Mutagenicity of Chemicals and Drugs

Tremendous advances in the understanding of genetics have come through the study of submammalian systems such as drosophila, bacteria, and viruses. Using a variety of well-characterized mutagens, scientists have been able to manipulate microorganisms in particular to produce selective mutations in the genes. Their methods are sophisticated enough to produce mutations in the genes governing the synthesis of the macromolecules involved in chromosome duplication (DNA synthesis) and gene expression (RNA and protein synthesis). With these advances has come the realization that similar mutations may be occurring in man by way of less controlled processes, such as radiation damage and alteration of chromosomes by chemicals and drugs. Many workers believe that chemical damage is now a more important problem than radiation hazard. Man has been able to detoxify foreign chemicals during the course of evolution. But in the last 50 years an enormous number of new chemicals has appeared that, unlike radiation, persist in the environment. Many of these have been shown to be mutagenic and to cause chromosome breaks. In addition, there are many examples now of amplification of the mutagenic action of a drug or chemical after it has been metabolized by man.

A group of scientists, concerned about the mutagenic effects of environmental chemicals and drugs on man, met in Washington, D.C., on 4 to 6 November to discuss present and potential hazards from these substances and methods of evaluating gene damage. The Conference on Evaluating the Mutagenicity of Drugs and Other Chemical Agents was sponsored by the Drug Research Board and four other groups* and demonstrated the widespread interest among the scientific community in the problem of mutation. E. A. Carlson of the State University of New York, Stony Brook, stated at the conference that people outside of science are also going to become concerned, just as they did when the hazards of radiation became apparent. The responsibility of the scientific community is to persuade government and industry, particularly those agencies with funds for research, that a potential problem does exist.

Scope of the Problem

Because of the genetic complexity of man, there are immense possibilities for mutation. This capacity for mutation is evidenced by the physical diversity of people. Individual genetic diseases occur infrequently, but there are very many of them—at least 1500 diseases are considered to be of genetic origin. Since genes control the synthesis of proteins, many of these diseases appear to be due to the absence of or abnormalities in proteins. At present, more than 70 variants of the enzyme glucose-6-phosphate dehydrogenase are known in man and 20 percent of these are harmful. Enzymatic deficiencies are being discovered, at an exponential rate, as fragments of old syndromes. This increase may not be due to a higher mutation rate, but rather to the development of techniques, such as electrophoresis, that readily detect changes in protein structure.

Barton Childs of Johns Hopkins University stated that between 4 and 20 percent of all conceptuses and 0.5 percent of all births now have chromosome abnormalities. In addition, he reported that 20 percent of the patients admitted to the pediatric service of the University hospital had chronic diseases in which the action of the genes is primary or very significant.

Although the mutation rate may not have increased in the population as a whole (and we have no way of knowing, since no monitoring has been done), mutation is becoming an increasingly important factor in human welfare. According to James Crow of the University of Wisconsin, "because of the reduction in the death rate due to parasitic and bacterial diseases, relatively more people suffer from genetic disease. In addition, there has been a reduction in the forces of natural selection, in the Darwinian sense, as a consequence of which we are accumulating mutations faster than they are being eliminated." Crow estimated that a mutation, once introduced, will remain in the genetic material for 40 generations, "producing an increasing burden of mutational damage with each succeeding generation, since each generation adds its own new mutations to those handed down from the past."

The insidious effects of mutations greatly concerned the participants of the conference. Although they agreed that a chemical or drug would probably never cause a genetic catastrophe on the scale of the teratogenic changes caused by thalidomide, most mutations are harmful or, at best, neutral and, according to Carlson, "will be expressed as lowered resistance to disease, lowered life span, increased infertility, and general physiological weakness."

Of particular importance are mutations occurring in the germ cells, since these are distributed to our offspring and retained in the population. Somatic mutations, which exist for only one generation, are considered to be of less importance, unless they are carcinogenic. And many mutagens have also been shown to be carcinogens. Although the mechanism of carcinogenesis is unknown, many workers believe that alterations in the chromosomes are part of the process. In addition, somatic mutations are very likely part of the process of aging.

Biochemistry of Mutation

Geneticists have defined two major categories of chromosome alterations—gene mutations and chromosome breaks or losses. A variety of chemicals and drugs have been identified which cause either or both of these changes. Scientists from many disciplines contributed to establishing the molecular basis for gene mutations by using microbial systems after the double helix model of DNA was developed. The sequence of amino acids in a protein is specified by a linear sequence of nucleotide base pairs in the DNA, with three base pairs corresponding to one amino acid since the genetic code is a triplet code. Thus a chemical change in a base pair or alteration of the linear sequence will generally lead to an abnormal protein.

For example, nitrous acid can cause oxidative deamination of cytosine, converting it to uracil; bromouracil, which can pair with guanine, can be incorporated into DNA in place of thymine, converting an adenine-thymine pair into a guanine-cytosine pair; and acridine dyes can intercollate between bases, causing an additional base to be incorporated into the DNA during replication.

* The conference was sponsored by the Drug Research Board, National Academy of Sciences; the Environmental Mutagen Society; the Food and Drug Administration; the National Institute of General Medical Sciences; and the Pharmaceutical Manufacturers Association Foundation, Inc.

These changes may be incorporated into the chromosome during normal DNA replication, since there is a finite capacity for error due to the complexity of the DNA and the enzymes involved in its replication. Some workers believe that breakage and reunion of DNA normally occur during transcription of messenger RNA, and hence mistakes may be introduced into the DNA during restoration of the covalent structure of the DNA. Introduction of mutations by these methods may have played an important role in evolutionary development and have had favorable effects. The mutation rate is abnormally increased, however, by mutagens which alter bases or decrease the fidelity of the replication and repair systems.

Experimental Testing of Mutagenicity

At the conference techniques were reported for determining whether a drug or chemical is mutagenic and even for determining what type of mutation it produces. Eight different tests were discussed in which submammalian systems (bacteria, drosophila, neurospora), mammalian cells in tissue culture, or whole animals as the test organisms were used.

The microbial systems were judged to be the easiest to use for quick mass screening. In the system described by Bruce Ames of the University of California, Berkeley, strains of bacteria with known genetic makeup are used; these strains undergo mutation in specific and quantifiable ways in the presence of certain mutagens. Thus a strain deficient for histidine because of a base substitution of an adenine-thymine base pair can be mutated to wild-type by bromouracil. Any potentially mutagenic chemical can be subjected to a petri plate assay for growth of wild-type bacteria; a variety of these tester strains can be used. Ames acknowledges that it is "absurd to extrapolate from bacteria to humans. But DNA has the same double helical structure and the same four nucleotides in all organisms, and it is logical to believe that mutagens of *Escherichia coli* DNA will also be mutagenic for animal DNA. In general, mutagens for higher organisms are mutagens for bacteria also. More than half of the mutagenic agents for bacteria are carcinogenic for animals." In addition, the assay on petri plates is cheap, simple, and rapid—"you can't afford not to use it." Procedures are now being developed by several laboratories by which genetic analysis of mammalian cells in tissue culture can be performed as easily as the bacterial tests.

Recessive lethal tests with *Neurospora crassa* and *Drosophila* were also described. Although these tests are more complicated than those in which bacteria are used, neurospora and drosophila are closer to man in the complexity of their chromosome structure. The tests with whole animals are the most complicated methodologically, but provide the most pertinent data on mutagenesis in man. In the dominant lethal assay, described by Samuel Epstein of Children's Cancer Research Foundation in Boston, male mice are treated with a potential mutagen and then mated sequentially for 8 weeks with groups of untreated females. The females are subsequently examined for deaths of fetuses due to lethal mutations in the male's sperm caused by exposure to the mutagen.

Marvin Legator of the Food and Drug Administration has developed an assay that bridges the bacterial and mammalian assays. In this host-mediated test, an animal is injected with a microorganism whose mutation rate can be easily measured and with a potentially mutagenic chemical or drug. After a period of time, the microorganisms are withdrawn, and the induction of mutants is measured. By this test, it is possible to determine whether the chemical is mutagenic and also whether the host animal has metabolized it to a mutagenic compound. Legator has found that both dimethylnitrosamine and cycasin are nonmutagenic in direct tests on microorganisms, but that they are mutagenic after being metabolized by the host animal. For this reason, many chemicals that may appear to be harmless may indeed be mutagenic.

Although major progress in testing has occurred over the last few years, the consensus of the conference was that more research is needed and that no single method, no matter how sophisticated, is sufficient to evaluate the hazards of a chemical. Legator does believe, however, that evidence of chromosome breakage is sufficient to ban a drug without resorting to the use of sophisticated techniques to demonstrate the specific type of mutational lesion.

Although more research is needed to improve testing methods, the currently known tests can be used to evaluate hazards. Many pharmaceutical firms are now looking at drugs in terms of their cytogenetic effects as well as their toxicity. Both James Neel of the University of Michigan and Crow feel that a burden of responsibility rests on the pharmaceutical industry, even though they are not the major producers of potential mutagens, and called on them to lead the way toward testing before use by the human population.

Population Monitoring

An essential part of protecting man from the harmful effects of environmental agents is the monitoring of the human population for genetic mutations. A precedent for population monitoring has been established in the mass screening of infants for the inherited disease phenylketonuria, commonly known as PKU. People afflicted with PKU lack the normal enzyme that converts phenylalanine to tyrosine. In these people, phenylalanine is metabolized to phenylpyruvic acid, a substance that produces mental retardation. With simple techniques, such as electrophoresis, scientists can now recognize abnormal proteins and enzymes that are the products of mutated genes. Although more sophisticated techniques are also available, electrophoresis is relatively inexpensive and is an efficient way of looking at subtle changes in protein structure.

According to Neel, a screening project should be inaugurated that would be capable of detecting a 50 percent increase in the human mutation rate. This project would involve electrophoretic testing of ten different proteins in blood samples from about 350,000 persons each year. Although this type of monitoring for mutation would not reveal the cause of a change in the mutation rate, it would serve as a public health warning system.

Epstein stated that we need a massive push toward population monitoring in order to put emphasis on anticipation of the problem of mutation rather than merely on detection. "Detection is limited to only one generation. Mutations are hazards for many generations to come." What is lacking is the money to support a program to monitor the mutation rate in man. Alexander Hollaender of Oak Ridge National Laboratory pointed out that the research effort directed toward the investigation of radiation hazards was made possible only by long-range guaranteed support. He also said that the same kind of support is needed in the field of chemical mutagenesis, and that hopefully industry, government, and private foundations will insure this support.—MAUREEN HARRIS

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