Kinds and Rates of Human Heritable Mutations

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INTRODUCTION

The genetic code specified in DNA directs the transcription of RNA, which is translated into polypeptide chains; these chains, in turn, are assembled into proteins, which are the body's tools for the regulation of physiological, biochemical, and behavioral functioning. Mutations, transmissible alterations in DNA, can change the messages, causing alterations in RNA molecules and proteins. Some of these changes can lead to subtle abnormalities, diseases, and disabilities.

Environmental factors and specific interactions between environmental and genetic factors are thought to account for the majority of diseases or susceptibilities to disease. Mutations alone are thought to account for only a fraction of the current burden of disease. However, there is a group of disorders for which new mutations are the primary causes. Among these "genetic" diseases is a group of rare clinical conditions, "sentinel phenotypes, " that occur sporadically in each generation; children with sentinel phenotypes are usually born to parents who do not have the same disease, indicating a new mutation that arose in a germ cell of one of the parents,

There are several other indicators of new mutations in human beings that do not rely on detecting diseases. The most widely used methods have been cytogenetic analysis of chromosomes and biochemical analysis of proteins.

The structure and number of chromosomes visible by light microscopy has been used to detect a certain class of heritable mutations. Gross chromosome abnormalities, often associated with high fetal and neonatal death rates, are identifiable with increasing precision by cytogenetic analysis. A large portion of these chromosome abnormalities arise anew each generation,

Studies of mutationally altered proteins have been done on a limited basis. The proteins most accessible for analysis are those circulating in blood; other body proteins are not routinely obtained for analysis. The method available for detecting abnormal proteins is electrophoresis. Heritable mutations have been detected by identifying electrophoretic variants of blood proteins. In addition, a combination of approaches has been used to estimate the mutation rate per nucleotide in genes coding for globin polypeptides, constituents of hemoglobin. These approaches to identifying mutations are discussed in this chapter.

The term "mutation rates" for particular types of mutations in specific regions of DNA is used frequently in this report. Mutation rates for different kinds of mutations, or corresponding to different overt effects of mutations, can be expressed as mutations per locus, per gene, per nucleotide, and per gamete. All of these indicate a specific type of mutation occurring *per generation,* reflecting mutations arising anew from one generation to the next. This chapter summarizes current knowledge about such mutation rates as they have been measured thus far; it is not yet known whether these rates apply also to other populations, to other gene regions, or to other types of mutations than the ones examined.

Rather than looking directly at germinal mutations—mutations in egg and sperm DNA—the methods described in this report focus on heritable mutations in liveborn offspring, a subset of all germinal mutations.[1] A larger subset of germinal mutations includes new mutations observed in offspring before birth. In recent years, cytogenetic techniques have been used to identify chromosome abnormalities in spontaneous abortuses. These investigations have shown that the frequency of new mutations in spontaneous abor-

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[1]At present it is not feasible to examine egg or sperm DNA for genetic damage, although some information on major chromosome abnormalities can be obtained by cytogenetic examination of sperm.

tuses is much higher than in liveborn infants; the observed incidence of new mutations at birth only partially represents the "true" incidence of such heritable mutations in all conceptuses, and may give an incomplete picture of the potential risk from environmental exposures to mutagenic agents. Nevertheless, the most lasting medical and social consequences of new heritable mutations are derived from those mutations that are compatible with life at least through infancy and childhood.

SURVEILLANCE OF SENTINEL PHENOTYPES

The United Nations Scientific Committee on the Effects of Atomic Radiation (144) and the Committee on the Biological Effects of Ionizing Radiations (87) estimate that 1 percent of liveborn infants carry a gene for an autosomal dominant disease; in 20 percent of these cases (0.2 percent of livebirths) their disease is due to a new, or "sporadic, " mutation that arose in the reproductive cells of one of their parents, Observing and recording the birth of infants with such sporadic conditions is the classical approach to identifying heritable mutations that lead to serious disease (36).[2]

A subset of the group of autosomal dominant and X-linked conditions, indicator conditions known as "sentinel phenotypes," maybe particularly useful in identifying heritable mutations. Sentinel phenotypes are clinical disorders that occur sporadically, probably as the result of a single mutant gene. They are manifested at birth or within the first months of life, and usually require long-term, multidisciplinary medical care (83). In addition, sentinel phenotypes are associated with low fertility, so their appearance suggests mutations that have been passed not from *affected* parents to offspring, but instead, have been passed from *unaffected* parents to offspring as a result of *a newly arising mutation in a germ cell* of one of the parents of that offspring. Affected infants would have this mutation in the DNA of all their somatic cells and 50 percent of their germ cells.

Mulvihill and Czeizel (83) compiled a list, shown in table 1, of 41 genetic disorders (36 dominant and 5 X-linked) that satisfy the above criteria. Each of these phenotypes is individually rare, some of them occurring in only one in a million liveborn infants.

Since children with sentinel phenotypes have serious health problems associated with their conditions, they may first come to the attention of the family practitioner or pediatrician, and then be referred to subspecialists for a complete diagnosis. Clinical expertise in dysmorphology,[3] medical genetics, pediatric ophthalmology, and oncology may be needed to diagnose accurately the small numbers of children with sentinel phenotypes present among the vast majority of unaffected children and among children with phenotypically similar, but nongenetic conditions (called "phenocopies") (71,83,165).

Reliable incidence data are available only for 13 dominant and 5 X-linked sentinel phenotypes (166); only those that are manifested in infancy, rather than in childhood or adolescence, Q are systematically recorded. Diagnostic records of sentinel phenotypes may be available in population-based registries of childhood cancers, birth defects, and genetic diseases, with initial entries based on birth certificates and later entries added as the conditions are diagnosed during infancy. After investigators rule out the possibility of a discrepancy between stated and biological parentage by genetic testing (166), sporadic cases of a sentinel phenotype are accepted as evidence for

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[2] Rates of a wide variety of new heritable mutations leading to severe diseases have also been estimated by an "indirect method. " This method is a theoretical approach, based on the loss of disease-causing genes from the population (due to reduced reproductive capabilities of individuals with these genes) and on the sporadic frequency of the genes in the population. The mutation rate necessary to maintain such genes in the population, despite their recurrent loss, is inferred from these observations. The types of populations and conditions suitable for this method are limited, and its precision is unknown (91,165).

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[3] Physicians who specialize in diagnosing syndromes characterized by unusual physical form and stucture, for example, congenital malformations.

[4] Such disorders include achondroplasia, acrocephalosyndactyly, osteogenesis imperfect, retinoblastoma, and Wilms' tumor (66).

Table 1 .—Candidate Sentinel Phenotypes'

	Inheritance[a]
Phenotypes identifiable at birth:	
Achondroplasia	AD
Cataract, bilateral, isolated	AD
Ptosis, congenital, hereditary	AD
Osteogenesis Imperfect type I	AD
Oral-facial-digital (Gorlin-Psaume) syndrome type I.	XD
Incontinentia pigmenti, Bloch-Sulzberger syndrome	XD
Split hand and foot, bilateral atypical	AD
Aniridia, isolated	AD
Crouzon craniofacial dysostosis	AD
Holt-Oram (heart-hand) syndrome	AD
Van der Woude syndrome (cleft lip and/or palate with mucous cysts of lower lip). . . .	AD
Contractual arachnodactyly	AD
Acrocephalosyndactyly type 1, Alpert's syndrome	AD
Moebius syndrome, congenital facial diplegia.	AD
Nail-patella syndrome	AD
Oculodentodigital dysplasia (ODD syndrome)	AD
Polysyndactyly, postaxial	AD
Treacher Collins syndrome, mandibulofacial dysostosis	AD
Cleidocranial dysplasia	AD
Thanatophoric dwarfism	AD
EEC (ectrodactyly, ectodermal dysplasia, cleft lip and palate) syndrome	AD
Whistling face (Freeman-Sheldon) syndrome	AD
Acrocephalosyndactyly type V, Pfeiffer syndrome	AD
Spondyloepiphyseal dysplasia congenita. . . .	AD
Phenotypes not identifiable at birth:	
Amelogenesis imperfect	AD
Exostosis, multiple	AD
Marfan syndrome	AD
Myotonic dystrophy	AD
Neurofibromatosis	AD
Polycystic renal disease	AD
Polyposis coli and Gardner syndrome	AD
Retinoblastoma, hereditary	AD
Tuberous sclerosis	AD
von Hippel-Lindau syndrome	AD
Waardenburg syndrome.	AD
Wiedemann-Beckwith (EMG) syndrome	AD
Wilms' tumor, hereditary	AD
Muscular dystrophy, Duchenne type	XR
Hemophilia A.	XR
Hemophilia B.	XR

aAD refers to autosomal dominant inheritance, XD refers to X-linked dominant inheritance, and XR refers to X-1inked recessive inheritance.

SOURCE: J.J Mulvihill and A. Czeizel, "Perspectives in Mutations Epidemiology 6: A 1963 View of Sentinel Phenotypes," *Mutat. Res.* 123:345-361, 1983.

a new germinal mutation originating in a germ cell of one of the parents (65).

In general, recording the incidence of sentinel phenotypes is not useful for monitoring a small population exposed to a suspected mutagen. Surveillance of sentinel phenotypes in large populations is potentially more useful for estimating the background mutation rates leading to dominant genetic disorders. Given the rarity of the individual sentinel phenotypes, the most reliable data for estimating mutation rates would be derived from the largest target populations with, for example, international cooperation among study centers (62,66).

Mutation Rates Estimated From Phenotypic Data

Data are available on the incidence of various sentinel phenotypes from different time periods in particular regions worldwide, though representation is incomplete. Crow and Denniston (22) summarized the most current data; these data have not been significantly updated since the 1940s and 1950s. The frequencies for any given phenotype are generally consistent between studies and the frequencies of occurrence of the different phenotypes vary over a thousandfold range, from 1 in 10,000 to 1 in 10 million depending on the particular disorder. The arithmetic mean of the rates for the different disorders is approximately 2 mutations per 100,000 genes per generation (17,89,166). This average rate is often cited as the "classical" rate of heritable mutations.

'Mutation rates corresponding to sentinel phenotypes are expressed as the frequency of mutations per gene. It assumes one gene per disorder and one disorder per person and it is designated "per generation" because the mutations are detected in offspring of individuals whose germ cells have incurred mutation. In general, the mutation rate is defined as the incidence of a sporadic case of some indicator (e.g., a sentinel phenotype or a protein variant) divided by two times the total number of cases examined (e.g., newborn infants or protein determinations). The two in the denominator is to express the mutation rate, by convention, as the rate "per fertilized germ cell, " instead of "per concepts," which is made from two germ cells **(162).**

Limitations of the Phenotype Approach

Several sources of error may affect estimates of heritable mutation rates based on the incidence of sentinel phenotypes. The most important one results from the choice of phenotypes studied: phenotypes that occur in the population most often are the ones most likely to be recorded. Phenotypes that are so severe as to cause prenatal death, or so mild as to be indistinguishable from the normal variety of phenotypic traits, are difficult or impossible to include in these population surveys. It is not known how large a bias this selection occasions. In addition, the mutation rates corresponding to clinically recognizable phenotypes may not be representative of mutation rates for other traits.

Other problems contributing to an over- or underestimated mutation rate may include: 1) incomplete ascertainment among certain populations of liveborn infants; 2) mistaken identification of parentage, a significant issue since the number of cases of mispaternity may be high enough to exceed the number of true sporadic cases of sentinel phenotypes; and 3) the occurrence of phenocopies (phenotypically similar nongenetic causes of the same condition) and genocopies (recessive forms of the same phenotype inherited from unaffected carrier parents). In addition, particular phenotypes do not necessarily correspond to a unique, single gene (163); often, mutations in any one of several genes can lead to the same phenotype. This is not likely to influence the rate estimate significantly unless a large number of different mutant genes are involved, in which case the rate of mutation of the particular phenotype would be the sum of the rates of mutation at each of the alternative genes.

Surveillance for sentinel phenotypes is an important part of assessing the clinical impact of new mutations. The number of sentinel phenotypes available to study is limited, however, so that surveillance of these phenotypes illuminates only a fraction, albeit an important one, of this overall impact.

Individually, the sentinel phenotypes are quite rare. Keeping track of the frequencies of the different phenotypes over periods of time would require large-scale surveillance of huge numbers of infants over many years. A large team of medical specialists and a well-organized database would be needed to obtain complete and accurate ascertainment (65). Unfortunately, the practical difficulties in screening huge populations of newborns, diagnosing these rare disorders, and organizing and maintaining the data, make it difficult, though not impossible, to generate reliable data. The first such effort is currently being organized by the European Collaborative Study (62).

CYTOGENETIC ANALYSIS OF CHROMOSOME ABNORMALITIES

The adverse effects of chromosome abnormalities on human health are well established and provide sufficient medical reasons for screening infants for them. Data on the frequency of chromosome abnormalities is also useful in assessing the impact of detectable new chromosome mutations on the current generation.

Chromosome abnormalities are defined as either numerical (extra or missing whole chromosomes) or structural (deletions, insertions, translocations, inversions, etc., of sections of chromosomes). In liveborn infants, the presence of an extra or missing sex chromosome is often associ-ated with physical, behavioral, and intellectual impairment. The presence of an extra autosome is even more detrimental: it is usually associated with severe mental and physical retardation and often with premature death (39,119).

There is no doubt that fetuses with recognized numerical chromosome aberrations are at a much higher risk for aborting spontaneously than fetuses without such defects. Such chromosome abnormalities contribute to a large proportion of spontaneous abortions and stillbirths, and in liveborn survivors may also contribute to repeated early abortions and fertility problems in adult-

hood (169). The significance to human health of structural rearrangements of the chromosomes is less well defined, but such defects have been associated with mental retardation, physical malformations, and a variety of malignant diseases (43). In addition, carriers of balanced translocations may produce offspring with unbalanced complements of DNA; these offspring may die in utero and may account for a high spontaneous abortion rate among such carrier parents.

It is believed that at least 5 percent of all recognized human conceptions have a chromosome abnormality, although this is a crude estimate because it is not corrected for factors which could influence the occurrence of such abnormalities (such as maternal age) or for factors which could affect the rate of embryonic or fetal death in some of the disorders (43). Numerical and structural chromosome abnormalities have been detected in so to 60 percent of recognized spontaneous abortions (data summarized in ref. 12), 5 to 6 percent of perinatal deaths (ibid.), and 0.6 percent of liveborn infants (42).

Chromosome abnormalities are useful for measuring heritable mutation rates since they are usually identified as new mutations, either because the defect causes virtual infertility or sterility (as in the case of numerical chromosome abnormalities) and therefore could not have been inherited from a parent with the same defect, or because the parents of an individual with a chromosome abnormality (e.g., a structural abnormality) have been shown not to carry the same mutation. Numerical chromosome abnormalities and some structural abnormalities are detectable with cytogenetic methods using current chromosome staining and banding techniques.

In general, little is known about the genetic mechanisms of either numerical or structural rearrangements. They appear to result from complex processes during gametogenesis in which various defects may lead to the same or different outcomes (18,119). Consequently, frequencies of the various cytogenetic abnormalities may correspond to a combined rate of several different mutational events or to a single mutational event.

Individuals with chromosome abnormalities can be identified on the basis of karyotypes (see

ch. 2). However, since the vast majority of conceptuses with chromosome abnormalities such as trisomies and unbalanced structural rearrangements are spontaneously aborted, many before a pregnancy is recognized, the frequency of these mutations *at birth* is known to represent only a small fraction of the presumed frequency of these mutations *at conception.*

Data on the frequency of chromosome abnormalities in fetuses at the time of amniocentesis (generally done at 16 to 18 weeks gestation) provides information not available in newborn screening. In addition, recent progress in cytogenetic analysis of chorionic villi cells makes it possible to identify chromosome abnormalities in fetuses at 8 to 10 weeks gestation (116). This technique provides even earlier information on possible chromosome abnormalities, before fetuses with certain abnormalities (particularly trisomies and unbalanced structural rearrangements) would have aborted spontaneously. The analysis of fetal cells obtained at amniocentesis has provided valuable data on the frequency of chromosome abnormalities in the second trimester of pregnancy (12,45,159.) However, estimates of mutation rates from those measurements may be inflated unless certain biases are corrected. Women who elect amniocentesis—generally older mothers, couples who have had previous reproductive problems, and/or couples who may have been exposed to mutagenic agents (101) —are at increased risk for chromosome abnormalities in their offspring.

Cytogenetic surveys of consecutive liveborn infants have provided the most extensive data on chromosome mutation rates, although it is known that these data underestimate the "true" rate of new chromosome mutations. It should also be noted that the prevalence of sentinel phenotypes and biochemical variants in liveborn infants may also underestimate their "true" rate at conception, but little is known about selection against these mutations in the developing fetus (e. g., molecular repair of the defect, or spontaneous abortion of the fetus), in contrast to the documented evidence for selection against *chromosome* mutations in the fetus (135.) However, the frequency at birth is probably the best indicator of events with the highest cost in terms of the social and economic burden of these diseases.

Mutation Rates Derived From Chromosome Abnormalities

At least 10 major research efforts worldwide have attempted to determine the prevalence of chromosome abnormalities in liveborn infants. It is possible to estimate the rate of new mutations underlying these defects, but difficult to compare with rates of sentinel phenotypes, since chromosome mutations, by definition, are multigenic changes and sentinel phenotypes correspond to single gene mutations.

The combined data from studies surveying a total of 67,014 newborn infants for various periods between 1974 and 1980 in the United States, Canada, Scotland, Denmark, the U. S. S. R., and Japan (120) are shown in table 2. Two of the studies used newer banding techniques that identify more abnormalities than conventional staining methods used in the remaining eight studies.

For conditions that are associated with virtual infertility, complete sterility, or prereproductive death, it is often assumed that each case is the result of a new mutation (unless there is evidence for gonadal mosaicism[6]). Such conditions include the autosomal trisomies (e.g., Down syndrome) and the sex chromosome aneuploidies (e.g., Turner's syndrome).

For these defects, the apparent mutation rate observed in liveborn infants is calculated as the number of individuals identified with a given abnormality divided by the total number of individuals examined. This mutation rate is expressed as the number of mutations per generation. For conditions which could be the result of either new mutations or inherited mutations, such as the various balanced and unbalanced structural rearrangements, it is necessary to karyotype the parents of infants found with these chromosome abnormalities, and to exclude inherited mutations from calculations of the de novo mutation rate. For these

conditions, the observed mutation rate is determined using the following formula:

$$\text{Mutation rate} + \frac{\text{Number of patients} \times \text{Proportion who are likely}}{\text{Total number examined}} \frac{\text{with abnormality} \quad \text{to have de novo mutations}[7]}{}$$

The data show that the frequencies of chromosome abnormalities in liveborn infants range from 2 in 100,000 newborns per generation for inversions to 121 in 100,000 newborns per generation for Trisomy 21 (Down syndrome), the most common newly arising numerical chromosome abnormality found at birth. Overall, the numerical abnormalities are much more frequent than the structural abnormalities. However, the rates for balanced structural rearrangements may be low in these data, since only two of the surveys used more sensitive banding methods which are needed to identify these more subtle aberrations.

Limitations of Cytogenetic Analysis

Screening for chromosome mutations in newborn infants gives only a partial picture of their incidence. The prevalence of a particular genetic mutation at birth can be thought of as the result of the incidence of the mutation at conception interacting with the probability of survival of conceptuses with the mutation. Fetal survival, in turn, depends on individual fetal and maternal attributes, as well as on the interaction between the two (136). Some mutations in the fetus may affect fetal survival, whereas other mutations may have no effect on survival. Major chromosome aberrations, in particular, numerical abnormalities and most unbalanced structural rearrangements, are often incompatible with fetal survival and are associated with an increased risk of prenatal loss (45).

Balanced chromosome rearrangements, however, may not lead to excessive prenatal loss. For this reason, the balanced structural rearrangements may be a useful indicator of new germinal mutations observed in liveborn offspring. Since some of these infants identified as having a bal-

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[6]Mosaicism refers to the presence of two genetically different cell populations in the same individual. This can result from a mutation during one of the earliest divisions in the zygote, a few days after conception, that led to the proliferation of a large number of cells that were derived from the original cell bearing the mutation.

[7]Such proportions are based on empirical data for each type of chromosome aberration. In this case, data from Jacobs, 1981 were used and are shown in table 2 in parentheses in the third column.

Table 2.— Prevalence of Chromosome Abnormalities at Birth

Chromosome abnormality	Total population	Number of new mutants [a]	New chromosome abnormalities per 100,000 newborns per generation
Numerical anomalies:			
Autosomal trisomies: [b]			
Trisomy 13	67,014	3	5
Trisomy 18	67,014	8	12
Trisomy 21	67,014	81	121
Male sex chromosome anomalies:			
47,XYY	43,048 males	43	100
47,XXY	43,048 males	42	98
Female sex chromosome anomalies:			
45,x . . . ., , ... , ..., ., ...	23,966 females	2	8
47, XXX	23,966 females	24	100
Balanced structural rearrangements:			
Robertsonian translocations: [c]			
D/D [d]	67,014	48(2/29) = 3.3	5
D/G	67,014	14(2/1 1) =2.5	4
Reciprocal translocations and insertions.	67,014	60(13/43) =18	27
Inversions	67,014	12(1/8)= 1.5	2
Unbalanced structural rearrangements:			
Translocations, inversions, and deletions	67.014	37(7/16) =16	24

[a] See discussion in text for proportion of new mutants among all those identified with Structural rearrangements.
[b] Trisomies refer t. conditions i, which there are 47 chromosomes (instead of the normal 46 chromosomes or 23 pairs of homologous chromosomes, indicated as 46,XX for females or 46,XY for males) because of the presence of an extra copy of one chromosome; three copies of this particular chromosome would be present instead of the normal two. Autosomal trisomies indicate an extra autosome, which includes any chromosome except one of the sex chromosomes
[c] Prearrangements in which the long arms of two chromosomes fuse, resulting in one "hybrid" chromosome and the loss of the short arms of each chromosome,
[d] D and G refer to groups of chromosome numbers 13-15 and 21'22, respectively

SOURCE: K Sankaranaravanan. *Genetic Effects of Ionizing Radiation in Multicellular Eukaryotes and the Assessment of Genetic Radiation Hazards in Man* (Amsterdam Elsevier Biomedical Press, 1982), pp 161-164

anced chromosomal mutation may have inherited the relatively subtle abnormality from one of their parents, it is necessary to determine whether the mutation observed in the child is actually a new germinal mutation or not.

Although it is not understood how bands observable under the microscope correspond to the structure and composition of DNA, mutations in DNA can cause a visible change in the banding pattern, particularly if such mutations involve a large section of a chromosome. Increased resolution and sensitivity of cytogenetic techniques, specifically the "high-resolution" banding methods (72), makes it possible to detect smaller and more subtle aberrations than was previously possible. Currently, routine cytogenetic methods can produce 200 to 300 bands (containing several hundred genes in each band) in a complete set of DNA, and the high-resolution methods can distinguish as many as 1,000 bands (containing about 100 or fewer genes per band) (59). With more smaller bands distinguishable, fewer genes are present per band, and a change in one gene has a greater likelihood of showing up as a change in the banding pattern.

At present, data available for large populations rely on only the routine staining and banding methods. Like the observations of sentinel phenotypes in newborn infants, cytogenetic methods focus on a subset of mutations, albeit those with serious clinical consequences. Even though chromosome abnormalities are not as rare as sentinel phenotypes, these two groups of abnormalities together probably account for only a small fraction of heritable mutations.

Mutations detectable by the methods discussed in the remainder of this chapter and in the following chapter do not necessarily have adverse clinical consequences. Their detection broadens the spectrum of identifiable mutations by including those that may not have an immediate adverse effect on the individual, and also offers more precise information about the nature of the genetic changes.

DETECTION OF BLOOD PROTEIN VARIANTS

In a single-gene, dominant genetic disease, if the causative mutant gene occupies one allele of the pair of genes and the normal form of the gene occupies the other allele, the one mutant gene is dominant over the normal gene and is sufficient to cause overt disease. *Dominant* diseases are said to be expressed in individuals who have a single dose, or are heterozygous, for the disease-causing gene. Autosomal *recessive* diseases, however, are clinically apparent only when the mutant gene is present at both alleles, or in the homozygous state; a single dose, the heterozygous state, can, for instance, destroy the activity of an enzyme or produce a defective gene product, but the corresponding normal gene supplies a sufficient amount of normal gene product to prevent overt clinical symptoms. It is difficult to infer the frequency of recessive genes by population studies of phenotypes since individuals with one copy of the recessive mutant gene are phenotypically indistinguishable from individuals with only normal genes for the particular protein. Biochemical means of identifying of heterozygotes, who are phenotypically normal, is one way of identifying new mutations that are expressed as recessive traits.

Biochemical studies on asymptomatic individuals with protein variants (indicative of single gene, recessive mutations) can be useful in expressing the cumulative, "invisible" effect of an increased mutation rate. Any protein in the body could be studied, but since peripheral blood is easily accessible, proteins from blood are most commonly used for these studies. Three major techniques for the detection and characterization of variant proteins have been used in studies of human populations:

1. electrophoresis of blood proteins,
2. quantitative and kinetic studies of enzyme deficiency variants from erythrocytes, and
3. molecular analysis of Hemoglobin M (HbM) and unstable hemoglobins.

One-Dimensional Electrophoresis

Electrophoresis separates proteins according to net molecular charge. A mixture of proteins is applied to a starch or acrylamide gel and exposed to an electric current. Each protein moves according to its own electric charge and separates from proteins with different charges. The subsequent addition to the gel of stains that bind to the proteins makes it possible to visualize the location of the proteins. Electrophoresis can be used to detect variant proteins in individuals and in their parents. The identification in an offspring of a variant protein that is absent from either parent is evidence for a new mutation. As in the other approaches, it is necessary to exclude the possibility of a discrepancy between stated and biological parentage so that only new, not inherited, mutations are considered.

Electrophoretic variants are detectable because a mutation results in a change in the net charge of a protein. On a theoretical basis it is expected that approximately one-third of amino acid substitutions produce a change in molecular charge of a protein, and there is also evidence that electrophoresis can detect changes in a molecule's configuration due to a nucleotide substitution. All in all, it is estimated that electrophoresis probably detects about 50 percent of nucleotide substitutions in expressed regions of coding genes (96).

The protein variants detected by electrophoresis are usually not associated with clinically recognizable problems. However, this technique has an important advantage for mutation research over the phenotypic approach; it measures changes at a level closer to the level of the mutational events and therefore may provide more accurate estimates of mutation rates. It also reflects more subtle genetic changes, broadening the spectrum of detectable genetic endpoints.

Mutation Rates Derived From Electrophoresis

Several large-scale studies to detect protein variants in human populations have been reported (excluding studies in Japanese atomic bomb survivors, which are described below). Neel, et al. (94), and Mohrenweiser (76) devised a large-scale pilot program to study mutation rates using placental cord blood obtained from newborn infants in Ann Arbor, Michigan (approximately 3,500 samples). They found no new heritable mutations

in 36 different proteins in a total of 218,376 locus tests in Caucasian and 18,900 such tests in black infants.[8] Harris and colleagues (38) summarized data from studies in the United Kingdom in which 43 different loci coding for blood proteins were analyzed, and no new mutations were found in 133,478 locus tests.[9] Altland and colleagues (5) examined blood from filter paper submitted originally for screening for phenylketonuria in approximately 25,000 newborn infants in West Germany. They identified one putative new mutation among five different hemoglobin proteins in approximately 225,000 locus tests.

Feasibility of Electrophoresis

The availability of human blood for analysis of variant proteins makes electrophoresis feasible for studies of large populations. In addition, it permits comparisons of the frequency of occurrence of variant proteins between humans and other species, since comparable proteins are usually associated with homologous gene sequences (93). Perhaps the most important advantage of this approach is that it permits the study of a defined set of genes expressing functional proteins and offers more precise information on the number of genes involved in the biochemical observations made and on the effect of mutation on some proteins (90).

However, electrophoretic analysis can provide information only on mutations in genes that are transcribed into RNA and subsequently translated into proteins, and only for mutations that alter the protein's net molecular charge or configuration. It cannot provide information on mutations in nontranscribed regions of the DNA, which comprise the larger portion of the genome (165).

Since the baseline frequency of new protein variants is still poorly defined, it is difficult to estimate the size and scope of a population study necessary to detect an increase over a period of time or to detect differences in rates between two populations. One-dimensional electrophoresis is limited by the number of proteins that can be examined for variants, approximately the same number of different phenotypes included in the list of sentinel phenotypes. However, one-dimensional electrophoresis in a large population is a more straightforward approach to studying new heritable mutations than is population surveillance for sentinel phenotypes.

Vogel and Altland (164) estimated that analysis of two populations of 10 million individuals each would be needed to have a 95-percent chance of detecting a 10-percent increase in spontaneous mutation rates. This calculation is based on a theoretical rate of 1 mutation per 1 million genes per generation and 50 loci tested per individual in the study. The population size could be reduced if the proteins that were examined mutated more frequently or if detection of only large increases in the mutation rate (e.g., 50 percent) were acceptable, or if many more proteins could be examined from each individual tested.

Quantitative and Kinetic Studies of Enzyme Deficiency Variants

Mutations that result in variant proteins with greatly reduced or no biological activity, when present in the heterozygous state, are not readily detectable in electrophoretic studies. Such "enzyme deficiency variants, " or "nulls,"[10] can be identified through automated biochemical tests that measure enzyme activity in red blood cells. These studies increase the detectable range of mutational events.

Loss of function of a protein may result from:

- the absence of a gene product,
- the presence of a protein which is nonfunctional catalytically, or
- abnormally unstable enzymes (75).

The types of mutations causing these abnormalities include chromosome rearrangement or loss, deletions, frameshift mutations, and nucleotide

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*The number of locus tests takes into account the number of proteins examined, the number of gene loci represented by each such protein, and the number of individual samples obtained.

°The number of individuals tested varies depending on the enzyme examined, but for some of the enzyme tests, more than 10,000 individuals were tested.

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[10] These mutations are characterized by the presence of an enzymatically inactive protein, or by an absence of a protein. These can be caused by gene deletions, nucleotide substitutions in critical locations in the gene, mutations in the "start" and "stop" codons for the protein, or even by nucleotide substitutions at splicing junctions for the introns.

substitutions in coding or noncoding regions of DNA. The range of genetic events underlying the production of enzyme deficiency variants is thought to be larger than for electrophoretic mobility variants, since mutations in noncoding regions (e.g., intervening sequences, flanking regions, etc.) as well as in coding regions could be detected (74).

Four studies have provided data on the frequency of enzyme deficiency variants. The frequencies of such variants have been measured in: 1) placental cord blood samples from approximately 2,500 individuals in Ann Arbor, Michigan (74,75); 2) blood samples obtained from participants of the studies in Japan coordinated by the Radiation Effects Research Foundation (122); 3) blood samples obtained from 3,000 hospitalized individuals in West Germany (3o); and 4) blood samples obtained from Amerindians of South and Central America (78). No new mutations were found in any of these studies. However, rare but not new enzyme deficiency mutations that have been inherited over many generations have been measured at an overall frequency of 2 variants per 1,000 determinations in a total of approximately 110,000 locus tests. If new mutations producing enzyme deficiency variants occur, as predicted, no more than twice as often as new mutations leading to electrophoretic variants, the lack of new mutations in these 110,000 locus tests is not inconsistent with electrophoretic data.

Molecular Analysis of Hemoglobin M and Unstable Hemoglobins

Stamatoyannopoulos and Nute (131) calculated mutation rates for certain nucleotides in the globin genes, which, when mutated, produce autosomal dominant disorders expressed as chronic hemolytic anemia or methemoglobinemic cyanosis. Their data provided the first direct measurements of heritable mutation rates for particular *nucleotides* in human DNA. Although these data are limited to the specific characteristics of the globin genes, they suggest the kind of information which may be possible to obtain for other genes as the genetic basis for various single gene disorders is discovered. Even if the mutation rates derived from these studies of the globin genes are not rep-

resentative of rates of mutation at other loci, the hemoglobin system described below provides a model for combining epidemiologic, clinical, and molecular information to estimate heritable mutation rates in human populations.

Structure and Function of Hemoglobin

Hemoglobin molecules, which exist in a number of slightly different forms, are the universal carriers of oxygen in the blood from the lungs to all cells of the human body. Different mixtures of hemoglobins are present at all stages of development from embryonic to adult, although a particular type of hemoglobin tends to predominate at any one stage. In addition, hundreds of abnormal hemoglobins have been identified, ranging in clinical severity from benign through serious hematologic disorders. Human hemoglobin is a protein consisting of four separate globin polypeptide chains and four iron-containing heme groups. In adults, the predominant form of hemoglobin is hemoglobin A (HbA), composed of two alpha-globin chains and two beta-globin chains each joined to a heme group.

Hemoglobin's primary function of binding, carrying, and releasing oxygen is closely regulated by several factors, including the maintenance of a precise three-dimensional conformation of the hemoglobin molecule, the composition and balance of globin chains, and the distribution of ionic charges in and around the molecule. Mutations which result in amino acid substitutions at particular sites in the globin polypeptide chain can markedly alter these specific functional properties of the hemoglobin molecule.

Two types of abnormal hemoglobin, HbM and unstable hemoglobin, usually occur sporadically as a result of a new mutation and are expressed as dominant mutations.[11] Stamatoyannopoulos and Nute (131) described the genetic changes leading to these particular abnormal hemoglobins and their incidence in human populations. From this

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[11] A heterozygous individual, having one mutant globin gene and one normal allele, would have clinically recognizable symptoms of the disease. This is in contrast to other, more common hemoglobin diseases, such as sickle cell anemia and thalassemia, where the heterozygote is asymptomatic and the disease occurs only when an individual is homozygous for the variant gene.

information, they were able to calculate the rate of mutation leading to these abnormal hemoglobins.

Unstable Hemoglobins

Various mutations in the globin genes can lead to an altered sequence of amino acids in the globin chains and to a major change in the three-dimensional structure of the hemoglobin molecule. Often these changes create a less stable hemoglobin molecule than its normal counterpart. "Unstable hemoglobin" can result in the hemoglobin precipitating within the red cell, leading to membrane damage and premature destruction of the red cells by the liver and spleen (165).

The degree of clinical severity of the unstable hemoglobin depends in part on the site at which the amino acid replacement occurs and on the properties of the amino acid that is substituted (37). However, manifestations of the disorder vary from mild hemoglobin instability which may not be clinically significant, to severe hemoglobin instability, which causes chronic hemolytic anemia.

Unstable hemoglobin is diagnosed by isolating and characterizing the abnormal hemoglobin, and by determining its molecular stability. Since the amino acid sequence of normal hemoglobin is known, and the sequences for some abnormal hemoglobins have been determined, the genetic mutation underlying these unstable globins have been identified.

Hemoglobin M—The Methemoglobinemias

A blue, cyanotic appearance is the chief clinical manifestation of methemoglobinemia, caused by HbM. Cyanosis occurs whenever a significant proportion of the hemoglobin in the circulating red blood cells is not carrying oxygen, and in this case it is because of an inability of the hemoglobin to bind and carry oxygen (37).

Five different mutations in the globin gene which lead to the production of HbM have been identified, All of these are expressed as dominant phenotypes. The common characteristic of these mutations is that each results in substitution of an amino acid in the globin peptide where the heme groups are attached. (These mutations are specific substitutions of histidine for tyrosine on the alpha-globin chain [at positions 58 and 87] or on the beta-globin chain [at positions 63 and 92], or by substitution of valine for glutamic acid on the beta-globin chain [at position 67].) By altering the site of attachment of heme on the globin chains, these mutations change the three-dimensional organization of the entire hemoglobin molecule and interfere with its ability to combine with oxygen (100).

Measurement of Mutation Rates in the Globin Genes

Using published data on the number of cases of hemoglobin disorders from 10 countries (134), Stamatoyannopoulos and Nute (131) determined the number of children with HbM and unstable hemoglobin among an estimated number of *live*-births in the defined populations. They recorded a total of 55 cases of de novo hemoglobin mutants, each of which derived from substitutions of single nucleotides in the coding portions of the globin genes: 40 children with unstable hemoglobin (all beta chain mutations) and 15 with HbM (10 beta chain and 5 alpha chain).[12] The requisite paternity data, also from published information on these mutations, had been collected on 19 of the 55 cases which were used to calculate mutation rates (131). Their data are shown in table 3.

The mutation rates derived from these data are expressed in mutational events per alpha- or beta-gene nucleotide per generation, and range from 5.9×10^{-9} to 19×10^{-9} for alpha- and beta-gene nucleotides, respectively. The rate for all beta-gene variants together is 7.4×10^{-9} per nucleotide per generation. These estimates correspond to mutation rates per globin chain gene per generation of 2.6×10^{-6} to 8.3×10^{-6}. The rate per gene is obtained by multiplying the rate per nu-

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[12] The number of alpha chain mutations detected was less than expected, given the existence of two alpha-globin genes per beta-globin gene in an individual. A possible reason for this may be that alpha-chain defects would produce physiological abnormalities at an earlier stage of fetal development than would beta-chain abnormalities, since the alpha gene is activated much earlier, and may be causing a greater loss of fetuses early in gestation.

Table 3.–Rates of Mutation Leading to HbM and Unstable Hb Disorders

Type of disorder	Mutation rate/ nucleotide/ generation	Mutation rate/ globin gene[b]
Unstable Hbs . . .	5.9×10^{-9}	2.6×10^{-6}/438 nucleotides
Alpha[M] variants .		4.2×10^{-6}/423 nucleotides
Beta[M] variants . .	18.9×10^{-9}	8.3×10^{-6}/438 nucleotides
Ail beta-chain variants	7.4×10^{-9}	3.2×10^{-6}/438 nucleotides

[a]The mutation rate per nucleotide is based on the rate of appearance Of the Particular disorder (number of mutants observed divided by 2 multiplied by the number of births in the generation(s) Included). This is the average mutation rate for the particular disorder expressed per globin gene affected per generation. To calculate the mutation rate per nucleotide per generation, Stamatoyannopoulos and Nute took the frequency of each mutant divided by 2 (for two beta genes in a genome) multiplied by 3 (since a given nucleotide can be substituted by any one of three other nucleotides), then divided that product by the number of different substituted nucleotides actually observed In their data.
[b]The estimated mutation rate per alpha-globin and beta-globin gene was calculated as the mutation rate per nucleotide multiplied by the number of nucleotides constituting the coding portion of the corresponding gene (423 in the alpha-globin gene and 438 in the beta-globin gene).

SOURCE: G. Stamatoyannopoulos and P.E.Nute, "De Novo Mutations Producing Unstable Hbs or Hbs M. II: Direct Estimates of Minimum Nucleotide Mutation Rates in Man," *Hum, Genet.* 60:181-88, *1982.*

cleotide by the number of nucleotides in the coding portion of the gene.

The rates calculated from these data are likely to be minimum rates corresponding to these disorders. The diagnosis of these hemoglobin disorders requires structural and functional analyses of the aberrant proteins, and not all cases would be identified initially by their phenotypic abnormalities; some degree of underascertainment is inevitable in these data. This is particularly true in the data for the unstable hemoglobins, where clinical manifestations of the genotype range from mild to severe. The more severe cases are likely to be identified and reported in the literature.

Overall, the order of magnitude of the estimates for gene mutation rates, 1 mutation in 1 million genes per generation, is consistent with that of the estimates of the spontaneous heritable mutation rate made by Neel and colleagues in the Japanese populations that comprised the control group in their ongoing study of the genetic effects of the atomic bombs (see below). This suggests that even though the rates derived from these data are based on mutations observed in only alpha- and beta-globin genes, they are not very different from mutation rates in other expressed genes.

STUDIES OF POPULATIONS EXPOSED TO ATOMIC RADIATION

The first demonstration that ionizing radiation could induce genetic mutation is usually credited to Muller (81) for his work with Drosophila, and since then, many scientists have examined the nature and frequency of induced mutations in various species of animals, including whole mammals (120). There is an extensive body of experimental data that suggests that exposure to radiation and to certain chemicals can induce mutations in mammalian germ cells. In humans, exposure to ionizing radiation is known to cause somatic mutation, and is suspected to enhance the probability of germinal mutation (165). To date, *however, the available methods have provided no documented evidence for the induction (by chemicals or by radiation) of mutations in human germ cells.*

The single largest population at risk for induced heritable mutations is the group of survivors of the atomic bombs dropped on Hiroshima and Nagasaki in 1945. Survivors of these bombs are estimated to have received doses of radiation considered to be "biologically effective"; in experiments with mutation induction by acute exposure to radiation in mammals, similar kinds and doses of radiation were sufficient to cause phenotypically apparent mutations in offspring (and presumably other nonexpressed mutations as well). On the basis of this kind of experimental data, the assumption was made that germinal mutations could have been induced in people exposed to the radiation.

Efforts to detect genetic consequences of the atomic bombs detonated in Hiroshima and Nagasaki have been in progress continuously since 1946, sponsored by the Atomic Bomb Casualty Commission and by its successor in 1977, the Radiation Effects Research Foundation. These studies have attempted to determine whether germinal mutations are expressed as heritable mutations in the survivors' offspring, particularly as "untoward

pregnancy outcomes," as single gene disorders, as chromosome abnormalities, or most recently, as abnormal blood proteins.

Early Epidemiologic Studies

The earliest clinical studies, as well as the continuing mortality surveillance study, sought to identify a variety of health problems in the offspring of exposed atomic bomb survivors. These studies consisted of epidemiologic surveys of 70,082 newborn infants born in Hiroshima and Nagasaki between 1948 and 1953, including about 38,000 infants for which at least one parent had been proximally exposed to the radiation (defined as being within 2,000 meters of the hypocenter, while distally exposed was defined as being greater than 2,500 meters from the hypocenter or not exposed at all). Stillbirths, infant mortality, birthweight, congenital abnormalities, childhood mortality, and sex ratio were examined. An "untoward pregnancy outcome" was defined as a stillbirth, a major congenital defect, death during the first postnatal week of life, or a combination of these events. The investigators found no statistically significant, radiation-related increase for any "untoward pregnancy outcomes" (123). One reason for this finding may be that these health problems each have some genetic basis, but that all are influenced by a variety of nongenetic factors as well, some of which may have obscured an effect of genetic mutation. In addition, a continuing study (the F1 Mortality study) of mortality among children born between 1946 and 1980 has not demonstrated a relationship between radiation exposure of the parents and mortality in the offspring (56).

Cytogenetic Studies

Since 1967, Awa and colleagues have screened the children of survivors of the atomic bombs for cytogenetic abnormalities to determine whether exposure of the parents' gametes to the radiation caused a higher frequency of chromosome abnormalities in the offspring. After first reporting a higher frequency of sex-chromosome abnormalities among 2,885 children of exposed parents compared with 1,090 children of the control group of parents, Awa (6) concluded that a mutagenic

effect of the radiation was not demonstrated in these data. The total frequency of chromosomally abnormal children of exposed parents (0.62 percent) was higher than in the control group (0.28 percent), although the difference was not statistically significant, and was similar to the frequency of such abnormalities observed in several studies of unselected, consecutive newborn populations (120).

Results of their more recent analysis of the data showed that 0.52 percent of the offspring of exposed parents were determined to have an abnormal chromosome constitution, compared to 0.49 percent of the offspring of the control group of parents, which suggests that there is no significant difference between the two groups of offspring in frequency of autosomal balanced and unbalanced rearrangements and of sex-chromosome numerical abnormalities (8). This study did not provide information on chromosome abnormalities which are associated with high mortality rates, such as most of the unbalanced structural rearrangements and numerical chromosome abnormalities involving the autosomes.

Biochemical Analyses

Beginning in 1976, one-dimensional electrophoresis was used to look for mutations affecting proteins found in red blood cells and blood plasma. Rare variants of these proteins, differing by a single constituent amino acid, are taken to represent mutations which occurred in the germ cells of one of the parents if the variant is detected in a child and is absent from both parents. Classification as a "probable mutation" is made after inquiry into possible errors of diagnosis and of stated parentage (124).

Neel and colleagues (93,96,121) analyzed 28 enzyme and serum proteins from blood samples obtained from children who were identified in the F1 Mortality study and who were born between 1959 and 1975 to survivors of the atomic bombs. Over 10,000 children were examined in each group of exposed and unexposed parents. These children were also participants in the cytogenetic studies, and the biochemical analyses made use of the same blood samples that were used for the

chromosome analyses. To date, two probable mutations have been identified among offspring of proximally exposed parents (estimated to have received an average conjoint gonadal exposure of approximately 600mSv [60 rem[13]]) in 419,666 locus tests, while 3 new mutations have been found among 539,170 locus tests of unexposed individuals. Although the number of new mutants is small, a crude mutation rate can be calculated from these data. The rate of mutation in the exposed group is approximately 5 mutations in 1 million genes, and the rate in the unexposed group is 6 mutations in 1 million genes per generation. Such rates have wide margins of error and are not statistically different (96).

In 1979, the tests of electrophoretic variants were expanded to include assays for reduced activ-

ity variants of certain red cell enzymes .14 The presence of such variants suggests a deletion in the DNA, producing "null" mutations which would not be identified by one-dimensional electrophoresis. No such variants were found among 11,852 locus tests of 10 erythrocyte enzymes in children of exposed and unexposed parents (122).

Mutation Studies in Populations Exposed to Radiation in the Marshall Islands

Neel and his colleagues (93) reported results of electrophoretic studies of blood proteins from offspring of parents exposed to radioactive fallout from the Bravo thermonuclear test explosion at Bikini in the Marshall Islands in 1954. No variant proteins indicating new mutations were found in 1,897 locus tests of 25 proteins measured in children of unexposed parents, and in 1,835 such tests in children of exposed parents.

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[13]The doses of radiation that the survivors are estimated to have received are currently being re-evaluated in light of new information concerning the quantity and quality of radiation released by the two bombs (125). The revised estimate of exposure will probably be lower than the current estimate of **60** rem.

[14]An enzyme level three standard deviations below the mean and/or less than or equal to **66** percent of normal was defined as a deficiency variant.

CONCLUSIONS

This chapter has described several currently feasible approaches for determining rates of heritable mutations. Each of the methods has its particular problems. The genetic defects underlying the sentinel phenotypes are largely unknown. These phenotypes may result from mutations within genes at one or several genes, and may be complicated by the presence of phenotypically similar but nongenetic traits, all serving to bias estimates of their frequency upwards and to create a wide margin of error. Even with a collection of sentinel phenotypes that can be reliably identified in newborn infants, population surveys of a sufficient size are difficult to perform. To detect a small or moderate *increase* of sentinel phenotypes, consecutive newborns of entire national populations would have to be monitored thoroughly for many years, perhaps as part of an ongoing surveillance program for birth defects and childhood cancers.

The routine staining and banding of chromosomes is the most straightforward of the current methods, but they allow only major chromosomal changes to be identified. More advanced banding techniques, which are not performed routinely, allow smaller chromosome abnormalities to be detected in some cases.

Among the current methods described here, the detection of electrophoretic protein variants comes closest to mirroring precise changes in DNA. However, one-dimensional electrophoresis is limited by the relatively small number of proteins it can examine and by the fact that it does not readily detect the absence of gene products, caused by deletions or by specific nucleotide substitutions. Detection of enzyme deficiency variants, although still in early stages of development, could complement electrophoresis by detecting loss-of-activity variants. In addition, large pop-

ulation surveys are required to generate statistically significant results, and many more paternity tests are likely to be needed as a result of the biochemical approach compared to the phenotypic and cytogenetic methods. (Since the protein variants are not associated with reduced fertility, as are most of the sentinel phenotypes and chromosome abnormalities, only a fraction of the protein variants will be new mutations.)

An interesting example of an approach for detecting mutations in a single gene or in a group of genes is provided by the analysis of mutations underlying unstable hemoglobins and HbM. Analyses of other accessible and well-studied genes may help to generate better estimates of mutation rates per nucleotide and per gene on the human genome, although it is not known how representative any one gene is of the remainder of the DNA. Moreover, characterizing the mutations at the molecular level provides important information, especially where specific mutagens are suspected to have caused the mutations.

The different approaches have led to various estimates of heritable mutation rates in human beings (see table 4). Sentinel phenotypes, representing the occurrence of a single dominant mutant gene, are detected in about 2 individuals, on average, per 100,000 examined per generation. Individual types of chromosome abnormalities are up to 50 times more frequent than the average frequency of sentinel phenotypes. Analyses of the mutant hemoglobins studied by Stamatoyannopoulos and Nute (131) give estimated spontaneous mutation rates of about three mutations per 1 million genes per generation. The rate measured in one-dimensional electrophoresis of blood proteins is also about three mutations per 1 million genes per generation, a figure based on the examination of over 1 million locus tests involving 30 to 40 different proteins.

The different approaches currently available for detecting heritable mutations (listed in table 5) in human beings generate data that are generally not comparable. Each set of data reflects the nature of the approach itself, not necessarily the nature of the different genes examined or their spontaneous mutation rates. It is particularly difficult to compare directly the results of studies of muta-

tion rates based on clinical syndromes with biochemical studies. Measurements from each of the current methods can be used to generate a separate mutation rate and, for the time being, those rates can be viewed as baselines for the frequency of various kinds of human mutations and as reference points for some of the most severe effects of heritable mutations.

Table 4.—Results of Studies To Detect Heritable Mutations by One-Dimensional Electrophoresis in Human Populations Not Known To Be at High Risk for Germinal Mutations

Population	Locus tests	Mutations
United States[a]: Caucasian	218,376	0
Black	18,900	0
United Kingdom[b]	133,478	0
West Germany[c]	225,000	1
Central and South America[d]	118,475	0
Japan[d]	539,170	3
Marshall Islands[e]	1,897	0
Total	1,255,296	4

Overall "spontaneous" mutation rate = 4 mutations/1 ,255,298 locus tests is equivalent to approximately 3 mutations/l million genes per generation

SOURCES

[a] J. V. Neel, H.W. Mohrenweiser, and M H Meisler, "Rate Of Spontaneous Mutation at Human Loci Encoding Protein Structure," *Proc Natl Acad Sci USA* 77(10):603743041, 1980; H W Mohrenweiser, 1986, unpublished data, cited in Neel, et al., 1986.

[b] H. Harris, D.A Hopkinson, and E B Robson, "The Incidence of Rare Alleles Determining Electrophoretic Variants" Data on 43 Enzyme Loci in Man, " *Ann Hum Genet.* Lend. 37 "237-253, 1974,

[c] K. Altland, M Kaempfer, M Forssbohm, and W. Werner, "Monitoring for Changing Mutation Rates Using Blood Samples Submitted for PKU Screen ing, " *Human Genetics, Part A: The Unfolding Genome* (New York Alan R Liss, 1982), pp 277-287.

[d] J.V. Neel, c, Satoh, K Goriki, et al , "The Rate With Which Spontaneous Mutation Alters the Elect rophoretic Mobility of Polypeptides," *Proc Nat Acad Sci USA* 83389-393, 1986, J V Neel, unpublished data.

[e] J.V Neel, H Mohrenweiser, S Hanash, et al., "Biochemical Approaches To Monitoring Human Populations for Germinal Mutation Rates 1. Elect rophoresis," Utilization *of Mammalian Specific Locus Studies in Hazard Evaluation and Estimation of Genetic Risk*, F J deSerres and W Sheridan (eds) (New York Pie. num Press, 1983), pp 71-93

Table 5.-Spontaneous Mutation Rates of Genetic Abnormalities in Human Populations

Genetic anomaly	Mutations/ generation	References
Sentinel phenotypes	2 mutations per 100,000 individuals	(166)
Chromosome abnormalities	2 to 121 mutations per 100,000 individuals	Table 3-2
Electrophoretic protein variants	3 mutations per million genes	Table 3-4; (96)
Analysis of unstable hemoglobins	3 mutations per million globin genes	Table 3-3; (131)

SOURCE: Off Ice of Technology Assessment

The different approaches currently available reflect the various forms that mutations and their consequences take. ***Each*** *of the current approaches to studying mutations is generally limited to particular kinds of mutations or to a particular set of outcomes. The* cytogenetic and biochemical approaches discussed are limited to detecting a small subset of mutations that occur in human DNA. Identification of sentinel phenotypes and chromosome abnormalities limits the observations to a particular set of outcomes, mostly in the severe end of the spectrum. The available data are based on mutations observed in a small fraction of the genes, about 40 to sO, out of a total of about 50,000 to 100,000 in the human genome.

Aspects of the current methods that limit their scope also make them inefficient. The proportion of all mutations that these methods can identify is relatively small and the limited number of suitable genetic endpoints (acceptable sentinel phenotypes, chromosome abnormalities, and protein variants) makes it difficult to sample more than a few mutational events per individual. As a result, huge populations of "exposed" and "unexposed" people would be needed to find out whether the exposure resulted in excess heritable mutations (71). Not surprisingly, perhaps, there is no documented evidence to date for a meaningful difference in rates for any type of mutation between two populations (or in the same population at different times).

Sentinel phenotypes and cytogenetic analyses provide a highly selective approach to some of the worst possible consequences of new mutations. However, they do not permit more than a partial view of the broader consequences of mutations in human beings. Detailed molecular, physiological, and clinical information on the kinds and rates of new mutations will eventually be needed to assess the immediate or potential impact of new mutations.