COMMISSION OF THE
EUROPEAN COMMUNITIES

Health and Safety Directorate
Luxembourg

D R A F T R E P O R T

BIOLOGICAL METHODS FOR MONITORING EXPOSURE TO MUTAGENIC OR CARCINOGENIC CHEMICALS

prepared by

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December 1986

CEC/LUX/V/E/3/41/86

**COMMISSION
OF THE
EUROPEAN COMMUNITIES**

Directorate-Generale
Employment, Social Affairs and Education
Health and Safety
Directorate
V/E/3

Luxembourg, 15. II. 1984
AB/sh/54

Dear Dr Sapir,

Re: **Biological methods for monitoring exposure to mutagenic or carcinogenic agents**

Please find under separate cover a draft report prepared on the above topic, at the request of the Commission of the European Communities, by A. Leonard, A. Bernard, R. Lauwerys, M. Duverger-van Bogaert and M. Lambotte-Vandepaer from the Catholic University of Louvain in Brussels.

This draft report is a follow-up of the conclusions of the Symposium on "Monitoring Human Exposure to Carcinogenic and Mutagenic Agents" held in Finland in December 1983 (also under separate cover).

It is intended that this draft report be the basis of a consensus meeting to be held later this year which should develop in particular:

- recommendations for an international validation programme for selected methods;
- agreed statements on the current knowledge regarding the health significance of the various end points on a group and individual basis.

At this stage we are requesting your comments and proposals for amendments to the draft report as well as suggestions regarding the recommendations to be developed at the consensus meeting.

Comments and documentation concerning your experience with these methods would also be welcomed.

You will be informed as soon as possible of the developments on the planned consensus meeting.

We are looking forward to receiving your comments by early March and thank you for your continued collaboration.

Yours sincerely,

Amicalement

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NOTE

This draft report is being distributed to all interested parties to solicit comments and suggested amendments.

These should be sent by 1 March 1987 at the latest to :

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Directorate General of Employment,
Social Affairs and Education
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CONTENT

INTRODUCTION

The consumption of chemicals by industrialized countries is doubling every seven years and approximately 60 to 70,000 chemicals are used in our everyday life. Industrialized societies are facing the problem of estimating the risk for human health of this rapidly growing number of chemicals.

In view of the impossibility to submit all of them to classical long term carcinogenicity experiments in animals, short term tests are currently used to assess their ability to alter the genetic material and so to indirectly evaluate their carcinogenic potency. So far, however, appropriate toxicity testing has been done for only about 10% of the chemicals present in the general or the work environment.

Even if all the currently used chemicals could be subjected to short term tests for mutagenicity and carcinogenicity, the problems remain how to extrapolate these data to the human situation in the form of occupational or environmental exposure guidelines. The magnitude of the difficulties encountered in this extrapolation is reflected by the very low proportion of occupational pollutants for which exposure limits have been proposed.

The situation is getting still more complex when one considers that human populations, particularly in the occupational settings are frequently exposed to mixtures of pollutants with a variable and often poorly characterized composition.

Finally, it must also be realized that the response of the human organism to a given exposure may be quite variable. Human cancers result from a complex interaction between host and environmental factors and can certainly not be predicted from environmental data only.

Whatever the sophistication of the experimental mutagenicity and carcinogenicity tests and the respect of the proposed exposure guidelines, hazardous exposures to genotoxic chemicals can never be completely excluded, particularly in industry. It is, therefore, important to be able to detect workers who have absorbed genotoxic chemicals.

The objective of the present report is to review the biological methods currently available for that purpose and to assess their validity and their ability to predict the cancer risk.

Part I

DESCRIPTION AND CHARACTERISTICS OF THE TESTS CURRENTLY PROPOSED FOR MONITORING EXPOSURE TO MUTAGENIC OR CARCINOGENIC CHEMICALS

CHAPTER I

DETERMINATION OF THE CHEMICAL OR ITS METABOLITES IN BIOLOGICAL MEDIA

§ 1. INTRODUCTION

This approach aims to estimate the amount of mutagenic or carcinogenic chemical absorbed by the organism. In practice, the biological samples most commonly used for analysis are urine, blood and alveolar air. The analysis of the chemical in other body compartments such as fat, milk, saliva or hair is rarely performed.

Depending on the kinetics of the measured parameter, its concentration in biological media reflects the uptake of chemical over a period which may range from a few hours (e.g. benzene in blood or alveolar air) up to several months or years (e.g. DDT or PCBs in adipose tissue).

This monitoring method is normally reserved to mutagenic or carcinogenic chemicals which do not cause their damage at the point of entry in the body. For carcinogens acting locally (e.g. some lung carcinogens), the determination of the chemical concentration or of the mutagenic activity in material collected from ambient air may be more relevant for risk assessment than measurements in biological media.

Furthermore, this type of monitoring is mainly relevant when sufficient information has been gathered on the metabolism of the chemical and on the relationships between external exposure, internal dose and adverse effects. Unfortunately, the latter conditions are fullfilled only for a limited number of carcinogenic chemicals. This could be for instance the case of benzene, although the risk of leukemia at low levels benzene exposure is still a matter of controversy.

Tests measuring the carcinogenic or mutagenic chemical or its metabolites in biological media can be classified in two broad categories : the selective tests and the non selective tests.

§ 2. SELECTIVE TESTS

Most exposure tests currently used in occupational or environmental medicine belong to this category. As a general rule, they measure the unchanged chemical in biological media when the substance is not, or poorly, biotransformed, when the exposure is too low for a significant amount of metabolites to be produced or when a high degree of specificity is required or preferred. However, the great majority of carcinogenic or mutagenic compounds which enter the organism are metabolized to more water soluble compounds that are readily eliminated via urine or bile.

Exposure to these chemicals is thus generally monitored by measuring specific urinary metabolites. The main advantage of these tests is the fact that they are easily accepted by the workers.

Table I.1. summarizes the chemical assays which could be used for monitoring exposure to some chemicals suspected or known to be carcinogenic for man. One limitation of these tests is the fact that in most cases no reference can yet be made to a maximum permissible value (i.e. Biological Limit Value). In other terms, these tests allow to detect the exposure to a chemical but they provide little information regarding the health risk associated with this exposure.

§ 3. NON SELECTIVE TESTS

These tests can be used as nonspecific indicators of exposure to groups of chemicals. The determination of the mutagenic activity in urine (discussed in Chapter II) is an example of such type of tests.

Another example of nonselective test for monitoring exposure to carcinogenic or mutagenic chemicals is the thioether analysis in urine. Electrophilic agents - a class of chemicals including most carcinogenic or mutagenic compounds - can be inactivated by reaction with glutathione or other SH containing molecules. The conjugates so formed usually appear in urine as mercapturic acid or

other thioether products. An increased urinary excretion of total thioethers has been observed in workers of chemical production plants (Seutter-Berlage et al., 1977) in rubber workers (Vainio et al., 1978; Kilpikari, 1981), in workers exposed to carbon disulfide (Henderson et al., 1984) or in operators of chemical waste incinerators (Van Doorn et al., 1981). The thioether assay has however several limitations. The sensitivity of the test is limited by the relatively high background of urinary excretion of thioethers. In the above-mentioned studies, the increase of thioether excretion was generally not more than twice the background level. Endogenous electrophilic compounds are indeed eliminated in urine as thioethers. There is for instance a difference in the urinary excretion of thioethers between male and female, because of the presence of female steroid hormones, especially estrogen conjugates (Lauwerys, 1983). In addition, several compounds for which neither carcinogenic nor mutagenic effects are known were found to substantially increase the urinary excretion of thioethers, e.g. bromobenzene, O-xylene, benzylalcohol, biphenyl and mesitylene.

Finally, smoking is a confounding factor. Cigarette smoke contains several substances which are eliminated in urine as thioethers (Van Doorn et al., 1979). In smokers, the urinary excretion of thioethers is related to the number of cigarettes smoked (Henderson et al., 1984), but not to the tar content of cigarettes (Heinonen et al., 1983). For all these reasons, the thioether assay in urine is

currently considered a nonspecific method whose sole interest could be to detect an excessive exposure to unsuspected electrophilic susbstances.

The determination of diazo-positive metabolites in urine is also a non selective test which has been applied to workers exposed to various aromatic amines and nitrocompounds (Ikeda et al., 1977; Linch et al., 1974b). As for the thioether assay, the interest of this test could lie in its signal function, allowing for the identification of groups at risk.

§ 4. CONCLUSION

Methods measuring the chemical or its metabolites in biological media can be used to estimate the amount of carcinogenic or mutagenic compounds absorbed by the organism. The evaluation is usually specific and sensitive when based on selective tests. The non selective tests such as the thioether assay are relatively unsensitive and applicable only on a group basis (e.g. identification of group at risk).

These tests, however, are of little value for quantifying the health risk since they provide no information on the importance of the active chemical interaction with the genetic material. In addition, in most cases, the results obtained cannot be interpreted by reference

to biological limit values. They may however be included in a biological monitoring programme to detect groups at risk.

able I.1.
arcinogenic chemicals (for man and/or animal) for which biological exposure tests ave been proposed.

HEMICAL	SUBSTANCE MEASURED	REFERENCES
crylonitrile	Acrylonitrile in urine thiocyanates in urine	Sakurai et al., 1978
flatoxin	Aflatoxin in urine and in tumors of liver and lung	Campbell et al., 1970 Martin et al., 1984 Sizaret et al., 1982 Dvorackova et al., 1981
-Aminobiphenyl	4-aminobiphenyl in urine	Lauwerys, 1983
rsenic (inorganic)	Sum of inorganic arsenic, monomethylarsinic acid, cacodylic acid (tentative BLV : 50 µg/g cr.)	Lauwerys, 1983
sbestos	Asbestosis fibers in sputum	Bignon et al., 1973
enzene	Phenol in urine (tentative BLV : 45 mg/g creatinine) benzene in blood and expired air	Lauwerys, 1979
enzidine	Benzidine and N,N'diacetylbenzidine in urine	Nony and Bowman, 1980
hromium	Chromium in urine (tentative BLV : 30 µg/g creatinine)	Lauwerys, 1983
yclophosphamide	Cyclophosphamide in urine	Hirst et al., 1984
3'-Dichloro- enzidine (DCB)	DCB and N-acetyl DCB in urine	Hurst et al., 1981
4-Dioxane	$-hydroxyethoxyacetic acid in urine	Young et al., 1976 Braun, 1977
4'-Methylene-bis 2-chloroaniline)	MOCA in urine	Ducos et al., 1985 Linch et al., 1971

(MOCA)		Van Roosmalen and Klein, 1979
2-naphtylamine	2-naphtylamine in urine	Lauwerys, 1983
Phenacetine	N-acetyl-para-aminophenol in urine	Smith and Timbrell, 1974
Polycyclic aromatic hydrocarbons (PAH)	Hydroxypyrine in urine	Jongeneelen et al., 1985
Vinyl chloride	Thiodiglycolic acid in urine	Müller et al., 1978
Chlorambucil	Chlorambucil in plasma or urine	Jakhammer et al., 1977 Chang et al., 1980
Nitrogen mustard	Nitrogen mustard in plasma or urine	IARC, 1975
Bischloroethyl nitrosourea (BCNU)	BCNU in plasma	Bartosek et al., 1978 Weinkam et al., 1978
Dacarbazine	Dacarbazine in plasma	Loo and Stasswender, 1967
Adriamycin	Biological fluids	IARC, 1976

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CHAPTER II

DETERMINATION OF MUTAGENIC ACTIVITY IN BIOLOGICAL MEDIA

§ 1. INTRODUCTION

The determination of mutagenic activity in biological media aims at detecting the exposure to mutagenic chemicals or to chemicals transformed by the organism into mutagenic metabolites. The mutagenic activity is detected by various short term tests relying on the interaction of the chemical with the DNA of bacteria (e.g. the Ames test), drosophila or mammalian cells. These tests are performed in the presence or absence of bioactivation systems (e.g. liver microsomes).

Mutagenicity tests are usually applied on urine but other biological samples such as serum, feces, amniotic fluid, sputum or milk can be used. These tests share some features which must be kept in mind when interpreting their results. They are not specific with respect to the exposure and are sensitive to many confounding factors such as smoking, drugs, diet or cosmetics. Furthermore, like most methods measuring chemicals in body fluids (Chapter I), they mainly reflect recent exposure (i.e. exposure over the previous 48-72 h).

§ 2. DETECTION SYSTEMS (Table II.1).

1. The bacterial mutagenicity assays - Salmonella typhimurium and Escherichia coli.

Ames and co-workers have developed a simple bacterial test for detecting chemical carcinogens or mutagens. The compounds are placed on petri dishes containing various histidine requiring mutants of S. typhimurium that can be reverted back to prototrophy by a variety of mutagens (Ames et al., 1975). Aliquots of urine or urine concentrates are mixed with the bacterial culture incubated on petri dishes. Urine specimen can be submitted to β-glucuronidase-arylsulfatase treatment before incorporation into the petri dish, or during the 48 h incubation period. This bacterial test has been successfully used to detect an increased mutagenicity of urine from smokers (Yamasaki and Ames, 1977), individuals handling chemotherapeutic agents (Nguyen et al., 1982; Barale et al., 1985), workers exposed to coal tar pitch volatiles in an aluminium reduction plant (Heussner et al., 1985), patients treated with various cytostatic drugs (Minnich et al., 1976) and workers from a coal liquefaction plant (Recio et al., 1984).

Some authors have used the bacterial fluctuation test of Green et al. (1977) with urine concentrates. This test has been described for E. coli but is also suitable for S. typhimurium. It has been used to detect mutagenic activity in the urine from workers in rubber industry (Falk et al., 1980; Sorsa et al., 1983). Fecal mutagens were also detected with S. typhimurium after sample extraction and purification (Reddy et al., 1984). The mutagenicity of human blood samples for S. typhimurium from volunteers treated with metronidazol was reported by Dobias (1980). The Ames S. typhimurium test has also been applied to breast fluid (Petrakis

et al., 1980). Amniotic fluids from smokers were tested for mutagenic activity toward S. typhimurium to determine the possible transfer of mutagenic compounds across the placenta (Rivrud et al., 1986).

A common drawback of these bacterial assays is the possible interference of urinary components such as histidine and tryptophan. The higher sensitivity of the bacterial fluctuation test may increase the variation due to possible presence of small amounts of histidine or tryptophane. Mutagenic agents may be present at levels too low to be detected because the sample is too diluted. Concentration of samples may lead to increased concentration of histidine and of toxic materials. Additionally, the resin columns which have been used to concentrate the samples may be unsuitable to detect certain substances (Anderson and Legator, 1984). Volatile metabolites may also be lost during the evaporation of the solvent used to elute the mutagens from the resin column.

2. Yeast assays : Saccharomyces cerevisiae

To perform the urinary assay, the yeast cells are incubated for 8 hours with urine, and induction of prototrophs is scored. Positive results have been obtained with urine from rats treated with high doses of cyclophosphamide (500 mg/kg) (Siebert, 1973). Another use of Saccharomyces cerevisiae is the induction of mitotic gene conversion by urine, using a diploid strain of Saccharomyces cerevisiae. This test is much more sensitive than the back-mutation system (Siebert and Simon, 1973).

3. Mammalian cells

The mutagenicity of urine towards mammalian cells has been little investigated. Guerrero et al. (1979) have demonstrated that urine induces sister chromatid exchanges (SCE) in mammalian cells in culture (human diploid fibroblasts, WI-38) and that the frequency of exchanges induced by the urine of smokers is higher than that of non-smokers. Beek et al. (1982) found that urine concentrates induces SCE and chromosome aberrations in Chinese hamster ovary cells in culture, but did not find an increased activity in the urine of smokers.

Dunn and Curtis (1985) performed experiments indicating that cigarette or coffee consumption results in the appearance in urine of compounds damaging chromosomes of CHO cells in culture.

§ 3. BIOLOGICAL MEDIA

1. Urine

Durston and Ames (1974) and Commoner et al. (1974) were the first to report independently an increased urinary excretion of bacterial mutagens in animals treated with carcinogens. As most carcinogens or mutagens absorbed by the organism are mainly eliminated by the urinary route, urine analysis is particularly indicated for detecting exposure to mutagenic substances. Urinary tests present also the advantage of being noninvasive and thus easily acceptable by the examined subject.

Urine can be tested directly but usually a pretreatment is required to concentrate mutagens and to eliminate interfering substances (e.g. histidine). In 1977, Yamasaki and Ames described a method for concentrating urine in which the sample is applied on a columm of XAD-2 resin (styrene divinylbenzene polymer) and the adsorbed material eluted with an appropriate solvent. The use of a resin such as XAD-2 is quite effective at adsorbing relatively non-polar compounds in urine, while amino acids are not retained. This concentration procedure has been applied to urine from smokers (Yamasaki and Ames, 1977; Recio et al., 1982), from individuals handling cancer chemotherapeutic agents (Minnich et al., 1976; Nguyen et al., 1982; Barale et al., 1985), from workers in rubber industry (Sorsa et al., 1983), from workers in coal liquefaction plant (Recio et al., 1984), etc... . The use of XAD-2 resin, however, suffers from poor recoveries of mutagenic material from urine and also from a lack of standardization (Aeschbacher and Ruck, 1982).

Other concentration procedures are available. Curtis and Dunn (1985) described a technique using preparative reverse phase HPLC to concentrate and fractionate urine samples from smokers. Urine can be concentrated and purified by TLC and HPLC before being tested in a mutagenicity assay (Connor et al., 1983). Another method for separating non polar mutagens takes benefit of the affinity of mutagens to a phtalocyanine derivative covalently bound to cotton (Hayatsu et al., 1983). The results from these various studies underline the need to critically appraise any extraction scheme used to concentrate organic material from urine for genotoxicity testing. Furthermore, metabolites may be excreted in urine in several conjugated forms such as β-glucuronides, mercapturic acids and sulfate esters. These conjugates are, with few exceptions,

biologically inactive. Any procedure used to screen urine must take this factor into account. Aliquots of urine should be submitted to appropriate enzyme treatment to cleave these conjugates (Aeschbacher and Ruck, 1982; Sousa et al., 1985a)

2. Feces.

Mutagenic activity in human feces extracts was first demonstrated by Bruce and co-workers (Bruce et al., 1977, 1979; Varghese et al., 1978). The search for mutagenic activity in feces has been mainly made in the framework of studies on intestinal tract cancer. Many reports suggest that colorectal cancer might be caused by carcinogenic chemicals generated endogenously in the intestinal tract mucosa or by the microflora (Venitt, 1982). Various methods for the extraction and purification of mutagens from feces have been described, (Reddy et al., 1984; Bruce et al., 1979; Wilkins et al., 1980). For obvious reasons there is much less published information on feces mutagenicity than on urine mutagenicity. It is difficult to avoid further bacterial metabolism once the sample has been voided and there is a natural reluctance to handle samples. Nevertheless, a few groups have carried out population-based studies on faecal mutagens (Ehrich et al., 1979; Reddy et al., 1980).

3. Blood

Samples of venous blood from volunteers treated orally with miridazole (about 10 mg/kg) were directly tested for mutagenic activity in the Ames test. The detection of mutagenic metabolites of miridazole in human blood suggests that the method might be used for the evaluation of newly

developed drugs (Dobias, 1980). Blood can also be lyophilized or eluted on XAD-2 resin in order to concentrate active compounds (Legator et al., 1975a). Using the S. typhimurium test, evidence of a mutagenic effect was found in both blood and urine of mice treated with one dose of miridazole (Legator et al., 1975b).

4. Amniotic fluid

Amniotic fluid from smokers has been tested for mutagenic activity to evaluate placental transfer of mutagenic compounds. Amniotic fluid samples were directly used in the Ames plate incorporation test (Rivrud et al., 1986).

5. Breast fluid

With the Ames S. typhimurium test, Petrakis et al. (1980, 1981) have detected mutagenic activity in breast fluid samples of non lactating women. Attemps to correlate clinical and epidemiological characteristics of the examined persons with mutagenesis tests were not informative.

§ 4. SOURCES OF INDIVIDUAL VARIATION AND CONFOUNDING

The urinary excretion of mutagens in the human population is subject to great variations. This variation may reflect different levels of occupational or environmental exposures to mutagens. But it may also result from acquired or inherited differences in the metabolism of xenobiotics. Furthermore, the urinary excretion of mutagens can be modulated by smoking habits, the use of cosmetics or various factors present in the diet.

1. Polymorphism of xenobiotic metabolism.

Genetic polymorphism has been described for several enzymes or enzymatic systems directly or indirectly involved in the metabolism of xenobiotics : N-acetylase (Vahakangas et al., 1984; Grant et al., 1983a,b); glucose-6-phosphate dehydrogenase (Beutler, 1984), cytochrome P450 (Distlerath et al., 1984) or epoxyde hydrolase (Oesh, 1972; Glatt and Oesh, 1984).

2. Factors modifying the activities of xenobiotics metabolizing enzymes.

The enzymatic system most widely implicated in the activation of xenobiotics is the cytochrome P450 dependent monooxygenase system. The level of cytochrome P450 and hence the ability of the host to metabolize xenobiotics can be influenced by a number of factors including smoking habits, drugs (e.g. cimetidine), diseases (e.g. liver diseases), nutritional status or exposure to some pollutants (e.g. pesticides) (Kato, 1977).

3. Confounding factors.

Smoking is certainly the most important interfering factor in the monitoring of exposure to mutagenic chemicals. The level of urinary mutagenicity is related to the type and the number of cigarettes smoked (Yamasaki and Ames, 1977; Falk et al., 1980; Sasson et al., 1985).

The consumption of various drugs such as metronidazole, niridazole and nitrofurantoïn, may also lead to an increased mutagenic activity of urine (Legator et al., 1975a; Connor et al., 1977; Wang et al., 1977). The mutagenic activity of urine can also be affected by dietary factors. For example, the consumption of fried beef, pork or bacon meals is followed by the excretion in urine of a significant amount of mutagens, as detected in the Ames test (Baker et al., 1982; Dolara et al., 1984; Sousa et al., 1985a). The consumption of red wine, grape juice and coffee does not lead to the excretion of mutagens in urine, even if previous studies have demonstrated that the concentrated beverages exhibited mutagenic activity (Sousa et al., 1985b; Stoltz et al., 1982; Aeschbacher and Chappuis, 1981). It has been shown that patients with cirrhosis excreted in urine mutagenic compounds of unknown origin (Gelbart and Sontag, 1980). Mutagens were also found in urine from patients suffering from bladder cancer (Garner et al., 1982).

§ 5. CONCLUSION

Mutagenicity tests have an adequate sensitivity for monitoring exposure to environmental or occupational carcinogens or mutagens. Confounding

factors, however, may arise from several sources, such as smoking, drugs, diet, cosmetics etc... This lack of specificity is an advantage to detect mutagenic compounds whatever the source. But the nonspecificity of mutagenicity tests restricts their application on a group basis and also obscures the relationship between the excretion of mutagens in urine and the presumed exposure. In addition, there is also the possiblity that urinary contaminants such as histidine in the Ames test interfere with the assay. Like the methods measuring the chemicals in body fluids, mutagenicity tests mainly reflect the recent exposure. A standardization of the methods currently used is also desirable.

With respect to the health significance of the results of mutagenicity tests, it must be stressed that the mutagenic activity measured in excreta is not necessarily relevant for what happens in the genetic material of the target organ. The compound responsible for the mutagenicity of urines may not be involved in the cancer induction at the target organ and inversely, the lack of increase of urinary mutagenicity does not necessarily mean an absence of genotoxic damage in the target organ.

Table II.1 : Example of applications of mutagenicity tests for monitoring human exposures.

Exposure to	Body fluids	Results	References
Rubber chemicals	urine	(EC)[a] +	Sorsa et al., 1981
		(ST) +	Falk et al., 1980
Coal liquefaction products	urine	(ST) -	Recio et al., 1984
Coal tar pitch volatiles	urine	(ST) -	Møller and dybing, 1980
Coal tar pitch volatiles	urine	(ST) +	Heussner et al., 1985
Antineoplasic drugs	urine	(ST) -	Barale et al., 1985
Cyclophosphamide	urine	(SC) +	Siebert and Simon, 1973
	urine	(HD) +	Guerrero, Rounds and Hall, 1979
	Peritoneal fluid	(SC) -	Siebert, 1973

a EC, Escherichia coli
ST, Salmonella typhimurium
SC, Saccharomyces cerevisiae
HD, human diploid fibroblast cells (WI-38)

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CHAPTER III

CYTOGENETIC OBSERVATIONS ON HUMAN SOMATIC CELLS

Occupational or accidental exposure to ionizing radiations is currently assessed by monitoring chromosome aberrations in peripheral blood lymphocytes, and the dose is estimated by comparison with dose-response relationships obtained in vitro (Lloyd et al., 1980 ; Lloyd and Purrot, 1981 ; Lloyd, 1984 ; Sasaki, 1983). On the assumption that agents increasing the yield of chromosomal aberrations are able to produce point mutations, this method is also one of the easiest direct estimation of the genetic damage induced by environmental mutagens in human somatic cells. The possibility of using this system for exposure to mutagens other than ionizing radiations is, therefore, very attractive and many papers have been published reporting an increase of structural chromosome aberrations in people exposed to chemicals. (Obe and Beek, 1982 ; Natarajan and Obe, 1982 ; Kucerova, 1982 ; Brewen and Stetka, 1982).

§ 1. METHODOLOGY

1. General characteristics of the peripheral blood lymphocytes system

About 90% of the peripheral blood lymphocytes are of the recirculating type, remaining in the bloodstream for less than one hour. Then they migrate to the lymphatic tissues to reappear in the several hours or

days later (Trepel, 1975, 1976). More than 99% of the circulating lymphocytes are in the GO or Gl stage of the cell cycle and have to be stimulated by a mitogenic agent, generally phytohemagglutinin, to replicate their DNA and to enter in division. The studies performed on irradiated persons have shown (Buckton et al., 1967 ; Dolphin et al., 1973) that the majority (90%) of the human lymphocytes have a life span exceeding 3 years, some surviving more than 20 years. Since lymphocytes accumulate genetic damages they allow to monitor the effects not only of an acute but also of a chronic or repeated exposure to clastogenic agents.

2. Structural chromosome anomalies produced by clastogens

Chromosome-type and chromatid-type are the most frequent structural aberrations which can be cytologically distinguished at metaphase after exposure to clastogenic agents. Chromosome-type aberrations occur at the same locus of both chromatids whereas chromatid-type aberrations usually involve only one chromatid of the metaphase chromosomes. Chromosome-type anomalies are terminal deletions, minutes or interstitial deletions, acentric rings, centric rings, inversions, reciprocal translocations and polycentric aberrations. The most common chromatid-type aberrations are chromatid and isochromatid gaps, chromatid breaks and interchanges. The chromatid and isochromatid gaps (achromatic lesion) are generally considered as unreliable indicators of real damage to the genetic material (Obe and Beek, 1982 ; Evans, 1976 ; Evans and O'Riordan, 1975 ; Evans, 1984). Some chemicals, especially those that inhibit synthesis of DNA, usually induce a high frequency of such achromatic lesions when cells are treated in S or G2 stage. As pointed out by Evans (1976) it is probable that gaps involve a mixture of phenomena some representing true lesions to genetic material. Their scoring is, however, extremely subjective, their

frequency is sometimes very high in control cells and may be increased by differences in methodology. In order to avoid a selective elimination of cells carrying unstable aberrations, the dividing cells are normally analysed in the metaphase stage of the first mitosis (M1) following their induction.

Comparative studies have shown that the structural changes produced by clastogenic chemicals are similar to those produced by ionizing radiations, the frequency of the different types of anomalies depending both on the stage of the cell cycle in which the exposure occurs and on the type of clastogenic agent. Ionizing radiations induce chromosome-type aberrations if the cells are exposed in G1, chromatid-type aberrations if the cells are exposed in G2 and a mixture of both types if exposure occurs in S phase. By contrast to ionizing radiations chemical mutagens have a wide spectrum of mechanisms by which they exert their mutagenic action. Most produce structural aberrations by a so-called delayed effect and after an intervening round of DNA replication give rise to chromatid-type aberrations. Some chemicals, however, mimic ionizing radiations and produce chromosome-type structural aberrations within a short time interval after exposure. Chemicals often are generally S-dependent or, more rarely, S-independent, but some compounds apparently possess both capabilities.

3. The micronucleus test on peripheral blood lymphocytes

The lack of centromere in acentric fragments produced by clastogenic agents as well as the possibility for a normal chromosome to be left behind because of a failure in the mitotic spindle can give rise to micronuclei. The production of such anomalies has been widely used to assess the production, by chemical clastogens, of chromosome aberrations in vivo in laboratory mammals.

More recently, it has been shown that, applied to human peripheral blood lymphocytes this technique may represent a useful and cheaper alternative to the observation of metaphase chromosomes (Abe et al., 1984 ; Goetz et al., 1975, 1976 ; Högstedt, 1984 ; Högstedt et al., 1981a,b ; Heddle et al., 1982 ; Krepinsky and Heddle, 1983 ; Pincu et al., 1984, 1985). A recent study by Norman et al. (1985) shows, indeed, that micronucleus count is an excellent predictor of the presence of cells with chromosome aberrations.

The assay of micronuclei in peripheral blood lymphocytes is to be preferred to the observation of such anomalies in bone marrow, a method which could hardly be used in routine tests in spite of its value as demonstrated by Högstedt et al. (1981).

Several attempts have been made to use the micronucleus count in other types of cells exposed to carcinogens. In a recent study, Stich and coworkers (1984) showed, for instance, an increase in the proportion of micronucleated buccal mucosal cells in Filipinos who chew betel nut.

4. Sister chromatid exchanges (SCEs)

The use of 5-bromodeoxyuridine (BrdU) (Perry and Wolff, 1974) has greatly simplified the detection of SCEs and explains the proliferation, during the last decade, of papers dealing with the induction of such cytogenetic changes by various physical and chemical agents. This technique which gives better resolution than autoradiography requires the exposure of the cells to BrdU during two consecutive replications. 5-bromodeoxyuridine is incorporated into both strands of one chromatid and one strand only of its sister chromatid. Subsequent treatment of the M2 chromosomes with a combination of the fluorochrome Hoechst 33528 and Giemsa results in a differential staining of the two sister chromatids allowing easy detection

of the exchanges. In vitro observations have shown that, in contrast to the S-independent agents, the S-dependent clastogenic agents are generally efficient in inducing SCEs (Carrano and Moore, 1982). No satisfactory mechanism has been proposed up to now for the production of SCEs and their biological significance is still unknown. Nevertheless, since the effects of many mutagenic agents can be readily detected at levels which cause almost no morphological chromosome damage, the induction of SCEs appears one of the most sensitive mammalian systems for detecting in vitro the mutagenic potential of chemicals.

The first results obtained on patients treated with cytostatic agents or after exposure to other toxic agents suggested that the scoring of SCEs in peripheral blood lymphocytes could represent an interesting method for detecting significant exposures of individuals to mutagens. Up to now, however, the method has not been proven to be a good monitor of human exposure for the majority of compounds analysed (Handsteen, 1982 ; and Table III 11).

5. Drawbacks of the cytogenetic analysis of cultured lymphocytes

Many of the studies that have been performed on persons occupationally exposed to chemicals do not permit to draw firm conclusions. The exposure to mutagens or suspected mutagens is generally low and, therefore, one can only expect a slight increase in structural aberrations in the peripheral blood lymphocytes. Nearly 50% of the approximately 500 x 10^9 lymphocytes of the human body are of the recirculating type with the consequence that a large number of cells must be analysed to get a representative sample of the lymphocyte population : the analysis of 250 cells corresponds roughly to a sampling of one cell per 10^9. When

few cells only are analysed, the presence or the absence of chromosome aberrations can result from the size of the sample.

Human lymphocytes not only vary with respect to their individual response to environmental mutagens but also with respect to their mitogenic activation. As early as 48 h after culture initiation, the culture time generally used, large numbers of second mitoses may already be present leading to an erroneous estimation of the yield of aberrations produced. At each cell generation about 50% of the unstable chromosome aberrations are eliminated (Conger, 1965 ; Sasaki and Norman, 1967 ; Carrano, 1973 ; Carrano and Heddle, 1973), and chromosome aberrations can develop from original chromatid-type aberrations as a result of the second division of cells (Dolphin et al., 1973). Furthermore, studies on normal and irradiated human blood cells have shown (Evans, 1984) that the incidence of gaps and chromatid aberrations in lymphocyte-chromosomes increases as cells are allowed to proceed through two or more cycles in culture.

Problems can also arise because human populations are often exposed to a mixture of potential mutagens. Any study should, therefore, take into account occupational history, age, previous or current exposure to drugs, toxic substances, ionizing radiations or viral infections. Such interferences probably explain the numerous reports of an increase of chromosome type aberrations such as centric rings or polycentric chromosomes in people exposed to S-dependent chemicals.

Proper controls are indispensable to detect an increase in the yield of structural changes above the normal aberration yield. Such control individuals should have a closely similar environmental background and be matched for sex and age with the exposed persons (Evans, 1984). Ideally, the best method would be to use cells from the same person before exposure but this is rarely feasible. Finally, the blood samples from control and

exposed groups should be processed together in order to minimize differences due to methodological factors (Evans, 1984).

Since the chromosome aberrations can be produced at any time during the employment (exposure) period, the disappearance of lymphocytes with aberrations has to be taken into account by multiplying the values observed by a correction factor, based on an exponential decline in aberrations corresponding to a half-live of 3 years and by averaging over the period of exposure (Table III.1).

Table III.1.

Correction factor to be used for the dicentric chromosome according to the duration of exposure.

Duration of exposure (years)	Correction factor
1	1.120
2	1.249
3	1.387
4	1.533
5	1.687
6	1.849
7	2.018
8	2.194
9	2.377
10	2.565
11	2.759
12	2.958
13	3.161
14	3.368

15	3.578
16	3.792
17	4.007
18	4.226
19	4.446
20	4.668
21	4.891
22	5.116
23	5.341
24	5.568
25	5.795
26	6.023
27	6.252
28	6.480
29	6.710
30	6.939
31	7.169
32	7.399
33	7.630
34	7.860
35	8.090
36	8.320
37	8.551
38	8.783
39	9.015
40	9.245

§ 2. BACKGROUND LEVELS OF CHROMOSOME ABERRATIONS IN PERIPHERAL BLOOD LYMPHOCYTES OF CONTROL POPULATIONS

1. Chromosome-type aberrations

The study of cytogenetic effects in humans exposed to ionizing radiations has provided a large amount of information on the yield of aberrations present in the peripheral blood lymphocytes of control populations. Table III.2. adapted from Lloyd et al. (1980) summarizes most data published up to 1980 on the background yields of dicentrics and acentrics (terminal and interstitial deletions). The mean levels of those anomalies are 0.78×10^{-3} and 3.7×10^{-3} respectively.

The baseline structural chromosome aberration frequencies (chromatid- as well as chromosome-type) can be influenced by age and by differences in the culture methodology (A. Léonard and Gerber, 1977 for review).

Table III.2.
Summary of published background levels for unstable chromosome aberrations in lymphocytes from control subjects (adapted from Lloyd et al., 1980).

Number of donors	Number of cells	Dicentrics	Acentrics	Reference
205	22 000	2	44	Sevankaev et al. (1974)
419	39 157	83	98	Awa et al. (1978)
105	16 267	11	116	Ivanov et al. (1978)

13	1 600	1	not given	Buckton et al. (1967)
16	1 600	-	2	Popescu and Stefanescu (1971)
9	227	-	-	El-Alfi et al. (1967)
10	1 738	1	9	Bocian and Ziemba-Zak (1972)
38	1 060	-	6	Buckton et al. (1967)
11	550	-	3	Abdulla et al. (1971)
3	527	-	6	Antoshchina et al. (1969)
1	580	-	8	
15	1 650	1	5	Bauchinger et al. (1976)
2	400	-	-	Bauchinger et al. (1975)
20	1 300	-	4	Bauchinger et al. (1972)
2	397	-	5	Bender and Barcinski (1969)
156	17 344	7	69	Blackwell et al. (1974)
2	3 202	1	10	Bocian et al. (1977)
1	400	-	-	Brewen and Gengozian (1971)
1	700	-	4	Brewen and Luippold (1971)
1	300	-	-	Brewen et al. (1972)
10	1 000	1	7	Brown and McNeill (1969)
2	600	3	1	Buckton and Baker (1972)
3	300	-	-	Chee and Ilberry (1975)
3	200	-	1	De Boer et al. (1977)
13	1 073	-	5	de la Chapelle et al. (1972)
10	1 000	1	12	Hoegerman et al. (1975)
5	928	2	5	Holmberg (1976)
10	1 000	-	3	Honda et al. (1969)
1	2 990	1	not given	Hori and Nakai (1978)
3	1 500	1	5	Kucerova et al. (1972)
4	400	1	2	Lilly (1975)

36	3 185	1	48	Liniecki et al. (1971)
1	230	-	-	Macintosh and Daev (1972)
26	2 600	2	6	McKenzie et al. (1977)
1	3 000	-	not given	Muramatsu and Matsuoka (1976)
1	2 295	-	not given	Norman (1967)
71	14 164	5	not given	Obe and Herha (1978)
316	6 207	4	20	O'Riordan and Evans (1974)
1	500	-	4	Preston and Brewen (1978)
50	5 000	1	13	Robinson et al. (1974)
11	9 510	2	39	Sasaki and Miyata (1968)
3	506	-	3	Schmid et al. (1972)
15	2 253	1	6	Schmid et al. (1976)
3	306	-	3	Schmid et al. (1974)
9	4 520	1	12	Stenstrand et al. (1979)
2	400	-	-	Stevenson and Wiernik (1974)
1	197	-	-	Suskov (1968)
11	791	-	-	Tamura et al. (1970)
1	2 000	-	5	Todorov (1975)
2	507	2	not given	Virsik et al. (1977)
6	300	-	3	Visfeld (1966)
3	5 400	-	15	Vulpis et al. (1978)
4	400	-	1	Watson and Gilles (1973)
8	1 700	-	4	Watts et al. (1972)
316	23 300	30	76	Lloyd et al. (1980)

2. Chromatid-type aberrations

As pointed out previously, most chemicals are S-dependent agents and in Go cells such as peripheral blood lymphocytes produce mainly chromatid-type aberrations. Table III.3. gives the yields of chromatid-type aberrations in control populations as reported in some recent studies.

Table III.3.
Examples of background levels for gaps and chromatid-type aberrations in lymphocytes from control subjects.

Number of donors	Number of cells	Gaps per 100 cells	Chromatid-type aberrations per 100 cells	Reference
23	11500	0.69	0.24	Léonard et al. (1984)
30	1312	1.14	0.8	Nordenson et al. (1979)
7	680	?	2.0	Bui et al. (1975)
298	4350	2.3	0.1	O'Riordan et al. (1978)
6	300	0	0.6	Shiraishi and Yoshida (1972)
11	1100	5.3	0.45	Waksvik et al. (1984)
20	1300	1.2	0.7	Schmid et al. (1972)
15	1500	2.1	0.7	Beckman et al. (1982)
12	1100	1.54	1.18	Forni et al. (1980)
32	3144	0.8	0.39	Clare et al. (1985)

3. Sister chromatid exchanges

Numerous methodological factors, such as the growth medium the sera used in the culture medium (Lambert et al., 1982 ; Kato and Sandberg, 1977), the culture temperature, the culture time or the amount of BrdU present in the culture medium relative to the amount of lymphocytes (Schneider, 1982 ; Lambert et al., 1982). Various environmental agents have been shown to influence also baseline SCE frequencies (Carrano and Moore, 1982 for review). For instance higher SCE levels have frequently been reported in smokers than in non-smokers (Lambert et al., 1978, 1982 ; Bala Krishna Murthy, 1979). Oral contraceptives, used actually by a large fraction of women, could also affect the measurement of human SCEs (Balakrishna Murthy and Prema, 1979). Severe protein malnutrition in children has also been reported to be associated with increased lymphocyte SCE frequencies (Balakrishna Murthy et al., 1980). However, the baseline SCE frequencies are, apparently, not influenced by age and sex (for reviews see Lambert and Olin, 1984 ; Carrano and Moore, 1982).

§ 3. INFLUENCE OF GENETIC CONSTITUTION ON THE SENSITIVITY TO CLASTOGENIC AGENTS

Several inherited diseases have been shown (for review Sasaki, 1982 ; Preston, 1985) to be associated with an increased chromosome instability and to display an hypersensitivity to environmental agents. The carriers usually show a high frequency of spontaneous chromosome aberrations in their bone-marrow cells, peripheral blood lymphocytes or cultured skin fibroblasts. Exposure to mutagens results in an abnormal incidence of

structural chromosome aberrations and/or sister chromatid exchanges due, in some cases, to defective DNA repair. An important feature is that most of these diseases are also associated with an unusually high frequency of cancers. Homozygotes for these inherited diseases are very rare but several observations (Swift et al., 1976) suggest that heterozygotes could be also more susceptible to mutagenic agents.

The best known mutations of this type are the Bloom's syndrome, Xeroderma pigmentosum, Ataxia telangiectasia and Fanconi's anemia.

Table III.4.

Examples of genetic diseases associated with chromosomal instability.

Disorder	Heredity
Bloom's syndrome	Autosomic recessive
Xeroderma pigmentosum	Autosomic recessive
Ataxia telangiectasia	Autosomic recessive
Fanconi's anaemia	Autosomic recessive
Cockayne's syndrome	Autosomic recessive
Werner's syndrome	Autosomic recessive
Chediak-Hygashi syndrome	Autosomic recessive
Kostmann's agranulocytosis	Autosomic recessive
Glutathione reductase deficiency anaemia	Autosomic recessive
Blacfan-Diamond syndrome	Autosomic recessive
Friedreich Ataxia	Autosomic recessive
Rothmund-Thomson syndrome	Autosomic recessive
Gardner's syndrome	Autosomic dominant
Polyomyositis	Autosomic dominant ?
Dyskeratosis congenita	X, recessive
Incontinentia pigmenti	X, dominant
Scleroderma	Multifactoriel
Rheumatoid arthritis	Multifactoriel ?
Systemic erythematosus	Multifactoriel ?
Down's syndrome	Trisomy 21

Table III.5.

Chromosomal susceptibility to mutagens and carcinogens, compared to controls (Sasaki, 1982).

Disorder	Mutagen						
	γ-ray	UV	4NQO	DCMMC	MMC	MMS	NNNG
Xeroderma pigmentosum							
classical	0.9	16.6	8.7	15.6	0.9	1.0	1.2
variant	1.0	1.1	1.0	1.2	1.0	1.1	-
Fanconi's anaemia	1.1	2.8	1.1	2.9	32.3	1.1	1.0
Ataxia telangiectasia	2.2	-	1.1	-	1.0	1.0	-
Werner's syndrome	0.8	1.0	1.0	-	0.9	0.8	-
Progeria	1.1	-	1.1	-	1.3	0.9	-
Incontinentia pigmenti	-	-	3.2	-	0.7	3.7	-
Dyskeratosis	1.1	1.0	1.0	-	1.0	1.0	-

4NQO = 4-nitroquinoline 1-oxide

DCMMC = decarbamoyl mitomycin C

MMC = Mytomycin C

MMS = methylmethanesulfonate

MNNG = N-methyl-N'-nitro-N-nitrosoguanidine

§ 4. RESULTS OBTAINED ON PEOPLE EXPOSED TO CHEMICALS

1. Structural chromosome aberrations

Two surveys (Ashby and Richardson, 1985 ; Léonard, 1986) of the papers published during the last decades on the incidence of chromosome aberrations in peripheral blood lymphocytes of persons exposed to chemicals have recently been made. Due possibly to the use of unappropriate methodology and because human populations are usually exposed to a mixture of potential mutagens the results obtained (tables III.8 and III.9) appear sometimes contraditory.

able III.8.
esults of cytogenetic observations performed on people exposed to heavy metals (adapted from Léonard, 1985).

Metal	Test	Type of exposure	Results	References
rsenic	Structural aberrations	Medical treatment	+	Petres et al. (1977)
			+	Nordenson et al. (1979)
			-	Burgdorf et al. (1977)
		Pesticides	+	Petres et al. (1970)
		Smelter	+	Beckman et al. (1977)
			+	Nordenson et al. (1978)
			+	Beckman et al. (1979)
			+	Nordenson and Beckman (1982)
	SCE	Medical treatment	+	Burgdorf et al. (1977)
		Medical treatment	-	Nordenson et al. (1979)
admium	Structural aberrations	Itai-itai disease	+	Shiraishi and Yoshida (1972)
			+	Shiraishi (1975)
			-	Bui et al. (1975)
		Pigment industry	-	O'Riordan et al. (1978)
		Zinc smelter	+	Deknudt et al. (1973)
			+	Deknudt and Léonard (1975)
			+	Schmid et al. (1972)
			+	Bauchinger et al. (1976)
		Battery manufactory	-	Bui et al. (1975)
hromium	Structural aberrations	Chromium plant	+	Bigaliev et al. (1977)
		Stainless steel welder	-	Littorin et al. (1983)
			-	Husgafvel-Pursianen et al. (1982)
	SCE	Chromium plant	-	Azhajev (1984)
		Stainless steel welder	-	Husgafvel-Pursianenet al. (1982)
			-	Littorin et al. (1983)

Lead	Structural aberrations	Lead oxide manufacture	+	Schwanitz et al. (1970, 1975)
			-	Sperling et al. (1970)
		Shipbreaking yard	-	O'Riordan and Evans (1974)
		Bus drivers	+	Högstedt et al. (1979)
		Policemen	-	Bauchinger et al. (1972)
			-	Schmid et al. (1972)
		Copper smelter	+	Nordenson et al. (1978)
			-	Nordenson et al. (1982)
		Lead smelter	-	Nordenson et al. (1982)
			-	Mäki-Paakkaner et al. (1980)
			-	Schmid et al. (1972)
			+	Bauchinger et al. (1976)
		Lead industry	+	Deknudt et al. (1977)
			+	Calugar and Sandulescu (1977)
			+	Forni et al. (1976, 1979, 1980)
			+	Forni (1967)
			-	Hoffman et al. (1985)
		Children living in contaminated area	+	Forni et al. (1979)
			-	Bauchinger et al. (1977)
		Lead intoxication	-	Biscaldi et al. (1969)
			±	Qazy et al. (1980)
		Volunteers	-	Bijlsma and De France (1976)
	SCE	Lead smelters	±	Mäki-Paakanen et al. (1980)
		Children living in contaminated area	-	Dalpra et al. (1983)
Organic Mercury	Structural aberrations	Contaminated fish	+	Skerfving (1974)
			+	Skerfving et al. (1970, 1974)
		Minamata disease	+	Kato et al. (1976)
Inorganic mercury		Mercury industry	+	Popescu et al. (1979)
			+	Verschaeve et al. (1976, 1978a,b)
		Chloroalkali plant	-	Verschaeve et al. (1979)
			-	Mabille et al. (1984)
Nickel	Structural aberrations	Nickel refineries	+	Boysen et al. (1980)
			+	Waksvik and Boysen (1982)
			+	Waksvik et al. (1984)
	SCE	Nickel refineries	-	Waksvik et al. (1984)

Table III.9.

Examples of cytogenetic observations (structural aberrations) made in persons exposed to organic compounds (for a more comprehensive review, see Ashby and Richardson, 1985).

Compound	Result	Reference
Pesticides	-	Dulout et al. (1985)
Rubber industry	-	Degrassi et al. (1984)
	-	Mäki-Paakkanen et al. (1984)
Ethylene oxide	-	Clare et al. (1985)
Asbestos	+	Srb et al. (1984)
Benzene	-	Clare et al. (1984)
	-	Sarto et al. (1984)
Ethylene oxide	-	Hansen et al. (1984)
	+	Laurent et al. (1984)

2. Micronuclei in human somatic cells

In spite of the fact that the yield of micronuclei can be considered as a good indicator of the structural chromosome aberrations induced by clastogenic agents very few studies have so far been published. The Table III.10 gives some examples of the results obtained recently with this method. Some additional results can be found in the review by Sorsa (1984). As discussed previously (§1) this technique can also be used to detect the production of chromosome aberrations in other types of cells than lymphocytes such as exfoliated cells from the mucosalcavity or the bladder.

Table III.10.

Micronuclei in somatic cells from subjects exposed to various chemicals.

Agent	% of cells with micronuclei			Reference
	Erythroblasts	Polychromatic erythrocytes	Lymphocytes	
Controls			7.7	Norman et al. (1985)
			2-7	Krepinsky and Heddle (1983)
			4	Hogstedt (1984)
Petroleum vapors				
High exposure	3.2	12.7		Högstedt et al. (1981)
Low exposure	3.4	7.0		
Control	1.0	3.2		
Cytostatics				
	Erythrocytes	Erythroblast		Abe et al. (1984)
Controls	7.2	0.4		
Exposed	253	2.9		

3. Sister chromatid exchanges

Numerous studies have investigated the increase of SCEs in subjects exposed to genotoxic agents (Table III.11, Sorsa, 1984 and Ashby and Richardson, 1985). Due probably to the existence of many confounding factors such as smoking habits and to the fact that the yield of SCEs is directly related to the amount of chemicals present in the vicinity of the cells at the time of the DNA replication the method, as pointed out previously (§1), has not been shown to be a good indicator of human exposure for the majority of compounds analysed.

Table III.11.

Examples of SCE frequencies associated with occupational exposures to various chemicals (for additional results see Sorsa, 1984 and Ashby and Richardson, 1985).

Agent	SCE/cell		References
	Control	Exposed	
Organic solvent	13.1	19.7	Funes-Gravioto et al. (1977)
	14.0	19.9	Lambert and Lindblad (1980)
Ethylene oxide	14.0	16.3	Lambert and Lindblad (1980)
	6.4	10.3	Garry et al. (1979)
Toluene	8.0	7.9	Mäki-Paakkanen et al. (1980)
Tetrachlorethylene	8.2	8.6	Ikeda et al. (1980)
Styrene	7.5	8.4	Anderson et al. (1980)
Epoxy resins	8.7	9.0	Mitchman et al. (1980)
Vinyl chloride	9.4	13.8	Kucerova et al. (1979)
Pesticides	5.5	6.4	Duclout et al. (1985)
Furfuryl alcohol and furfural	7.4	7.5	Gomez-Arroyo (1985)
Vulcanizers	5.2	5.2	Degrassi et al. (1984)

§ 5. BIOLOGICAL SIGNIFICANCE OF CYTOGENETIC CHANGES

1. Relation between chromosome aberration induction and probability of cancer occurence

1° Structural and numerical chromosome aberrations

Numerous studies have demonstrated that chromosome aberrations are regularly associated with cancer process and, if we except some acute leukemias in man and certain tumors induced in rodents by RNA viruses, nearly every mammalian neoplasm is characterized by chromosome anomalies. These aberrations, however, have to be considered as indicators rather than as the cause of the disease. It should be pointed out that clastogenic agents could play a role not only at early stages of tumourigenicity but could also act as promotors. Promotors such as phorbol esters display clastogenic properties and have also been shown to cause aneuploidy (Parry et al. 1981). Progression of tumours involves also generally structural and numerical changes (Klein and Klein, 1985).

To date, however, we are still far from being able to establish, for man, a quantitative estimate of the relationship between the yield and type of chromosome aberrations induced in somatic cells by exposure to a clastogenic agent and an increased risk to develop a cancer although some observations made on mice (Sugiyama, 1973) show a close correlation between the amount of chromosome damage induced in bone marrow by various benz(a)anthracene derivatives and the ability of these substances to cause fibrosarcomas.

In 1972, King et al., reported the lack of association between chromosome aberrations and clinical findings on postexamination for atomic

survivors. More recently Kamada and Tanaka (1983) confirmed that the presence of abnormal clones in the bone marrow does not apparently contribute to leukemia progress. One cannot exclude, however, the possibility that the presence of cytogenetically abnormal cells in the bone marrow, T and B lymphocytes, clone formation, clonal selection and functional deviations may be related, in some way, to leukemia development, cancer, and other types of malignancies among atomic bomb survivors.

The same conclusions can be drawn from observations on people exposed to chemicals. Epidemiological studies have firmly established, for instance, that cigarette smoking causes anaplasic and squamous cell bronchial carcinomas whereas cytogenetic studies has previously shown an increase in chromosome aberrations in blood lymphocytes of heavy smokers compared with non-smokers (Hopkin and Evans, 1980).

2° Sister-chromatid exchanges

As pointed out previously SCEs are cytogenetic events that can be related to a number of processes involving DNA damage and/or repair but, so far, their biological significance has not been elucidated. In spite of the fact that different S-dependent clastogenic agents displaying carcinogenic properties have been shown to be able to produce SCEs in peripheral blood lymphocytes after acute or chronic exposure to various chemicals no correlation has been established between an enhancement of the yield of such anomalies and an increased risk of cancer.

2. Relation between the anomalies observed in somatic cells and the production of transmissible chromosome aberrations into germ cells

Quantitative correlations between somatic damages and changes in the germ cells would allow extrapolation from observations on peripheral blood lymphocytes. Several attempts have been made to estimate the production of transmissible chromosome aberrations into germ cells from the yield of anomalies observed in somatic cells. A review of the literature made recently by van Buul (1982) demonstrates however, that, for man, such extrapolation cannot be made on the basis of results obtained with experimental mammals. Several factors, such as species, chromosomal constitution, or genetic constitution have differential effects on the induction of chromosomal anomalies in somatic cells and in germ cells. Since observations on human germ cells are extremely scarce it is currently impossible to estimate from the yields of aberrations observed in peripheral blood lymphocytes the risk of an increased incidence of offspring carrying genetic defects.

In that respect, it is of interest to point out that a survey of the chromosomes from 2908 children of A-bomb survivors (Awa et al., 1976) did not reveal a statistically significant increase of children carrying chromosome aberrations. The authors themselves conclude, however, that the sample size of their controls (1 127) was "not large enough to obtain valid estimates of the true frequency of individuals with various chromosome abnormalities of either inherited or spontaneous origin in the Japanese general population".

3. The use of chromosome aberrations observed in human somatic cells to assess the probability to develop teratogenic effects

It is evident that agents displaying clastogenic properties can interfere with the development in several ways other than by directly damaging genetic material (German, 1979). No serious attempt has been made to correlate the yield of aberrations observed in peripheral blood lymphocytes with embryotoxic or teratogenic effects. It should be stressed, however, that increases of miscarriages and stillbirths have frequently been reported in populations exposed to abnormally high levels of certain genotoxic chemicals (Nordstrom et al., 1978a,b ; Hemminki et al., 1983).

§ 6. CONCLUSIONS

During the last decade, numerous studies have been published which attempt to monitor human exposure to genotoxic chemicals by the detection of structural chromosome aberrations and sister chromatid exchanges in peripheral blood lymphocytes. The approach is certainly promising for detecting exposure to genotoxic chemicals. Better standardizations of test protocols, however, is required.

Chromosome damages observed in blood samples can be considered as indicators of mutagenic actions of environmental agents. Furhtermore, it should be remembered that clastogenic agents are generally able to produce also gene mutations. In spite of the close relationship between the carcinogenicity and mutagenicity of chemicals it is, at the present time, impossible to use the yield of aberrations observed in peripheral blood lymphocytes to estimate the probability for the carrier to develop a cancer or to give birth to offspring displaying genetic defects.

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CHAPTER IV

THE PRODUCTION OF GENE MUTATIONS IN SOMATIC CELLS

§ 1. PRINCIPLES OF THE METHODS

Various methods to detect gene mutations in somatic cells of humans exposed to chemicals are presently under development.

(1) One of the most promising techniques is based on the detection of 6-thioguanine-resistant (TG') cells in peripheral blood lymphocytes (Albertini, 1980, 1982, 1985 ; Albertini and De Mars, 1974 ; Albertini and Sylwester, 1984 ; Albertini et al., 1981, 1982a, 1982b).

Purine analogue resistance is determined by the capacity of the variant TG^r cells to incorporate ^3H-thymidine in response to stimulation by phytohemagglutinin in the presence of the purine analogues which normally inhibit such incorporation. The culture interval is short (30 hr of culture plus 12 hr of labeling) and is insufficient for the stimulated lymphocytes to complete their first cell cycle and to be in their second DNA synthesis phase. Therefore, the in vitro TG^r frequencies will directly reflect the in vivo frequencies.

Two different methods are currently proposed (Albertini, 1985) to detect the production of TG^r cells :

- the first one which involves autoradiography (Strauss and Albertini, 1977, 1979 ; Albertini, 1982 ; Albertini et al., 1984) is relatively inexpensive and can be adapted for automation (Zetterberg et al., 1982 ; Amneus et al., 1982 ; Stark et al., 1984) but the mutational origin of the TG^r cells cannot be verified ;
- a newer method involves clonal assay and allows unequivocal identification of mutant cells (Albertini et al., 1982a, 1985 ; Morley et al., 1983b ; Morley et al., 1985) ;
- an extension of the Albertini's method for in vivo studies consists in growing cells in the presence of 6-thioguanine for the selection of HGPRT mutants, and then pulsing the cells with bromodeoxyuridine (BrdU) - containing medium before termination of the culture (Gratzner, 1984).

(2) The detection of human red cells containing hemoglobin mutants could represent an interesting method to study the production of gene mutations arising in the hemopoietic stem cells of subjects exposed to mutagens (Papayannopoulou et al., 1976, 1977 ; Stamatoyannopoulos, 1979 ; Stamatoyannopoulos et al., 1980, 1984). The method is based on the detection of circulating red blood cells containing S or C variant hemoglobins in addition to the normal forms i.e. heterozygous for an abnormal hemoglobin. Two antibodies, one specific for hemoglobin S and the other for hemoglobin C recognize and bind tightly to these hemoglobin types but not to the normal A one. These variant hemoglobins differ from the normal hemoglobin amino acid sequence by single amino acid substitutions. Initially the scoring of mutant cells was performed manually under a fluorescence microscope but this method was not practical because the determination of the mutation rate of a single gene implies the screening

of very large number of cells. According to Stamatoyannopoulos et al. (1984) visual screening by one person of 10^8 red cells takes 2 months ! The development of a hemoglobin antibody staining method for red cells in suspension and the use of high speed sorter technology capable of processing large numbers of cells with precision and quantitatively sorting antibody labeled, presumptively mutant, cells for subsequent analysis could open new interesting perspectives (Bigbee et al., 1981 ; Bigbee and Branscomb, 1984). According to Bigbee and Branscomb (1984) a total processing time well under 1 hr per sample could be achieved by using flow cytometric processing but it would remain necessary, probably, to perform a final count manually in order to detect possible false positive artefacts.

(3) Another technique is the glycophorin test, which measures the expression loss of the glycophorin A gene in human erythrocytes (Bigbee et al., 1984 ; Bigbee and Branscomb, 1984). Glycophorin A is a glycosylated red cell membrane protein present at about 5-10 x 10^5 copies per cell (Gahmberg et al., 1979). This protein is responsible for the M and N blood group determinants defined by a polymorphism in the amino acid sequence of the protein coded for by a pair of co-dominantly expressed alleles (Furthmayer, 1978). The assay aims at detecting erythrocytes in the blood of glycophorin A heterozygotes which fail to express one or the other of the two allelic forms of the pattern. These assays, however, must still be validated by determining the background rates in unexposed control populations, the dose-response relationship in mutagen-exposed individuals and by a direct biochemical verification of sortant variant cells (Bigbee, 1984).

§ 2. RESULTS OF OBSERVATIONS PERFORMED ON HUMANS EXPOSED TO CHEMICALS

1. TG^r mutant in peripheral blood lymphocytes

The TG^r peripheral blood lymphocytes frequencies are of the order of 10^{-6}-10^{-5} in normal individuals (Albertini, 1985 ; Strauss, 1982 ; Morley et al., 1983, 1985 ; Vijayalaxmi and Evans, 1984a,b) and could be influenced by age (Trainor et al., 1984 ; Vijayalaxmi and Evans, 1984a). Their incidence is significantly increased in cancer patients receiving chemotherapy (Dempsey et al., 1985), in persons handling cytotoxic drugs (Chrysostomou et al., 1984) or in PUVA treated psoriatic patients (Albertini et al., 1982). A quantitative estimate of the incidence of mutant cells induced in those persons is, however, difficult because the method used in some studies does not discriminate true mutants from phenocopies (Albertini, 1985).

2. Detection of hemoglobin mutants

Due to the time required to perform such studies, few observations have been made on the occurence of S- and C-like cells in humans (Stamatoyannopoulos et al., 1984). In a study on 15 healthy males and females the frequency of S-like or C-like cells ranged from 3×10^{-7} to 4×10^{-8} (mean 1.1×10^{-7}). The yield of S-like cells increased : to a mean of 1.7×10^{-7} (range 3.1×10^{-7} to slightly less than 1×10^{-7}) in five subjects who received X-irradiation of the spine during treatment of ankylosing spondylitis ; to 5×10^{-7} in a subject given a combination of X-ray and chemotherapeutic treatment of Hodgkin's disease several years

prior the screening. The yield of mutant cells was, however, normal in the four other persons examined.

Promising results have been obtained also by Zetterberg (1984) in the detection of red cells containing hemoglobin mutants in blood samples of control donors, cancer patients treated with cytostatics and patients treated with psoralen and UV light.

§ 3. BIOLOGICAL SIGNIFICANCE OF THE GENE MUTATIONS INDUCED INTO SOMATIC CELLS

It is probable that mutations induced in vivo in human somatic cells are due to gene alterations (Turner et al., 1985). These assays, however, quantitate somatic mutants but not necessarily somatic mutations (Albertini, 1985).

Many questions remain, also, about the translation of these results into genetic risks and actually it is not known whether such mutations are predictable of neoplasic transformation or cancer promotion.

§ 4. CONCLUSIONS

Several systems for detecting mutations in somatic cells appear promising, but the lack of sensitivity of these tests currently available makes them only useful to identify population with high exposure to genotoxic chemicals.

Furthermore, the detection of gene mutations in vivo in human somatic cells is extremely laborious and time consuming.

It appears, therefore that the use of such methods for routine monitoring of persons occupationally exposed to suspected or known mutagens is closely dependent on the possibility of developing methods allowing the scoring of high numbers of cells in short periods of time together with a distinction between true mutants and phenocopies.

It should also be stressed that these assays must still be validated by determining the background rates in control populations, the dose response relationship in mutagen-exposed individuals and also by direct biochemical verification of sorted variant cells.

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CHAPTER V

DETERMINATION OF PROTEIN OR NUCLEIC ACIDS ADDUCTS (MOLECULAR DOSIMETRY)

The most probable mechanism of tumour induction by genotoxic carcinogens involves the covalent binding of the compound or its metabolites to DNA in the target organ. Most genotoxic carcinogens are electrophilic compounds or are biotransformed to electrophilic metabolites which can bind to nucleophilic sites on biological macromolecules such as DNA or proteins. The binding of carcinogens to DNA and the resulting formation of DNA adducts can be directly demonstrated by identifying these adducts either in cellular DNA or in degradation products of DNA excreted in urine, or indirectly by measuring the adducts formed with non target macromolecules such as proteins.

§ 1. DETERMINATION OF PROTEIN ADDCUTS

A number of studies in which animals were exposed to radioactively labeled carcinogens indicate that for various types of genotoxic compounds the ratio between DNA-adducts formation in blood cells and binding to hemoglobin is constant over a wide dose range (Neumann, 1983). Under these conditions, the hemoglobin

binding test might be used as an indirect estimate of DNA binding. Hemoglobin and albumin are the two most promising proteins for dosimetry. Hemoglobin is abundant and contains amino, sulfhydryl and aromatic groups with which carcinogens can react. In addition, the lifetime of hemoglobin in the red blood cell being about 120 days, the circulating level of hemoglobin adducts can reflect the exposure over several months. Albumin is also abundant and reactive, it has a half-life of 20-24 days and is synthetized in the hepatocyte, a site where many carcinogens are metabolized to their reactive forms.

A list of some directly or indirectly alkylating agents binding to hemoglobin or albumin is given in Table V.1. The directly acting carcinogens usually bind to hemoglobin to a greater extent than indirect acting carcinogens (Perera and Chang, 1981). This is probably due to the fact that the reactive metabolites of indirect carcinogens usually formed in the liver are detoxified or bind to liver macromolecules before entering the bloodstream and reacting with hemoglobin.

Table V.1.

Protein binding of directly acting or metabolically activated carcinogens (from Farmer et al., 1984).

Compound	Protein	Species
Methyl methanesulfonate	Hb	mouse
	Hb	rat
	Hb	rat
N-nitrosodimethylamine	Hb	mouse
	serum	rat
	Hb	rat
	Hb	rat
	erythrocyte[a]	human
Methyl bromide	Hb	mouse
Methyl chloride	erythrocyte[a], plasma	human
Dichlorvos	Hb	mouse
Ethylene oxide	Hb	mouse
	Hb	human
	Hb	rat
	Hb	mouse
Propylene oxide	Hb	rat
Vinyl chloride	Hb	mouse
Ethylene	Hb	mouse
Benzo(a)pyrene	Hb	mouse
	Hb	mouse
	Hb	rat
Chloroform	Hb	rat, mouse

2-Acetylaminofluorene	Hb	rat
	Hb	rat, mouse
	Serum	rat
Benzyl chloride	Hb	mouse
Aflatoxin B_1	Hb	rat
trans-dimethylaminostilbene	Hb, plasma	rat
trans-4-aminostilbene	Hb, plasma	rat
4-Aminobiphenyl	Hb, albumin	rat

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[a] In vitro experiment

To be meaningful, protein adduct determination requires that the degree of binding to blood proteins is related to the exposure dose, or more importantly to DNA-binding in the target organ. Animal data available so far suggest that these conditions might be satisfied for some alkylating agents. A relationship between exposure dose of alkylating agent and the production of alkylated aminoacids has been demonstrated in animals treated with methyl methanesulfonate (Bailey et al., 1981; Segerbäck et al., 1978), trans-4-dimethylamino-stilbene (Neuman, 1980), chloroform (Perera and Chang, 1982) and acetylaminofluorene (Perera et al., 1981). In mice and rats treated with ethylene oxide, the extent of DNA-alkylation in liver, spleen and testis can be estimated from the degree of hemoglobin alkylation (Segerback, 1983; Osterman-Bolbar et al., 1983).

Relationships between liver DNA alkylation and hemoglobin alkylation have also been demonstrated in rodents treated with trans-4-dimethylaminostilbene and 2-acetylaminofluorene (Neuman, 1980; Perera et al., 1981).

So far, the only human data available on protein adducts are that of Calleman et al. (1978) in workers exposed to ethylene oxide. The results obtained by these authors suggest that the degree of N-3-hydroxylation of histidine of hemoglobin can be used to estimate the integrated exposure to ethylene oxide during the last 4 months.

The main advantage of protein adducts determination as a monitoring method for genotoxic cancerogens is that it can provide an exposure index integrated during the lifespan of the protein. The major limitation of this method is that the analytical procedure is time consuming, requires rather sophisticated instruments (e.g. mass spectrometer) and is thus not yet really suitable to routine application. Furthermore, relatively high background levels have been reported for some alkylated aminoacids (Bailey et al., 1981, Farmer, 1982, Osterman-Golkar et al., 1983) which may constitute an additional limitation to the method, particularly for monitoring low exposure levels. The source of this background is unknown but its importance seems to be species dependent.

§ 2. DNA ADDUCTS

DNA adduct determination is currently considered as one of the most promising monitoring method for genotoxic carcinogens. It has the potential of estimating directly the biologically effective dose of initiating carcinogen.

The extent of DNA binding expressed as the covalent binding index* can be quantitated "in vivo" in animal after administration of radioactive chemicals. So far more than 100 chemicals have been shown to bind to DNA "in vivo" among which well known carcinogens such as aflatoxins, various polycyclic aromatic hydrocarbons (e.g. benzo(a)pyrene), acetylaminofluorene, nitrosamines, benzidine, 2-naphtylamine (Lutz, 1979).

For a series of polycyclic aromatic hydrocarbons, their ability to bind to DNA in mouse skin is correlated with their carcinogenic potency (Brooks and Lewley, 1964, Gashman and Heidelberger, 1967). In rat, the ability of hepatocarcinogens to bind to liver DNA also correlates with their carcinogenic potency (Table II.2). These data show that potent genotoxic carcinogens usually have a high affinity for DNA. This suggests that for these compounds,

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* CBI : $\frac{\text{micromole chemical bound per mole nucleotides}}{\text{millimole chemical administered per kg animal}}$

monitoring DNA adducts may assess the biological effective dose and possibly the associated cancer risk.

Covalent adducts to nucleic acids have different levels of stability. Some are removed spontaneously through chemical depurination whereas others are removed enzymatically during the process of DNA repair. A small proportion of adducts remains in DNA.

Two complementary approaches are available to estimate DNA adducts. Firstly, DNA adducts can be quantitated in cells of accessible tissues (e.g. white blood cells, biopsy). Provided that the chemical nature and stability of the DNA adducts are well characterized, this approach could provide an indication of the exposure history of the monitored person and of his ability to activate the procarcinogen to electrophiles capable of binding to DNA.

Table V.2.

Correlation of hepatocarcinogenicity of chemicals in the rat with the covalent binding index, CBI, for rat-liver DNA (from Lutz, 1979).

Compound	Range of CBI
Strong hepatocarcinogens	
Aflatoxin B1	17 000
Aflatoxin M1	1 600
Dimethylnitrosamine	6 000
Diethylnitrosamine	42-430
Moderate hepatocarcinogens	
Aflatoxin B2	560
2-Acetylaminofluorene	560
Vinyl chloride	525
o-Aminoazotoluene	230
Nitrosopyrrolidine	180
Nitrosopiperidine	120
Nitrosomorpholine	44
Weak hepatocarcinogens	
Urethane	29-90
4-dimethylaminoazobenzne	6
p-Aminoazobenzene	2
Ethionine	1
Non-hepatocarcinogens	
Saccharin	< 0.005

Another approach for monitoring DNA adducts takes advantage of the fact that adducts removed from cellular nucleic acids are excreted in urine. Their determination might provide information on recent exposure to genotoxic chemicals and perhaps on the individual capability for DNA repair.

Three types of methods to detect DNA-adducts in human are currently under development : i) immunological methods; ii) physico-chemical methods; iii) postlabelling method. Immunological methods are currently at the most advanced stage of development. Table V.3. lists structurally modified DNA-compounds for which polyclonal or monoclonal antibodies have been produced.

Some of these antibodies have already been used to screen potentially exposed individuals. For instance, benzo(a)pyrene-DNA adducts have been detected in the DNA of lung tissue of patients with lung cancer (Perera et al., 1982). Using an antibody against cis-diamminedichloroplatinum (II)-modified DNA, Poirier (1984) has detected adducts in circulating lymphocytes of patients treated with cis-platinum. O^6-methyl-deoxyguanosine adducts have been found in oesophageal tissue obtained from Chinese patients living in an area with high oesophageal cancer incidence (Garner, 1985). Immunoassays for DNA adducts are sensitive, specific and suitable to routine monitoring. However, their drawback is the fact that the structure of the adducts must be known. Furthermore, a specific and high affinity antibody must be raised for each type of adduct.

Table V.3.

Modified DNA components for which immunoassays have been described.

O^6-Methyl-2'-deoxyguanosine	Wild et al. (1983)
O^6-Butyl-2'-deoxyguanosine	Shaffhill et al. (1982)
O^4-Butyl-2'-deoxythymidine	
O^2-Butyl-2'-deoxythymidine	
O^6-Ethyl-2'-deoxyguanosine	Rajewsky et al. (1980)
O^6-Isopropyl-2'-deoxyguanosine	
O^4-Ethyl-2'-deoxythymidine	Adamkiewicz et al.(1982)
8-Acetoxy-N-2-acetylaminofluorene-2'-deoxyguanosine	Kriek et al. (1984)
8-Acetoxy-aminofluorene-2'-deoxyguanosine	Poirier et al. (1979)
Benzo(a)pyrene-modified DNA	Poirier et al. (1980) Kriek et al. (1984)
Aflatoxin B_1-modified DNA	Haugen et al. (1981)
Ultra-violet-modified DNA	Strickland & Boyle (1981)
Cis-diamminedichloroplatinum (II)-DNA	Poirier et al. (1982) Fichtinger-Schepman et al. (1985)

A few physio-chemical methods have been developed for measuring some DNA-adducts such as gas chromatography-mass spectrometry (Shucker et al., 1984), luminescence spectrometry (Rahn et al. , 1980) or fluorescence spectroscopy (Daudel et al., 1975). Using the latter technique, Autrup et al. (1983) detected a putative aflatoxin B1-guanine adduct in 6 of 81 urine samples from people living in an area of Kenya where contamination of food with aflatoxin B1 is known to occur frequently.

The ^{32}P postlabelling method is a nonspecific procedure for detecting adducts in cellular DNA (Randerath et al., 1981). This method can thus be used for a preliminary screening of DNA adduct formation. Briefly, the method consists of digesting purified DNA to deoxyribonucleoside-3'-monophosphates which are then enzymatically labeled with ^{32}P and subjected to thin-layer chromatography followed by autoradiography. So far, the only application of this method to man is the detection of smoking related DNA adducts in placenta (Everson et al. in press, cited by Hemminki and Randerath, 1985).

§ 3. CONCLUSION

The determination of protein or nucleic acids adducts (i.e. molecular dosimetry) is currently considered as one of the most promising methods for monitoring human exposure to carcinogenic or mutagenic

chemicals. It has the potential of estimating directly (DNA adducts) or indirectly (protein adducts) the biological effective dose of initiating carcinogens. In addition, in the case of protein adducts, this method may provide an integrated index of exposure.

Presently, the major limitation of this approach seems to be the analytical aspect which is still too elaborate for routine monitoring, particularly in the case of DNA adducts. But besides this methodological difficulty, other problems must be solved before considering the application of these tests for biological monitoring. For instance, little is know about the background level of certain protein or DNA adducts in man. The health significance of adduct formation remains to be established. Adducts formed with proteins are non-specific with respect to the biological consequences. They may however permit to assess the tissue dose if their occurrence correlates with adduct formation in DNA of target cells. There is also so far no evidence that an increased incidence of DNA adducts can always be quantitatively translated in a higher cancer risk.

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CHAPTER VI

UNSCHEDULED DNA SYNTHESIS

The majority of the alterations produced by mutagens to DNA are, normally, repaired. Although our knowledge of repair systems in mammals is still limited, several methods are currently available to study these processes (Regan and Setlow, 1973 ; Cleaver, 1977). Measurement of the patching step of repair (unscheduled DNA synthesis ; UDS) is, probably, the only one applicable for screening purpose, the most commonly used technique being based on differences of ^{3}H uptake between cells from control and exposed subjects (Cleaver, 1984).

Several genetic disorders (see Chapter III) which increase the sensitivity to environmental mutagens are associated with DNA repair deficiencies as shown by in vitro experiments with Xeroderma pigmentosum cells, Ataxia telangiectasia or Cockayne syndrome cells (Cleaver, 1984 for review). Different studies reveal also, the existence in the normal population of an interindividual variation in the level of UDS induced by genotoxic agents (for review Vakakangas et al., 1984 ; Laval and Huet, 1984). In a total of 266 individuals Pero (1984) reported a 3-fold interindividual variation in N-acetoxy-2-acetylaminofluorene (NA-AAF) induced UDS. Comparable interindividual variation in repair has been reported by Lake (1984) after in vitro exposure of monocytes to MNNG (small patch repair) and/or NA-AAF (long patch repair), by Lambert et al. (1979) in human leucocytes and also in the bromodeoxyuridine photolysis test in normal fibroblast cultures (Settlow, 1983).

In addition to genetic variability (Lake, 1984) variability, exogenous factors such as smoking habits, season of sampling and drug consumption might influence UDS (Madden et al., 1984). In the study of Madden et al. (1984) the sex, use of alcohol, caffeine or marihuana did not influence the level of UDS. The authors were unable to confirm the observations of Lambert et al. (1979), Collins et al. (1977) and Erixon and Ahnstrom (1979) with regard to the influence of age on UDS changes. Neither age nor cigarette-smoking were found to affect the UDS response in workers of the rubber industry (Benigni et al., 1984).

Since the method is still under development very few papers on UDS have been published so far but significant effects have been reported in workers exposed to 1-40 ppm styrene (Pero et al., 1982a) or to less than 12 ppm of ethylene oxide or propylene oxide (Pero et al., 1982b). Significant decrease in the ability to repair damage to DNA has also been reported in patients having undergone treatment for leukemia (Bohr and Køber, 1985) and among workers in the rubber industry (Benigni et al., 1984).

As described previously, DNA repair deficiencies have been shown to be associated with a number of clinical syndromes (Friedberg et al., 1979) and with the increased incidence of cancers frequently encountered in those genetic defects (Setlow, 1978) even though the relationships between sensitivity to mutagens and genetic disorders appear rather complex (Arlett and Lehman, 1978 ; Generoso et al., 1980 ; Pointer and Young, 1980). For most mutagens, there seems to exist a correlation between their ability to produce chromosome aberration and SCEs and their ability to interfere with DNA repair (Generoso et al., 1980 ; Natarajan et al., 1982 ; Paterson, 1978 ; San and Stich, 1977).

CONCLUSION

The study of unscheduled DNA synthesis appears to be applicable for monitoring exposure to mutagenic or carcinogenic chemicals. Several genetic defects can interfere with the results obtained and the data available are still limited. It is, therefore, difficult to estimate actually, to which extent the method can be used for routine screening.

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CHAPTER VII

DETERMINATION OF TUMOR MARKERS IN BIOLOGICAL FLUIDS

§ 1. DEFINITION

Tumor markers can be defined as substances which are significantly associated with neoplasic diseases. The association might be qualitative in the case of a marker detectable in neoplasic disorders only. But so far, no tumor marker specifically associated with neoplasms has been described and the existence of such marker is questionable. For all tumor markers currently available, the association with the occurrence of transformed cells is quantitative and results from an increased production of a substance usually present in trace amounts in the healthy organism. More than 100 substances have been proposed as potentially useful tumor markers. These substances do not belong to a specific biochemical category. They fall into several biochemical groups depending on their structure, origin and function : antibodies, enzymes, hormones, antigens, nucleosides, amines, ...

Most tumor markers are measured in blood, but in some circumstances, their determination in other biological fluids such as urine or even cerebrospinal fluid is more justified.

§ 2. MECHANISMS OF APPARITION OF TUMOR MARKERS

Several mechanisms can be involved in the appearance of tumor markers in biological fluids (Franchimont et al., 1982) :

1. Derepression of silent genes resulting from the neoplasic transformation.

This mechanism is responsible for the appearance of the oncodevelopmental tumor markers i.e. the markers which are produced by both developmental (i.e. foetal, trophoblastic, placental...) and neoplasic tissues. The most commonly used oncodevelopmental tumor markers are the carcinoembryonic antigen (CEA), α-foetoprotein (AFP) and the chorionic gonadothrophic hormone (HCG). These markers are also called universal markers because they can derive from the 3 germinal layers (ecto- endo- and mesoderma).

2. Specific production by the tissue from which derives the neoplasm.

Ferritin in hepatocellular carcinoma and calcitonin in medullar carcinoma of the thyroïd are examples of markers resulting from an increased production by the tissue of origin. However, the production by the cancerous cell is often qualitatively and quantitatively different from that by the normal cell. The protein produced has not always exactly the same physicochemical characteristics of that synthetized by the normal tissue. The ferritin of malign origin is, for instance, more acid than that present in normal plasma. The production by the cancerous cell is also usually lower than that by the normal cell. The increased production of these markers results in fact from the proliferation of neoplasic cells.

3. Increased cellular proliferation or turnover.

Modified nucleosides, which are degradation products from nucleic acids and particularly from t-RNA, are normal constituants of urine. Their determination may be used to detect an increased turnover of t-RNA. The tissue polypeptide antigen (TPA) is also an indicator of cellular proliferation released in extracellular fluids during cellular division.

4. Reaction of the organism to the tumor.

C-reactive protein, antibodies directed against some tumor antigens, immune complexes, β-microglobulin are examples of markers appearing by this mechanism.

§ 3. CRITERIA FOR A USEFUL TEST BASED ON THE DETERMINATION OF TUMOR MARKERS

To provide a useful information in the detection, diagnosis or treatment of neplasms, a test relying on the determination of a tumor marker must meet the following criteria :

1. Specificity

A good diagnostic test must have a high degree of specificity. There should be very few false positive results i.e. few individuals without neoplasic cells who have a positive test. A high specificity may also mean the capability of the test to differentiate malignant from small benign tumors. The criterion of specificity is particularly important in a

screening programme. An excessive number of false positive results may indeed give rise to psychological and economical difficulties. If for instance, the frequency of a cancer in a given population is 0.5 ‰ and the frequency of positive results is 5 ‰, most individuals identified as positive will be unnecessarily worried and subjected to additional medical examinations.

2. Sensitivity

The test should also be very sensitive and yield very few false negative results. In other terms, it must be able to detect the presence of neoplasic cells in a large proportion of cancer patients, including those with small localized tumors.

3. Organ specificity

For the diagnosis of cancer, it is desirable that the test provides some information regarding the localisation of the neoplasic process.

4. Applicability

To be suitable for monitoring or screening programmes, the test must be relatively inexpensive and easy to perform. The majority of tests using tumor markers are based on radioisotopic or enzymatic immunoassays, which are costly and required a skilled personnel. It is clear that the financial resources necessary for these tests are a serious limitation of their application for survey of large populations.

Current tumor markers are neither highly sensitive nor tumor specific. The lack of specificity of tumor markers can partly be overcome by taking into account the possible confounding factors such as age, sex, smoking habits and benign diseases giving false positive results. The probability of detecting a given neoplasm can be increased by combining different markers. The combined use of CEA and tissue polypeptide antigen allows for an earlier detection of malignant recurrency and also a better discrimination of patients with stable tumors from those with progressive cancer (Lüthgens and Schlegel, 1983). The combination of CEA and ferritin may be useful in the diagnosis (particularly in the staging procedure) of both breast and lung cancer (Blockx et al., 1984). The simultaneous determination of AFP and ferritin seems also to be useful for detecting hepatocellular carcinoma in high risk patients (Nakano et al., 1984). Combination of tests is however a valuable approach only if the tests have a similar and relatively high sensitivity. No improvement in sensitivity can indeed be expected by combining one high and one low sensitivity test or by using a battery of poorly sensitive tests (Fischer and Oehr, 1984).

§ 4. CLINICAL APPLICATION OF TUMOR MARKERS

During the last 15-20 years, considerable effort has been devoted to the development of tests allowing a specific and early detection of neoplasic process. Although significant technical advances have been made in the identification of several tumor-associated substances, the clinical usefulness of tumor markers is still limited by their insufficient sensitivity and/or specificity (Pohl et al., 1983; Wagener, 1984).

Even CEA and AFP which are widely used markers are far from being absolutely specific. Increased serum levels of CEA may for instance be observed in heavy smokers (MacCartney and Huffer, 1974) in patients with benign neoplasms (Logerfo et al., 1971) and in 15 to 20 % of patients with inflammatory disorders, pulmonary infections, liver diseases, pancreatitis, ulcerative colitis and Crohn's disease (Constanza et al., 1974). Serum levels of AFP can also be increased in nonmalignant liver diseases such as viral hepatitis or cirrhosis.

Because of their lack of specificity, currently available tumor markers are in general of little value for screening of malignancies. The CEA determination has revealed unsuitable for cancer screening in the general population (Cullen et al., 1976) or in high risk groups such as hospital patients (Doos et al., 1975).

There are however a few markers that can possibly be considered as appropriate for screening purpose in some circumstances. AFP, which in absence of pregnancy is relatively specific of hepatocellular carcinoma, has been successfully used as a screening tool in some areas of Asia and Africa and in patients with liver cirrhosis (see § 5). The chorionic gonadotrophic hormone β-HCG level is in males an excellent indice of trophoblastic tumors, which is easily detectable in urine before the appearance of clinical signs of cancer. As a marker of testicular germ cell tumors, it has a specificity of 100 % (Winship-Ball et al., 1983). Calcitonin is also an excellent marker in medullary thyroid carcinoma.

At the present time, the major clinical usefulness of tumor markers lies in the monitoring of therapeutic effectiveness and in the determination of malignancy recurrence. The existence of residual neoplasic tissues and/or

distant metastases following therapeutic intervention can indeed be detected by monitoring the time course of the concentration of a tumor marker, provided, of course, that this marker was elevated before the onset of therapy. In this application, tumor markers represent the most sensitive diagnostic procedure to detect resistance to therapy or recurrence of the malignant process. In some situations, tumor markers may also help in the staging or classification of the disease or in estimating the prognosis.

§ 5. EARLY DETECTION OF SUBJECTS BEARING MALIGNANT TUMOR

One question which deserves some consideration is whether the monitoring of tumor markers might be useful for the early detection of malignant tumors in subjects known to have been exposed to carcinogenic chemicals. Modern diagnostic techniques (e.g. isotopic scanning, ultrasonography) are likely to permit an earlier detection of small tumors than the currently available markers. It is however evident that the former techniques cannot be applied routinely on workers who have been in contact with potentially carcinogenic agents. Few studies have examined the usefulness of tumor marker monitoring for this specific purpose. An example of such an application is the determination of serum AFP for screening of hepatocellular carcinoma in areas of Asia or Africa where this cancer has a high incidence. For instance, the Shangai coordinating group (1979) compiled statistics on 1,967,511 staff members of factories and companies screened from 1971 to 1976. Three hundred patients were detected as positive during these years which corresponds to a detection rate of 15.4/100,000. Among the AFP positives patients, 60 were found to be at an advanced stage of hepatocellular carcinoma by isotope scanning and ultrasonic detection and 134 cases were in the early stages. The

patient resection rate was 58.5 %. The 1-, 2-, and 3-years survival rates after operation were 86.7 %, 75.0 % and 57.1 % respectively. In addition, 1,223,912 individuals were screened in three consecutive years (1974-1977) in Qidong County, Jiangnu province, a high endemic area (Zhu, 1981). Four hundred seventy five patients with liver cancer were detected (detection rate 38.5/100.000). Among these positive subjects, 167 were in the early stage. Two years and five years after the operation, their survival rate was 69 % and 29 % respectively.

The determination of AFP has also been successfully applied to patients with chronic liver diseases. In these patients, a high serum level of AFP is indeed highly suggestive of the presence of hepatocellular carcinoma. For instance, Masseyef et al. (1976) reported the determination of serum AFP in 195 cirrhotic patients, 72.8 % of them having AFP levels lower than 10 µg/l, 22.1 % showing values between 10 and 2000 µg/l and 5.1 % having concentrations above 2000 µg/l. Follow-up examination of these subjects has shown that all subjects with the highest level of AFP have developed an hepatocellular carcinoma while about 25 % of the group with mid-levels developed the disease and none with normal AFP levels had a malignant disease. These studies demonstrate thus that the AFP determination is an useful test for the early diagnosis of hepatocellular carcinoma, increasing the resectability and survival rates and permitting an earlier therapeutic intervention. The AFP test has however some limitations. Firstly, it does not respond to all types of hepatocellular carcinoma. As mentioned earlier, the combined use of other markers such as ferritin improves the sensitivity of the screening. The test also does not respond in case of small tumors. Chen et al. (1984) have for instance reported that among 17 patients with

liver tumor < 3 cm in size, only 11 subjects had an increased level of AFP in serum.

The use of tumor markers for monitoring populations exposed to carcinogenic chemicals may be a useful approach for the early detection of chemical-induced neoplasms. This possibility should be further explored by undertaking prospective studies in groups of workers who are or have been exposed to suspected carcinogens. The few studies realized so far on this topic were limited to a comparison of the prevalences of abnormal levels of some tumors markers between exposed and control subjects. These cross-sectional studies provide little information on the predictivity and pronostic value of these increased levels of some markers.

Page et al. (1976) reported an increased prevalence of abnormal CEA levels in workers exposed to vinyl chloride monomer. 48 % of the studied population had abnormal levels in serum (i.e. higher than 2-5 µg/l). However, no specific control group was included in this study and the influence of confounding factors such as tobacco, alcohol consumption or liver diseases was not taken into account.

Anderson et al. (1978) measured CEA levels in 1115 workers exposed to vinyl chloride monomer in 3 polymerization plants and in 248 workers of a PVC extrusion plant where no vinyl chloride monomer was used. The authors found multiple factors affecting the CEA levels in serum among which cigarette smoking, past illness and alcohol consumption had the strongest effects. After controlling for these possible confounding factors, the distribution of CEA levels in the polymerization plants workers still differed significantly from that observed in the extrusion plant workers or from that of an additional control group. Among the various

subcategories of workers, those of the maintenance and production had the CEA levels which differed the most from that of controls. The activity of alkaline phosphatase was also measured in these workers and a correlation with CEA levels was found. In persons exposed to styrene, an increased serum level of CEA was reported by Anderson et al. (1976) but this observation was not confirmed by Järvisalo et al. although the group of workers examined by the latter authors was heavily exposed to the solvent.

In asbestosis patients, who are subjected to a high risk of malignancy, Jarvisälo et al. (1984) observed a high prevalence of abnormal serum levels of CEA, β-microglobulin and ferritin. These elevated values could not be explained by diseases of the liver or kidneys. A follow-up study will probably shed more light on the origin and significance of these pathological levels of tumor markers. Kumar et al. (1978) have measured the concentrations of tissue polypeptide antigen (TPA) in 108 male workers previously exposed to bladder carcinogens (naphtylamine and benzidine). The prevalence of abnormal TPA levels in serum was significantly increased by comparison with a group of 63 healthy male blood donors (37 % vs 9.5 %). Since TPA is a proliferative antigen, it is possible that a raised level of this marker constitutes an early sign of hyperplasic bladder epithelium.

More recently, Kotlar et al. (1982) have applied the leukocyte adherence inhibition assay (LAI) in a group of 51 workers employed in a nickel refinery. The principle of this test is that when leukocytes sensitized by a tumor antigen react with this antigen, their normal adherence to glass is inhibited. The frequency of positive response against a lung carcinoma

antigen was higher among the refinery workers (21/51) than in the controls (3/17). Moreover, among workers employed for 10 years or more, the response was higher than that found in workers with shorter employment. Of the nickel workers with nasal dysplasia 56 % (15/27) gave a positive reaction against the lung carcinoma antigen compared to 25 % (6/24) of the workers without dysplasia (P = 0.03). The same trends were also found for the nasal carcinoma antigen. This study indicates that the humoral inhibition test may be useful in the identification of individuals with an increased cancer risk. This test does not seem to be more specific of cancer than other tumor markers, but it could be more sensitive. It is indeed very likely that in the course of cancer, the immune response to tumor antigens is detectable at a much earlier stage than the products of tumor released in the circulation. Positive results have, for instance, been reported with the LAI-test in serum samples drawn up to 5 years before the clinical diagnosis of cancer. Another approach of cancer screening with tumor markers is to use them for detecting cancerous cells in biological samples collected by biopsy or cytology. For instance, tumor markers based on the use of specific antibodies could identify cells bearing tumor antigens in the sputum.

§ 6. CONCLUSION

Despite considerable research efforts made in the field of tumor markers, the tests currently available still lack sufficient sensitivity and/or specificity to be used in the early diagnosis of cancer. At the present time, the major clinical usefulness of tumor markers is the monitoring of therapeutic effectiveness of oncologic treatments. In most

cases, they represent the most sensitive and quantitative parameters to follow up the evolution of neoplasms in cancer patients.

But another potential application of tumor markers, which so far has been little explored is the monitoring of populations exposed or who have been exposed to occupational or environmental chemical carcinogens. The available information is insufficient to assess whether such an approach would be worthwhile and could contribute to an early detection of neoplasic processes. Follow-up studies of populations subjected to a known risk, using several markers possibly in combination with other indicators of the presence of a carcinogenic risk, are needed to assess the predictive value of tumor markers and hence their practical relevance in a biological monitoring programme.

Table VI.1.

Studies on tumor markers among workers occupationally exposed to established or suspected chemical carcinogens

Substances	Authors	Number of workers	Markers	Observations
Vinyl chloride	Page et al. (1977)	200 exposed	CEA	48.3 % with values > 2.5 µg/l
	Andersson et al. (1978)	1114 exposed and 248 control	CEA alkaline phosphatase	Significant difference between both groups for CEA
Styrene	Andersson et al. (1976)		CEA	Higher concentrations in the exposed group
	Jarvisälo et al. (1984a)	31 exposed	CEA β-m ferritine	CEA (µg/l) 2.8 vs 2 in control ($p > 0.05$). No significant difference for $\$_2$-m and ferritine
Asbestosis	Jarvisälo et al. (1984b)	90 patients with asbestosis	CEA β-m ferritine	17 % with CEA > 5 µg/l; 48 % with $\$_2$-m > 3 mg/l 22% with ferritine > 400 µg/l.
	Stenman et al. (1982)	103 patients with asbestosis	tumor-associated trypsin inhibitor	no effect
	Borek et al. (1983)	.13 subjects without asbestosis symptoms . 9 subjects with mesothelioma	modified nucleosides	8 positive all positive

bstances	Authors	Number of workers	Markers	Observations
naphtylamine naphtylamine enzidine	Kumar et al. (1978)	108 exposed 68 control	tissue polypeptide antigen	a 4-6 fold increase in the prevalence of positive results in the exposed group
ickel refinery	Kotlar et al. (1982)	51 exposed 17 controls	Leukocyte adherence inhibition test against lung & nasal carcinoma antigen	Higher response rate against lung carcinoma antigen in refinery workers (21/51) than in controls (3/17) (p=0.07). The increase is related to duration of exposure and presence of nasal dysplasia.

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CHAPTER VIII

SPERM ASSAYS IN MAN

§ 1. PRINCIPLES OF THE METHOD

Many experimental and epidemiological studies have shown that anomalies of the sperm can be used to estimate the antispermatogenic effects and the production of genetic damages by chemicals in mammalian male germ cells (Wyrobek, 1981, 1982, 1984a,b ; Wyrobek et al., 1980, 1981a,b, 1982a,b). Four measures of sperm quality are actually utilized : sperm count ; motility morphological changes and YFF test.

Sperm count, (i.e. the number of sperms per milliliter of ejaculate) and sperm motility are two of the best indicators of fertility and the first one is also commonly used as a measure of human spermatogenic toxicity. Various factors, such as duration of continence before sampling, collection of incomplete ejaculate, interindividual variability or methodological factors may, however, interfere with the results (Schwartz et al., 1979).

Experiments with mice suggest that changes in the sperm morphology resulting from exposure to chemicals are correlated with the ability of these agents to induce genetic changes such as dominant lethals, heritable translocations and gene mutations. The classification of the sperm into

shape categories has greatly decreased the variability between scoring persons and laboratories.

The presence of two fluorescent bodies, corresponding to two Y chromosomes, in spermatozoa of normal size can be used to test for Y-chromosomal non disjunction. It should be pointed out, here, that various other approaches have also been proposed to assess chemically induced spermatogenic dysfunction including testicular biopsies (Whorton et al., 1979), questionaire surveys (Levin et al., 1981), blood levels of gonadotrophins (Eliasson, 1976), meiotic chromosome analysis or sperm chromosomes vizualized by in vitro penetration of hamster oocytes (Hulten et al., 1984 for review). Methods implying testicular biopsies cannot be used in routine and, actually, vizualization of sperm chromosomes encounters too many technical difficulties.

§ 2. RESULTS OF OBSERVATIONS ON MAN EXPOSED TO CHEMICALS

According to the recent reviews of Wyrobek (1984a,b) more than 100 papers covering approximately 90 different human exposures studied with one or more of these tests, have been published. About 90% of them deal with exposure to experimental or therapeutic drugs. An increase of 300-600% in sperm with two F bodies has been observed, for instance, after treatment of individuals with adriamycin, flagyl plus diagnostic radiation or after X-ray therapy (Legator and Kapp, 1984). A few studies have been performed on people exposed to occupational and environmental agents (Table IX.1). Higher proportions of sperm with shape abnormalities than controls but no differences in sperm counts have been found in men occupationally exposed

to carbaryl (Wyrobek et al., 1981b) and increased Y-chromosomal nondysfunction (Kapp et al., 1979) and depression of sperm counts (Takahashi et al., 1981 ; Whorton et al., 1977) were observed in workers exposed to dibromochloropropane. Positive results (Wyrobek, 1984b) have also been obtained on men exposed to carbon disulfide, toluene diamine and dinitrotoluene, or lead. Exposure to anesthetic gases, apparently, has no effect on sperm (Wyrobek et al., 1981a) and no statistically significant difference was observed by Coldiron et al. (1983) and Ward et al. (1984) between controls and workers exposed to formaldehyde in a hospital autopsy service with respect to sperm count, abnormal sperm morphology and 2F-body frequency. Negative results have been reported, also, on people exposed (Wyrobek, 1984) to epichlorohydrin, in workers employed in glycerine production or handling polybrominated biphenyls and (Rosenberg et al., 1984) in waste water treatment workers. Some attempts have also been made to correlate age, lifestyle characteristics or personal drug use with sperm anomalies. Positive results were reported with marijuana (Hembree et al., 1979 ; Coldiron et al., 1983 ; Ward et al., 1984) and alcoholic beverages (Wyrobek, 1984) but the results were not very demonstrative for tobacco smoke (Wyrobek, 1984a).

§ 3. CONCLUSION

Major reductions in sperm counts and motility and some sperm shape abnormalities have been shown to be associated with reduced fertility, sterility or higher incidence of spontaneous abortions (Furuchjelm et al., 1962 ; Czeizel et al., 1967 ; Nistal et al.,

1978). The association between sperm abnormalities and infertility observed in dibromochloropropane exposed workers (Whorton et al., 1977) suggest that damage to semen quality produced by environmental chemicals could result in the same effects. There exists, however, no indication that those sperm parameters are predictive of induction of transmissible genetic defects.

Observation of meiotic and sperm chromosomes could provide more information in that respect but currently, these methods do not appear of practical value for genetic monitoring of people exposed to environmental or industrial pollutants.

Table IX.1.
Results of sperm assays after occupational or environmental exposure.

Compound	Response	Reference
Alcoholic beverages	+	Wyrobek (1984a)
Carbaryl	+	Wyrobek et al. (1981b)
Carbone disulfide	+	Wyrobek (1984b)
Dibromochloropropane	+	Kapp et al. (1977)
		Takahashi et al. (1981)
		Whorton et al. (1977)
Dibromochloropropane ethylene dibromide	+	Wyrobek (1984b)
Lead	+	Wyrobek (1984b)
Marijuana	+	Coldiron et al. (1983)
		Hembree et al. (1979)
		Ward et al. (1984)
Toluene diamine + dinitrotoluene	+	Wyrobek (1984b)
Kapone	±	Wyrobek (1984b)
Tobacco	±	Wyrobek (1984a)
Anesthetic gas	-	Wyrobek (1984a)
Epichlorohydrin	-	Wyrobek (1984b)
Formaldehyde	-	Coldiron et al. (1983)
		Ward et al. (1984)
Glycerine production	-	Wyrobek (1984b)
Waste-water treatment	-	Rosenberg et al. (1984)

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Part II

OVERALL ASSESSMENT

CHAPTER I

VALIDITY AND HEALTH SIGNIFICANTCE OF THE BIOLOGICAL METHODS CURRENTLY AVAILABLE FOR MONITORING EXPOSURE TO CHEMICALS

In this chapter we shall attempt to assess the validity of the various tests described in part I whereas chapter II will be devoted to recommandations regarding their application.

§ 1. METHODS MEASURING THE CHEMICAL OR ITS METABOLITES IN BIOLOGICAL MEDIA

The objective of these methods is to estimate the amount of carcinogen or mutagen effectively absorbed by the organism (i.e. the internal dose). When based on a selective determination of the chemical in biological media, this approach presents the advantage of being sensitive, specific and in theory applicable to all potential industrial carcinogens. In addition, the determination can, sometimes, be performed in urine, which is easily accepted by the worker. So far, however, tests relying on the determination of the unchanged chemical or its metabolites in biological fluids have been described for a limited number of carcinogenic or mutagenic compounds.

The thioether assay is a nonselective test, which has been proposed for monitoring exposure to electrophilic chemicals or chemical giving rise to electrophilic metabolites. The sensitivity of this test is, however, limited due to a relatively high and fluctuating background. Smoking is a possible confounding factor. Some compounds devoid of carcinogenic or mutagenic properties can also produce an increased thioether excretion.

Currently, the main value of the thioether test seems to lie in its capability to identify groups exposed to unsuspected electrophilic compounds. But the lack of increase of thioether excretion does not permit to conclude that no exposure to electrophilic substances has occurred.

The specific and non specific tests measuring the chemical concentration in biological fluids are of limited value for assessing the carcinogenic or mutagenic risk because they provide no information on the extent to which the chemical has interacted with the genetic material. An indirect assessment of health risk can be made if quantitative dose-response relationships exist, but this is rarely the case in human carcinogenesis.

§ 2. DETERMINATION OF MUTAGENIC ACTIVITY IN BIOLOGICAL MATERIALS

This test can be used to detect exposure to mutagenic substances or substances giving rise to mutagenic substances. Performed on urine it appears interesting because collection of samples is easy, the cost and the time required appear acceptable even for a large scale study and demonstrative results have been obtained with urine of people exposed to various types of chemicals.

It has an adequate sensitivity for monitoring exposure to environmental or occupational carcinogens or mutagens. Confounding factors, however, may arise from several sources, such as smoking, drugs, diet, cosmetics, etc... This lack of specificity is an advantage when one is trying to determine whether there is an exposure to mutagenic compounds whatever the source. But the nonspecificity of mutagenicity tests restricts their application on a group basis and also obscures the relationship between the excretion of mutagens in urine and the presumed exposure.

In addition, there is alo the possibility that urinary contaminants such as histidine in the Ames test interfere with the assay. Like the methods measuring the chemicals in body fluids, mutagenicity tests reflect mainly the recent exposure, at best over the previous 48-72 hr. A standardization of the methods currently used is also desirable.

With respect to the health significance of the results of mutagenicity tests, it must be stressed that the mutagenic activity measured in excreta is not necessarily relevant for what happens in the genetic material of a target organ. The compound responsible for the mutagenicity of urines may not be involved in the cancer induction at the target organ and inversely, the lack of increase of the urinary mutagenicity does not mean the absence of genotoxic damage in the target organ.

§ 3. CYTOGENETIC OBSERVATIONS ON HUMAN SOMATIC CELLS

Cytogenetically visible damage in human chromosomes can be detected as chromosomal aberrations, sister chromatid exchanges (SCEs) or as micronucleated cells.

The search for chromosomal aberrations in peripheral lymphocytes has proved sensitive and specific enough to be used as a biological dosimeter in cases of exposure to ionizing radiations. The induction of chromosomal aberrations in vitro has been demonstrated for a large number of compounds. There are, however, relatively few compounds that have been shown to increase the numbers of chromosomal aberrations in lymphocytes of occupationally or environmentally exposed persons. It is not yet established whether the method is sufficiently sensitive for chronic low level exposures to chemicals. By contrast to the preceding tests,

chromosomal aberrations are cumulative and may integrate the exposure over a relatively long period of time. Chromosome aberrations, however, are nonspecific. It is thus important to take into account all the possible factors which may influence the background of spontaneous aberrations such as excessive alcohol consumption, cigarette smoking, age, viral infections, X-ray examination, etc... . A major disadvantage of this test for routine monitoring is that, with the present methodology, scoring of chromosomal aberrations is laborious.

The detection of SCEs is easy to perform but the method has not been proven to be a good indicator of exposure to genotoxic chemicals.

The main advantages of the micronucleus test are its speed and its lack of requirements for mitotic cells. Furthermore, micronuclei are indicators of structural chromosome aberrations and also of interference of the chemicals with the distribution of the chromosomes in the daughter cells. The micronucleus test can be applied to lymphocytes and also to exfoliated cells, which in the latter case may sometimes offer the possibility of monitoring genotoxic effects in target organs such as the bladder, the lung or the buccal cavity.

The induction of chromosomal aberrations by chemical agents, in vitro in mammalian cells or in vivo in laboratory animals, can be considered as reasonably good indicators of carcinogenic potential. If one add that many types of human cancer display specific chromosomal aberrations, an increase of chromosomal aberrations in peripheral blood lymphocytes could indicate, therefore, a higher probability for the exposed group to develop cancer. At the individual level, however, several studies performed on human populations, mainly on the survivors of Hiroshima and Nagasaki, suggest that there exist no correlation between the frequency of chromosomal abnormalities in the peripheral blood lymphocytes and the risk of cancer.

It is not known, also, whether chromosomal changes in blood cells reflect the presence of such damage in internal organs. Finally the association between chromosome damage and blood cell diseases is speculative and no studies on man or animal has demonstrated a possible cause-response relationship between induced cytogenetic changes and immune function.

§ 4. DETECTION OF GENE MUTATIONS IN SOMATIC CELLS OR OF PROTEIN VARIANTS

The genotoxic risk can be evaluated by searching for point mutation either in DNA of peripheral lymphocytes or in blood proteins (e.g. hemoglobin). The methods are at an early stage of development. Their potential for human monitoring cannot yet be assessed.

§ 5. DETERMINATION OF PROTEIN OR NUCLEIC ACID ADDUCTS (MOLECULAR DOSIMETRY)

This approach is currently considered as one of the most promising for human monitoring to carcinogenic or mutagenic chemicals. It has indeed the potential of estimating directly (DNA adducts) or indirectly (protein adducts) the amount of chemicals bound to the target molecules from which the cancer process can be initiated. In addition, in the case of protein adducts, this method may integrate the target dose over a relatively long period corresponding to the biological half life of protein and of the adducts. The results obtained so far suggest that this method might be sensitive enough for biological monitoring in occupational setting but a thorough validation of this approach is required before considering its application for human monitoring.

In general, the adduct determination is very specific with respect to the chemical group bound as well as to the site of the macromolecule (aminoacid or nucleic acids base) where this binding occurs. In the case of DNA, a nonselective detection is however possible by using the ^{32}P-postlabelling method. The latter technique might therefore be used for a preliminary screening, but its sensitivity might be a limiting factor.

The major limitation of this approach, particularly for DNA adducts, is the methodology which at this stage is too elaborate for routine monitoring. The development of immunological methods for the determination of adducts could make these monitoring techniques more suitable for routine monitoring. But the drawback of immunoassays is that the structure of the adducts must be known and that for each type of adducts a specific antibody must be raised. Another limitation of this method is that it is only applicable to compounds that can bind to macromolecules either directly (direct alkylating agents) or after metabolic activation. Furthermore, little information is usually available on the background level of protein or DNA-adducts in man which may limit the sensitivity of this test.

Adducts formed with proteins are non-specific with respect to the biological consequences. They are only relevant for monitoring genotoxic risk if their occurrence correlates with adduct formation in DNA of target cells. There is so far, however, no absolute evidence that an increased incidence of DNA-adducts can be quantitatively translated in a higher cancer risk. It is indeed likely that only alterations at a specific site of DNA coding for a particular gene (oncogen) are probably related to the cancer induction. Although this approach is very promising for cancer risk estimation, presently, it cannot be used for this purpose.

§ 6. DETERMINATION OF DNA REPAIR

The determination of DNA repair (e.g. in lymphocytes) can be used to indirectly estimate the extent of damage to the genetic material. The applicability, sensitivity and specificity of this approach for monitoring purpose is unknown. The method which so far has been the most commonly used, is the determination of the unscheduled DNA synthesis. It is however questionable whether the DNA repair determination can provide an accurate estimate of the genetic damage. One of the most serious limitation is the fact that the method quantifies only the damage susceptible to excision repair. Mutagenic lesions which are reversible are not detected. The usefulness of this monitoring method is also limited by the fact that it reflects probably the very recent exposure preceding immediately the assay.

§ 7. DETERMINATION OF TUMOR MARKERS

Tumor markers can be defined as substances which are significantly associated with neoplasic diseases. Tumor markers can belong to very different biochemical groups : oncodevelopmental antigens, enzymes, hormones, antibodies, nucleosides, ...

The current tests based on tumor marker determination still lack sensitivity and/or specificity to be useful for the early diagnosis of cancer. Alfa-foetoprotein, however, seems to be an exception since this marker has been successfully used for the early detection of hepatocellular carcinoma in some groups at risk.

The possibility of using tumor markers for monitoring populations occupationally exposed to potentially carcinogenic chemicals has been rarely explored. It would be worthwhile to determine whether this approach

could be used to identify groups or subjects at risk. In that application, the lack of specificity of tumor markers could be partly overcome by taking into account the possible confounding factors while the sensitivity of the screening could be increased by using combinations of markers.

§ 8. SPERM ASSAYS

These tests may be useful to evaluate genotoxic effects which are particularly relevant for male fertility and possibly reproductive outcome. But for obvious practical reasons, this method cannot be considered for routine biological monitoring except in very specific situations.

§ 9. CONCLUSIONS

The ultimate objective of biological monitoring is to assess the health risk. Presently, none of the methods described above is capable to predict the cancer risk at an individual level. Some methods, however, can be used to identify groups at risk. But it must be emphasized that even at the group level, the health significance of the biological effects is still uncertain. Selective tests measuring the chemical or its metabolites in biological media are specific with respect to the agent but not to the biological response while the opposite is true for methods searching for genotoxic effects. The latter are indeed influenced by a number of non occupational factors, such as smoking, eating habits, drugs or cosmetics which may increase the background level. Therefore, it is probably desirable to use a combination of methods, some reflecting the exposure and others the biological response, in order to detect groups at risk.

In addition, the performances of the tests (i.e. specificity, sensitivity, precision and accuracy) and hence their suitability for monitoring occupational or environmental exposure have not yet been sufficiently established. In some cases (e.g. macromolecule adducts), this results from the fact that the test is still at an early stage of development. But for tests which are already being used for several years (e.g. mutagenicity of urine), the uncertainties which persist concerning their precision and specificity are probably due to methodological reasons (lack of standardization).

In conclusion, several methods reviewed in the present report can be used as early warning system. They can indicate that exposure to mutagens and/or carcinogens has occurred and that the groups so exposed could present an increased risk of developing diseases, particularly cancer.

CHAPTER II

RECOMMENDATIONS

Research efforts must be encouraged to develop new assays for monitoring exposure to carcinogenic or mutagenic chemicals and to improve the performances (i.e. the sensitivity, specificity, applicability to routine monitoring ...) of the tests currently available. The latter must still be validated from an epidemiological and analytical point of view before routine application.

The objective of the analytical validation is to determine to which extent the results obtained with the proposed method are sufficiently reliable to be used for detecting groups at risk. This requires assessment of the accurary of the methods (i.e. lack of analytical bias or of confounding factors) and of their reproducibility within and between laboratories. Intercomparison studies, development of standardized procedures, implementation of external and internal quality control programmes, and organization of training programmes should be stimulated. A collaborative project between several laboratories is already underway to assess the validity of the methods currently used for monitoring chromosomal aberrations and SCEs in a group of about 400 subjects. It should be extended to other methods.

The epidemiological validation aims at determining (1) the sensitivity and specificity of the method for detecting groups exposed to occupational or environmental carcinogens or mutagens (2) the health significance of the

test i.e. its ability to predict the carcinogenic risk on an individual or a group basis. The validity and health significance of the tests can be assessed only through well planned epidemiological studies among groups potentially at risk.

The tests to be evaluated in these studies must be selected by considering their characteristics (applicability, specificity, sensitivity) and also the exposure conditions. In this regard, two different situations may be encountered :

(1) Exposure to one or a few well defined carcinogenic or mutagenic chemical(s) (e.g. : ethylene oxide, benzene, 4-aminobiphenyl, vinyl chloride).

Among the tests currently available, those which seem the most appropriate for such situation are :

- determination of the chemical or its metabolites in biological media ;
- search for cytogenetic alterations (chromosome aberrations and micronuclei in lymphocytes or exfoliated cells) ;
- detection of specific adducts with blood proteins or DNA.

(2) Exposure to a mixture of poorly characterized carcinogenic or mutagenic chemicals.

In such situation, only the following nonspecific tests can be considered at the present time :

- detection of mutagenic activity in urine ;
- search for cytogenetic alterations ;
- determination of urinary thioethers ;
- ^{32}P-postlabelling method.

Table III.1 provides some estimate of the cost of some of these tests. Except for the tests measuring specifically the chemical or its metabolites in biological media, all the precited tests may provide false negative

results. Therefore, whatever the type of exposure, it would be worthwhile to study also whether the determination of some tumor markers could be used as early indicators of cell transformation.

The selection of the groups to be monitored should be based on our current knowledge on the carcinogenicity or mutagenicity of chemicals handled at workplaces. Various groups potentially at risk can already be suggested : coke oven workers, groups of workers exposed to asphalt tar or bitume fumes, such as roofing applicators, graphite electrode production workers and road construction workers.

Because of the lack of specificity of most monitoring tests, a great attention must be devoted to the various possible confounding factors such as age, sex, smoking habits, alcohol and drug consumption. Therefore these tests should presently be used only in the framework of a well planned study which includes a control group. In the planning of these studies, the ethical aspects are of paramount importance. The informed consent of the worker must be obtained before collecting biological specimens. Sufficient information must be given to the worker regarding the objective of the study and our current knowledge on the significance of the various tests. The confidentiality of the results must also be guaranteed.

Table III.1.

Estimate of the cost of the biological methods proposed for monitoring exposure to genotoxic chemicals.

Methods	Approximative cost in ECU (per individual)
Determination of the chemical or its metabolites in biological media	< 50
Determination of the mutagenic activity of biological materials	200 - 300
Cytogenetic observations on somatic cells	200 - 300
Protein or nucleic acid adducts	?