Part III
An Assessment of the State of the Art

Chapter 6

Genetic Monitoring in the workplace

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Although humans have been exposed to chemicals and radiation for thousands of generations, the numbers and amounts of these potentially hazardous agents in the environment have increased dramatically since the industrial revolution. Moreover, certain occupational groups are exposed to these substances over many years at much higher concentrations than is the general population.

While the contribution of toxic chemicals and ionizing radiation to the human genetic burden has not been directly shown, some of these agents do cause mutations and chromosomal damage in laboratory animals. Geneticists are concerned that exposures to new mutagenic agents could increase the number of gene mutations in the human population and hence the incidence of disease. Because of the increasing chances for exposure to harmful agents, it is desirable to develop tests that identify the mutagenic and clastogenic (chromosome-damaging) potential of chemicals and ionizing radiation. When hazards are identified, prevention programs can he considered that will reduce exposures to the hazards. In addition, genetic tests may be useful to monitor human populations for exposure.

Ideally, an occupational monitoring technique would provide early, reliable, and quantitative information regarding "biologically significant" exposure * to hazardous agents. Once a biologically significant level of exposure has been established, intervention measures to eliminate or significantly reduce worker exposure and prevent untoward biological effects could be implemented.

Genetic monitoring in the workplace involves the periodic testing of employees to assess damage to their deoxyribonucleic acid (DNA) or chromosomes from exposure to hazardous agents. Currently, genetic monitoring techniques are most useful for identifying or monitoring exposure to a chemical that causes genetic damage. The objective of these techniques ultimately is to predict risk of disease due to genetic damage.

There are two types of techniques covered in this assessment. The first type, cytogenetic techniques, looks for gross changes in chromosomal structure. These techniques, including tests for chromosomal aberrations and sister chromatid exchange (SCE), represent the currently used methods for monitoring for genetic damage. Other monitoring techniques that also look for damage to the genetic material have been proposed for human populations. These newer noncytogenetic techniques may offer advantages in sensitivity, cost, and performance time over the cytogenetic methods, often because of automation, but most of them have had little actual application in human monitoring programs at the present time. This chapter reviews the occupational studies done to date, first examining the use of cytogenetic monitoring and then noncytogenetic monitoring.

*The definition of biologically significant" exposure is a difficult 011(1, and there is by no means a concurrence of opinion among health care professionals It normally refers to an exposure level that can cause detectable damage or disease

Cytogenetic monitoring

The empirical association between chromosomal damage and mutagenic/carcinogenic agents established from animal studies implies that chemically induced chromosomal aberrations and SCEs may be used as possible biological dosimeters (measures of exposure) for human exposure to these agents. There are three natural extensions of this dosimeter hypothesis. One is

the idea that studies using cytogenetic endpoints* may be used to identify human carcinogens or mutagens.** The second is that these studies might identify populations at risk for cancer and other diseases as a result of exposure to these clastogens. Last, the studies may identify individuals in those populations who, because of a defect in DNA repair mechanisms, may be particularly predisposed to chromosomal damage. In the workplace, the goals of such studies would be to determine acceptably safe levels for occupational exposure to chemicals and radiation (that is, those levels associated with minimal, or acceptable, risk of disease) for the average population and to insulate susceptible individuals from the chemical.

This section examines the appropriateness of chromosomal endpoints to detect occupational exposures to chemicals and radiation. This critical review of the literature seeks to determine whether chromosomal endpoints can be used as a measure of occupational exposures and whether the results of cytogenetic monitoring are a predictor of risk for disease for either groups or individuals. The discussion is limited to studies that are pertinent to adult somatic effects resulting from occupational exposures.

Associations between chromosomal aberrations and disease

Many diseases are thought to involve somatic cell genetic defects. Interference with immune function, manifested as autoimmune disease, allergy, or increased susceptibility to infectious agents, may involve somatic cell genetic changes. Hence, induced chromosomal aberrations may prove to be related to these clinical states. However, there are no studies, either in animals or in man, that address the possible association between induced chromosomal aberrations or SCEs and immune function,

Photo *credit: National Institutes of Health*

Cytogenetics involves the examination of chromosomes under a microscope

other diseases of the blood/lymph system, such as aplastic anemia and multiple myeloma, also may be the result of somatic cell genetic changes. Because cytogenetic monitoring usually utilizes blood lymphocytes as the chromosomal source, cytogenetics may prove useful in predicting whether individuals exposed to high levels of clastogens may be candidates for these blood cell diseases, but this association is only speculative.

Cancer is the disease most commonly hypothesized to be associated with induced chromosomal aberrations, undoubtedly because of the large animal literature linking carcinogens with chromosomal aberrations. Additionally, many types of human cancer cells contain specific chromosomal aberrations (31,34,63,76,93,99,141). Chromosomal aberrations have been found for both lymphoproliferative disorders, such as leukemia,

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'The term endpoint refers to the biological response to exposure being monitored. In this section, two endpoints are discussed, chromosomal aberrations and SCEs.

•*Studies would not be done primarily to investigate this issue, but results identifying human carcinogens or mutagens could derive from cytogenetic studies investigating risk for disease. The identification of mutagens and carcinogens is done with animals and extrapolated to humans; cytogenetic studies would either verify the animal data or refute the extrapolation.

and solid tumors. The strength of the association between the specific aberration and the presence of cancer varies from one cancer to another. At one extreme, the Philadelphia chromosome (a translocation from the long arm of chromosome #22 most often to the long arm terminus of chromosome #9) correlates highly with chronic myelogenous leukemia, with 85 to 95 percent of patients having this marker chromosome in their affected cells. On the other hand, in the case of a pair of identical twins, each carrying the specific deletion associated with Wilms tumor, only one of the twins developed the disease (76). Thus, even when specific chromosomal markers are involved, other nongenetic factors can play a role in the development of cancer.

Correlation between chromosomal aberrations, SCEs, and carcinogenicity

The extensive literature on animal studies that use chromosomal endpoints has recently been appraised and reviewed by the U.S. Environmental Protection Agency's (EPA) Gene-Tox Program committees (80, 113). The Gene-Tox committee for mammalian in vivo and in vitro cytogenetic assays (chromosomal aberrations) reviewed 177 papers published through October 1978 using several somatic cell systems (113). For one or another of these systems, 150 chemicals were reviewed.

According to the Gene-Tox findings for chromosomal aberrations and SCEs, the data base for chemicals that have been adequately studied in animal or in in vitro systems is quite limited, and the number of the chemicals for which carcinogenicity is known is even more limited. In addition, the carcinogenicity data were generated in separate studies from the chromosomal data and are not directly comparable. But within these restrictions, it would appear that the induction of chromosomal aberrations and SCEs by chemical agents are reasonable indicators of carcinogenicity, with the former possibly being the better predictor. However, for both endpoints, examples exist of chemicals that cause chromosomal damage but not cancer, and vice versa. In order to relate genotoxicity more closely to humans, the Gene-Tox review recommended human cells as being suitable for in vitro studies. The review is still is progress, and EPA has yet to issue any recommendations on the predictive value of any of the techniques.

A recent review by Gebhart (57) summarizes the world's literature concerning the agreement between chromosomal aberrations and SCEs. Gebhart found a 30 percent disagreement between these two endpoints where the same chemical had been evaluated for aberrations and SCEs both in vitro and in vivo. This indicates that the fundamental way in which a particular chemical interacts with the DNA to produce SCEs may be different from the mechanism that produces chromosomal aberrations.

Chromosomal studies on groups

This section provides a comprehensive review of occupational studies using chromosomal endpoints. Many of the studies fall short of ideal. For instance, confounding factors, such as cigarette smoking, were not always determined, and some studies examined populations too small to produce reliable results. In general, more recent studies are better designed and executed. However, rather than discard the evidence of the older studies, the strengths and weaknesses of all the studies are reviewed, and overall conclusions are drawn.

The review covers studies addressing the normal range of values for chromosomal endpoints, studies on the chromosomal endpoints and health status of atomic bomb survivors exposed to ionizing radiation, and occupational studies on the chromosomal endpoints of workers exposed to ionizing radiation and specific chemicals.

For the occupational studies, the following questions were asked:

- Was there substantial evidence of an increase in the endpoint that could be associated with exposure to a specific agent? was there a dose response? Were chronic as well as acute exposures monitored?
- Was there evidence to link any of these endpoints with increased risk for disease?
- Are these tests sufficiently sensitive to permit detection of effects at current occupational exposures?

- For each of the agents discussed in detail, is there evidence that cytogenetic endpoints can detect susceptible individuals?

STUDIES ON UNEXPOSED POPULATIONS

Several large studies have examined the range of chromosomal aberration and SCE frequencies and the variables affecting them in unexposed populations (13,15,28,29,35,59,71,79,86,88,90, 95,100). Seasonal variations, age, sex, smoking, and alcohol effects have been reported, although not consistently for every study. From these studies, it is apparent that background frequencies for chromosomal aberrations and SCEs may fluctuate greatly. The background frequencies reported in several studies are shown in appendix D for chromosomal aberrations and in appendix E for SCEs. Comparison of findings from studies with varied laboratory methodology is difficult but useful to the extent that it conveys a sense of possible variability for these endpoints.

The range of reported frequencies per cell for individual aberrations found was:

- chromatid breaks: 0.11 to 6.72 percent,
- chromosome breaks: 0.1 to 3.0 percent,
- exchange aberrations: O to 0.34 percent,
- cells containing any aberration: 0.2 to 8.5 percent, and
- SCEs: 5.8 to 16.2 per cell.

These wide variations suggest that the range of "normal" values for chromosomal endpoints may be dependent on the particular laboratory methodology. Certainly the fortyfold differences seen for background values of chromosomal aberrations are much greater than those reported in individual studies between occupationally exposed and unexposed groups.

STUDIES ON ATOMIC BOMB SURVIVORS

Since the end of World War II, the Japanese survivors of the atomic bombs and their offspring have been extensively monitored by the Atomic Bomb Casualty Commission, recently redesignated the Radiation Effects Research Foundation, for residual and latent biological effects, These studies have been a joint effort between eminent Japanese and American scientists and are ongoing (102). Refinements are still being made in dosimetry estimates and epidemiology.

This Japanese population has been observed to have an increased risk for many types of cancer (14). Leukemias were the first evident cancers, with incidence peaking 7 to 8 years after exposure, The incidence of all leukemias for this population has subsided with time, but it is not clear if the risk for leukemias has declined to background values.

With the decline of leukemias, the onset of other types of cancer has become apparent. To date there is a clearly increased risk for cancers of the thyroid, female breast, and lung. Excess stomach and salivary gland cancers are suspected but not yet confirmed. In contrast to the leukemias, the time of onset for breast cancer in exposed populations has not been earlier than would be predicted on the basis of studies in unexposed populations. Rather, breast cancer has appeared at a higher, dose-related frequency at the age when it usually occurs. All of these cancers have shown a dose dependence for radiation, but the shape of the dose-response curves differs with different cancers. These differences in patterns of cancer incidence are consistent with the widely held opinion that the mechanisms of radiation-induced cancers are complex and perhaps different from one cancer to another.

In conjunction with these morbidity and mortality studies, extensive cytogenetic investigations on these survivors have revealed a dose-dependent increase in chromosomal aberrations (14). These cytogenetic studies have been possible because some of the chromosomal abnormalities induced by ionizing radiation are very long lived. The frequencies of these aberrations seen in the atomic bomb survivors are very high compared to the aberration frequencies found in occupational exposures, even in individuals receiving small doses (less than 1 rad *). The reason for this is unknown.

The ongoing epidemiological studies on these same individuals provide an unparalleled opportunity to examine directly the relationship be-

*A rad is a measure of absorbed dose of ionizing radiation,

tween chromosomal aberrations and cancer. In an extensive study reported by King, et al. (75), where chromosomal aberrations, malignancy, and other clinical findings were tracked for individuals in the Hiroshima population, the tentative conclusion was that such correlations do not exist.

with populations or groups, however, the studies on the Japanese clearly demonstrate a relationship between estimated radiation dose and certain cancers and between radiation dose and chromosomal aberrations. But for individuals, elevated frequencies of chromosomal aberrations are not reliable predictors for risk of cancer or other somatic cell diseases. Thus, even these ex - tensive studies do not provide evidence that radiation-induced chromosomal aberrations mean that individuals with these aberrations will necessarily develop cancer.

Several points should be borne in mind in relating these studies to the ionizing radiation studies discussed below. First, the Japanese studies reported quite high frequencies for rare aberrations, even in groups receiving less than 1 rad. Second, because of the manner in which the studies were designed, little or no cytogenetic information is available on the group receiving exposures between 1 and 100 rads. This group is probably the most comparable, in terms of equivalent genotoxic dose, to groups receiving occupational exposures to radiation. Thus, because of the different types of chromosomal aberrations involved and, because of probably larger radiation doses in the exposed Japanese populations, it is difficult to relate the significance of these findings to occupational studies. Finally, the Japanese studies deal with the effects of a single, large, acute dose of radiation. In the majority of occupational situations, the effects of chronic lower doses of radiation may not be so easily detected.

OCCUPATIONAL STUDIES ON ION1ZING RADIATION

If chromosomal aberrations induced by radiation are indicative of some health risks, albeit for a group, then occupational cytogenetic studies on workers exposed to chronic lower doses of radiation may be of some value in setting acceptable standards for occupational exposure to radiation. Several occupational studies have been done on uranium miners, workers in a plutonium processing facility, and nuclear powerplant workers (17,20,21,22,23,46,89,98). Every study reviewed has shown that increases in chromosomal aberrations are associated with occupational exposures to ionizing radiation. If these aberrations are as stable as those in the Japanese, they would not necessarily indicate recent exposures under current occupational exposure standards, but rather accumulated exposure over several years.

From these studies, it is not clear if the chromosomal aberration endpoint is sensitive enough to detect chronic exposures within the current occupational exposure standard of 5 reins* per year. occupational groups would have to be studied at low-level chronic exposures to determine if individuals exposed to the current occupational standard have increased frequencies of aberrations.

OCCUPATIONAL STUDIES ON ARSENIC

Arsenic is a ubiquitous element that can occur in several chemical forms, some of which have commercial uses as fungicides, insecticides, and herbicides. It also has been used for medicinal purposes, for instance, in the treatment of ailments ranging from asthma to psoriasis and syphilis. When arsenic is eaten or inhaled, about 50 percent of the dose is absorbed. Of the absorbed dose, roughly half is excreted within 2 days. The remainder is eliminated more slowly, and a fraction can accumulate in the body, where it is distributed in many different tissues (85). Arsenic is classified as a human carcinogen (72). Several types of cancers have been associated with occupational exposure to arsenic in smelters, in the chemical industry, and in gold mining (103).

Both chromosomal aberrations and SCEs have been reported to be elevated in individuals exposed to arsenic, with SCEs possibly being the more sensitive indicator (26,97,1 10). Chromosomal effects of arsenic exposure are long lived and possibly reflect cumulative exposure. It is not clear if chromosomal endpoints can detect low-level chronic exposure to arsenic, because the exposures in the studies reviewed here were relatively high. The one study on arsenic in an oc-

*~ rem is a rad multiplied by a number that takes into account the potential damage-causing ability of a particular type of ionizing radiation in a biological system.

cupational setting (97) is not sufficient to permit a decision on the suitability of cytogenetic endpoints for measuring exposure.

OCCUPATIONAL STUDIES ON BENZENE

Benzene is a constituent of fossil fuels and also is a major industrial and laboratory chemical. As a result, a substantial number of people are chronically exposed to benzene at work, and many more receive transient exposures outside the workplace, for instance, while pumping gasoline. As with arsenic, benzene is classified as a human carcinogen (72), with an excess of leukemias, particularly erythroleukemia, associated with high exposures (73,134), Benzene is also a potent blood cell poison, with manifestations including pancytopenia (reduction of all blood elements) and aplastic anemia, The current occupational exposure standard for benzene in the United States is 10 parts per million (ppm), a time-weighted average for 8 hours.

Benzene is one of the most widely studied chemicals in occupational cytogenetics. Several studies consistently have shown that exposure to high doses of benzene (greater than 40 ppm) is associated with chromosomal aberrations, even though frequencies are low in comparison with radiation-induced effects (52,53,54)111)112,131) 132,136). The aberrations appear to be stable for years and most likely reflect cumulative rather than recent exposure. Whether exposure to benzene within the current occupational standard of 10 ppm induces chromosomal aberrations has yet to be determined.

OCCUPATIONAL STUDIES ON EPICHLOROHYDRIN

Epichlorohydrin is a highly reactive major industrial chemical that has been extensively studied for genotoxic activity (126). It is mutagenic in microorganisms, causes chromosomal aberrations in mouse bone marrow, and induces chromosomal aberrations in human lymphocytes in vitro (126). Epichlorohydrin causes nasal tumors in rats by the inhalation route at levels higher than 10 ppm (N. Nelson, unpublished study). One study has indicated a slight, but not statistically significant, excess in respiratory cancers among workers exposed to this chemical (45), and IARC ***(72)*** found that it "could not be classified as to . . . carcinogenicity for humans."

The only adequate cytogenetic study on epichlorohydrin (127) showed that occupational exposure to the chemical may be associated with low frequencies of chromosomal aberrations. No studies have been done linking chromosomal aberrations with risk for disease in a population exposed to epichlorohydrin.

OCCUPATIONAL STUDIES OF ETHYLENE OXIDE

Ethylene oxide is an extremely reactive chemical whose commercial uses are primarily as a sterilizer of medical products and as a chemical intermediate. Ethylene oxide has been shown to cause leukemias in rats by the inhalation route at doses higher than 33 ppm (58). It can induce chromosomal aberrations in vitro and mutations in tester micro-organisms (46). Because of ethylene oxide's volatility, there is a high potential for occupational exposure in situations where it is not properly contained. The current Occupational Safety and Health Administration's (OSHA) occupational exposure standard is so ppm, time-weighted average. Two reports (.57,68) suggest an increase in leukemia among workers exposed to this chemical.

Three published studies associate present levels of ethylene oxide found in the workplace with chromosomal aberrations and possibly SCEs (56, 109, 130). However, frequencies for these endpoints were low. Two additional studies on ethylene oxide (S. Galloway and A. Carrano, personal communication) are now being conducted. Preliminary reports on a study being conducted at three Johnson & Johnson plants indicate a consistent dose-response for both chromosomal aberration and SCE endpoints. Statistically significant increases in cytogenetic aberrations were seen even in a plant where exposures range from 1 to 10 ppm. No effects were found at less than 1 ppm (32, 101).

OCCUPATIONAL STUDIES ON LEAD, CADMIUM, AND ZINC

Lead, cadmium, and zinc tend to occur together in mineral deposits in nature and, therefore, in occupational exposures. High exposures to lead or cadmium can produce both acute symptoms

of poisoning and chronic effects. Lead poisoning involves nerve degeneration, interference with some metabolic processes, and, in its severest form, mental retardation. Lead acetate is carcinogenic in rats, but has been negative for chromosomal aberrations and SCEs in vitro (80,113). Cadmium has been shown to cause birth defects and cancer in rodents, and there is some evidence for human carcinogenicity (72). Occupational exposure to cadmium has been associated at least tentatively with lung and prostate cancers (36). Both cadmium and lead tend to accumulate in the body with chronic exposure, a point which may be important in interpreting the occupational studies on these agents because damage may reflect a cumulative exposure.

Zinc, unlike lead and cadmium, is necessary for the function of some of the enzymes involved in DNA replication and repair, and it is possible that cadmium and lead, when present in high amounts, can replace zinc in these enzymes. If this happened, the enzymatic activity would be greatly reduced.

Photo credit Occupation/ Safety and Healfh Administration

Cytogenetic monitoring has been explored as a possible technique for monitoring worker exposure to lead

Many conflicting findings exist in the literature addressing occupational exposure to lead, cadmium, and zinc (16)25,37,38,39)50,51)69)89, 104, 105,120, 132). Increased frequencies of chromosomal aberrations and correlations with blood lead values have been reported often enough to provide some credibility to the correlations, but many good studies have failed to find such relationships. Perhaps one reason for such discrepancies is the diversity of occupational exposures studied. Different work situations could have entailed quite different kinds of exposures with respect to the specific compounds in the work environment and possibly to different primary routes of exposure. The relationship between the three elements and chromosomal aberrations clearly is complex and awaits further elucidation.

OCCUPATIONAL STUDIES ON VINYL CHLORIDE MONOMER

Vinyl chloride monomer is a major industrial chemical used to make polyvinyl chloride plastics. It is mutagenic for tester micro-organisms and causes chromosomal aberrations in rats treated with 1,500 ppm by the inhalation route, a very high level (7). Vinyl chloride is a human carcinogen (72) and has been implicated in several types of cancer (103).

Elevation of chromosomal aberrations to relatively high levels by occupational exposure to viny I chloride is consistent and well documented (5,8,41,49,55,62,74,77,1 14,1 18,129). The chromosomal aberration endpoint seems to be more sensitive than the SCE endpoint to vinyl chloride exposure. In contrast to the aberrations seen with benzene, arsenic, and ionizing radiation, the aberrations associated with vinyl chloride exposure are short lived, disappearing over days or weeks. The mechanism for this difference is unknown. Chromosomal aberrations, in this case, could be used to document recent exposure but not necessarily cumulative exposure. Elevations in chromosomal aberration frequencies have not been detected when documented exposures have been less than 5 ppm. (The OSHA standard states that

the vinyl chloride level shall not exceed 1 ppm during any 15-minute period). Thus, chromosomal aberrations and SCEs do not seem to be sensitive enough to detect chronic low-level exposures to vinyl chloride for the number of cells usually scored (200 or fewer) per individual.

Conclusions

Elevated chromosomal endpoints are associated with occupational exposures to ionizing radiation and may be associated with exposure to some chemicals (arsenic, benzene, and vinyl chloride), particularly where long- lived aberrations (arsenic and benzene) are involved. The nature and longevity of the aberrations vary from one agent to another. For some chemicals such as benzene, the aberrations may persist for years. In these instances, the aberrations would indicate a cumulative exposure. For others, such as vinyl chloride monomer, the aberrations disappear quickly after reduction of exposure, and the cytogenetic tests could monitor exposure only over short time periods. It is not known whether cytogenetic monitoring will detect chronic low-level exposure. Hence, the appropriateness of chromosomal endpoints for occupational monitoring needs to be determined on a case-by-case basis for each chemical. In addition, a monitoring program should only be instituted when bacterial and animal tests have proved that the chemical in question is mutagenic or carcinogenic,

No occupational studies directly relate positive findings for any chromosomal endpoint with increased risk for any disease. Therefore, the clinical significance of a positive occupational cytogenetic study is unknown; nor is it known whether cytogenetic monitoring can be used to determine "safe" levels of exposure.

Retrospective cytogenetic studies done in conjunction with morbidity and mortality studies on populations of survivors of the atomic bomb attacks in Japan have found both high frequencies of stable chromosomal aberrations, particularly complex aberrations, and increased risk of cancer. On the other hand, there is no known correlation between an individual's chromosomal aberrations and his or her risk for cancer.

Cytogenetic monitoring, or any other test based on a single endpoint, may never be sufficient to predict health risks for an individual (with the possible exception of the Philadelphia chromos$_{ome}$), because the causes of cancer and other chronic diseases are complex and multifactorial, with some genetic and some environmental components. As more is understood about the molecular basis of each disease, an appropriate battery of tests may be designed with a variety of endpoints, each reflecting some aspect of the potential causes, Given the present information, any single endpoint, such as chromosomal aberrations or SCEs, may have some predictive value for a group, Even findings about groups with increased chromosomal damage require epidemiological studies on the populations to determine if increased risk for disease accompany the damage.

More research is needed to identify any relationships between chromosomal aberrations, SCEs, and disease in populations. At the present time, genetic monitoring may be most useful for detecting exposure to harmful agents. The most pressing questions yet to be answered concerning the use of monitoring in the workplace are: What is the biological significance of small elevations of aberrations or SCEs? Is there consistency between a given frequency of aberrations or SCEs induced by different agents and risk for disease?

Priorities for future research

Additional occupational cytogenetics studies are needed, combined with epidemiological investigations, to define further the meaning of induced chromosomal aberrations and SCEs, There is also a need to develop faster and easier tests for occupational genetic monitoring. Discussions with scientists involved in this work led to the following suggestions for future research:

- There is a need to standardize the laboratory conditions for cytogenetic tests.
- The best method of categorizing chromosomal damage for analysis has not been determined. Perhaps more comprehensive and critical analysis of results already available could contribute to the understanding of

both the effects of confounding variables and the biological mechanisms involved in the induction of chromosomal aberrations and SCEs. It maybe worthwhile to single out the percentage of cells with large numbers of SCEs and to display the chromosomal aberrations of each category for each individual in a study, including a distinction between the percentage of cells with aberrations and the percentage of aberrations per ceil.

- New cgytogenetic tests that are less labor intensive and that possibly could be automated are essential if cytogenetic testing is to be conducted on a large scale. Manual scoring, such as is done now, is so labor intensive and time-consuming that most cytogenetics laboratories in the United States are now working at capacity. Test scorers require several years of training to reach the point of consistent scoring. The use of a fluorescent-activated cell sorter has been studied as a possible means of automating chromosomal analysis, but this technique has intrinsic insensitivities and has not successfully been used to detect low frequencies of aberrations in human chromosomes (107,140).
- Further research needs to be done on in vitro sensitivity of human lymphocytes to chemicals encountered in the workplace. This approach could eventually have some value in predicting human clastogens as well as individual sensitivities to clastogens.
- The variables influencing baseline (normal) frequencies of chromosome] aberrations and SCEs need to he elucidated.
- There apparently has not been a prospective study done that looks for the association between chromosomal aberrations and risk for somatic disease in the same individual. There is a critical need for such studies, where individuals with no previous occupational radiation or chemical exposure are tracked, with concurrent comparisons. The National Institute for occupational Safety and Health has developed protocols for studies on radiation-exposed workers, but has yet to begin them. Any study addressing biological effects of current occupational exposure standards for radiation or chemicals should examine some individuals whose entire occupational history has been under the current standards.

Noncytogenetic monitoring

Because of the need for inexpensive and rapid tests to monitor human exposure to mutagens, many tests originally developed to screen chemicals for mutagenic activity have been modified to identify human exposure to mutagens. * All of these tests, either directly or indirectly, identify the presence of mutagens or DNA damage resulting from the presence of mutagens. Most of these developments are recent and, for the most part, have not been used for routine human monitoring.

*The extensive literature on animal studies using noncytogenetic endpoints for mutagenicity}' or carcinogenecity has recently been reviewed and evaluated by EPA's Gene-Tox program (61). Most of the papers have yet to be published, but two reviews have been published on specific mutation analysis in Chinese hamster cells (1970). Both of these papers state that the correlation between mutagenicity in this type of assay and animal carcinogenicity is high

Survey of monitoring methods

Virtually none of the tests described in table 15 has as yet been established as a reliable technique for monitoring human populations. The only test applied to population monitoring on more than a single trial basis has been the analysis of urine for mutagens. A few of the remaining tests have been used in human pilot studies, but these studies were for baseline analysis and not actual monitoring,

MUTAGENS IN BODY FLUIDS

The assumption is made that the presence of mutagens in body fluids represents a genetic hazard. Mutagenic activity in these fluids can be shown by using the rapid screening tests developed for bacterial or in vitro cell culture systems.

Table 15.—Summary of Noncytogenetic Monitoring Techniques

Test type	Description
Mutagens in body fluids:	
1. Urine	Body fluids used as test materials in bacterial or in vitro cell culture mutagenicity assays.
2. Feces	
3. Blood	
Somatic cell damage:	
1. Binding of mutagens to hemoglobin	Alkylation of the hemoglobin protein
2. Specific mutation analysis in lymphocytes	Technique that measures gene mutatation
3. Unscheduled DNA synthesis in lymphocytes	Technique that measures DNA damage
4. Hemoglobin gene mutations	Immunological technique that measures gene mutation
5. Chemically damaged DNA bases	Technique that measures DNA damage
6. Lymphocyte transformation	Technique that probably measures gene mutation
Germ cell (sperm) damage:	
1. YFF test	Detection of abnormal number of chromosomes
2. LDH-X variants	Immunological technique that measures gene mutation

SOURCE: Office of Technology Assessment

Since blood and urine are routinely collected in medical examinations, these types of tests could be integrated into a medical monitoring program.

A number of studies have been performed with body fluids, primarily urine, from humans presumably exposed to mutagens in their occupation or exposed to mutagens in the course of medical treatments (82,83,123,124). Lifestyle factors such as cigarette smoking also have been studied (2, 133,139). It is anticipated that urine analysis increasingly will be used for human epidemiological studies, both because it successfully identifies mutagens and because this test probably can be automated. Less commonly used tests of fecal sources and blood are not expected to be useful in general screening because of inherent technical problems.

Analysis *of urine for the* presence *of* mutagens.–The use of urine collected from humans as a test material is readily applicable to human monitoring situations for the following reasons (18):

- Studies have demonstrated that mutagenic activity can be detected in the urine of individuals exposed to various therapeutic drugs and industrial chemicals and of individuals with specific lifestyles or occupational experiences.
- Collection of urine samples is easy and can be obtained from both males and females on a regular schedule.
- Both mutagenic and chemical analysis can be performed simultaneously from a single collection of urine.
- Urine samples can be tested for the presence of mutagens not only in bacterial cells but also in mammalian cells.
- The costs and performance time associated with this approach are amenable to large-scale sampling studies.

There are also drawbacks to the use of this test in occupational settings. For example, only recent exposures can be measured. Moreover, the presence of mutagens in urine has not been translated into a known risk to the individual. Presence of mutagens in the urine can be considered evidence of exposure to a mutagenic chemical or to a chemical that forms mutagenic metabolizes which are eventually excreted. However, excretion of mutagens may be a protective process. In fact, the absence of mutagens in the urine could be interpreted as evidence that the mutagens are bound to cellular molecules (thus potentially causing damage) and are not available for urinary excretion. Consequently, knowledge of the metabolic fate of the suspected mutagens is critical to the proper interpretation of this monitoring technique. Moreover, there may be many confound-

Photo credit: National *Institutes of Health*

Noncytogenetic monitoring involves the use of biochemical tests

ing variables in urine analysis. For instance, the urine of cigarette smokers has been shown to contain mutagens **(139).**

Analysis *of feces fez" the presence of mutagens.*–Because cancer of the colon is a major cause of cancer mortality in Western countries, the incidence of the disease has been associated with diet (12,96, 103). Examination of human feces for mutagens is gaining some attention following a report (24) that showed that feces from individuals on typical Western diets contain high levels of mutagens. Consequently, analysis of human fecal samples might represent a monitoring approach for examining the relationship of dietary factors to specific types of cancer. Because of technical difficulties, such as concentration of the feces, the use of this procedure in occupational settings currently is limited.

Analysis of blood serum for the presence of mutagens.—There are only two reports on blood serum analysis for mutagenicity (40,82). Because of the difficulty of obtaining large quantities of serum, it is doubtful that serum analysis will contribute to the array of human monitoring techniques unless the detection tests could be made more sensitive.

SOMATIC CELL DAMAGE

Binding of mutagens to hemoglobin. —The possibilities for using hemoglobin from red blood cells as a biological dosimeter have been explored by Ehrenberg and coworkers in a series of experiments on mice using alkylating agents (43, 106,121). The assay is designed to measure alkylated amino acids in hemoglobin from exposed individuals. This phenomenon is not a genetic alteration, but protein alkylation strongly implies concurrent alkylation of DNA, a presumed first step in the production of mutations. The assay seems to work well for several alkylating agents. It can be used as a dosimeter, that is, it gives a positive dose-response curve, and it is a measure of accumulated damage over a period of a few months. This latter point is important because many other tests have to be done within a day or two of exposure.

The accumulation of alkylated groups in hemoglobin and the relatively large amounts of hemoglobin that can be isolated from one blood sample and analyzed, together with the availability of sensitive chemical and analytical techniques, make it feasible to determine small quantities of alkylated amino acids formed as a consequence of exposure to mutagens in the environment. For the most part, however, the procedures have been used only for mice injected with known mutagens. In the single report on the practical application of these procedures to human monitoring, Ehrenburg (42) showed that hemoglobin molecules were alkylated in workers exposed to ethylene oxide,

Before these techniques can be used in routine monitoring, more extensive validation studies are needed to standardize protocols, evaluate reproducibility, and determine intrinsic individual

variability. Once these factors are established, the procedure might have wide applications for specific chemical classes.

Specific mutation analysis in lymphocytes. —The production of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT) in humans is controlled by the hpt gene located on the X-chromosome (65). Cultured cells in which hpt has mutated are easily detected because of their resistance to normally poisonous guanine analogs, such as 6-thioguanine (TG). Thus, cells lacking HGRPT can be selected and grown by exposing them to one of these analogs in the culture medium. The frequency of TG-resistant (presumed mutant) cells in the peripheral blood lymphocytes of normal persons is very low (128), but the frequency increases among cancer patients being treated with known mutagens (4,128). Although the technique has been hampered by high background levels, recent modifications appear to have resolved some of these problems (3).

Although no studies have been undertaken, it is speculated that the detection of TG-resistant cells that arise in vivo could play a role in occupational monitoring. This technique could provide a sensitive assay for the induction of genetic mutations in human somatic cells. An increase in the percentage of TG-resistant cells in individuals exposed to toxic agents in the workplace might indicate exposure to a mutagen.

Unscheduled DNA synthesis in lymphocytes. — The damage to DNA by chemicals or radiation is often repaired by cellular mechanisms that remove the damaged area and replace it with new nucleotides (33,116), A test that measures this DNA repair, referred to as unscheduled DNA synthesis (UDS), has been suggested as a good indicator of exposure of chromosomes to mutagenic agents since the amount of DNA repair induced should be proportional to the amount of DNA damaged. In fact, reported data with human lymphocytes suggest that UDS is associated with mutation and chromosomal aberrations (108).

This assay also can be used to identify agents that inhibit DNA repair. Chemicals capable of inducing DNA damage while simultaneously inhibiting DNA repair may be especially hazardous. Results of a preliminary study of workers exposed to ethylene oxide show increases in both chromosomal aberrations and suppression of DNA repair synthesis (109).

There have been few UDS studies on lymphocytes in vivo. Limited studies of normal human lymphocytes (108) and lymphocytes from exposed humans (109) indicate that sex, age, and blood pressure may affect both background and chemically induced levels of UDS.

At present it appears that this assay can best be used to study the nature of tissue specificity of chemical DNA damage. For instance, compounds that cause stomach cancer may induce UDS in stomach tissue but not in liver tissue. On the other hand, it may not be a very good assay for mutagenesis because it is a measure of DNA repair, not damage, and repair may not correlate with genotoxicity.

Hemoglobin gene mutations. —Hemoglobin proteins can be altered by single gene mutations, and specific antibodies prepared against altered hemoglobin proteins can be used to detect these rare variants. Normal individuals generate the rare variants at a rate of about one variant per 10 million red blood cells. If the specific antibodies are linked to a fluorescent molecule, an automated, fluorescent-activated cell sorter can detect these rare cells with high sensitivity and specificity (92), Presumably, individuals exposed to mutagens would have an increased rate of production of the variants. This method could provide an excellent tool for evaluating human populations since it can be conducted objectively, quantitatively, and economically. Some preliminary pilot studies, using blood samples from individuals on cancer chemotherapy drugs or radiation therapy, found that the frequencies of abnormal hemoglobin were within the normal range but statistically higher than the frequencies for the corresponding controls (Omenn, personal communication).

Detection of chemically damaged DNA bases. — The detection of chemically damaged DNA bases is a direct measure of binding of a chemical to DNA. This binding can interfere with accurate DNA base pairing, thus causing mutations during DNA replication. Several detection methods have been described recently (1)64,66)138). All these methods are extremely sensitive and some,

depending on the chemical, can detect as few as one event per cell. This level is in the range necessary for a test to be predictive for chronic low-level exposure.

There are limitations to the study of chemically damaged bases. It is a measure of an early event and a base change may not result in a mutation. For instance, the mutated bases could be repaired prior to cellular DNA synthesis and be of no consequence at all. It also is not known how persistent the damaged bases are. For instance, in monitoring a population, it is necessary to know when to collect samples and then whether the damaged bases found are a result of recent or prior exposure.

The detection of damaged DNA bases has moved beyond the laboratory. A prospective epidemiological study was begun recently on coke oven workers; the researchers are using specific antibodies to detect benzo(a)pyrene bound to DNA bases (137).

Lymphocyte transformation assay. —Several reports (30)60) 117) suggest that mammalian cells exposed to mutagenic chemicals in vitro exhibit an enhanced susceptibility to transformation, a condition that has many similarities to tumor cells. It is possible that a modification of this transformation assay could be used to monitor exposure to harmful chemicals. The lymphocytes from exposed individuals are grown in culture under conditions that select for transformation. Presumably, increased exposure will -yield more transformed cells.

GERM CELL DAMAGE

Studies on germ cells have focused exclusively on sperm. The advantage of monitoring sperm, aside from the ease of obtaining viable cells, is that studies using sperm have tested about 10 times the number of chemicals that have been tested using any other cell type. Whether toxicological studies of sperm will reflect the genetic status of somatic cells is unknown, but, assuming that they will, two assays show promise in an occupational setting.

YFF' *test.* —The YFF test purports to identify an extra Y chromosome. Sperm with increased fluorescence under a microscope in the presence of quiniline dye are scored as having more than one Y chromosome. They are abnormal and presumably arise due to abnormal chromosome segregation during cell division. Yet it has not been shown conclusively that the increased fluorescence is not due to a change in the fluorescence pattern of the other chromosomes. Thus, this change may be an important indicator of exposure to chemicals, but cannot be taken as a result of abnormal segregation. This sperm assay, because of its relative experimental ease, might be most useful in determining priorities for longer term studies of chemical agents.

LDH-X variants. —The principle on which this assay is based is the same as that of the test for mutant hemoglobin. Lactate dehydrogenase-X (LDH-X) is a protein found on the tail of sperm and is detectable by specific antibodies (27). Experiments with rats and mice have shown that LDH-X mutants are detectable with different antibodies (10,11). Presumably, LDH-X variants could be detected with a battery of different monospecific antibodies. One experiment with mice showed a linear relationship between increased dose of mutagen and increased mutant LDH-X molecules (9).

The presence of LDH-X mutants presumably will reveal whether an individual is sensitive to an environmental mutagen in an analogous fashion to the hemoglobin gene mutation assay. As in that assay, the method could be automated by using fluorescent-labeled antibodies and a fluorescent-activated cell sorter. The human LDH-X mutants need much better characterization, however, before this assay will find applicability in a routine monitoring situation (H. Mailing, personal communication).

Conclusions

At present, there is not enough research experience using humans for most noncytogenetic techniques to determine accurately their usefulness in workplace monitoring situations. The detection of mutagens in urine is the only assay that has been used with human subjects often enough to show that spurious results will not be generated. The other techniques will require considerably more development to be considered of

value as monitoring techniques. The most obvious deficiency in these tests is the lack of the availability of the normal baseline response. Without a good estimate of the range of responses in unexposed humans, the data from test populations will be difficult to interpret. Table 16 summarizes all of the human studies that used noncytogenetic methods.

Priorities for future research

Several of the noncytogenetic techniques may have potential for use in human monitoring. The characteristics necessary for a good workplace monitoring technique include the ability to detect accurately low levels of the abnormality being assayed, the likely prospects for automation, and low cost. Six of the tests discussed in this section potentially have these necessary characteristics, and their development could lead to better workplace monitoring. These tests include the detection of:

- mutagens in urine,
- alkylated hemoglobin,
- a specific mutation (HGPRT) in lymphocytes,
- hemoglobin mutations,
- chemically damaged DNA bases, and
- LDH-X variants in sperm.

Table 16.—A Summary of Noncytogenetic Methods Used in Human Monitoring

Technique	Population monitored	Reference
1. Mutagens in body fluids		
A. Urine	Rubber industry workers	Falck, et al. (47)
	Coke plant workers (smokers v. nonsmokers)	Moeller and Dybing (94)
	Nurses administering cytostatic drugs	Falck, et al. (48)
	Chemical workers in ink and solvent plants	Mazzoll (91)
	Patients receiving cancer therapeutic drugs	Siebert and Simon (123,124) Legator, et al. (82,83,84) Speck, et al. (125) Wang, et al. (135) Roxe, et al. (1 19)
	Patients receiving drugs	Legator, et al. (81)
	Cigarette smokers v. nonsmokers	Yamasaki and Ames (139)
B. Blood	Persons dosed with an antiparasitic drug which has mutagenic and carcinogenic activity	Legator, et al. (82) Dobias (40)
C. Fecal	Comparison of humans with different diets and geographical origin	Ehrich, et al. (44) Reddy, et al. (115) Kunhleln, et al, (78)
2. Somatic cell damage		
A. Hemoglobin alkylation	Workers exposed to ethylene oxide	Ehrenberg (42)
B. Specific mutations in lymphocytes	Cancer patients treated with chemotherapeutic drugs	Strauss and Albertini (128) Albertini and Allen (4)
C. Unscheduled DNA synthesis	Factory workers exposed to ethylene oxide	Pero, et al. (109)
D. Hemoglobin gene mutations	Cancer patients treated with chemotherapeutic drugs	Mendelssohn, et al. (92)
E. Chemically damaged DNA bases	Coke oven workers exposed to benzo(a)pyrene	Weinstein and Perera (137)

SOURCE: Off Ice of Technology Assessment.

Chapter 6 references

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