Epidemiological Studies of Germ Cell Mutation in Human Populations, with Particular Reference to Groups with Unusual Chemical Exposures*

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ABSTRACT

Most studies of the potential genetic effects of chemical exposures will be retrospective. It is suggested that under these circumstances, the most objective indicators are certain classes of chromosomal aberrations (sex-chromosome aneuploids, reciprocal translocations) and protein variants detected through electrophoresis or activity studies. Because spontaneous mutation is thought to be a rare event (frequency of the order of $1 \times 10^{-5}$/locus/generation), very large sample sizes are necessary to detect increases of the order of 50 or 100%. As examples of efforts at genetic monitoring, the design of the follow-up studies on the potential genetic effects of the atomic bomb is briefly presented, as is the design of two studies in Japan on the potential genetic effects of two chemical mutagens, namely tobacco smoke and sulfur mustard gas. Technical innovations which could substantially increase the efficiency of genetic monitoring are discussed. Finally, the necessity of international collaboration in genetic monitoring is urged.

1 INTRODUCTION

Our assignment for this workshop is to consider how best to monitor human populations for possible transmitted genetic damage following an exposure to a known or potential chemical mutagen. The arguments for such monitoring have been developed on numerous occasions and need little elaboration here. The United Nations Scientific Committee on the Effects of Atomic Radiation (1977)

* The financial support of the U.S. Department of Energy, the Japanese Ministry of Health and Welfare, the US National Cancer Institute, and the Japanese Ministry of Education is gratefully acknowledged.
has, with many qualifications, suggested that the frequency of disease maintained in human populations by the pressure of recurrent mutation was approximately 1950 per 100,000 live births. This figure encompasses not only simple dominantly and recessively inherited traits, but some traits with more complicated (multifactorial) modes of inheritance. In addition, it has been suggested that as many as 7% of recognized conceptions are characterized by serious chromosomal abnormalities, most of which result in early fetal loss (Jacobs, 1975), an estimate reinforced by the recent demonstration that 9% of sperm are characterized by a gross chromosomal abnormality (Martin et al., 1982). Roughly 1 in 14 of these children with chromosomal defects reach term and present with major defects. Another substantial fraction of early developmental losses are due to homozygosity for alleles responsible for serious enzyme defects, perhaps 3% of all conceptuses being characterized by such homozygosity (Neel, 1978; Mohrenweiser and Neel, 1981). The fraction of these reaching term is more difficult to estimate, but it is probably below one-third. A doubling of the mutation rate would double these various frequencies, rather quickly for the serious, dominantly inherited diseases, much more slowly for diseases with a recessive or complex mode of inheritance. The number of different genes which can mutate to produce alleles associated with disease is unknown, but it is commonly placed at 10,000–50,000, with the spontaneous mutation rate at any one gene locus of the order of $1 \times 10^{-5}$/locus/generation (Neel, 1983). Incidentally, when, as above, we refer to the consequences of a doubling of the mutation rate, we do not mean to imply we consider this a possible consequence of any but a few extreme exposures to chemical mutagens. On the other hand, questions concerning the genetic effects of chemical mutagens are being raised by responsible persons; we are here to consider how to meet them.

2 SOME GENERAL CONSIDERATIONS

At the outset, let us draw a clear distinction between monitoring processes concerned with the somatic cells of exposed persons which, if the results are positive, will create the presumption of genetic damage and generate programs designed to search for evidence of increased mutation rates in the children of such persons. Studies designed to demonstrate an increase in chromosomal damage, sister-chromatid exchanges, DNA adducts, or even in vivo somatic cell mutations in exposed persons can all yield results suggesting that the genetic material of these persons has been adversely affected; but in the present state of our knowledge, these studies in no way permit us to extrapolate to the next generation. One of the urgent needs in the field of chemical mutagenesis is for studies so organized that data are collected simultaneously on somatic cell genetic damage and transmitted damage. Only such studies will permit building
the intellectual bridges, the conversion factors, so that eventually we may indeed be able to extrapolate from somatic cell studies to the next generation.

A characteristic of most studies of the children born in human populations exposed to a chemical mutagen is that the studies will be initiated some time after the exposure, be it accidental or occupational. One of the first decisions to be made is whether the study should be retrospective or prospective. The latter is always preferable, but the temptation to acquire some retrospective data usually proves irresistible. The issue then becomes how to design any retrospective study so that bias is either minimized or recognized. A well-known source of bias stems from the use of any indicator that significantly impairs survival. At the moment, we feel that the most appropriate indicators of mutation, characterized by relatively little ambiguity of interpretation even in a retrospective study, are the occurrence of certain cytogenetic and biochemical traits present in a child but absent in its parents. In addition, it will usually be desirable to attempt to determine the frequency of ‘untoward’ pregnancy outcomes and the death rate among live-born infants—but only, if this is a retrospective study, under carefully defined conditions which the bias which creeps into so many studies of this type. All of these indicators should be increased in frequency by an increase in mutation rates.

The cytogenetic indicators of choice for surveys not performed at birth appear to be the sex-chromosome aneuploids and reciprocal, balanced translocations or pericentric inversions. The former, an indicator of non-disjunction, have relatively little impact on postnatal survival, in contrast to the autosomal aneuploids. In addition to their obvious and well-known phenotypic effects, those aneuploids are usually characterized by sterility or greatly reduced fertility, so that from the genetic standpoint they are self-limiting. The latter, on the other hand, are indicators of chromosomal breakage and are associated with chromosomal mispairing at meiosis and the formation of both ‘balanced’ and ‘unbalanced’ gametes. The balanced gametes result in normal-appearing individuals, but these individuals may produce abnormal gametes which result in abnormal children. The sex-chromosome aneuploids are so very rarely the result of a cytogenetic abnormality of the parent, i.e., transmitted, that when one is encountered, it may be presumed due to a chromosomal mutation. On the other hand, the occurrence of a translocation in a child always requires an examination of the parents, to determine whether, although phenotypically normal, one of them carries a predisposing chromosomal rearrangement.

The biochemical indicators of choice in the detection of ‘point’ mutations appear to be certain electrophoretic variants of proteins, or, for proteins functioning as enzymes, variants resulting in extreme loss of activity. The latter type of indicator is restricted to enzymes whose mean activity has a relatively low coefficient of variation; the search for half-normal enzyme levels is greatly facilitated by the so-called centrifugal fast analyzer (Neel et al., 1979). For
reasons of convenience, these proteins will usually be those present in a blood sample. Numerous examples are available of electrophoretic and activity variants associated with disease, i.e., these are relevant markers of genetic damage. Currently it is believed that the ratio of mutation resulting in electrophoretic variants (with near-normal activity): mutation resulting in uncharged amino acid substitutions (with near-normal enzyme activity): mutation resulting in loss of enzyme activity, is roughly 1:2:3 (Neel, 1983). Thus, the combination of electrophoretic and activity studies, where feasible, should detect about two-thirds of the mutations occurring at a locus encoding for a protein. Some of the variants detected by the electrophoretic approach normally occur in relatively high frequencies in populations, and such known, common variants are usually disregarded in mutation studies. On the other hand, when a rare variant is encountered, rare defined as a phenotype seen less frequently than 1 in 100 determinations, family studies must be carried out to determine whether it is inherited from a parent, the usual situation, or whether it results from mutation.

The third type of possible indicator of genetic effects mentioned was ‘untoward pregnancy outcomes’ and survival through childhood and adolescence of live-born infants. An ‘untoward pregnancy outcome’ is defined as a stillbirth and/or major congenital defect and/or death during the first postnatal week. These are to parents very graphic and personal events. Unfortunately from the monitoring standpoint, and in distinction to the previously described indicators, it is usually quite impossible to tell which specific untoward event or early death (other than those due to chromosomal abnormalities) results from newly arisen mutations in the parents. We have recently suggested, in the study to be described in the next section (Schull et al., 1981; Neel et al., 1982), that to a first approximation 1 in 400 newborn infants will either be classified as an ‘untoward pregnancy outcome’ or will, if live-born, die prematurely because of a mutation in a gamete of one or both parents. This is thought to be a conservative estimate (Hook, 1981), but we feel a conservative position is indicated at this point.

Past studies, including our own, have also included such other indicators as sex ratio and physical growth and development, but we now feel the ambiguities in the interpretation of changes in these indicators are such that they should no longer be pursued (see especially Schull et al., 1981). We have also not mentioned studies of ‘pregnancy wastage’. Such studies are notoriously difficult to carry out in an epidemiological setting. Furthermore, an adverse outcome may either reflect a direct effect on the mother or on the developing fetus, if the mother herself is under exposure (this is also true for congenital defects). Finally, to the extent that ‘pregnancy wastage’ is more or less synonymous with abortion, we suggest that tragic though it is to a woman to lose a wanted pregnancy, it is even more tragic to devote a large share of one’s life to caring for a genetically defective child, so that it is towards the latter possibility that surveys of pregnancy outcome should be directed.
3 SOME NUMERICAL CONSIDERATIONS

Spontaneous mutation, as noted earlier, is a rare event. To detect even a 100% increase requires a large series of observations. Figure 1 presents the 'power curves' for the detection of specific increases in cytogenetic abnormalities or protein variants. This figure, prepared several years ago, was based on the assumption that the cytogenetic abnormalities being studied had a baseline, mutational frequency of 5 per 1000 newborn infants, and the protein abnormalities, a mutation frequency of 1 per 100,000 newborn infants \((5 \times 10^{-6} / \text{locus/gamete})\). In developing these curves we accepted a type I \((\alpha)\) error of 0.05 and a type II \((\beta)\) error of 0.20. With these assumptions, it required two samples of approximately 14,700 each to demonstrate, for example, a 50% increase in chromosomal abnormalities due to mutation, and two samples of approximately 740,000 each to demonstrate a similar 50% increase in electrophoretic variants (Neel, 1980).

Since that figure was prepared, it has now become clear that for electrophoretic mutations, the baseline is probably closer to \(2 \times 10^{-6} / \text{locus/generation}\) than to \(5 \times 10^{-6}\) (Neel, 1983). On the other hand, survey methods are becoming available for detecting loss-of-activity variants, for which we expect mutation rates greater than for electrophoretic variants (Mohrenweiser and Neel, 1981;

![Figure 1](image)

Figure 1 The numbers of determinations necessary to detecting 50% differences in mutation rates between two samples of protein indicators or of karyotypes. The assumptions on which the curves are based are discussed in the text (see section 3)
Satoh et al., 1983). Thus, more information concerning mutation can now be extracted per blood sample, but with more effort; the figure of $5 \times 10^{-6}$/locus/gamete may yet prove realistic. Recent studies suggest that the frequency in 7 to 8 year-old children of chromosomal abnormalities of the type previously mentioned remains about 5/1000 determinations (Patil et al., 1977; Awa, personal communication). Since the numerical requirements increase exponentially as the frequency of the baseline event decreases, for electrophoretic variants any downwards revision in baseline mutation rates results in disproportionately impressive numerical requirements. With the assumptions concerning ‘untoward effects’ and ‘early death’ mentioned earlier, the numerical requirements in studies using these markers are very substantially less, but now the interpretation of the findings is much less clear cut.

These numerical requirements have tended to discourage investigators from contemplating studies of the requisite size. We would like to propose a contrary viewpoint. If we only consider a study successful if it yields a statistically significant result, not only will we frequently be led astray by falsely positive results (discussion in Land, 1980), but, since one seldom proves a negative, many studies will have to be labelled indeterminate. We will suggest an alternative approach, that for an appropriately sized study we take the results at face value and ask what they imply concerning genetic risk. This is the approach we have recently employed in evaluating the results of the study of the genetic effects of the atomic bombs, to be discussed in the next section.

What now is an appropriate size? Any number will be arbitrary, but we would suggest for children judged to be at relatively high risk of a mutational event because of a chemical exposure of their parents, a minimum of 400000 observations in both the exposed and control groups in a study based on electrophoresis, and 10 000 in both groups in a cytogenetic study. The latter number corresponds to children examined, but for the former number, the number of children examined depends on the number of determinations per child. Thus, if one examined 30 different proteins per child, and both parents had sustained a potentially mutagenic exposure, this is 60 observations per child. With 60 observations per child, the minimum number of 400000 just mentioned corresponds to some 67 000 children. These are by current standards still very large numbers. The acquisition of such numbers will almost surely require combining the results of a number of studies, and also the development of improved technologies, both of which topics will be discussed later.

4 THE APPLICATION OF THESE APPROACHES TO THE STUDY OF RADIATION EFFECTS

The most clearly defined need to date for the application of these various technologies to the evaluation of a mutagenic exposure arose in the aftermath of the atomic bombings of Hiroshima and Nagasaki. The ensuing genetic studies
have been described in considerable detail (Neel and Schull, 1956; Schull et al., 1981, 1983; Neel et al., 1982). A brief summary will suffice for present purposes, with emphasis on certain methodological features of the study. The study which was initiated in 1946, under the auspices of what is now termed the Radiation Effects Research Foundation (RERF), was greatly facilitated by the fact that, for ration purposes, in post-war Japan over 90% of pregnant women were registered at the completion of the fifth lunar month of pregnancy. It was thus possible to develop a truly prospective study of a defined cohort, concerning such aspects of pregnancy termination as viability at birth, birthweight, sex ratio, presence of congenital defect, physical growth and development at age 9 months, and survival, the latter still being followed even though accessions to this cohort ceased in 1954. The amount of radiation received by an individual parent of a child in the series varied from the maximum consistent with survival to zero, the latter in the instance of a parent who was not in the city at the time of the bombing. In 1968 cytogenetic studies of the children in the cohort were initiated, along the lines mentioned earlier in this presentation, and in 1976 biochemical studies of 30 enzymes, also along the lines mentioned earlier.

We have recently reanalyzed much of the evidence accumulated to date, with a type of analysis designed to estimate the magnitude of the genetic doubling dose for humans, i.e., the amount of radiation which results in a frequency of mutation equivalent to that occurring spontaneously. For this purpose, the indicators most appropriate to generating such an estimate have been grouped under four categories, namely:

1. **Untoward pregnancy outcomes**, consisting of stillbirth and/or congenital defect and/or death during the first postnatal week,
2. **Survival among live-born infants** through an average age of 17 years,
3. **Sex-chromosome aneuploidy** (assumed always to be of mutational origin), and
4. **Protein variant resulting from mutation**.

All of the indicators vary according to expectation on the presumption of a genetic effect of the exposure, but none of the findings is of statistical significance. On the thesis that we may assume that the atomic bombs had a genetic effect, i.e., that since radiation has had a genetic impact wherever and however properly tested we were not in a hypothesis testing mode, we have explored the implications of our findings for the human genetic doubling dose. As indicated earlier, this involves some difficult assumptions concerning the contribution which newly arisen mutation makes each generation to indicators 1 and 2; attempts to improve that estimate have high priority.

The doubling-dose estimate which we have generated is between 139 and 258 rem, the range depending on how we weight our various indicators. The variance of that estimate is large but difficult to calculate. Until now, most of our thinking concerning the genetic risks of radiation has been guided by exper-
ments with mice for which the doubling dose of acute radiation of sper-matogonia, the germ cell of most pertinence, is commonly placed at about 35 rem. Thus, our very preliminary estimates suggest that a heterogeneous human population may be some 4–7 times more resistant to the genetic effects of radiation than the current guidelines. Should this estimate be correct—and we wish to emphasize how preliminary is the conclusion—it has important implications for how we view the genetic risks which increased amounts of radiation pose for our species.

Elsewhere we have recently discussed the several sources of error inherent not only in the human estimates, but also in the mouse estimate (Neel, 1983). These errors are of sufficient potential magnitude that there might in fact be no difference between the two species, but whether in that case the higher or lower estimate is more appropriate remains unclear. On the other hand, and this is the view towards which we incline at present, the species difference may be correct in principle although of uncertain magnitude. The presumed reason for the difference would be the evolution of superior genetic repair systems in humans, a prerequisite to our longer life-span and relatively low reproductive potential. Fortunately, the study in Japan is continuing, and within the next several years there should be important acquisitions to the present data bank.

If the findings in Japan are sustained, they have important implications for monitoring programs all over the world. Let us assume we are also more resistant to the chemical mutagens than the mammals so far employed in such studies, so that here too the dose required to produce a 50 or 100% increase in mutation rates is relatively high. The numbers necessary to demonstrate a 50% increase remain the same as in the previous example, but now a 50% increase for any given exposure has a lower probability. In other words, the numerical requirements of a statistically significant difference between two cohorts, if desired, become very large indeed.

Incidentally, even 37 years after the exposure, there is conspicuous cytogenetic damage in the survivors of the atomic bombings, the amount of damage of course being proportional to the degree of exposure (Awa et al., 1978). In time, this study should thus provide one of those opportunities to build an intellectual bridge between genetic damage in the somatic cells of exposed persons, on the one hand, and transmitted damage in their children, on the other hand.

5 TWO CURRENT PROTOCOLS FOR THE STUDY OF THE EFFECTS OF A POTENTIAL CHEMICAL MUTAGEN

Difficult though the development of an appropriate protocol for the study of the potential genetic effects of radiation has been, developing similar protocols for the study of chemical mutagenesis could be much more difficult. With radiation exposures, the calculation of gonadal doses is relatively easy. Not so with chemical exposures, for which there are many barriers between the portal of
entry and contact of the chemical mutagen with a germ cell. The decision regarding the desirability and/or necessity of a study on the genetic effects of a chemical mutagen will usually be guided by the kinds of presumptive evidence for genetic damage mentioned in our introductory remarks, and as we emphasized then, our bases for extrapolation are not strong. Despite these reservations, we have recently embarked on two studies of the possible effects of a known chemical mutagen on humans, both of these studies under unusual circumstances not apt soon to be repeated.

5.1 Protocol 1: Cigarette Smoke as a Mutagen

In 1977 Yamasaki and Ames reported that an extract of the urine of persons who smoked between 15 and 44 cigarettes a day increased the rate of mutation of bacteria in the Ames test on average about 3-fold. Urine from non-smokers revealed no such effect. Cigarette smoke condensate has been shown to produce many dose-related lesions in the cellular DNA of cultured human lymphocytes, as measured by sister-chromatid exchange (SCE) induction (Hopkin and Evans, 1972). Heavy smokers show an increase in chromosomal aberrations in blood lymphocytes relative to non-smokers (Obe and Herha, 1978). Finally, there is evidence that cigarette smokers show an increase in SCEs (Lambert et al., 1978; Hopkin and Evans, 1980) although there is not complete agreement on this point (Hollander et al., 1978). On a world-wide basis, the introduced chemical mutagen to which exposure is most ubiquitous is probably cigarette smoke.

Several years ago, Dr Richard B. Everson of the National Institute of Environmental Health Sciences (personal communication) directed our attention to the potentialities for the study of the mutagenic effects of smoking inherent in the follow-up study in Japan just described. If it were possible to obtain accurate smoking histories from all the parents whose children entered into the study, these data could readily be collated with those on mutation becoming available through the study of radiation effects. In effect, the difficult and expensive biochemical portion of the study will be accomplished for other reasons, and the data on the relation between smoking and mutation could constitute an inexpensive add-on. In this connection, it should be mentioned that an earlier study of the epidemiology of cancer of the lung at RERF had already indicated a relatively high rate of response to a postal questionnaire that included a smoking history, so that we have reason to believe we can obtain the necessary smoking histories by post. An effort to obtain such data has just been initiated. Since this questionnaire will not be linked to any particular health outcome, we do not see any opportunity for bias in the retrospective approach. This particular set of circumstances is somewhat unusual. Nevertheless, if in the future studies are undertaken on the effects of chemical mutagens, it would seem to be appropriate from the very beginning to collect data on smoking habits. In fact,
such is the expense of epidemiological studies that it would be well to make them as multipurpose as possible.

5.2 Protocol 2: Sulfur Mustard Gas as a Mutagen

During World War II, the Japanese Government maintained on Ohkuno-jima, an island 'ward' of Takehara City, in Hiroshima Prefecture, an installation for the manufacture of a variety of 'war gases', including sulfur mustard. The wartime employees of this installation who worked with mustard gas have in subsequent years shown a highly significant increase in the frequency of cancer of the stomach and lung, and, more recently, liver (Nishimoto, 1961; Wada et al., 1962, 1968; Kambe, 1967; Shimura et al., 1978; Nishimoto, unpublished observation). The latter suggests that the mustard exposure was not limited to sites of primary contact. Sulfur mustard is of course one of the classical chemical mutagens (Auerbach and Robson, 1946; Auerbach, 1949, 1978). It is, then, a reasonable presumption that the children born to these workers are at increased risk of mutation.

These former workers are encompassed within a program of long-term medical surveillance and care directed by one of us (Y.N.). They can be divided into three categories, according to the probable degree of exposure, as follows:

(1) individuals engaged in the manufacture of the gas,
(2) individuals responsible for the maintenance of the equipment, and
(3) other employees—clerical, transportation, etc.

The first two categories are considered to have received considerably higher exposures than the last.

In 1977 a census was undertaken of the number of living children of these workers, subdivided as to work category of the parents. The results are shown in Table 1. The data are restricted to children both of whose parents were alive as of that date. As for the protocol governing the study of the potential genetic effects of the atomic bombs, this constraint is necessary for any investigation of this type. Unfortunately, because of the increased death rates in the parents with the higher exposures, as well as natural attrition among parents of this age, this results in the loss from the study of a substantial number of children.

As many of these children as possible will be studied with respect to the same 30 proteins being analyzed for electrophoretic variants in the investigation in progress at RERF. The same set of 11 enzymes studied for quantitative variation in the RERF protocol will also be examined.

The controls for this study will be supplied by the children of non-radiated parents who serve as the controls for the study of atomic bomb effects. Since Ohkuno-jima is only 50 miles from Hiroshima, there is the requisite ethnic similarity. The ability to draw on a suitable control sample is of course an
Epidemiological Studies of Germ Cell Mutation in Human Populations 337

economy in this particular study which will not be true of most such undertakings.
Not all these children will be available for study, many having left the area (although we hope to contact some of these when they return on holiday occasions). Thus it is clear that this sample cannot, on any reasonable projections of presumptive effect, yield a decisive insight into the effects of children whose parents received a mutagenic exposure, a fact to which we will return later, in the discussion of opportunities for international collaboration.

6 TOWARD BETTER TECHNOLOGIES

Clearly there is an urgent need for improved technologies for the detection of mutations, both chromosomal and point. Although it is now possible in theory to extend studies of mutation to the DNA itself, effective technologies for so doing look to be some years away. Furthermore, most of the DNA scanned for mutation by the most likely technique, the use of restriction site enzymes, would be non-translated (and even not identified as to function) and so actually not relevant to the question of the phenotypic impact of mutation. On the other hand, it may be possible to expand by an order of magnitude, with relatively little increase in cost, our search for mutational changes in proteins. Our group is currently in midstream of an effort to achieve this objective through the use of two-dimensional polyacrylamide gel electrophoresis. With this technique, the proteins of a complex mixture, such as serum plasma or an erythrocyte or lymphocyte lysate, are first separated on the basis of molecular charge by isoelectric focusing and then, in a second dimension, further separated on the basis of molecular weight by electrophoresis (cf. Klöse, 1975; O'Farrell, 1975). When such preparations are subjected to the recently developed, very sensitive silver stains (Merril et al., 1981; Sammons et al., 1981; Wray et al., 1981), as many as 1000 protein moieties can be visualized in a single preparation. In each preparation, a subset of these moieties—perhaps 50–100—will be sufficiently clearly demonstrated that, just as in the previously described one-dimensional approach, one can detect the presence of rare variants, after which if one is detected, the appropriate family studies as to the possibility of mutation can be carried out. By full utilization of the various components of a blood sample and their fractions—plasma, erythrocyte and leukocyte cytosols and membranes, platelets—it is possible that each child studied could yield data concerning the status of several hundred proteins (discussion in Neel et al., 1983). An attempt is being made to develop computer programs capable of relieving much of the tedium of scanning the gels for variants (Skolnick, 1982; Skolnick et al., 1982).

7 OPPORTUNITIES FOR INTERNATIONAL COLLABORATION

It is difficult to conceive of a scientific question which offers better opportunities for international collaboration than the one we are discussing here. Every
industrialized country, and many in the process of industrialization, can identify one or more groups of individuals who have inadvertently been exposed to a potential mutagen. Such groups have in some instances already been surveyed for presumptive evidences of genetic damage, i.e., chromosomal findings in the exposed persons (discussions in Berg, 1979). If the results of such surveys are positive, then in our opinion it is mandatory that their children be appropriately studied. Not to study such children after the demonstration and disclosure of somatic cell changes can only create long-lasting anxieties which, as we will mention in the next section, are in the end more expensive than proper studies. On the other hand, with limited resources, it seems important at the outset to concentrate on groups felt to be at highest risk, i.e., a 'worst cases' approach.

A prerequisite for an international study is a 'standard' protocol. By 'standard' we do not mean a protocol to which every study should adhere in every detail. Rather, we imply a listing of possible observations and a description of the conditions under which they are to be made. A group involved in a collaborative study could choose to implement only one or several aspects of the protocol, but whatever is done should be with uniform standards permitting pooling of the results of all similar studies. Several international groups have made a start on such a protocol (Appendix, 1979; WHO, 1982).

8 COSTS AND COST-EFFECTIVENESS

What is the cost of a monitoring program of the requisite size? We believe it is appropriate to turn the question around, and ask, 'what is the cost of not mounting an appropriate monitoring program?' In many countries, guidelines are in place for compensation of the occupationally exposed persons, both for radiation and for chemicals. The US Congress is currently considering an extension of this principle to permit, under carefully defined conditions, compensation to individuals developing certain diseases following inadvertent exposure to fallout from the testing of atomic weapons, using the principle of 'causative odds', with compensation at some arbitrarily fixed value of the odds. The principal basis for developing the risks on which this compensation is based, incidentally, is the follow-up study on Japanese exposed to the atomic bombs. We suspect that, once procedures for redress for somatic cell damage from radiation exposures have been established, the question of compensation for presumed genetic damages will receive increasing attention in the next decade, and that both the somatic and genetic issues will be broadened to include chemical exposures. Large sums of money are involved. We simply do not have the data necessary for developing the probabilities on which just genetic settlements should be based. Without this data base, there will be continuing confusion and litigation.

It is important at this juncture to emphasize that there is no such thing as a
'negative study' of this type. Every properly defined study contributes to our knowledge. Furthermore, one can always use a study in which there is no significant difference between the control and the test group, to place an upper limit on the effect which might exist but go undetected. This is especially important in the context of a 'worst cases' approach, such as would be pursued if the industrialized countries concentrated their study efforts on the children of their most exposed groups.

We estimate that the laboratory costs per determination are $0.75 for either the electrophoretic or the enzyme activity tests currently in use (Neel et al., 1983). Thus, the cost for two minimal panels of 4000 000 determinations each could be $6000 000. How much that would be reduced by the development of the two-dimensional gel technology mentioned earlier is difficult to estimate at present. There would, in addition, be what we term the 'front-end' costs, of collecting the specimens and ensuring their arrival in the laboratory in good condition. Although these are considerable costs, they are far below those committed to screening chemicals for carcinogenicity in various countries. The costs are considerably less if advantage can be taken of samples routinely being submitted to facilities screening for phenylketonuria or other diseases (Vogel and Altland, 1982).

With respect to cytogenetic studies, the quest for better automation of karyotyping continues (Oosterlinck et al., 1977; Vanderheydt et al., 1980). In a survey (research) setting, a karyotype (5 cells examined) without the benefit of automation costs approximately $100; perhaps the cost could be halved with a semiautomated system.

9 DISCUSSION

We have in this presentation considered both the problems and the promise of monitoring for the transmitted potential genetic effects of chemical mutagens. Because the endpoints for such monitoring are either not well collected or not collected at all in the vital statistics procedures of any country, appropriate studies will require large and expensive undertakings. Studies of exposed individuals for the frequency of chromosome observations or sister-chromatid exchanges in somatic cells are much less demanding and, if they yield positive results, can create the presumption of transmitted genetic damage, but this presumption is not subject to quantification. Thus, studies on somatic cell effects (in contrast to germ line effects) if positive raise many unanswerable questions. Particularly desirable are studies in which both somatic cell genetic endpoints in exposed individuals and transmitted genetic effects in their children are recorded; once the conversion factors between the two types of studies have been established, studies of somatic cell end-points, rather than creating ill-defined fears, will become much more valuable.
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Epidemiological Studies of Germ Cell Mutation in Human Populations


