Part II
Underlying Scientific Principles

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Chapter 4

Essentials of Genetics

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Chapter 4

Essentials of Genetics

The role of genes in disease is still not fully understood. Many diseases--cancer and heart disease, for example—appear to have some genetic influence. These genetic variations may be inherited or may arise from environmental sources. In other cases, it is hypothesized that a person with inherited types of gene variations may suffer harmful effects when exposed to hazardous substances.

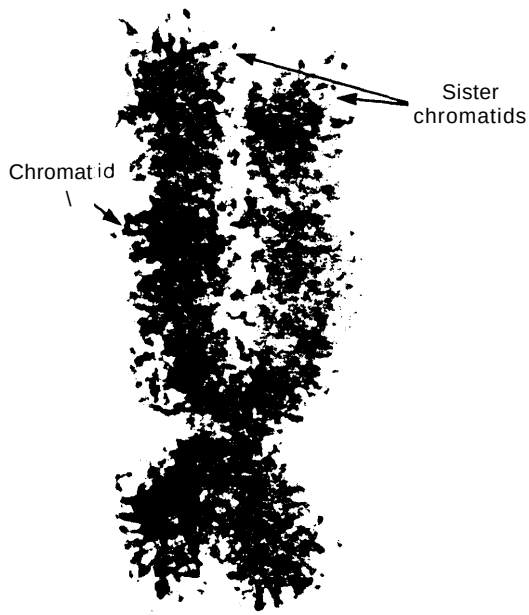
The occupational studies assessed in Part 111 of this report rely on genetic analyses. An understanding of genetics—the basic structure and function of genes and their connection with disease—will enable the reader to comprehend more readily the report's interpretation of those studies. This chapter, therefore, attempts to provide an introduction to the complex subject of genetics.

Chromosomes

In higher organisms, the nucleus of each cell contains the genetic material DNA (deoxyribonucleic acid), which directs all the functions of the cell such as metabolism and growth. The DNA in its normal state in the nucleus is joined with proteins to form a set of complicated structures called chromosomes. Each human cell contains 46 chromosomes, half derived from the mother and half from the father. These 23 pairs of chromosomes mean that all genetic material is represented twice in each cell. One of the twenty-three pairs is a pair of sex chromosomes. Females have two X chromosomes and males have one X and one Y. When the cell is in a resting stage, all the chromosomes are tangled and difficult to distinguish; just prior to cell division, however, the chromosomes condense and replicate to appear in a light microscope as dual structures, each chromosome consisting of two identical chromatics (fig. 2). These two sister chromatics are held together by a central constriction, the centromere. At cell division, the sister chromatics are pulled apart at the centromere, and one chromatid goes to each of the two daughter cells, thus ensuring a full complement of DNA in each cell.

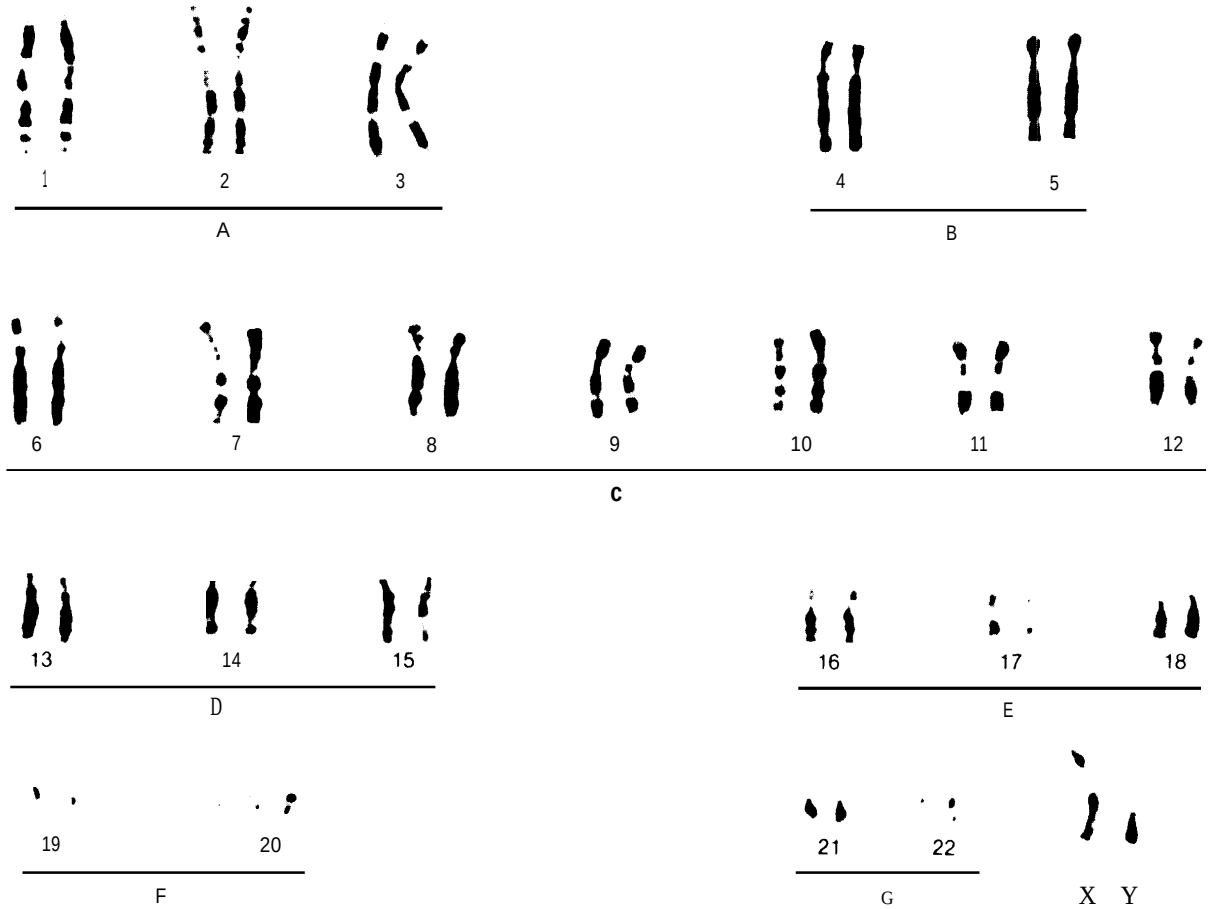
Each of the 23 pairs of chromosomes is a unique size and shape, permitting the chromosomes to be distinguished from one another, In addition, various staining treatments have been developed that reveal, for each chromosome, a characteristic sequence of "bands" composed of alternately dark and light staining regions. From the combination of size, shape, and banding patterns, the 46 chromosomes from a single cell can be arranged into a systematic picture called a karyotype (fig. 3). Chromosomal abnormalities, which alter the

Figure 2.—A Chromosome



SOURCE: Office of Technology Assessment

Figure 3.—Normal Human Male Karyotype



SOURCE" Cytogenetics Laboratory, The Johns Hopkins University

banding patterns or size or shape, can be detected using this technique.

The study of cytogenetics compares the appearance of the chromosomes in the karyotypes to identifiable traits in the individual. Several chromosomal abnormalities have been identified, and they fall into two classes: a change in the number of chromosomes and a change in the chromosome structure. Changes in the number of chromosomes occur during germ cell (egg or sperm) formation and are detected in the offspring. For example, Down's syndrome is a result of an extra chromosome 21 in all cells. Although it is possible for the number of chromosomes to change in somatic cells (all cells other than germ cells) by a mistake made during cell division, these cells are usually nonviable; thus, this type of change will not be discussed further.

Chromosomal aberrations, or changes in chromosome structure, can occur spontaneously or can be caused by chemicals or ionizing radiation. They are important because, whatever their origin, they can be replicated and passed on to succeeding generations of somatic cells. How chemicals or ionizing radiation causes these aberrations is not well understood.

Everyone has some chromosomal aberrations in his or her somatic cells. In lymphocytes (white blood cells) grown under laboratory conditions, roughly 2 out of every 100 cells contain at least one structurally abnormal chromosome. Similar levels of aberrations have been seen in preparations of bone marrow cells and fibroblasts (connective tissue cells) and presumably are present to some extent in every kind of somatic cell (see app. D). These "background" or "spontaneous"

chromosomal aberrations are thought to be the consequence either of failure to repair rare replication errors or of postreplication chromatid exchanges, which may be a normal part of the cell cycle. However, an increase in the number of aberrations may imply the existence of certain rare chromosomal instability diseases (discussed below) or exposure to clastogens (chromosome-damaging agents) in the environment. In the latter case, such an increase may serve as a method for monitoring exposure to harmful agents.

Another type of chromosomal change is a sister chromatid exchange (SCE). SCEs are exchanges of apparently equivalent sections of the sister chromatics of the same chromosome (fig. 4). This phenomenon, which can be seen only under special laboratory conditions, occurs at a much higher frequency than do chromosomal aberrations, with most reported background frequencies being in the range of 5 to 15 SCEs per cell (see app. E). * The biological or genetic significance of SCEs is unknown. While the presence of SCEs in a cell is not necessarily indicative of damage to that cell, some empirical evidence suggests a relationship between SCEs and agents which damage DNA (7). The detection of SCEs thus is seen as another way to monitor damage to chromosomes. A major exception is that SCEs are not usually induced by ionizing radiation (3).

● 'I'hese higher background frequencies are thought to be due to the laboratory procedures necessary for visualization of SCEs.

Figure 4.—Sister Chromatid Exchanges

SOURCE: Biomedical Sciences Division, Lawrence Livermore National Laboratory

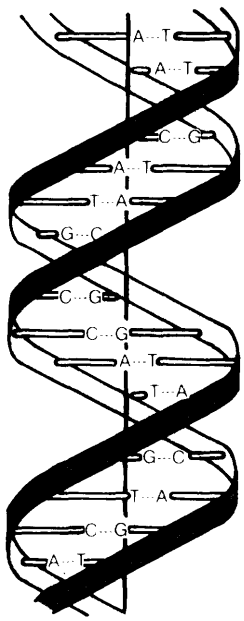
DNA, genes, and proteins

The genetic information contained within the familiar DNA double helix (fig. 5) is completely defined by the linear order of four chemical compounds known as nucleotide bases, adenine (A), guanine (G), cytosine (C), and thymine (T). These bases, attached to the double strands of the helix, interact in a specific fashion to form the rungs of the DNA ladder: A's can pair only with T's, and G's only with C's. Therefore, the two sides of the ladder are not identical but are "complementary." The chemical nature of the complementary base pairing is vital to the function of DNA. The pairing is specific, which ensures that the genetic information will be maintained, but not very strong, so that sections of the helix can "unzip" to expose the bases, thus making the genetic information available for use,

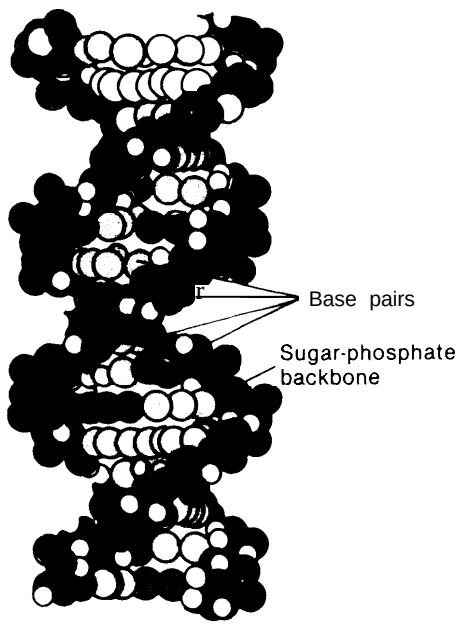
An ordered sequence of a few thousand nucleotide bases is the unit of heredity known as the gene. A gene has regulatory signals at either end specifying its beginning and end. The signals themselves are a series of nucleotide bases, usually on the order of 10 to 100 bases long. For the most part, one gene contains the information for the synthesis of one protein. Thus, the four bases, A, G, T, and C, depending on their order, contain the information for the synthesis of proteins. The genetic code is the same for all organisms, The difference between organisms, therefore, is not the inherent chemical nature of the genetic material, but the different sequences of nucleotide bases.

The DNA present in every cell of every living organism has the capacity to direct the functions of that cell. Gene expression is the way in which the genetic directions in any particular cell are decoded and processed into the final functioning product, usually a protein (fig. 6). In the first step,

Figure 5.—The Structure of DNA



A schematic diagram of the DNA double helix.

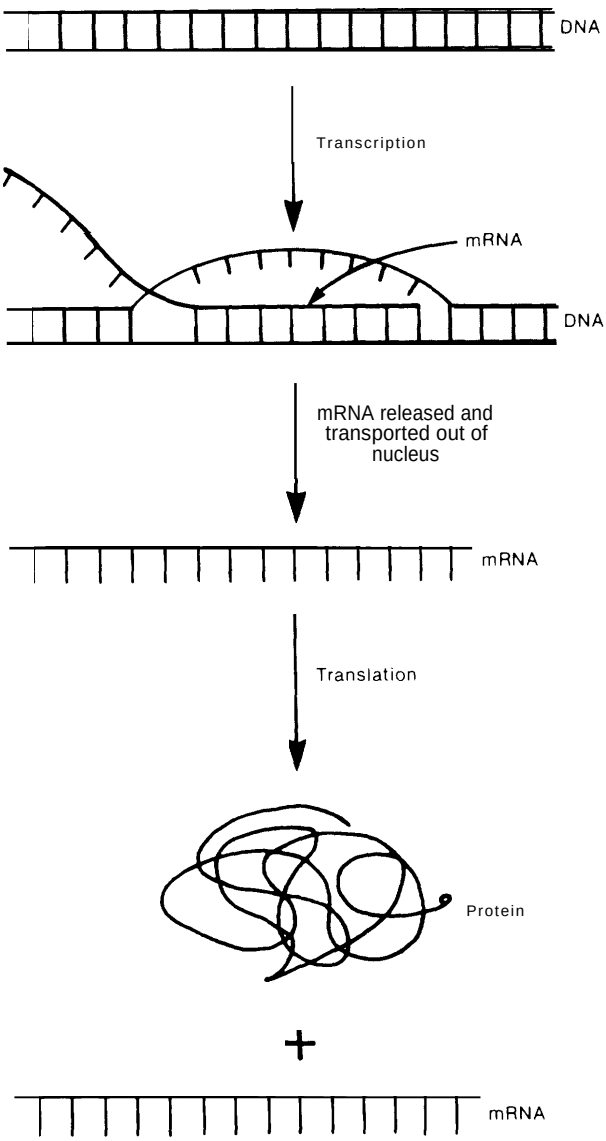


A three-dimensional representation of the DNA double helix.

The DNA molecule is a double helix composed of two chains. The sugar-phosphate backbones twist around the outside, with the paired bases on the inside serving to hold the chains together.

SOURCE" Office of Technology Assessment

Figure 6.—Mechanism of Gene Expression



SOURCE Office of Technology Assessment.

called transcription, the DNA double helix is locally unzipped, in the region of the gene of interest, and the intermediate product, messenger RNA (mRNA), a single-stranded, linear sequence of nucleotide bases chemically very similar to DNA, is synthesized. The transcription process dictates the synthesis of mRNA that is complementary to the section of unzipped DNA. The second step is translation. The mRNA, after release from the DNA, becomes associated with the protein-synthesizing machinery of the cell, and the sequence of nucleotide bases in the mRNA is decoded and translated into a protein. The protein goes on to perform its particular function, and when the protein is no longer needed, the protein and the mRNA coding for that protein are degraded. This mechanism allows a cell to "fine tune" the quantity of its proteins while keeping its DNA in a very stable and intact form.

Proteins actually perform the necessary functions of the cell. By far the most diverse group are the enzymes, or the proteins that catalyze all biological reactions. Another group, the structural proteins, are found, for instance, in cell membranes. Other proteins, such as hormones, have regulatory functions; still others have highly specialized functions—for example, hemoglobin carries oxygen from the lungs to the rest of the tissues.

Genetic variability in humans

Because all humans need to perform the same life-supporting tasks, they all have genes that code for the same types of proteins. But for any given protein, there may be many variants, some "normal" and some deleterious. This means that the genes that code for these variants are also slightly different. These forms of the same basic gene are called alleles. It is the variation in these alleles that forms the basis for diversity within species.

Variants have been discovered for many human genes. For example, of the 319 possible detectable variants of beta-globin, 104 had been observed by 1976. More than 80 different variants of glu-

cose-6-phosphate-dehydrogenase (G-6-PD) have been identified, and some of these differ in their metabolic and clinical effects.

Although most variants are rare, a few occur with frequencies of at least 2 percent. The products of such variable alleles include beta-S globin (which produces a sickling of red blood cells), the A- G-6-PD allele, blood groups, and histocompatibility substances. One study estimated that for the "average" gene coding for an enzyme, approximately 6 percent of individuals will be easily detected as carrying a variant gene. Other variants, which are not so readily detectable, probably occur more frequently. Thus, it may be that for a given gene, 20 percent of the population might possess two variant alleles. If this is true, the prospects for detecting individuals with susceptibility-conferring genotypes by mass screening seem high.

At first glance, it would appear unlikely that genes conferring susceptibility to chemical or physical agents in the workplace could gain high frequency; they should have been selected against. This is not necessarily the case.

The frequency of an allele will decrease over the course of generations if the individuals who possess it have, as a result, lower reproductive fitness (that is, die before having children or have children who die before reproductive age). However, those who possess alleles whose only harmful effect results from workplace exposures may be well into the reproductive phase of their lives before their first exposure. If, in addition, a latent period occurs between exposure and harmful effect, as is the case for most cancers, those individuals may have completed their reproduction before the disease appears, Thus, the allele would not reduce reproductive fitness. Such alleles could attain high frequency by random genetic drift or by an advantage that they confer to the reproductively active members of the population or that they conferred in an earlier evolutionary setting. For example, the hemoglobin alleles (such as sickle cell) gained in frequency because of the protection they conferred against malaria.

On the other hand, in situations where the harmful effect of an allele only occasionally manifests itself during the reproductive phase, some individuals with that allele would have their reproductive years shortened, Therefore, the average reproductive fitness of those possessing the allele would be slightly less than those lacking it. Over many generations, even a small decrease in reproductive fitness would diminish the frequency of the allele and, in the absence of other effects, result in its eventual disappearance. Thus, there may not be very many alleles present today with high frequency whose harmful effects are usually manifested later in life.

Harmful reactions to newly invented chemicals also may occur frequently simply because most individuals lack genetically determined mechanisms for detoxifying them or repairing the damage they cause. It remains to be determined whether biological detoxification methods have already evolved for many chemicals that cause disability today. If they have not, only rare individuals may be able to cope with those chemicals. Thus, the susceptibility-conferring alleles then would be the predominant types.

Even when a genetically determined detoxification mechanism has evolved, it might protect against an acute effect of the chemical (and consequently confer a reproductive advantage), but the mechanism itself might cause a change in the chemical that increases the chance of a latent harmful effect. If the latency period exceeds the reproductive period, this long-term effect would not have a selective disadvantage.

Mutation

A mutation is a heritable change in the sequence of nucleotide bases. * A mutation can be an insertion or deletion of a base or a base change. A base change is usually caused by a mispairing reaction during DNA replication which effectively changes one base pair to the other one. For instance, if a modified A mispairs with a C, the C will correctly pair with a G during replication and will have effectively converted an A-T base pair to a G-C base pair in one of the daughter cells.

Mutations normally occur at a low rate and, indeed, are the raw material for the evolutionary process. They can occur in spaces between genes and thus be neutral, or they can occur within genes. Occasionally an intragene mutation will cause the gene to encode a protein that is better adapted to its function, but most mutations within genes are deleterious (l). Mutations within genes are well documented in humans. In fact, at least 1,000 human diseases are known to be genetic in origin (5), and many more are thought to have a significant genetic contribution.

Induced mutations can be caused by genotoxic (gene-damaging) chemicals or ionizing radiation. Mispairing of nucleotide bases during DNA replication can be caused by chemical modification of bases or by the incorporation of compounds that look like bases. Some compounds insert themselves between base pairs, distort the helix, and thus cause additions or deletions of nucleotide bases during DNA replication. Radiation is thought to cause mutations by damaging the structure of the nucleotide base or the backbone of the DNA helix.

DNA damage that can lead to mutations can often be repaired. A mismatched base pair or chemically modified base will distort the double helix and alert cellular repair mechanisms. The damaged section of the DNA strand can be excised and new DNA synthesized using the other strand as a template. It is important for DNA to be repaired prior to replication because mismatched bases become fixed during replication.

*Some of the chromosomal aberrations discussed above are actually mutations, but for simplicity, the use of "mutation" in this report will refer to nucleotide base changes whereas "chromosomal aberration" will refer to gross structural changes visible in the light microscope

Single gene traits

A genetic trait is any detectable condition that is known to be inherited. The easiest case to study genetically is a trait specified by a single gene. Examples include sickle cell anemia and Tay-Sachs disease. Suppose a hypothetical single gene trait B determines the normal condition and the rarely occurring allele b results in an observable deficiency. Because there are pairs of each chromosome, a normal individual will be either BB (both chromosomes of the pair have the B allele) or Bb (one chromosome has the B allele and the other has the b allele). An afflicted individual will be bb (both chromosomes of the pair have the b allele). When both chromosomes carry the same allele, the trait is homozygous (that is, BB or bb); when the two chromosomes carry different alleles, as in the Bb individual, the trait is heterozygous. In a heterozygous trait where only

one allele appears to be contributing to the observable condition (in this case, B), that allele is said to be dominant. The allele (b) that is masked by the dominant allele is recessive. Only when both chromosomes of a pair carry the recessive allele will the deficiency be detected. A heterozygote for the trait is called a carrier.

The actual genetic constitution of a trait (or an individual) is the genotype, In the case of BB and bb individuals, the genotype is reflected in the observed trait, or phenotype. In general, a specific phenotype associated with the heterozygous genotype is not observed. In this simple case, because B is dominant, the phenotype of the heterozygote is the same as the homozygote (BB). In addition, the phenotype reflects the interaction of the genotype with the environment,

Occasionally, a heterozygous trait is expressed at an intermediate level, that is, neither allele is fully dominant or recessive. In these cases the alleles are said to be codominant, and the observed phenotypes reflect both homozygous and heterozygous genotypes.

The deficient phenotype (from genotype bb) may be expressed by various symptoms, but it is the result of a single protein deficiency. The result may be due, for example, to a nonfunctional structural protein, the lack of a protein or hormone, or a deficient metabolic enzyme. Moreover, there are many traits whose phenotypic expression is the result of several gene products working together. An example of a polygenic trait is a person's height,

Mutations, chromosomes, and cancer

Both mutations and chromosomal aberrations are thought to play a role in the set of diseases known as cancer. Many (about 90 percent) of the chemicals known to cause cancer (carcinogens) in animals are also known to cause mutations in in vitro tests (6). In fact, chemicals being tested for their potential carcinogenic effects are first screened for their ability to cause mutations. One theory of carcinogenesis postulates that one or more mutations causes a cell to reproduce out of control and, subsequently, form a tumor. Strongly supporting a mutational origin of many cancers is the fact that they arise from a single somatic cell; that is, the cancer genotype, once present, is stable and passed on to daughter cells during tumor growth.

Most cancer cells have abnormal karyotypes; there are frequently changes in chromosome number, and more recently, it has been shown that there are an unusually high number of chromosomal aberrations. It is not known whether these abnormalities are a cause or an effect of the malignant state, but several inherited diseases, * in which there is an increased aberration frequency, are associated with an increased risk for developing cancer (9). These single gene recessive diseases are thought to be due to deficiencies in DNA repair processes, and the chromosomes of afflicted individuals are much more susceptible to breakage caused by radiation and chemicals. Clearly, these chromosomal instabilities precede any malignancy, because these individuals also have many aberrant cells that are not malignant. Still, some types of cancer (for example, myelocytic leukemia) correlate with specific chromosomal abnormalities.

One hypothesis holds that mutations and/or chromosomal aberrations are precancerous events, but, as yet, there is very little definitive scientific evidence to support this. A recent report has shown that the gene presumably responsible for one type of human bladder cancer differs from its normal counterpart by a single base mutation (10).

A conspicuous feature of carcinogenesis is the generally long period of time that elapses between the initial exposure to the carcinogen and the appearance of the disease. Why this time course is so long is unknown, but based on extensive animal experimentation, carcinogenesis can be separated into two distinct steps, initiation and

*Bloom's syndrome, ataxia telangiectasia, Fanconi's anemia, and xeroderma pigmentosum.

promotion. Evidence has accumulated to suggest that initiation may be nothing more than mutagenesis, the most powerful initiators being the most potent mutagens. However, initiation alone apparently is not enough to produce the disease; promotion is also necessary. * The nature of promotion is still obscure. Various agents or physical insults (for example, wounding) can act as promoters, often a long time after initiation. The feature all promoters have in common is that they provoke increased cell multiplication of initiated cells; generally, they do not affect noninitiated cells. How rapid cell proliferation in the presence of a mutation could lead to a malignant state is unknown. At any rate, both genetic and environmental factors can influence whether one develops cancer, but the elucidation of these factors has not yet been achieved (6).

If cancer indeed has one or more genetic components, individuals who have the "wrong" combinations of genes may be genetically more susceptible to cancer. Several gene products that could be involved in determining one's inherited predisposition to cancer are genes for DNA repair, immune function, and carcinogen metabolism.

Certain complex pathways of DNA repair are beginning to be understood in higher animals. If damaged DNA is repaired in such a way that the nucleotide base is the same as the old, no permanent change has taken place. On the other hand, if mistakes are made during DNA repair due to deficiencies in the repair enzymes, the new base sequence will be different from the original one, and, by definition, a mutation will now exist. Hence, this deficient repair may play an intimate role in the mechanism of formation of some kinds of mutations, Individuals who have deficient pathways of DNA repair might then be more susceptible to cancer.

Immune deficiencies, or the inability to fight disease, also might predispose one to cancer. If initiation is the result of a somatic cell mutation, then potential cancer cells would continually be formed in our bodies at a low frequency. An efficient immune system would recognize these cancer cells as "foreign" and kill them. Hence, reduced immune function may increase the risk for cancer. Indeed, many chemical carcinogens are also immunosuppressants, and this has been speculated to be part of their carcinogenic mechanism. Organ transplant patients who receive massive doses of immunosuppressants are at increased risk for developing cancer later (8).

Finally, genetic differences in the ability to metabolize chemical agents to carcinogens may be involved in determining an individual's predisposition to cancer. Many chemicals alone are not harmful, but in mammals a metabolic activation by complex enzymatic systems can occur to form an active carcinogenic compound. However, there are also enzyme systems that deactivate potential carcinogens by forming compounds that are safely eliminated from the body.

Hence, a person who is deficient in DNA repair or cellular immune function or who has higher levels of the activation enzymes or lower levels of the deactivation enzymes may be more susceptible to chemically induced cancer than someone who is competent in DNA repair or immune function or who does not activate specific chemicals to carcinogens very well or who efficiently eliminates potential carcinogens by deactivation. Because each of these critical functions may show a wide spectrum of activity, cancer susceptibility may be quite variable as well (2,4).

● Som[? agents, known as "complete carcinogens ," ran act as both initiators and promoters.

Body fluids used in genetic testing

The detection of genetic traits or abnormalities in humans presents special problems because tests for these factors must be noninvasive, that is, they must use easily obtainable material. Sev - eral sources of body fluids are available, but usually blood, urine, and feces have been used in genetic tests. Because they are waste material and do not participate in the normal functions of the

body, urine and feces have limited application in genetic testing. Their current use is as sources of material for the detection of mutagens. The assumption is that the presence of mutagens in waste material indicates that those mutagens also were present in body tissues.

Only blood serves as an easily obtainable source of body fluid and cells for genetic tests, and, again, an assumption is made that blood reflects events happening in the other parts of the body. An argument against this assumption is the fact that chemicals act in tissue-specific manners. On the other hand, in experimental situations with animals, it has been found that blood cells do reflect exposure levels. In addition, because blood is so easy to obtain, much of the research on genetic mutations in humans has been done on blood proteins.

Blood can be divided into two components: cells and serum, the fluid in which they float. Serum can be assayed for three types of compounds: proteins normally found in serum such as clotting factors, protein degraders, and antibodies; proteins from liver cells, some of which are important in carcinogen metabolism; and mutagens.

The two types of blood cells, red and white, can be used to detect genetic traits or abnormalities. Red blood cells, or erythrocytes, have both enzymatic functions and an oxygen-carrying function, handled by the protein complex called hemoglobin. Because the red cells are so easily obtained, a great deal is known about both the normal and genetic variants of erythrocyte proteins, In addition, hemoglobin is probably the easiest protein in the body to isolate in large quantities. For these reasons, tests to identify genetic or chemical variants of hemoglobin have been used to detect the presence of mutagens and are included in this assessment.

White blood cells, or leukocytes, are a heterogeneous set of cells involved primarily in the immune functions. A subset of these cells, the lymphocytes, are the cells most often used to detect mutagenic activity. It is the lymphocytes that are assayed for chromosomal aberrations and SCEs. Many other biochemical tests to detect mutagenesis also use lymphocytes.

Chapter 4 references

1. Crow, J. F., "How We]] Can We Assess Genetic Risk? Not Very," *Lauriston S. Taylor Lectures in Radiation Protection and Measurement,* Lecture No. 5. (Washington, D, C.: National Council on Radiation Protection and Measurements, 1981).
2. Harris, C. C., et al., "Individual Differences in Cancer Susceptibility, " *Ann. Int.* Med. 92:809-825, 1980.
3. Latt, S. A., et al., "Sister Chromatid Exchanges: A Report of the Gene-Tox Program)" *Mutat. Res. 87: 17-62, 1981.*
4. Marks, P. A., "Genetically Determined Susceptibility to Cancer," *Blood* 58:415-419, 1981.
5. McKusick, V., "Mendelian Inheritance in Man" (Baltimore: Johns Hopkins University Press, 6th cd., 1982).
6. Office of Technology Assessment, US. Congress, *Technologies for Determining Cancer Risks From the Environment* (Washington, D.C.: U.S. Government Printing Office, OTA-H-138, June 1981).
7. Perry, P., and Evans, H. J., "Cytological Detection of Mutagen-Carcinogen Exposure by Sister Chromatid Exchange, " *Nature* (London) 258:121-125, 1975.
8. Siskind, G. W. (cd.), *Immune Repression and Cancer (New* York: Grune & Stratton, 1975).
9. Swift, M., et al., "Reassessment of Cancer Predisposition of Fanconi Anemia Heterozygotes, " *JNCI* 65:863-867, *1980.*
10. Taparowsky, E., et al., "Activation of the T24 Bladder Carcinoma Transforming Gene is Linked to a Single Amino Acid Change, " *Nature* (London) 300:762-765, 1982.