene, which occupies a specified region or locus on the chromosome, is responsible for the formation of a polypeptide. This may be, for example, a component of an enzyme or of the protein moiety of haemoglobin—globin—and in this way the metabolism of the body is directly under genetical control. Nevertheless, a large part of our genetical material is not devoted to the synthesis of proteins needed for metabolism. Apart from the *structural* genes, which are concerned with the synthesis of single essential proteins, a large part of the chromosomes is occupied by loci concerned in the regulation, control, and integration of metabolic processes.

Thus one or more structural genes may be under the control of a so-called *operator* gene in their immediate proximity, and yet other genes may produce substances which act as *repressors* or *inducers* of the action of the operator gene. Mechanisms of this type have been much studied in bacteria, but these or analogous control systems must be of fundamental importance in the vast but as yet little-explored fields of mammalian embryogenesis and cell differentiation.

The Haemoglobinopathies

Much of the work on genetical control of protein synthesis and its various regulatory mechanisms has been done in bacteria and other unicellular forms of life. Nevertheless, man has provided at least one important model for the study of changes in proteins brought about by mutations or chemical changes in genes. Normal adult haemoglobin (haemoglobin A) is composed of four pyrrole rings and a protein, globin. Globin is made up of four polypeptide chains, two known as α chains and two as β chains. Other types of haemoglobin have also been found in man, and are usually called by the letters of the alphabet—for example, haemoglobin C, haemoglobin D, etc. Analyses of the sequence of amino-acids in the polypeptide chains of the globin fractions from these other haemoglobins showed that this differed from the sequence in normal haemoglobin A. Some of these differ from haemoglobin A in their α chain, others in their β chain.

In many cases the properties of the haemoglobin are drastically altered by the mere substitution of one amino-acid for another. Thus haemoglobin S, which is found in persons with sickle-cell anaemia and sickle-cell trait, contains a β chain which differs from the normal in only one amino-acid—valine being substituted at a certain point for glutamic acid. This substitution is presumably caused by a change or mutation in the chromosomal D.N.A., in which a change in a single base pair may alter the code in the triplet containing it—thus giving rise directly to the incorporation of valine rather than glutamic acid at a certain position into the haemoglobin β chain molecule. Though this is a substitution of only one of the

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† Reader in Genetics, University of Adelaide, South Australia.

140 amino-acids in the β chain, it leads to drastic changes in the properties of the haemoglobin molecule. Not only is it far less effective in performing its normal functions, but in an oxygen-deficient atmosphere the red cells containing it assume a sickle shape. In homozygotes, whose red blood cells contain no normal adult haemoglobin at all, this produces the serious condition of sickle-cell disease, with anaemia, crises produced by infarction of organs by sickled cells, and early death. In heterozygotes, who have one normal allele producing a normal β chain, the red cells contain a mixture of sickle-cell haemoglobin and normal haemoglobin. These heterozygous people are said to possess the sickle-cell trait, and though they may be apparently normal their red cells will still sickle if they are deprived of oxygen. When two such heterozygous individuals marry (Fig. 5) a quarter of their offspring will

of sickle-cell anaemia is well known and is altogether different and far worse than the heterozygous state of sickle-cell trait. Hence the strict definition of dominance does not apply to this situation.

If the level of observation of the phenotype is extended to include biochemical and haematological criteria, as well as purely clinical ones, heterozygotes and both normal and abnormal homozygotes may be readily and definitively identified; and all the above ambiguities regarding dominance, recessivity, and penetrance are clarified. The boundaries between dominance and recessivity are rapidly being broken down as the level of observation moves towards the gene itself—and the concept of penetrance, too, is being modified in the process.

Sickle-cell anaemia arises because of an actual amino-acid substitution in the haemoglobin molecule, presumably reflecting a change in a chromosomal D.N.A. base pair which cannot as yet be verified experimentally. Many other "haemoglobinopathies" where other substitutions are concerned, both in the α and the β chain, have been detected and intensively studied.

Apart from conditions associated with the presence of a haemoglobin in which an amino-acid substitution has taken place, there is another class of red-cell abnormalities, collectively known as thalassaemias, in which haemoglobin synthesis is abnormal. Despite intensive investigation, no amino-acid substitutions have been detected in this group, and it is thought that the thalassaemias may be associated with mutations in genes controlling the rate of haemoglobin synthesis—such as the operator genes mentioned above. As in the case of haemoglobinopathies associated with amino-acid substitutions, a "dominant" thalassaemia trait and a "recessive" disease (thalassaemia minor and major respectively) exist.

Molecular Disease

The substituted haemoglobin conditions and the thalassaemia group, though very rare in the native English population, form excellent models of so-called molecular diseases in man. These are diseases which are marked by qualitative or quantitative abnormalities of an essential constituent—usually an enzyme. They are generally recessive, but they may be dominant if one normal chromosome cannot produce adequate amounts of the essential protein or if the pathological product produced by one abnormal chromosome interferes with normal metabolic processes. For example, we have seen that persons with sickle-cell trait are occasionally not entirely normal clinically, and this could therefore be regarded as a "dominant" disease. In the case of many other "recessive" molecular diseases, with increasing technical refinements detection of a large proportion of heterozygotes is becoming possible, thus reproducing the situation seen with "recessive" sickle-cell anaemia and "dominant" sickle-cell trait.

People with such molecular diseases often seem normal at birth. Nevertheless, many genetical diseases in man which follow simple Mendelian laws of inheritance are associated with gross congenital malformations. There are at least two explanations for the occurrence of such diseases. First, they may be true molecular diseases which concern a protein which plays an essential, and possibly temporary, part in embryogenesis and which cannot be supplied by the mother. Secondly, they may be due to more extensive chromosomal changes affecting more than one base unit. Such diseases may be dominant and be transmitted from one generation to the next, or they may be recessive and one or more sibs are born of clinically normal parents. At the other end of the scale some diseases in man inherited in a Mendelian manner—for example, various types of retinal degeneration or Huntington's chorea—do not seem to become manifest until relatively late in life. Appearances may be deceptive, however—for example, objective tests of visual function in retinitis pigmentosa may reveal abnormalities long before symptoms occur. In this type of disease there is

FIG. 5

on the average inherit the abnormal gene from both parents and produce only the β chains of haemoglobin S—that is, they will have recessive sickle-cell anaemia.

Penetrance

A study of the pattern of inheritance shown in Fig. 5 illustrates the lack of exactitude in the light of modern concepts of the old ideas of recessivity and dominance. At the clinical level sickle-cell anaemia is inherited as a recessive disease. Sickle-cell trait could be described also at the clinical level as a dominant disease, since minimal or slight symptoms occur in carriers in successive generations. Symptoms are not clinically manifest in all carriers, however; the gene responsible could be said to have reduced "penetrance."

This is an over-simplification, since the strict definition of dominance discussed above includes an identical effect in the heterozygote and one of the homozygotes. In the case of most "dominant" traits in man this cannot be tested, since even if they do not preclude reproduction in carriers altogether they are so rare that matings of affected persons never take place and abnormal homozygotes never occur. In the case of the sickling phenomenon, however, the abnormal homozygous state

probably a gradual degeneration of a specialized tissue owing to an inherent, though mild, defect in its metabolism. Hence we cannot exclude the possibility that single-gene effects of this type have a role in the aetiology of some of the common degenerative diseases of middle life and old age.

Disease of Single-gene Inheritance

Diseases due to single-gene inheritance, whether dominant or recessive, are individually rare, but there are so many of them that collectively they are relatively common. Furthermore, their apparent number is increasing yearly as new ones are discovered. In many cases some progress has been made in the elucidation of the pathways between the genotype and the phenotype, and in the study of the inborn error of metabolism (to use Garrod's term) concerned. These problems and those of detecting the heterozygotes are of the greatest importance from the point of view of prevention and treatment. Thus in phenylketonuria, phenylalanine cannot be metabolized by the patient, and exclusion of this from the infant's diet, when early enough, changes the prognosis radically for the better.

Another aspect of genetics is the study of the reasons for the prevalence of the large number of pathological genes in the population as a whole. The average "normal" member of our society possesses several genes which, though harmless in heterozygous form, would give rise to serious or fatal disease in homozygotes. It must be remembered that an exceedingly rare recessive condition (occurring, say, in only one per million of the population) is represented in heterozygous form in 1 in 500 people. A more common one (say, with a prevalence of 1 in 20,000, such as phenylketonuria) is represented in no less than 1 in 70. Since there are so many such recessive conditions, all of us are likely to carry one or more of the genes that cause them. This explains the well-known dangers and disadvantages of consanguineous marriages—since relatives are more likely to carry the same pathological genes than unrelated members of the population.

Two questions might be asked. First, what are the mechanisms which retain this large store or "load" of pathological genes in the human population? Secondly, why have the forces of evolution (which presumably tend to improve the species) not acted to eliminate this burden? Two main sorts of explanation have been advanced.

Balanced Polymorphism

The first of these explanations is that a gene that may be disadvantageous or even lethal in homozygous form may be actually advantageous in heterozygotes. This situation is known as a *balanced polymorphism*. Thus in patients who are heterozygous for some abnormal haemoglobins there seems to be a certain amount of protection against malaria. Certainly the areas of the world where malaria is common seem to correspond very closely to those populations which have a high frequency of the genes for abnormal haemoglobins; in the English and other North European populations, on the other hand, these conditions are uncommon.

Another genetical trait that also affects the red cell profoundly, and which also occurs in high frequencies in the same areas, is the deficiency of the enzyme glucose-6-phosphate dehydrogenase (G.6-P.D.). The gene determining this character is on the X chromosome, and the mechanism of its inheritance is being investigated very fully at present, since it may shed light on an apparent anomaly (Fig. 6). This is that the Y sex-chromosome in males seems to carry very few genes—implying, therefore, that the female (possessing two X's) would seem to have an extra active chromosome. It has been suggested that some time during embryogenesis the whole or at least a large part of one of the X chromosomes in cells of females becomes inactivated and gives rise to the so-called sex-chromatin body

in the nucleus. The inactivated chromosome takes no further part in the control of development, thus redressing the balance between female and male (*dosage compensation*).

SEX LINKED INHERITANCE AND G.6-P.D. DEFICIENCY

FIG. 6.—Sex linked inheritance and G.6-P.D. deficiency. The male hands on his X chromosome to his daughter and the Y to his sons; the female hands on one of her two X chromosomes to each of her offspring, male and female. Thus (1) affected males will have only normal sons and only carrier daughters, and (2) half the daughters of carrier females will be carriers and half normal; half their sons will be affected and half normal. Owing to random inactivation of one X chromosome in each female cell, female carriers may have a very wide range of values of the enzyme G.6-P.D. Any value between these characterizing normal and deficient males is possible, depending on the relative proportions of normal and abnormal X-chromosomes inactivated in enzyme-producing cells. Other conditions inherited in this manner include haemophilia and colour-blindness.

Mutation

The second type of mechanism which may be concerned in the maintenance of harmful or potentially harmful genes in man is mutation. It is well known that mutations may be produced in animals by radiation or chemicals, and by analogy, therefore, natural or artificial exposure to these agents may have the same effect in man. These agents would presumably act by changing the composition of the base pairs of the deoxyribonucleic acid in the chromosome. Such mutations at any one gene locus take place in only about 1 in 100,000 of the germ cells which take part in fertilization. This infrequency is fortunate, since they often produce grossly pathological conditions. It is because of this possibility of unfavourable genetical effects that so much anxiety has been felt about the artificial increase of background radiation because of atomic explosions.

Sometimes the nature of gross congenital malformations can be determined only by inference as being due to mutation, since those affected cannot reproduce. Thus transmission from one generation to the next may never be observed. In such cases a study of the parental ages at birth may be helpful, since the frequency of new mutations due to natural radiation or to exposure to chemical agents is probably higher with increasing parental age.

Both balanced polymorphism and mutation are thought to be necessary for the continued evolution of the species. Thus balanced polymorphism enables man to adapt himself to an unfavourable environment and to live there. Mutation is the very building stuff of evolution—for, while it usually gives rise to grossly pathological conditions, the occasional favourable mutation provides the substrate on which evolution can act. The "load" of deleterious genes, therefore, can be regarded as a price which individuals, families, and sometimes entire communities pay for the continued survival and improvement of the species.

(To be concluded.)