Biochemical Genetics and Man: Accomplishments and Problems

Man manifests most of the kinds of gene action found in other organisms and some additional complexities.

H. Eldon Sutton

Too often, in the history of genetics, man has been considered the poor relation of the fruit fly or, as times have changed, of *Neurospora* or *Escherichia coli*. The utility of these organisms in elucidating various aspects of genetics has sometimes obscured the possibilities offered by human beings as objects of genetic studies. This attitude has changed significantly, due in part to the advance in knowledge that came from the study of human hemoglobins and in part to the greater interest shown recently in genes as factors in human disease. It is now generally recognized not only that man can be studied genetically but also that he offers opportunities to investigate problems which are difficult to approach with other organisms, particularly organisms lacking the degree of differentiation of mammals.

This article considers some of the ways in which the study of man has contributed to basic ideas of biochemical genetics. It points out the possible occurrence in man of some mechanisms which are known primarily through study of other organisms and discusses some of the problems in human biochemical genetics which have yet to be solved. No attempt is made to list the many inherited conditions in man for which biochemical information is available. These have been the object of several extensive reviews (*1*).

Primary Genetic Information

It is nearly 10 years since Ingram first demonstrated that hemoglobin S (Hb S) differs from the ordinary hemoglobin A (Hb A) by substitution of a single amino acid (*2*). These two forms of hemoglobin, shown by Pauling *et al.* (*3*) to differ in electrophoretic mobility, were known, from the work of Neel (*4*) and Beet (*5*), to reflect variation at a single locus, the types of hemoglobin present corresponding to the types of alleles present. This 1:1 relationship was in accord with the one gene, one enzyme theory derived from studies of metabolic blocks in microorganisms and, to some extent, in man. However, a metabolic block involves loss of enzymic activity, whereas the hemoglobin variants involve an altered but still recognizable protein. It was thus possible to conclude that one effect of gene action (mutation) is that of changing the primary amino acid sequence of proteins. A corollary is the conclusion that one of the functions of genes is that of specifying primary protein structure.

Many variants of human hemoglobin are now known; the chemical change has been identified in about 20 of them (*6*). Nearly all of these 20 variants involve substitutions of single amino acids in the primary structure, although the position and nature of the substitutions vary. They occur both in the α- and in the β-chains, the two kinds of polypeptide chains which constitute adult hemoglobin. In addition, variants of the normal minor component Hb A_2 (consisting of α- and δ-chains) are known, although the amino acid substitutions have not been worked out (*7*).

The genetic control of the primary amino acid sequence of proteins has been extensively verified in other organisms and in other proteins of man. The existence of structural genes is now accepted, and it has long been assumed, and now experimentally demonstrated, that the sequence of DNA and RNA bases which code for amino acids is arranged colinearly with the polypeptide sequence (*8*). In other words, starting at one end of the structural gene and proceeding to the other end corresponds to starting at one end of the polypeptide chain and proceeding to the other end.

This simple picture of a 1:1 correspondence between structural gene and polypeptide chain explains most of the genetic variation in proteins which has been studied in detail. It is important to consider, though, whether the systems studied are typical of all systems or whether the systems which have proved most amenable to study are those with fewest complexities. Particularly in the case of a highly differentiated metazoan such as man, it would seem necessary to consider to what extent the soma expresses only the regular Mendelian events of the germ line and whether variation in the genetic information of cells may change during development.

Perhaps the protein system most likely to diverge from the hemoglobin pattern so far as genetic control is concerned is gamma globulin. In the production of antibodies as part of the immune response, gamma globulins clearly reflect information contributed by the environment. Challenge with a foreign substance induces synthesis of antibodies specific for that foreign substance. The array of variants of the gamma globulin structure must be very large to account for the wide range of possible responses. A central question of immunology has long been: Does the antigenic stimulus provide information for the primary or tertiary structure of proteins or does the antigen only trigger the synthesis of certain proteins, the structures of which are determined by genetic information already in the organism? A consequence of the latter hypothesis would be the necessity either to have a large number of genes responsible for specifying structures of different antibodies or to have a few genes which undergo somatic mutation with high frequency. In this case, no one cell would have the potential for producing all the antibodies which the organism may produce.

The importance of the primary structure of γ-globulin molecules has only recently been demonstrated (*9*). By complete reduction of all the disulfide bonds and unfolding of the polypeptide chains, all specific anti-

The author is professor of zoology and a member of the Genetics Foundation at the University of Texas, Austin.

body activity is lost. Slow oxidation of this inactive preparation leads to restoration of a large portion of the specific antibody activity. In the words of one group of investigators (*10*), "It is evident, therefore, that the information required to create antibody specificity survives when all noncovalent interactions are disrupted and all disulfide bonds are broken. The information must therefore lie in the amino acid sequence of the protein."

Still unanswered is the question of whether the zygote has many or only a few structural genes for gamma globulins. Of importance to these considerations are recent studies of inherited variations in human gamma globulins. In 1956, Grubb and Laurell (*11*) reported inherited variation in the human gamma globulins, expressed as antigenic differences. The rather complicated reaction has been extensively investigated and is capable of revealing very small differences in gamma globulin types. Two genetically distinct systems have been detected thus far. The first, known as the Gm system, reflects variation in the larger of the two types of polypeptide chains which constitute 7*S* γ-globulin molecules (Fig. 1) (*12*). This type of H (heavy) chain is replaced in 19*S* and β_{2A} globulins by other polypeptides lacking Gm specificity. The other γ-globulin locus for which genetic variation is known, the *Inv* locus (*13*), involves differences in the L (light) chain, which is common to all the classes of antibodies (*12*). At present, only a few alleles are known at the *Inv* locus, but a large number have been detected at the *Gm* locus (*14*).

Studies of the Gm and Inv types provide partial answers for some important questions regarding synthesis of γ-globulins. First, how many structural loci direct synthesis of γ-globulins? If the antibody response involves selective synthesis of molecules from information which already exists in the organism, does each cell have a separate locus for all the different forms of H- and L-chains? Antibody response in this case would involve activation of the appropriate structural locus. On the other hand, it has been suggested that there is only one locus per haploid complement for each polypeptide chain in γ-globulins. The variability would arise through a very high rate of somatic mutation, so that the population of cells would have variability but individual cells would not (*15*). Sequential mutation of the

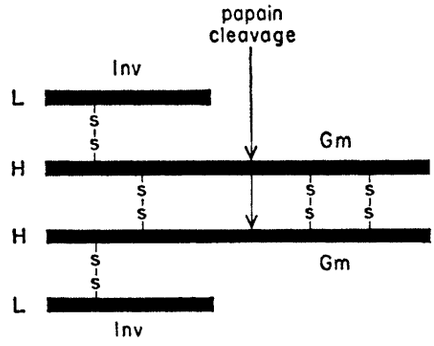

Fig. 1. Structure of 7*S* γ-globulin, showing two types of polypeptide chains (light, L, and heavy, H) bound together into a tetramer by disulfide bonds (*41*). The Gm antigenic specificity is located on the fragment of H-chain cleaved by papain. The Inv antigenic specificity is on the L-chain.

structural gene would lead to a population of alleles, most of which would be similar to the original alleles of the zygote. The mass of antibodies produced by this family of related loci would be expected to show simple Mendelian inheritance for variation in the primary structure, although the locus in a particular cell might have mutated away from the characteristic type of the zygote.

Present results suggest that the number of structural loci for γ-globulins is very limited and that the hypothesis of a single locus for each of the chain types is at least plausible. The evidence consists in the demonstration that a majority of 7*S* antibody molecules possess the Gm specificity known to be transmitted as a single Mendelian trait (*16*). Failure to observe recombination among the Gm subtypes is evi-

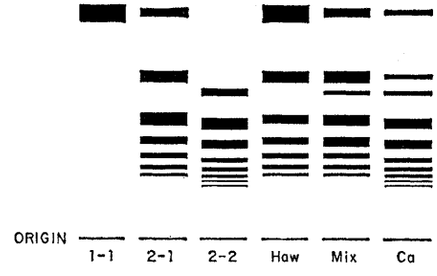

Fig. 2. Diagram of three common haptoglobin types and the three rare types which appear to be somatic mixtures of the common types. The protein bands are separated by electrophoresis at alkaline *p*H in starch gel. The patterns shown represent the hemoglobin complexes of haptoglobin. Haptoglobin Haw appears to be a mixture of 1-1 and 2-1, and haptoglobins Mix and Ca both appear to be mixtures of 2-1 and 2-2, with more of the 2-2 in haptoglobin Ca.

dence that all reflect genetic variation limited to a small region of one chromosome (*14*). Myeloma proteins depart somewhat from this pattern, in that they do not always exhibit all the Gm factors known to be present in the host genome (*17*). However, if one views myeloma cells as arising clonally from a single abnormal cell, then it may be that myeloma proteins merely reflect the somatic "mutation" which, it is postulated, gives rise to γ-globulin variation.

As yet, full understanding of the control of γ-globulin structure and synthesis has not been achieved. Whatever the ultimate resolution of these problems, it seems likely that the γ-globulin system will not be merely another "example" of gene action as typified in hemoglobulin.

It is ordinarily assumed that all diploid cells of the body contain identical genetic information, even though part of the information is not expressed. The mutational origin of γ-globulin variability would lead to genetic mosaicism, in which not all cells do contain identical information. Chromosomal mosaicism has been observed on many occasions, particularly XX/XO mosaicism, the affected persons expressing some degree of abnormality usually associated with the abnormal chromosome complement. Most persons are probably mosaic for a variety of abnormal chromosome complements, the defective mitosis having occurred so late in development as to give rise to only a small clone of abnormal cells.

The evidence is now strong that certain persons and families have increased risk of nondisjunction of chromosomes (*18*). The possibility that somatic mosaicism may contribute significantly to phenotypic variation has not been fully explored, owing largely to lack of systems which would permit investigation (*19*). While mosaicism involving major chromosome changes may be readily detectable, minor morphological changes would, at best, be difficult to recognize, and changes affecting only a few loci could not ordinarily be detected cytologically. One must therefore search among functional properties of genes for an expression of mosaicism.

The analysis of clones from persons heterozygous for distinguishable protein variants would seem ideal for the demonstration of mosaicism. This technique was used effectively by Davidson, Nitowsky, and Childs to demon-

strate mosaicism of X-chromosome activity (*20*). Some unusual haptoglobin types can be interpreted as resulting from mosaicism and may be a means of studying this phenomenon further. Diagrams of the haptoglobin types are shown in Fig. 2 (*21*). Types Ca and Haw have the appearance of mixtures of the usual heterozygous haptoglobin 2-1 with homozygous haptoglobin types 2-2 and 1-1, respectively. The intermediate type (Mix) between haptoglobins 2-1 and Ca can also be explained as a mixture of 2-1 and 2-2, with less of the latter than in haptoglobin Ca. This intermediate type has been observed both in a known mosaic resulting from double fertilization (*22*) and in normal persons (*23*).

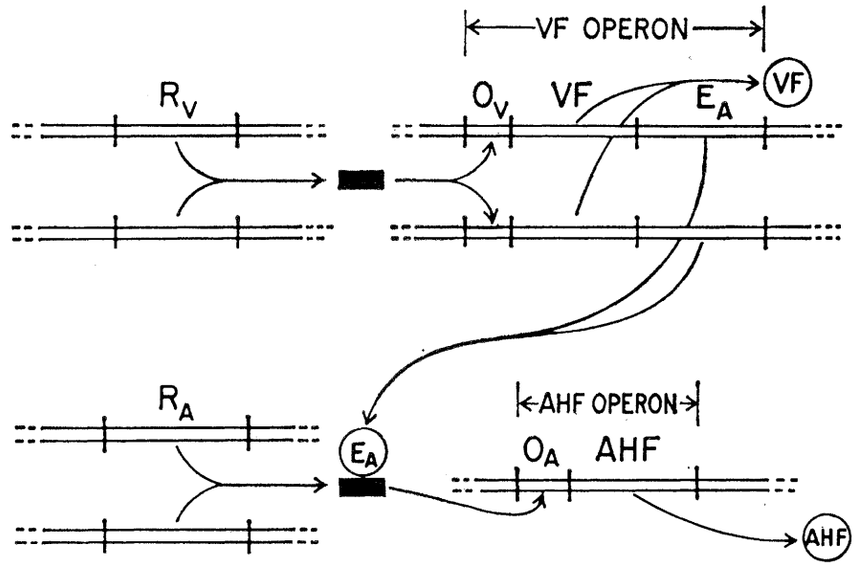

Fig. 3. Diagram of relationships postulated for genes involved in synthesis of antihemophilic factor (AHF) and vascular factor (von Willebrand's factor, VF). Hemophilia A would result from a defect in the AHF operon, possibly in the structural gene for AHF. Von Willebrand's disease would result from a defect in the VF operon, resulting in a deficiency of both VF and E_A, the effector substance for the AHF regulator. R_V and R_A are the regulator genes for the VF and AHF operons, respectively, and O_V and O_A are the operators for these operons. The E_A deficiency permits the repressor produced by R_A to inhibit activity of the AHF operon.

Regulation of Gene Action

Proteins vary quantitatively as well as qualitatively, and investigations of the ways in which protein synthesis is regulated have become widespread. In these studies, for the most part, the investigators have made use of microorganisms, from which enzymes can be readily isolated and in which genetic analysis can be carried out with high resolution. There is indication, however, that mechanisms may exist in higher organisms which are unknown in *Escherichia coli*.

The development of the operon theory by Jacob and Monod (*24*) stimulated interest both in applying this theory to many problems other than that of the β-galactosidase of *Escherichia coli* and in searching for other means of regulating protein synthesis. Attempts to apply the operon hypothesis to human variations have led to a number of proposals, few of which can be approached experimentally as yet (*25*). The genetic analysis necessary for establishing relationships among control and structural genes has not been possible in man. It is tempting to cite all inherited quantitative variations in proteins as possible examples of control-gene mutations —at least until a structural difference is demonstrated in the variant protein.

An interesting example of a mutation in man which can be explained by the operon hypothesis is von Willebrand's disease (vascular hemophilia) (*26*). This rare condition is inherited as an autosomal dominant. Affected persons have a defective clotting mechanism due to reduced activity of antihemophilic factor (AHF), the same activity which is reduced in classical hemophilia A, a sex-linked trait. In addition, there is a prolongation of bleeding time which is not corrected by administration of AHF but is corrected by transfusion of hemophilic plasma.

The lack of in vitro complementation, evidenced by poor clot formation in a mixture of plasma from the two types of patients, indicates the common AHF defect. A distinction is revealed by transfusion studies. Plasma from a patient with von Willebrand's disease has no effect when injected into a patient with hemophilia A. On the other hand, transfusion of plasma from a patient with hemophilia A into a patient with von Willebrand's disease leads to synthesis of AHF by the latter (*27*). It is obvious that the basic defect of von Willebrand's disease is not in the structural gene for AHF. Rather, it is in part the failure to evoke synthesis of AHF.

Several explanations are possible. These must account for the following observations. (i) Synthesis of AHF has not been induced in hemophilia A; therefore, a defect in the structural gene or at least in the function of the operon for AHF synthesis is likely. (ii) Since synthesis of AHF can be readily induced in von Willebrand's disease, the AHF operon is probably intact. (iii) Transfusion of hemophilia-A plasma into a patient with von Willebrand's disease produces transient correction of the vascular defect, but the correction appears not to persist as long as induction of AHF synthesis does (*28*). (iv) In two patients homozygous for von Willebrand's disease, induction of AHF synthesis by transfusion was much less than is observed in heterozygotes (*29*).

The foregoing observations are accommodated by the hypothesis shown diagrammatically in Fig. 3. Von Willebrand's disease is pictured as a defect in an operon responsible for synthesizing the von Willebrand's factor (vascular factor) and the effector protein responsible for AHF induction. It is not known at present whether two separate proteins are involved or whether there is a single protein having both functions. In a patient heterozygous for von Willebrand's disease, some vascular factor and some effector substance are synthesized, but these ordinarily are inadequate to maintain normal clotting. Transfusion of hemophilia plasma, which is normal or possibly enriched with respect to levels of vascular factor and effector, corrects the vascular defect directly and, by means of the effector, derepresses the AHF operon. The low response of AHF synthesis in patients homozygous for von Willebrand's disease could be explained as due to the fact that in

homozygotes transfusion was the only source of effector, whereas in heterozygotes effector was available by endogenous origin from the normal operon and also through transfusion. This hypothesis is not likely to stand the test of time, but it is the simplest which is consistent with present observations (*30*).

Another example of possible control-gene mutation in man has been identified in the very rare autosomal recessive disease oroticaciduria. Only three cases have been identified, the first in 1959 (*31*). The metabolic nature of the defect was recognized through identification of crystals of orotic acid in the urine of the patient. A block in the metabolism of orotic acid was proposed and subsequently demonstrated in heterozygous normal relatives of the patient.

Direct assay of enzymes revealed a defect in two enzymes, orotidylic pyrophosphorylase and orotidylic decarboxylase, both on the biosynthetic pathway of uridylic acid (*32*). Subsequent studies in another patient have confirmed these results (*33*). Although many examples are known of quantitative changes in several enzymes as a result of a single gene effect, these have all been explained as secondary responses. Oroticaciduria is thus the first example in man of a primary defect in two separate enzyme activities. This could occur through several mechanisms—for example, the presence of a common polypeptide chain in the two enzymes or the deletion of a chromosome segment containing portions of the two structural genes corresponding to the two enzymes. That neither of these is the explanation is suggested by the observation that the enzyme level in heterozygotes is appreciably less than 50 percent (*34, 35*). Thus, the normal structural allele must be inhibited also. These relationships are consistent with the view that there is a defect in a control gene, causing inhibition of both sets of normal structural genes. Such a defect might be in a regulator gene, with formation of a repressor with increased affinity for the operators associated with the structural genes (*35, 36*).

There are other situations in man which seem not to reflect control solely through an operon mechanism. Rather, synthesis appears to occur at a rate near the intrinsic maximum rate. The evidence for this is indirect, consisting of demonstrated differences in relative amounts of product produced by two structural alleles in heterozygous combination. The best-known example is hemoglobin. Persons heterozygous for abnormal hemoglobins ordinarily produce less of the variant hemoglobin than of Hb A (*37*). Presumably in such persons the feedback control is identical for the two alleles. The only difference is in the structural genes. Therefore, the structure of a gene (or of the corresponding messenger RNA) may influence significantly the rate of expressed gene activity.

Jacob and Monod were careful not to assign too many physical properties to the operator (*24*). It was defined as a region lying at the "beginning" of a structural gene, responsible for turning the structural gene on and off through formation of complexes with a repressor. Conceivably, an operator could include a portion of the structural gene, and structural mutations would therefore also be operator mutations. While this indeed may be the case for some proteins, it is stretching the utility of the hypothesis to apply it to hemoglobin. The current picture of the Hb_β locus is shown in Fig. 4. The β and the δ structural genes of hemoglobin are located on the same chromosome, apparently very close to each other (*38*). The Lepore hemoglobins, a family of rare hemoglobins, appear to result from a deletion of part of the β structural gene and part of the δ structural gene (*39*). The resulting polypeptide chain has a sequence of amino acids on the C-terminal end characteristic of the corresponding amino acids of the β-chain. Similarly, the N-terminal amino acids are characteristic of the N-terminus of the δ-chain. The deletion would therefore involve the N-terminal part of the β structural gene and the C-terminal part of the δ gene. This requires the alignment shown in Fig. 4.

The abnormal hemoglobins involve substitutions in various locations in the

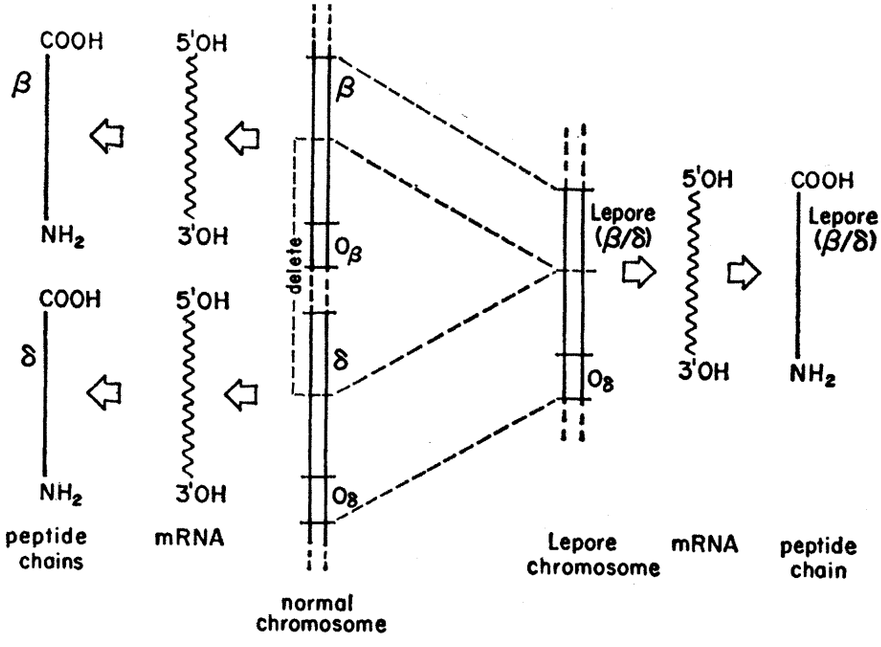

Fig. 4. Diagram of the proposed relationship of Hb_β and Hb_δ structural loci. These two structural loci are known to be on the same chromosome very close to each other (*38*). The deletion which leads to Lepore-type hemoglobins indicates that the DNA which codes for the C-terminal position of the β-chain is on the opposite end from the N-terminal segment of the δ-chain. From the studies of microorganisms, it seems probable that each operon produces a single messenger RNA (mRNA) molecule (*42*), the synthesis of which is initiated at the operator. Translation of mRNA into protein occurs in the same direction, as indicated by the decrease in amount of product formed by each structural gene as a function of distance along the mRNA molecule (*43*). The synthesis of polypeptide chains has been shown to proceed from the N-terminal end (*44*). It therefore follows that the operators must be at the lower end of the structural genes, as shown in the diagram. The existence of separate operators for the β and δ loci is indicated by the much greater production of β-chains than of δ-chains, something which would not be possible if the β gene were the distal structural gene of a single β–δ operator (*43*). The deletion which produces the Lepore gene must also eliminate the β operator, leaving the Lepore gene under control of the δ operator.

peptide chains. For the most part, these variants are synthesized at a lower rate than normal α- and β-chains. Since the only difference in the structural genes may be a single nucleotide substitution far to the interior, it is obvious that the structural gene (or its corresponding messenger RNA) must play a significant role in determining the rate of protein synthesis.

This hypothesis, which has been designated by some as the "structure-rate" hypothesis, explains only the upper limit of gene activity. The obvious fact that not all human cells synthesize hemoglobin testifies to the existence of other mechanisms, possibly of the operon type, which can shut off function.

One additional mechanism has been demonstrated in man. This is the so-called X-chromosome inactivation, the best-known statement of which is by Mary Lyon (*40*). The hypothesis states that in XY males the single X chromosome is active in all cells. However, in XX females only one of the two X's is active in any cell. Inactivation occurs randomly in early embryonic development, but, once a particular X chromosome is inactivated, that same X chromosome is inactive in all descendant cells.

There are various indirect lines of evidence supporting the hypothesis. Convincing evidence came from a study of structural variants of human glucose-6-phosphate dehydrogenase (*20*). Women heterozygous for the electrophoretically distinct forms A and B, whose structure is determined by genes on the X chromosome, ordinarily have both forms present in the tissues which produce this enzyme. If individual cells are isolated and allowed to form clones of cells in culture, all of the cells in the clone will be either form A or form B (not both), depending on the active chromosome present in the original isolated cell.

X-chromosome inactivation is a means of preventing gene imbalance in some persons who have two chromosomes in place of the one necessary for normal function. To what extent similar mechanisms exist for other chromosomes or, more likely, for regions of other chromosomes is not known. Possibly evolution has favored genes on the X chromosome which can be regulated in this way. Understanding the nature of X-chromosome inactivation promises to be an important step toward understanding the general problem of gene control.

Summary

Genetic studies in man have given insight into the mechanism of gene action and have provided examples of mechanisms first investigated in other organisms. Studies of inherited hemoglobin variants first demonstrated that determination of the primary structure of proteins is a function of genes. Investigation of γ-globulins has led to the concept that all proteins may not show the somatic stability of gene structure characteristic of hemoglobins. In addition, somatic mosaicism for more stable characteristics may increase the phenotype variability in some persons.

Two examples of mutations in man which can be most readily explained by systems of gene regulation of the Jacob-Monod type are von Willebrand's disease (vascular hemophilia) and oroticaciduria. In addition, some structural genes seem to be regulated in part by the structure of the gene itself, including portions far removed from the regions where an operator would be expected. Still another system of regulation, chromosomal inactivation, accounts for balance of genes on the X chromosome in females.

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