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Prevention of avoidable mutational disease: Memorandum from a WHO Meeting*

About 1% of children are born with a serious disorder which is the direct result of a mutational event in a parent or a more distant ancestor. These disorders, of which several thousand are known, mainly afflict the blood, bone, brain, ear, eye or muscle and the changes are usually irrevocable by the time of diagnosis. Another 1% of individuals will develop a serious genetic disease some time after birth. In addition to these direct consequences of a mutant event, far higher proportions will suffer from the indirect effects of one or several mutations.

In view of their chronic and severe nature most of these disorders impose a burden disproportionate to their frequency, and it is sound public health policy to avoid the birth of babies known to have the established mutations and prevent further cases in the immediate or distant future by minimizing the exposure of people at risk to known mutagens. The advantages in permitting certain mutagenic exposures must be assessed against the later costs.

Owing to the natural mutation rate and the vast backlog of previous mutations, the prospects of prevention are limited to preventing an increase, rather than to achieving any substantial decrease. This Memorandum describes progress in the ability to dissect and interpret the mutational process, to identify populations at risk, and to evaluate the consequences of the various types of mutational event and emphasizes that the current approach to prevention of mutational disease must involve improving our ability to study populations that appear to be at increased risk.

INTRODUCTION

The effects of radiation on human heredity, the first WHO report on this subject, was published in 1957 in response to increasing public concern over the genetic consequences of radiation (*1*). Since then, this concern over the hazards of radiation has continued; in addition, industrial and agricultural practices have been exposing human populations to an increasing variety of potentially mutagenic chemicals. While the reports on the Biological Effects of Ionizing Radiation (BEIR) (*2*) and of the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) (*30*) discussed the radiation aspects, the problem of mutagens as a whole in relation to the recent advances in molecular biology and clinical genetics have not been considered.

During the past three decades, our knowledge of both the nature of the gene and its functions has greatly increased; the chromosomal disorders, whose cumulative impact on health is comparable to that of the Mendelian disorders, have been defined; numerous new genetic disorders have been identified, and many old ones recognized as heterogeneous. It is now possible to define both normal and pathological variability in chromosomes, in proteins, and in DNA itself, and to test substances for mutagenicity by methods of high sensitivity but uncertain relevance to human health.

At present, knowledge of the likely consequences of exposure to radiation and other mutagens is limited because of ignorance of the basic mechanisms involved and of the ways in which these can be disturbed by mutation. Besides the inference that any small addition to the background level of radiation will be followed by a small increase in the mutation rate, the evaluation of possible mutagens other than radiation is difficult because there is no well-characterized background exposure as a baseline. The very major advances in the last twenty-five years have, to some extent, increased the magnitude of the problem by revealing unexpected complexities. Against this widespread ignorance and understandable public anxiety, considerable resources are being deployed to protect populations and occupationally-exposed individuals against hazards that may well be relatively

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small. On the other hand, there is little information available on the genetic consequences of massive population exposures to any mutagenic agent.

BIOLOGICAL BASIS FOR THE GENETIC EFFECT OF RADIATION AND OTHER MUTAGENS

Intensive studies during the last ten years, including the application of the techniques of molecular genetics to the analysis of eukaryotic genomes, have resulted in an increasing knowledge of genetic fine structure and, in particular, a detailed insight into the organization at the DNA level of many individual genes, including many human genes. It is now clear that only a small proportion of the genome of higher eukaryotes, including man, is transcribed and translated into a protein. Presumably most of the DNA has an architectural or regulatory function or has had some such function in the past. Our knowledge about most of this DNA, a substantial part of which is highly repetitive within the genome, is still very limited.

The fraction of DNA coding for specific proteins is estimated at between 1% and 10% of the total genome. Assuming an average coding sequence length of about 1500 base pairs (bp), some 15 000 to 300 000 transcribing units, or genes, can be accommodated in the haploid human genome which has a total size of about 3×10^9 bp. Many of these exist in families of almost identical or closely related genes. In the eukaryotes most genes are divided into regions corresponding to the sequence of messenger RNA (mRNA) (exons) and segments interrupting these sequences (introns or intervening sequences). After transcription the intervening sequences become excised from the nuclear or pre-messenger RNA, the remaining segments being spliced together to produce the mature cytoplasmic RNA.

Knowledge of these sequences, and of the functional significance of specific sequences in the intervening and flanking regions of eukaryotic genes, is increasing rapidly, so that it is possible to start to elucidate the regulation of the transcription and processing of messenger RNA at the molecular level. The detailed knowledge of several human genes at the DNA level, including partial and complete nucleotide sequences, is the necessary basis for understanding mutational events. These can be:

— base substitutions (transversions or transitions);
— deletions or insertions of one or several base pairs;
— extensive deletions including the complete loss of a gene;
— rearrangements within or between chromosomes;
— insertions of mobile sequences.

The extent of the alteration at the DNA level is not necessarily reflected by the extent of phenotypic disturbance. For example, the loss or gain of a single base in an exon will lead to a frame shift in the translation process, with mostly severe consequences, while the same occurring to a whole triplet, or a larger event such as a balanced translocation, may have no effect on the phenotype. Studies on the molecular pathology of the globin genes by the use of restriction enzymes and sequencing techniques have revealed the whole range of the different mutational events considered above, except for mobile sequences, which have not yet been found to cause mutations in man.

Base exchanges in coding regions may lead to no change in the protein owing to the redundancy of the genetic code while the similarity of some amino acids may lead to harmless protein variants. Single base changes can also lead to abnormal proteins, as in disorders involving the haemoglobins Hb S, Hb E and Hb C. They can also create or eliminate normal splicing sites in the pre-mRNA and several such defects have been shown to cause a severe beta-thalassaemia (*3*).

Although substantial progress has been made in the analysis of genetic fine structure, knowledge about gene organization at this level is still very limited. In particular, nothing is known about its changes during the various stages of gametogenesis, or about the influence of structure on the susceptibility to mutation. Almost nothing is known about mutations between the gene regions with respect to either their biological effect or their frequency.

HUMAN DISEASE DUE TO SPONTANEOUS MUTATIONS

Germinal mutations

All inborn variability is due to mutations, mostly from the very remote past, and no disorder is likely to be uninfluenced by the genetic background. Some disorders are demonstrably due to additional or missing chromosomes, while others show patterns of transmission of a simple Mendelian nature. These are respectively the chromosomal and genic disorders. At least 5% of human conceptions fail owing to chromosomal disorders; of those surviving to birth, about 0.5% have a chromosomal abnormality and about as many have recessive or X-linked Mendelian disorders, most of the dominant disorders not being evident in infancy.

The exact lesion in the DNA in some recessive disorders is now known and includes deficiencies of segments of DNA and changes in single bases. No example is yet known of a Mendelian disease due to mutations in the DNA between the gene loci, except

in their immediate flanking regions, although intergenic sequences represent the bulk of the DNA.

Most chromosomal mutations are new while most genic disorders represent mutations that occurred one to ten generations ago in the dominants and X-linked recessives and tens to thousands of generations ago in the autosomal recessives. The nature and frequency of germinal mutations might appear to be a simple matter of diagnosis and enumeration. In fact, the matter is far from simple, as is shown in the wide range of estimates from various sources (Table 1).

The bulk of human disorders are, of course, neither chromosomal nor simple Mendelian but are strongly influenced by the genetic background, and this may be dominated by one locus at which a strongly predisposing phenotype is determined by the joint effects of two or more loci. In general, whenever human disorders have been studied, familial predispositions have been found. The congenital malformations pose a particular problem: they are widely regarded as highly relevant to mutational damage, yet there is little evidence to suggest that most fetal disorders are more "genetic", or have a simpler genetic basis, than most disorders of later life.

Estimating the contribution of genetic factors to common disorders is a very difficult problem, usually resolved by attributing arbitrary degrees of proportional representation to the various disorders, as a result of which it is possible to compute totals to the contribution of genetic factors to disease. Clearly there are likely to be many loci where phenotypes will be determined which are peculiarly susceptible to some extrinsic factors, such as the predisposition to lung disease of smokers with alpha-1-antitrypsin deficiency. In this example, the frequency of disease could be roughly estimated by multiplying the proportion of the population with this deficiency by the proportion that smoked. It is also likely that the predisposition to many disorders results from the combined effects at numerous loci, none having a very strong effect on its own.

In the present state of knowledge it is very difficult to integrate such vague estimates of genetic predisposition with the more precise, but far from exact, estimates based on the Mendelian disorders. Little more can be done than to assume that this is an iceberg type of problem, and that, as with an iceberg, the visible fraction is of the order of a tenth of the whole.

The cumulative incidence of *chromosomal mutations* at birth is about 0.5%, fairly evenly divided between the autosomes and the sex chromosomes, almost all being new mutations with far higher incidences at conception.

The *dominant disorders*, more correctly termed disorders manifest in the heterozygote, are usually

Table 1. Incidence of genetic disease at birth (excluding chromosomal disorders) per 1000 live births

	Source, year and reference								
	Stevenson, 1959 (*4*)	UNSCEAR, 1972 (*27*)	BEIR, 1972 (*5*)	Edwards, 1974 (*6*)	Trimble & Doughty, 1974 (*7*)	UNSCEAR, 1977 (*28*)	BEIR, 1980 (*2*)	CMCF,[a] 1981 (*8*)	UNSCEAR, 1982 (*29*)
Dominant	33.2[b]	+ + +	10.0	0.6	0.8	10.0[c]	10.0[c]	1.85–2.62	10.0[c]
Recessive	2.1	+	1.5	2.5	1.1	1.1	1.1	2.23–2.54	2.5
X-linked	0.4	+	0.4	0.5	0.4	–[c]	–[c]	0.78–1.98	–[c]
Subtotal	35.7	10.0[d]	11.9	3.6	2.3	11.1	11.1	4.9–7.1	12.5
Malformations	14.1	–	25.0	–	42.8	90.0	90.0	37.4–44.5	90.0
Others	14.8	–	15.0	–	47.3			20.0–29.0	
Subtotal	28.9	20.0	40.0	–	90.1	90.0	90.0	57.04–73.5	90.0
Total	64.6	30.0	51.9	3.6	92.4	101.1	101.1	62.3–80.6	102.5

[a] Committee on Mutagenicity of Chemicals in Food.

[b] Includes many conditions no longer regarded as dominant.

[c] X-linked included with the dominants.

[d] Mostly dominant.

regarded as the commonest genic disorders in man, and also those most likely to increase under an increased mutation rate (*2, 29*). Most are not evident at birth. Achondroplasia, a classical dominant disease with an incidence of about 1 in 20 000 births, is usually obvious but the diagnosis may be missed for several months; the early studies included a minority of disorders now known to be distinct (*10*). Its high mutation rate, exceeding 20×10^{-6}, places it in an unusual category of disease. Tuberose sclerosis, another classic disorder, is now recognized to be substantially commoner than achondroplasia, and, as it is also usually sporadic, must have an even higher mutation rate (*11, 12*). Neurofibromatosis has a similar incidence but exact estimates are difficult owing to the presence of mild forms, and occasional very mild manifestations in a parent, which makes the recognition of new mutants difficult except in specialized centres (*13*). In Marfan's syndrome the problems are similar, and it is common to find relatives who are so mildly affected that they would otherwise escape notice (*10*).

In Huntington's chorea and myotonic dystrophy, which have a similar incidence, new mutations are exceptional; this has led some investigators to postulate explanations other than mutation for the maintenance of these debilitating disorders, such as behavioural changes before severe symptoms develop (*14*). Recently a dominant type of hypercholesterolaemia, with an incidence of up to 3/1000, has received considerable attention (*15*); it seems impossible for such levels to be maintained by mutation, and there are no serious obstacles to postulating some heterozygote advantage, at any rate in the past. Disorders that do not appear to be maintained by mutation can hardly be used as a basis for estimating the consequences of increases in mutation rate. Adult polycystic disease of the kidney, which is dominantly inherited, is common at autopsy (incidence, about 1/1000), but only a minority of individuals are seriously incapacitated. Disorders resembling these seven conditions have not been recognized in the mouse.

In later life as many as 10% of individuals are seriously disabled by deafness, cataract or dementia, and a substantial proportion of these disabilities may be dominant, but are not easily recognized because of the obvious difficulty in obtaining appropriate family histories. The incidence figures relevant to mutationally-maintained dominant disease are well below 0.25% for disease manifest at birth, although reaching several times this level for diseases manifest later.

Recessive disorders, even excluding the haemoglobinopathies, which on a world scale are the most common cause of death from recessive disease, are the major form of genic disease in infancy and childhood. The commonest forms in many ethnic groups that have been studied are too common to be maintained by mutation, sometimes providing over half the cases of recessive disease. It seems likely that most forms are maintained by mutation, and that this group of diseases, in particular, would be increased by a higher mutation rate. Excluding the haemoglobinopathies, the incidence of these disorders at birth is of the order of 0.25% (see Table 1).

The commonest *X-linked conditions* are Duchenne's muscular dystrophy and the haemophilias, with an incidence of 1 in 3000 and 1 in 5000 boys, respectively (*16, 17*). The former is lethal, and must have a mutation rate exceeding 100×10^{-6}. A form of X-linked mental deficiency, which is associated with a visible peculiarity at the tip of the long arm of the X chromosome in a proportion of cells (the fragile-X syndrome), occurs at a frequency similar to Duchenne's disease, and the affected males rarely reproduce (*18*). All other X-linked disorders are unlikely to exceed the combined incidence of these, which is about 0.2%.

In summary, genetic disorders, which may be divided into chromosomal and genic disorders, are manifest in about 1% of births, each category being responsible for about half. Of the 0.5% due to chromosomal disorders, about half are very severe and autosomal, and half are fairly mild and due to an extra or missing sex chromosome. Of the 0.5% or so due to genic disorders maintained by mutation, those that are manifest in infancy and childhood are mainly X-linked or autosomal recessive, and those that appear later are mainly dominant. An indeterminate proportion of total morbidity and mortality is a necessary consequence of eliminating mutations that are not manifest as Mendelian disorders. It is likely that this exceeds the proportion above, possibly about tenfold.

Somatic mutations

Mutational events also occur in somatic cells: some mutational events lead to a loss of growth inhibition, with the production of either benign or malignant tumours, or of leukaemias, collectively termed "neoplasms". Recent work suggests that a substantial proportion of neoplasms are due to changes, or rearrangements, involving specialized genetic units, the oncogenes (*19*). Such mechanisms are not known to be involved in germinal mutations. Based on correlations between the ability of some agents to produce neoplasia and their ability to damage germ cells in the experimental situation (*8*), it might be reasonable to suspect that an agent related to neoplasia in man would usually damage the germ-line cells of exposed persons, though more data are needed before reliable predictions can be made.

AGENTS CAUSING MUTATIONS IN MAN

Radiation

Radiation is the classical mutagen. So far, population studies on humans exposed to this mutagen fall under two main categories: those on populations living in areas with a relatively high background radiation level, and those on children born to parents surviving the atomic bombing of Hiroshima and Nagasaki. A major study on this has been continuing in Japan since 1946. The most recent analysis of the accumulated data, which included an effort to generate an estimate of the genetic doubling-dose of radiation, was published in 1981 (*20*). The analysis dealt with four indicators:

(1) Frequency of untoward pregnancy outcomes (major congenital defect, stillbirth, or death during the first week after birth).

(2) Deaths among liveborn infants, through an average life expectancy of 17 years.

(3) Frequency of children with sex-chromosomal aneuploidy.

(4) Frequency of children with mutations altering the electrophoretic mobility of proteins.

In no instance was there a statistically significant difference between the children of so-called proximally exposed parents, who received an estimated dose of between 1 rem (0.01 Sv) and the maximum dose compatible with survival (about 500 rem or 5 Sv),[a] and the children of distantly exposed parents, who received essentially no excess radiation. However, the findings were in the direction of the hypothesis of a genetic effect of the exposure. To derive from these data an estimate of the doubling dose it was necessary to estimate to what extent "spontaneous" mutations in the unexposed parents contributed to the first two of the previously-mentioned indicators under the conditions of post-war Japan.

With what seemed to be reasonable assumptions, and averaging together the findings of the four indicators, the doubling dose for acute radiation exposure was estimated at 156 rem (1.56 Sv), with a large and somewhat indeterminate error. Unfortunately, just as the estimate was completed, it became clear that the radiation exposures of the survivors had been overestimated. This means that the above estimate of the doubling dose must be revised downwards, but no revision is possible until the current re-evaluation of radiation exposure has been completed.

[a] The conversions to SI units are as follows: 1 R (röntgen) equals 0.258 mC/kg (milli-coulomb/kg); 1 rem equals 0.01 Sv (sievert); 1 rad equals 0.01 Gy (gray). For practical purposes in the context of this report, sieverts and grays are approximately equal.

Populations that have been exposed to high natural radiation levels from monazite in the soil and are sufficiently large for the monitoring of possible mutational effects, both somatic and germinal, exist in various places, such as the coastal area of the State of Kerala in India (*21*) and Yangjian county of Guandong Province in China (*22*). The mean background radiation exposure in these areas is five to ten times the average background elsewhere (*21*, *22*). In China the cancer mortalities in this area have been investigated for more than twelve years and no correlation with exposure has been documented. However, the number of person-years observed is not yet sufficient to draw a definite conclusion.

For many of the northern European countries, Canada, the USA and the USSR, a significant source of radiation is radon and its products in houses, and this could be diminished by changes in house construction.

Non-disjunction leading to abnormal numbers of chromosomes is a recognized consequence of radiation. A doubling dose of about 100 rad (1 Gy) has been used (*23*) but further data are needed. The human data on trisomy 21 (Down syndrome) following diagnostic radiation are suggestive of a small but inconsistent effect. The studies in Hiroshima and Nagasaki mentioned above, involving acute radiation exposure, failed to reveal an increased frequency of Down syndrome.

The principal experimental mammal for studying the genetic effects of radiation is the house mouse, on which an extensive body of information is available. The genetic doubling dose for acute gamma radiation, yielded by the average for several different mutational systems, is approximately 40 rad (0.4 Gy). On the basis of the generally lower yield of mutations from chronic or divided radiation exposures, it has been suggested that the doubling dose of chronic radiation for the mouse (and by extrapolation for humans) is within the range of 50–250 rad (0.5–2.5 Gy). There are, however, substantial differences both between the results yielded by different mutational systems and between different genetic loci. In addition to the difficulties in extrapolating from one species to another, there are important questions concerning the most appropriate value for the mouse.

Human data provide very little information on the contribution to the "spontaneous" mutation rate from the background radiation of about 0.1 rad/yr (0.001 Gy/yr) or about 3 rad (0.03 Gy) per generation. However, extrapolation from experimental data, especially from the mouse, leads to the conclusion that only a small fraction, about 3% of the naturally occurring mutations, could be caused by background radiation. On the assumption that there is linearity, this proportion must be the ratio of the

background level to the doubling dose. So far, no estimate of the proportion of mutations that might be caused by other naturally occurring mutagens is feasible.

Chemical exposure

Although numerous chemical agents have been shown to be mutagenic in bacterial systems, only a few have been tested for the induction of germ-line mutations in the mouse and there is no information at all for man (*24*). A particular problem with the study of chemical mutagens is the estimation of gonad dose (in contrast to radiation where regional dosimetry is fairly simple).

The possibility has often been raised that a substantial fraction of naturally occurring germ-cell mutations can be caused by mutagens in the human diet (*8*). These may include plant products as well as substances resulting from fungal contamination of food and from hydrocarbons produced by frying and roasting. The influence of dietary mutagens cannot be estimated even approximately. It is possible that spontaneous mutations, including chromosomal mutations, may be commoner in some primitive populations than in those living under more advanced conditions owing to nutrition or infection. However, the evidence is uncertain and the interpretation unclear.

Biological factors that influence germ-cell mutation

There are a number of biological factors that influence the rate of some mutations in human germ cells. In trisomy 21, which is due to meiotic non-disjunction, usually at the first meiotic division at oogenesis, the incidence increases strongly with maternal age, the risk to women aged 40 years being about twenty times higher than to women aged 20 years. The influence of age on non-disjunction in the male is unclear. Other biological factors that might influence non-disjunction include season and auto-antibodies.

Some classical presumptive point mutations are influenced by the age of the father. This effect was first discovered in achondroplasia (*25*) and has since been found in several other dominant conditions (*15, 26*). An age effect has also been reported in the maternal grandfathers of sporadic cases of classical haemophilia and of the Lesch-Nyhan syndrome. It is less pronounced in some other dominant conditions such as bilateral retinoblastoma. In achondroplasia and other conditions with a similar paternal-age effect the risk of mutation occurring from fathers over 40 years might be increased about sixfold compared to fathers of 20 years (*15*).

In all monitoring schemes for chromosomal aberrations and gene mutations the age distribution of the parents should be given due consideration. In numerical chromosomal aberrations, concomitant variables such as auto-immune status or seasonal factors may need consideration. Discussions in recent literature on a possible paternal-age effect in trisomy 21 show that the statistical problems are by no means trivial.

SUSCEPTIBLE POPULATIONS

A variety of populations may be unusually susceptible to mutation for different reasons, some of which have been mentioned in the preceding section. Efforts to minimize mutational disease might concentrate on the following specific factors or groups.

Pregnancy and early childhood are the commonest situations in which there is a widespread belief that radiation imposes a greatly increased hazard. There is no doubt that exposure to high doses of radiation in early pregnancy will disturb the development of the embryo and that later exposures will disturb rapidly growing tissues. Even the small dose used in obstetric X-rays (about 2 rad (0.02 Gy) or about the cumulative background dose up to a first pregnancy) has been claimed to increase the risk of leukaemia and trisomy in the developing child. However, there is no reason to expect any peculiar sensitivity to germinal mutations from very low doses of the order of the annual background rate, beyond the obvious fact that two individuals are at risk. The major preventable mutational hazard from X-rays is gonad exposure in the years between birth and parentage.

Adverse environment. There are a variety of environments in which populations have unusual mutagenic exposures, e.g., those living in areas with high background radiation levels, those exposed to aflatoxins, and those living near chemical dump sites. Occupational exposures to potential mutagens are generally well controlled where the risk is recognized. Nevertheless, there are potential mutagenic exposures resulting from employment in the nuclear energy industry, in the smelting industry, in pulp and paper mills, in industries using chemical sterilants, and from the agricultural use of pesticides.

Use and abuse of drugs, other substances and smoking. Drug use has become an everyday event in developed countries. It is worth separating the following groups of persons who may be exposed to potentially mutagenic drugs. (1) *Occasional drug use* owing to acute disorders. This is common, and may involve a large proportion of the population in epidemics. (2) *Regular drug use:* (*a*) for the treatment or

control of chronic disease (e.g., diabetes, epilepsy, rheumatoid arthritis, auto-immune diseases, depression, hypertension, etc.); with respect to malignant disease some treatments are used in spite of their presumed mutagenic consequences, e.g., cytostatic drugs; however, fortunately these treatments are mainly used after the child-bearing age; (*b*) for purposes unrelated to disease (e.g., contraception and drug addiction); (*c*) for the prevention of disease by exposing whole populations (e.g., antimalarial prophylaxis, immunization). (3) *Self-poisoning and accidental overdosage*: individuals who attempt suicide through self-poisoning with extremely large doses of drugs and survive may comprise a high-risk population. Unsuccessful suicide attempts demand particular attention because of their high frequency in young adults in some communities. (4) *Smoking*: there are reports that smokers exhibit increased chromosomal damage in cultured lymphocytes and produce urine which is mutagenic to bacteria. They may be at an increased risk of germinal mutations.

Individual variation in susceptibility. Not everybody exposed to a given environmental agent reacts in the same way, and some of this variation is genetically determined. Numerous examples of genetic variation in susceptibility are known. In the present context, variation related to the inherent susceptibility to carcinogens is most relevant because many carcinogens are mutagenic. For example, if substantial subpopulations with an increased tendency to develop cancer upon exposure to certain environmental factors exist, such as heterozygotes for DNA-repair deficiency conditions, it would be an important preventive measure to protect their members from unfavourable exposure—thus, protecting these individuals from cancer and their descendants from mutational disease.

Populations exposed to vaccines containing live virus. Part of the life-cycle of many viruses includes their incorporation within chromosomal segments and, in principle, this could result in a mutation or even disturb mitosis or meiosis. Conspicuous chromosomal disturbances are demonstrable in some viral infections.

The data linking epidemics of influenza and hepatitis with trisomy 21 are limited and inconsistent. No clear-cut epidemics of chromosomal disorders have been described at the population level, although many remarkable small clusters have been reported.

Groups with heavy diagnostic and therapeutic exposure. The exposure of patients undergoing treatment with ionizing agents (X-rays, isotopes, etc.) usually exceeds the recommended dose limit for populations. A higher rate of chromosomal aberrations in somatic cells, and an increased incidence of some types of cancer have been reported. Fortunately these treatments are mainly used after the usual age of parentage, and only in small proportions of any population. However, there are now many survivors of childhood malignancy who received radiotherapy or chemotherapy.

Finally, the possibility of accidental exposure of populations to high levels of radiation or chemicals should be noted; these have occurred in most industrial countries. Such accidents provide unusual opportunities for learning from mistakes.

STUDIES THAT CREATE THE PRESUMPTION OF HERITABLE GENETIC CHANGE

These include the use of cancer registers and other relevant data bases, clinical or pathological studies suggestive of carcinogenic effects of certain compounds, chromosomal studies in cultured cells after exposure (including analysis of chromosomal breaks and rearrangements and of increased sister chromatid exchanges), detection of mutant proteins in single somatic cells, biochemical detection of changes in body fluids or cells reflecting exposure to mutagenic substances, biological detection of these substances in body fluids, and studies on animal models.

CURRENT POSSIBILITIES OF EVALUATING INCREASES IN THE FREQUENCY OF MUTATIONAL DISEASE

As should be apparent by now, the spectrum of mutational changes ranges from nucleotide substitutions with no known phenotypic effects to major chromosomal abnormalities resulting in early fetal death or severe malformation. The investigation of this spectrum presents many problems as most of the approaches currently available will detect only a limited part of the whole.

In principle, the evaluation of whether mutation rates are changing (or have changed in some specific subpopulation) proceeds in one of two ways. On the one hand, one can follow with appropriate studies over an extended period of time a population thought to be entering on a period of increased risk. The terms "monitoring" or "surveillance" are often applied to observations of this type. On the other hand, most efforts to understand possible mutagenic influences will be *post hoc*, i.e., will be undertaken after it is realized that a subpopulation has been exposed to a potential mutagen. In a prospective study, the population serves as its own control, whereas in the *post hoc* study, it is vital that a suitable control population be established.

Since no population will knowingly be exposed to

the risk of a greatly increased rate of mutation (except for the radiation effects resulting from military action), the increase in mutation rate to be anticipated in a prospective study will usually be small, and the numbers of persons who must be screened if the study is to be informative are correspondingly increased. On the other hand, the *post hoc* studies will usually involve small groups with relatively large exposures, so that the number of persons required is less. Five approaches to the evaluation of an altered mutation rate are discussed below.

(1) *The use of vital statistics and related data.* Traditional vital statistics are of relatively little value in evaluating a change in mutation rate, although, in the extreme, a rise in perinatal deaths might raise this possibility. However, several countries now require notification of congenital defects at birth. To the extent that chromosomal abnormalities and the mutationally-derived dominantly inherited syndromes contribute to congenital defects diagnosable at birth, such notification could be a first step towards the detection of altered mutation rates. Such certification could be integrated with clinical studies.

(2) *Clinical studies.* There are a few dominantly inherited disorders which arise *de novo* on a sufficient scale as a result of mutation, and which can be diagnosed with sufficient accuracy to justify population studies. Some of these can be diagnosed at birth on the basis of a careful physical examination (e.g., achondroplasia, aniridia), while others usually manifest themselves some years after birth (e.g., retinoblastoma, neurofibromatosis). Such syndromes are sometimes referred to as "sentinel phenotypes", i.e., phenotypes resulting from a simple highly penetrant allele that confers low fitness and a distinctive phenotype which can be diagnosed accurately with minimal effort. The accumulation of data on such syndromes can proceed in either of two ways. Some countries now maintain registries of handicapped children, with initial entries based on birth certificates, and later additions as specific handicapping diseases are diagnosed. Such registries, if properly maintained, could constitute a source of data for initiating studies of mutation rates, particularly in small communities. Other sources of data might be specialized registers of diseases and hospital records. However, in whatever way the data are gathered, these registers will contain a mixture of new cases, cases due to mutation in preceding generations, and cases due to mutation in more remote generations, so that their use in the context of mutation studies demands a detailed genetic evaluation of each case.

(3) *Chromosomal studies.* Studies evaluating the frequency of chromosomal abnormalities can be based on blood samples at birth or on cells acquired by amniocentesis or chorion biopsy. However, allowance must be made since the population sample will be selected by hospitalization, illness or age. Given the high early mortality associated with aneuploidy and unbalanced translocations, studies of older children are valid only if life expectation is not greatly affected. While numerical aberrations are almost always the result of a fresh mutation, establishing the origin of a balanced translocation in the preceding generation requires family studies. The rate with which chromosomal mutation leads to structural abnormalities of a sufficiently gross nature to be detected with current techniques is approximately 1/1000 per individual per generation (*9*).

(4) *Studies of protein abnormality.* The increasing ease with which variant proteins can be detected and characterized suggests that this may become a useful approach to measuring mutation rates. The principal method available for detecting such variants are electrophoretic (either one-dimensional or two-dimensional) or activity analyses. These studies cannot utilize the results of medical practice (as do the chromosomal disorders and the dominantly inherited genetic syndromes), but require an independent organization. In principle, the approach is the same as in the use of the dominant disorders and the chromosomal abnormalities: one searches for a type of variant not present in either parent. Because the frequency of mutation resulting in an electrophoretic variant is of the order of 5×10^{-6} per locus per generation, the numerical requirement of such studies are rather extreme. Attention must be directed to all possible economies and efficiencies. Where feasible, one can reduce costs by making use of established medical procedures. For example, in the situation of neonatal screening for phenylketonuria, the remainder of the blood spot may be used for screening the products of some 10 to 20 loci. Two-dimensional gel electrophoresis requires a special blood sample, but has the advantage of permitting the examination of very many more gene products from a single individual. This field is evolving very rapidly and it may soon be possible to read these gels for presumptive mutants using computers.

(5) *Direct methods of gene analysis.* So far, the developments in molecular genetics, permitting direct investigation of the DNA using restriction enzymes or nucleotide sequencing, have not been used for screening purposes to detect new germinal mutations.

The large amount of DNA needed for digestion with several combinations of restriction enzymes, the work involved to isolate DNA samples from a large population, and the number of probes needed set severe limitations to the restriction-hybridization approach. This is in part because DNA procedures test alleles rather than loci.

Any approach depending on the sequencing of DNA is even more limited by the necessity to have to clone and select particular gene sequences and to include both parents as well as the index case. Even if this is only done for already established mutants of proteins in order to determine the precise nature of the mutation, it still involves a large amount of work and is not yet a suitable procedure for screening.

Finally, both these approaches need considerable technical expertise and a broad range of expensive reagents, many of which are unstable or radioactive. The techniques of molecular genetics are developing very fast and improvements in some of them, including automation of standard procedures, the introduction of methods for multiple use of DNA-filters, the development of non-radioactive and more sensitive means of probe-labelling, and finally methods for direct sequencing of genomic DNA, might make the direct screening of mutational events economic.

There are no direct estimates of spontaneous mutation rate at the nucleotide level. A very approximate estimate for one type of mutation, nucleotide substitution, can be obtained from the rate for electrophoretic variants given in the preceding section. If we assume that electrophoresis will detect roughly half the mutations characterized by nucleotide substitutions, and that the average protein requires 1500 nucleotides for its specification, then the minimal rate per nucleotide is, very approximately, the mutation rate per locus $\times 2$/number of nucleotides, or $4 \times 10^{-6} \times 2/1500$ which is about 1×10^{-8} mutations per generation per nucleotide. This is a very minimal estimate, since it does not include mutations in exons which lead to unchanged proteins or do not lead to a polypeptide product. There are multiple problems to be solved in the development of techniques appropriate to the efficient study of mutation at this frequency. It would therefore be prudent to establish a few centres of excellence to work out relevant strategies and techniques for the use of DNA for the screening and detailed elucidation of germinal mutations.

This work should be developed in parallel with the established techniques of protein analysis, until there is direct information on the costs and informativeness of the DNA studies in comparison with protein analysis. Even then, since collection and documentation will always be a major cost, and the protein techniques involve relatively minor amounts of material of the order of a thousandth of the blood volume needed for DNA studies, it would seem uneconomical to undertake large-scale DNA studies on populations without protein studies on the same samples. To establish an accurate dose–effect relationship, experimental models using animals are indispensable to both the protein and the DNA studies considered above.

TECHNICAL AND OTHER CONSIDERATIONS

Technical problems. The techniques discussed in the preceding section, particularly those on protein abnormality and gene analysis, are in a state of rapid evolution. Although one-dimensional gel electrophoresis and enzyme activity studies have been used in investigations of mutation in experimental animals and man, the use of two-dimensional gels is only now being introduced on a pilot study basis into the programme in Hiroshima and Nagasaki, and although the theory of an approach utilizing DNA is clear, the actual technical details remain to be worked out. It may seem premature to devote too much attention to an unvalidated technology, but at present these are the technologies that promise to offer the most decisive insights into the vexing question of the genetic effects of environmental mutagens, since they permit obtaining several mutational events to be defined from each individual, and thus the accumulation of the number of observations necessary for a reliable evaluation of the problem. An additional advantage of these techniques is that they can be carried out on cell lines. This raises the possibility that repositories of cell lines from children at risk, their parents, and suitable controls should be established. The cells could also be studied by techniques yet to be developed.

Once all technical errors have been excluded, the principal alternative to mutation to account for a child exhibiting a genetic trait not present in either parent, is a discrepancy between legal and biological parentage. This possibility must always be examined in detail using marker studies. The need to do such studies—and the certainty that some putative mutations will be attributed to discrepancies between legal and biological parentage—necessitates maintaining strict confidentiality.

Other considerations. The numerical requirements of a study seeking to demonstrate an increase in mutation rate may be approached in two ways. On the one hand, a lower boundary to the magnitude of the type I and type II errors that are acceptable can be set, and the necessary size of the two samples (e.g.,

children of exposed persons and children of controls) calculated. To reach significance at the 0.05 level, a doubling of the mutation rate requires numbers that would currently be considered very large indeed, no matter which indicator trait is used. These numbers are so large that they will probably never be supplied by a single study, and coordination of several studies employing identical protocols becomes necessary.

An alternative approach, especially when the null hypothesis does not seem to apply (e.g., following substantial exposure to a known mutagen), is to consider that, in the present state of concern and uncertainty over the possibility of exposure-related increases in mutation rates, any properly implemented study whose results can be combined with those of other studies is a contribution to knowledge. The combined results of such studies can always be used to set an upper limit on the magnitude of the effect compatible with the data. In this approach it would be appropriate to study the most flagrant cases of a potential mutagenic experience that can be identified. From the levels of exposure under study, one could then, on the assumption of a linear relationship between dose and effect, extrapolate to what can be excluded at lesser levels of exposure. Alternatively, in cases of exposure to an agent like radiation, which is demonstrably mutagenic in all experimental systems, and where the gonadal dose in human exposures can be estimated, the data from a substantial study of an exposed human population can be accepted as the best available estimate of the effect. Further, one can estimate the most likely value of some parameter, such as the doubling dose, even in the absence of a significant difference in the findings in the children of controls and of exposed persons, as in the Hiroshima and Nagasaki study.

COSTS AND BENEFITS

It is neither feasible nor desirable to exclude the use of many mildly mutagenic substances to which populations are exposed, or to control within defined limits certain industrial and common diagnostic procedures which are necessary for present standards of living in developed countries. Nor is it feasible to withhold the use of mutagens for treating certain patients who are mainly above the reproductive age.

An evaluation of the benefits in terms of the health and prosperity of populations against the cost in terms of the small number of serious genetic casualties in the immediate or distant future should, however, be attempted. While extensive investments in fundamental research are needed to allow estimates of these casualties within even one order of magnitude, these costs are likely to be small compared with the financial consequences of a total ban on mild mutagens that are now essential to agriculture and industry, or an imposition of unrealistically low thresholds on industrial exposure to these agents. The cost of supporting these casualties, without reference to the more serious and uncostable problems of such disorders, is likely to be more than the cost of identifying and withdrawing common mutagens with a small adverse effect or of identifying new forms of industrial or medical misadventure.

Although there is disagreement among experts about whether most casualties resulting from contemporary mutations will appear in the near or distant future, it seems unlikely that more than a small proportion of such mutations can be recognized before they appear as a seriously disabling mutant. It should not be assumed that the effects of present mutations could be safely exported to the distant future on the grounds that our descendants will have the resources as well as the abilities to recognize and repair such damage.

The number of persons exposed to specific agents in any one country may be so low that this information will have to be pooled from several countries, through collaborative studies, in order to detect even a highly significant effect. Even if the number of exposed persons with indicator conditions is relatively high, collaborative studies in several countries could ensure reliable conclusions to be drawn from the results. Finally, for valid comparisons to be made, it is important that the study protocols should be harmonized between centres and countries, and quality control should be maintained in laboratory procedures.

CONCLUSIONS AND RECOMMENDATIONS

(1) Public concern over the genetic effects of exposure to radiation and chemicals has been expressed in many countries. Studies on this problem are therefore necessary for the control of exposures to real hazards as well as for reassurance where the hazards are imaginary or trivial.

(2) Carefully executed controlled studies are needed to facilitate evaluations of the potential problem of increased mutation rates, due consideration being given to the identification of groups that may be at special risk for mutation.

(3) Proper genetic follow-up studies, in the event of positive findings with somatic tests, should be encouraged. In this connection there is an urgent need for better techniques for detecting genetic damage in

human somatic cells.

(4) Efforts to evaluate whether new industrial developments have resulted in an increase in mutation rates requiring specific action are currently compromised by the inadequacies of present methods of evaluation. Among these are clinical chromosomal and biochemical studies (including protein and DNA analysis), each requiring the development of better procedures.

(5) Because of the potentially high cost of such studies, wherever possible they should be undertaken as part of an established screening programme or of a programme evaluating the effects of occupational or environmental exposure.

(6) Since the number of children born to parents with unusual mutagenic exposures from therapy or nuclear accidents will generally be small, special emphasis needs to be placed on the development of methods to extract maximum information from each subject. The most promising methods in this respect involve the examination of protein and DNA. Repositories of cell lines from children at risk and their parents, and from suitable controls should be established to enable studies by better techniques, when these are available.

(7) The newer techniques for genetic studies of protein and DNA offer important possibilities for comparing the effects on animal models with the human situation; these studies should be carried out on a broad front using more than one strain of each of several species.

(8) The question of whether a significant proportion of the population is particularly susceptible to genetic damage, including persons who are carriers of rare alleles, needs clarification. Screening for such carriers is not recommended until properly evaluated methods are available.

(9) Efforts to prevent mutational disease should be accompanied by analyses of the costs and benefits, taking into consideration the risks society now accepts in other contexts.

(10) Coordinated international effort in the prevention of avoidable mutational diseases is required to facilitate technical developments and collaborative arrangements.

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