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Contributions from Molecular/Biochemical Approaches in Epidemiology to Cancer Risk Assessment and Prevention

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Introduction

The origin of cancer in the humans is considered to be a complex and multistep process. In all cases, however, the cancer cell contains a genome in which gene(s) are either altered or wrongly expressed. Therefore, alterations in DNA have been thought to be a crucial first step (initiation) in the long chain of events leading to cancer.

The occurrence of DNA lesions in cells in the human body is not a rare phenomenon. They can occur due to exposure to many exogenous agents present in our food, environment, and occupational situations. A major additional cause of DNA damage in cells is the action of endogenous factors like the intermediates of metabolic and detoxifying processes in our body (*1*). *In toto* the human body contains about 10^{14} cells; endogenous factors alone may induce around 4000 DNA lesions/day/cell, i.e., 4×10^{17} DNA lesions/body/day. Since the frequency of the transformation of cells into cancerous ones in the body is orders of magnitude lower than that of the formation of DNA lesions, it is obvious that only a limited number of DNA lesions are related to the process of neoplastic transformation. Some of the DNA lesions in cells may be converted to DNA sequence alterations (i.e., mutations), but still the number of DNA alterations is considerably greater than the number of cells that are ultimately converted into cancer cells. A partial explanation is that the majority of DNA lesions induced by exposure of mammalian cells to genotoxic agents, like X-rays, are more lethal than mutagenic, although other agents such as the alkylating agent ethyl methane sulfonate cause DNA lesions which, at equal level of survival, are considerably more mutagenic in the same kind of mammalian cells than X-ray-induced DNA lesions (*2*). Furthermore, when DNA lesions form mutations, these are not necessarily those that lead to cancer.

Another explanation for the difference in the mutagenic potency of genotoxic agents is variability in the fidelity with which individual DNA lesions in cells are repaired. The vital role of DNA repair in the etiology of cancer was first established by Cleaver (*3*), who found that cells from patients with the hereditary, cancer-prone disease xeroderma pigmentosum (XP) had defective repair of ultraviolet radiation (UV)-induced DNA damage. XP is an autosomal recessive disorder occurring at a frequency of about 1–10 per 10^6 individuals. Patients suffering from this disease have a greater chance of developing neoplasia on parts of the body that are exposed to solar radiation. In several other genetic diseases there is increased susceptibility to DNA damaging agents; these include ataxia telangiectasia, Fanconi's anemia, Bloom's syndrome, Cockayne's syndrome, and trichothiodystrophy. The first three diseases are also associated with an increased incidence of cancer, but the other two are notably not. On the basis of these observations it can be hypothesized that either *a*) not all DNA lesions lead to neoplastic transformation or *b*) the existence of high-fidelity DNA repair processes is an essential prerequisite for preventing tumor formation.

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Prediction of the Carcinogenic/ Mutagenic Potency of a Chemical

Weight-of-Evidence Evaluation and Interpretation of Results from Short-Term Tests for Genotoxicity

Over the past 20 years, the genotoxic activity of chemicals has been assessed typically by conducting a series of short-term tests using various organisms. Most of these tests were designed to be rapid and comparatively inexpensive; some were proposed as surrogates for rodent carcinogenicity or rodent germ-cell bioassays. It is clear, however, that the end points and target cells used in short-term assays are dissimilar to the phenomena of carcinogenesis and germ-cell mutation. The notion that the universe of chemicals is dichotomous toward the carcinogenic/mutagenic potential of individual chemicals has been the led to the development of tier or battery approaches for determining genotoxic properties in short-term assays. A weight-of-evidence approach to the analysis of a large database of short-term test results showed an almost total absence of results indicating a division of the chemical universe into mutagenic and nonmutagenic chemicals (*4–6*). Rather, genotoxicity appears to be a consistent property throughout this universe. The genotoxicity of a chemical can be expressed in terms of a so-called genotoxic agent score, and a wide variety of chemicals can be ranked in this way, using the results of short-term test with a minimal number of end points *in vitro* and *in vivo*. This approach is demonstrated in Figure 1 and Table 1. It has been applied to halogenated organic air pollutants identified under the Clean Air Act, amended in 1990, in the United States. The results contradict the hypothesis that the mutagenicity of chemicals can be divided into a simple positive/negative dichotomy. They also demonstrate that a

Table 1. Hazardous halogenated organic air pollutants.

No.	Sa[a]	SD	Chemical	CAS no.
1	−24.28	17.17	Tetrachloroethylene	127-18-4
2	−16.67	12.11	Polychlorinated biphenyls	1336-36-3
3	−15.27	9.56	Chloroform	67-66-3
4	−13.58	23.86	Dichloromethane	107-06-2
5	−13.48	11.57	1,1,1-Trichloroethane	71-55-6
6	− 7.29	14.90	Bromoform	75-25-2
7	− 7.22	17.80	2,4,6-Trichlorophenol	88-06-2
8	− 6.18	15.47	Carbon tetrachloride	56-23-5
9	− 5.06	6.93	Heptachlor	76-44-8
10	− 2.70	25.02	Hexachlorocyclohexane	58-89-9
11	− 2.38	12.62	Vinylidene chloride	75-35-4
12	− 2.34	11.96	Trichloroethylene	79-01-6
13	− 0.76	15.20	Vinyl chloride	75-01-4
14	1.09	14.54	Pentachlorophenol	87-86-5
15	1.38	12.49	2,3,7,8-TCDD	1746-01-6
16	3.14	19.90	2,4-D	94-75-7
17	3.58	14.53	Benzyl chloride	100-44-7
18	4.58	17.02	1,2-Dichloroethane	107-06-2
19	4.87	16.90	Dimethylcarbamoyl chloride	79-44-7
20	5.71	12.26	Dibromochloropropane	96-12-8
21	6.37	12.47	Methyl chloride	74-87-3
22	6.60	19.50	Ethylene dibromide	106-93-4
23	9.95	10.87	Epichlorohydrin	106-89-8
24	18.67	16.20	Chloroprene	126-83-9
25	20.66	23.73	Methyl bromide	74-83-9

CAS, Chemical Abstracts Service Registry number.
[a]Sa, agent score according to Mendelsohn et al. (*5*).

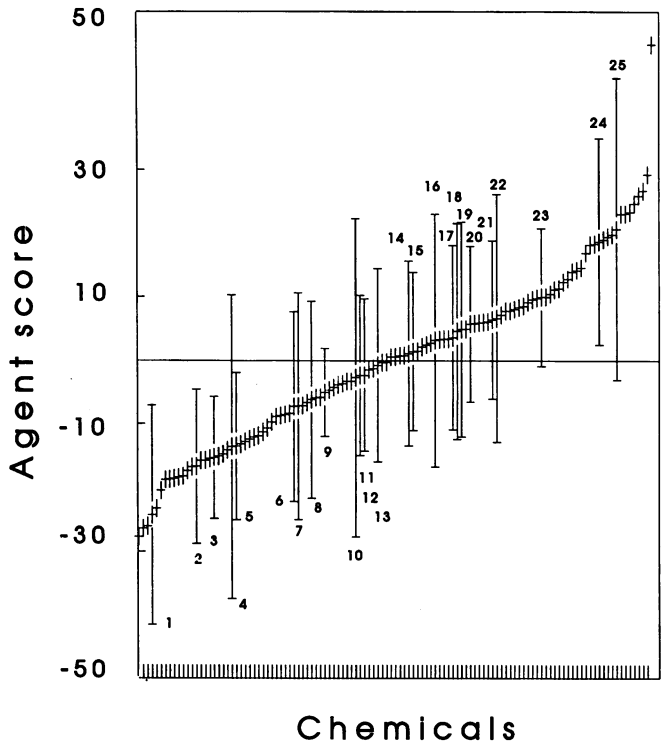

FIGURE 1. Ranking of the genotoxic potential of chemical agents according to their agent scores, calculated as described by Mendelsohn et al. (*5*). The agent scores of the chemicals listed in Table 1 are numbered and shown with the standard deviation; other agent scores (+) are those of genotoxic agents described by Lohman et al. (*4*).

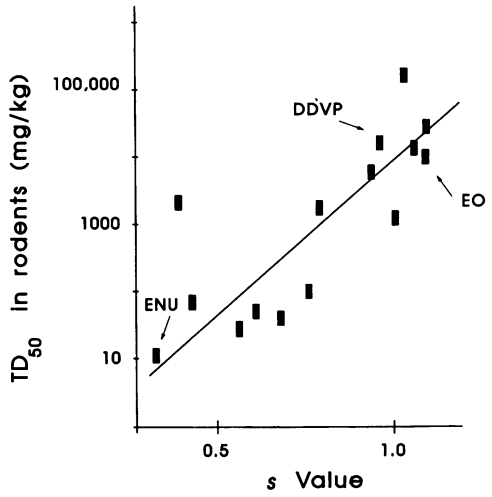

FIGURE 2. Relationship between the carcinogenic potency in rodents (median TD_{50} estimate, expressed as lifetime dose in mg/kg body weight) of monoalkylating agents and their nucleophilic selectivity [Swain-Scott constant, *s* (*8*)]. ENU, *N*-ethyl-*N*-nitrosourea; EO, ethylene oxide; DDVP, 2,2-dichlorovinyldimethylphosphate.

Table 2. *s*-Values, ratios of ring-X loss (CA) to recessive lethal mutations (RL) and TD_{50}-values for a series of 15 genotoxic agents.

Chemical	CAS no.	*s*	CA/RL	TD_{50}	Classification
N-Nitrosodiethylamine	55-18-5		<0.04	56	Monofunctional
Dacarbazine	4342-03-4	0.42	0.06	684	Monofunctional
Procarbazine, Natulan	366-70-1	0.42	<0.14	262	Monofunctional
N-Nitrosodimethylamine	62-75-9		<0.16	139	Monofunctional
1-Phenyl-3,3-dimethylitriazene	7227-91-0	0.22	0.18	1580	Monofunctional
1-(2,4,6-Trichlorophenyl)-3,3-dimethyltriazene		0.29	0.42	7000	Monofunctional
1,2-Dibromoethane	106-93-94	1.2	0.37	1650	Monofunctional
Mitomycin C	59-07-7	0.81	1.14	0.68	Cross-linking
Thio-TEPA	52-24-4	1.10	2.83	131	Cross-linking
Busulfan	55-98-1	0.90	5.43	<140	Cross-linking
Cisplatin	15663-27-1		6.79	20	Cross-linking
Cyclophosphamide	79-19-4		8.54	2080	Cross-linking
Hexamethylphosphoramide	680-31-9		11.97	27	Cross-linking
TEPA			14.60	110	Cross-linking
2-Chloroacetaldehyde	107-20-0	1.3	19.00	13,000	Cross-linking

CAS no., Chemical Abstracts Service Registry Number.

ranking of the genotoxic potential of halogenated air pollutants is possible; however, whether this genotoxic potential also reflects the carcinogenic potential of a chemical needs further evaluation.

Structure–Activity Relationships

Not only short-term tests but also the physicochemical properties of a chemical have been used to predict its relative genotoxic potential. This approach has been especially successful for a range of simple monofunctional alkylating agents, such as *N*-ethyl-*N*-nitrosourea, ethylene oxide, and 2,2-dichlorovinyldimethyl-phosphate. The way in which these compounds react with DNA can be described by their nucleophilic selectivity, as expressed by the Swain-Scott *s* value. As first described by Barbin and Bartsch (*7*), a relationship exists between the *s* value and the carcinogenic potency of monoalkylating chemicals in rodents (median TD_{50} estimates). That study was extended by Vogel et al. (*8*), and a limited summary of the data obtained by those investigators is presented in Figure 2. For the monofunctional alkylating agents studied, the relationship depicted in Figure 2 holds not only for nucleophilic selectivity but also for the initial *N*7-alkylguanine/O^6-alklyguanine ratio in DNA. Those observations indicate that the modifications at base oxygens in DNA by monoalkylating agents are key DNA lesions for determining their carcinogenic potency in rodents (*8*).

The relationship shown in Figure 2 was not found when polyfunctional alkylating agents were studied. Polyfunctional alkylating agents possess more than one active ligand that can react with nucleophilic centers in DNA. These agents, including a considerable number of antitumor drugs such as cisplatin and cyclophosphamide, can cross-link DNA strands. As Vogel et al. (*8*) describe, their carcinogenic potential is not directly related to their nucleophilic selectivity (see also Table 2) but to their ability to cross-link DNA, with the possible exception of cross-linking agents with a very high *s* value. Agents such as 2-chloroacetaldehyde (Table 2) have such a high affinity to other macromolecules (e.g., proteins) present in cells and/or body fluids that hardly any damage to DNA occurs. Therefore, DNA is not the main target and these agents have low carcinogenic potency.

The cross-linking property appears to be the main determinant of the low TD_{50} of polyfunctional carcinogens. Interestingly, the group of cross-linking compounds can be recognized relatively easily by their high efficiency in inducing clastogenic effects in cells. The relative clastogenic efficiency of genotoxic agents can be determined in the fruit fly, *Drosophila melanogaster*, by measuring the ratio of induced ring-X loss (a chromosomal damage end point, CA) to sex-linked recessive lethal mutations (RL) (CA/RL). Compounds with a CA/RL ratio greater than about 1.2 are likely to be cross-linking agents (*8*) (Table 2). Thus, this method has prognostic value for discriminating monofunctional alkylating agents from those with cross-linking properties.

Processing of DNA Damage in Human Cells

Mechanisms of DNA Repair

A mammalian cell contains about 50 cm of DNA, which has to be reduced some 50,000 times in size to fit into the cell nucleus. Although not always recognized it is obvious that the processes behind DNA repair are strongly influenced by the organization of the nucleus in the cell. Considerable evidence is now available to suggest that regions of the chromatin that are engaged in DNA replication and transcription are in a more open configuration than the bulk of the chromatin. The nonrandomness of the functioning of DNA repair processes and, *ultimo*, the alteration of DNA (i.e., mutations) can be recognized at least the level of the chromatin, the individual genes, the DNA strand in genes actively involved in transcription of RNA, and at the individual type of DNA damage.

The complexity of DNA damage processing can be demonstrated in experiments in which normal human fibroblasts and fibroblasts from patients with the DNA repair-deficient syndrome XP complementation group C (XP-C) are exposed to low doses of 254 nm UV. At the chromatin level, the DNA is organized in loops attached to

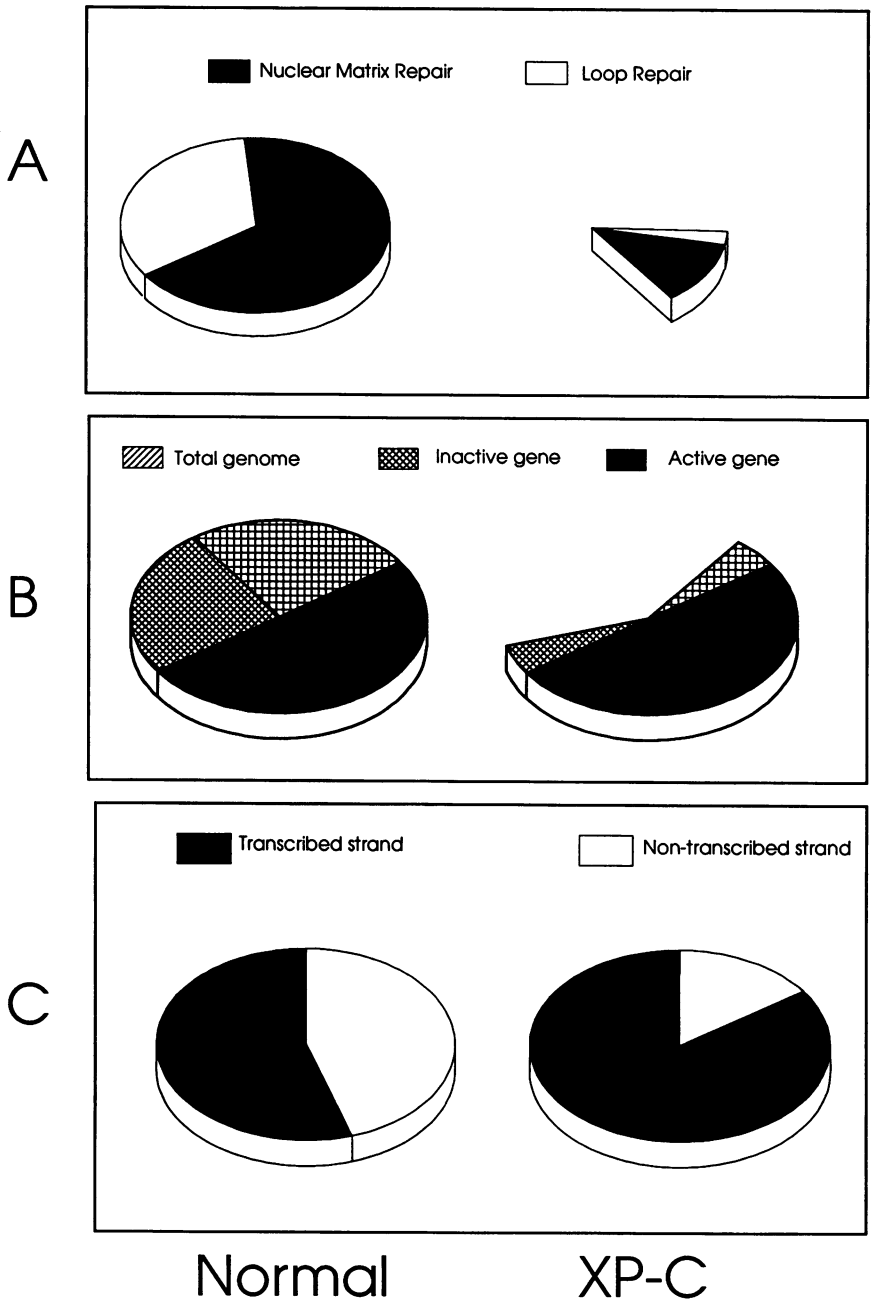

FIGURE 3. Matrix-, gene-, and DNA strand-specific DNA repair in normal human fibroblasts and fibroblasts from xeroderma pigmentosum, complementation group C, patients (XP-C) after exposure to 5 J/m^2 254 nm ultraviolet light (UV). (*A*) Excision repair of DNA at the nuclear matrix and chromatin loops 60 min after exposure to UV. (*B*) Removal of cyclobutane pyrimidine dimer (CPD) from an active housekeeping gene and an inactive X-chromosomal DNA sequence (754) 4 hr after exposure to UV. (*C*) Removal of CPD from the transcribed and nontranscribed DNA strand of the *ada* gene 24 hr after exposure to UV.

a nuclear matrix. As depicted in Figure 3*A*, we found that in normal human fibroblasts exposed to 5 J/m^2 UV, DNA repair closely associated with the nuclear matrix was approximately 2-fold more frequent than in loop DNA. Although XP-C exhibited only 15% residual DNA repair compared to wild-type cells, DNA repair activity at the nuclear matrix was about four-fold greater than in loop DNA (*9,10*). This finding indicates that in human cells different DNA repair mechanisms are associated with the functional status of the chromatin.

Evidence exists that transcriptionally active DNA is associated with the nuclear matrix. A DNA repair mechanism associated with transcriptionally active DNA was identified by measuring removal of the (UV-induced) cyclobutane pyrimidine dimer (CPD) from the active adenosine deaminase (*ada*) housekeeping gene and an inac-

tive X-chromosomal sequence designated 754 (located in the proximity of the Duchenne's muscular dystrophy gene) in human cells. As shown in Figure 3*B*, CPDs are repaired more quickly in active housekeeping genes than in inactive genes or in the (bulk) total DNA of the genome. XP-C cells can efficiently repair active genes but are defective in repairing CPD in transcriptionally inactive DNA (*11*).

The relationship between a specific DNA repair process and RNA transcription in active genes was further elaborated by studying the repair of CPD in the transcribed and nontranscribed DNA strand in the *ada* gene of normal and XP-C fibroblasts [Figure 3*C* (*12,13*)]: XP-C cells efficiently repaired the transcribed DNA strand but were inhibited from repairing the nontranscribed DNA strand.

The fourth level at which different DNA repair processes operate in human and other mammalian cells is at the kind of lesion that is induced in DNA. Discussions have been going on for many years about which is the most significant lesion (key lesion) for mutagenesis in mammalian cells after exposure to UV. The two major types of DNA lesion induced by UV are CPD and the 6–4 pyrimidone photoproduct. Zdzienicka et al. (*14*) found that a revertant of the UV-sensitive Chinese hamster cell line VH1, which has similar mutation induction frequencies as wild-type cells after exposure to UV exhibits complete deficiency in dimer removal from the X-chromosomal hypoxanthine phosphoribosyl transferase (*hprt*) gene and a normal level of 6–4 pyrimidone photoproduct repair in the genome overall. The level of UV-induced mutations was similar to that found in normal cells, which indicates a minor role for dimers in mutagenesis; however, this may not be a general rule for all cell types from all mammalian species. The main conclusion is that different repair processes and/or different efficiency in the DNA repair process exist for different DNA lesions and that this may influence strongly which DNA damage can be considered to be the key lesion for mutation induction after exposure to a genotoxic agent. Of course, this still leaves open the question of whether a DNA lesion for which no repair process exists within cells should be considered to be a key lesion for possible adverse biological effects.

Organ- and Tissue-Specific Carcinogenesis/Mutagenesis

Tumor formation in both rodents and humans after exposure to genotoxic agents commonly occurs in specific organs and tissues. This may indicate that several groups of precarcinogens need metabolic activation and that activation differs in the various organs of the body. The organ and tissue specificity of tumor formation by many other genotoxicants, which are capable of inducing DNA lesions in all cells of the body, is still not understood.

Few methods are available to study the organ- and tissue-specific effects of DNA lesions *in vivo*, but transgenic mouse model systems have been proposed recently which allow study of mutations *in vivo* (*15,16*). One of these strains of transgenic mice harbors multiple copies of a bacteriophage λ shuttle vector containing the prokaryotic *lac Z* gene (λgt10LacZ vector), integrated in a head-to-tail arrangement in the genome, which provides a target for mutagenesis in all organs and tissues of the transgenic animal (*17*). The integrated vectors can be rescued from total genomic DNA with high efficiency by packaging *in vitro* and the induction of mutations can be analyzed after propagation of the phages in a $LacZ^-$ strain of the bacterium *Escherichia coli* C (*18*). Several strains of transgenic mice have been produced, each with a different number of shuttle vectors at a different integration site in the genome (*17*).

In Figure 4, an example is given of the induction of mutations in the brain and liver of the strain 20.2 transgenic mouse (80 copies of the λgt10LacZ vector on an autosomal chromosome), after exposure to the directly acting DNA alkylating agent *N*-ethyl-*N*-nitrosourea. After administration of 250 mg/kg body weight of the alkylating agent, the mutation frequencies in brain cells

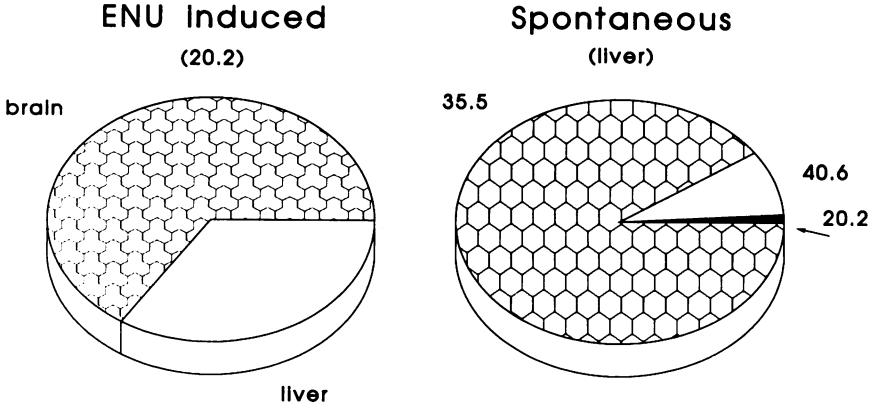

FIGURE 4. Induction of mutations in the λgt10LacZ vector in transgenic mice. (Left) Relative number of mutations induced in the brain and liver of strain 20.2 after exposure to 250 mg *N*-ethyl-*N*-nitrosourea (ENU) per kg body weight. Mutation induction was analyzed 7 days after treatment with ENU (mutation frequency: brain cells, 7.1×10^{-5} liver cells, 2.1×10^{-5}). (Right) Spontaneous mutation frequencies in the DNA of liver cells from three transgenic mouse strains (20.2: 80 copies, autosomal, mutation frequency 0.1×10^{-5}; 40.6: 35 copies, autosomal, mutation frequency 0.7×10^{-5}; 35.5: 15 copies, X-chromosomal, mutation frequency 15.5×10^{-5}).

appeared to be three times greater than those in liver cells from the same animal. Since in this case the vectors were isolated from postmitotic brain tissue of adult transgenic mice, DNA replication had not taken place after exposure, and the observed mutation may have been the result of an on-going error-prone DNA repair process in nondividing cells. This experiment shows that even after exposure to a directly acting agent like *N*-ethyl-*N*-nitrosourea, which induces a similar kind and similar amounts of DNA lesions in all cells of the body, there is a striking difference in mutation induction in different organs.

Of course, it must be realized that the λgt10LacZ vector is heavily methylated *in vivo* and should be considered an inactive gene; no information can be given on the possible differential response of mutation induction in an active housekeeping gene. Still, this kind of experiment provides a first approach to mechanistic studies at the organ and tissue level in an individual mammal. Recently, Gossen et al. (*19*) provided evidence that the integration site of the vector is an important parameter in the occurrence of spontaneous mutations. They found that the background mutation frequencies in three transgenic mouse strains (20.2: 80 copies, autosomal; 40.6: 35 copies, autosomal; 35.5: 15 copies, X-chromosomal) varies considerably. Especially noteworthy was the high spontaneous mutation frequency in cells of the liver (and also the brain) of strain 35.5 (Fig. 4). These results indicate that the mutability of genes present in the genome of mammalian cells is not uniform and, therefore, clearly indicates the presence of mutational "hot spots" in the genome of somatic cells *in vivo*.

Biomonitoring Exposure to Genotoxic Agents in Human Populations

Cytogenetics

It is generally recognized that chromosomal changes are associated with the process of tumor development. Although the molecular basis for the relationship between specific chromosomal changes and cancer is not well understood in all cases, cytogenetics is one of the most frequently used methods to establish human exposure to mutagens and/or carcinogens. Conventional techniques for measuring chromosomal changes require that the cells (in humans, usually stimulated peripheral blood lymphocytes) be proliferating, so that chromosomes in interphase can be seen at mitosis or as micronuclei (*20*). This, however, limits application of this approach to a specific class of cells.

A method for studying chromosomal aberrations in nondividing cells has been improved and made available for screening of large human populations. This involves the technique of premature chromosome condensation (PCC); i.e., when mitotic cells are fused with interphase cells, the nuclei of the interphase cells undergo an immediate prophaselike reaction, such that the nuclear envelope is disassembled and the chromatin condensed into chromosomes (Fig. 5). This technique allows sensitive monitoring of accumulation of chromosomal damage in slowly dividing or nonproliferating cell populations of human origin. The sensitivity of the PCC method in comparison with that of

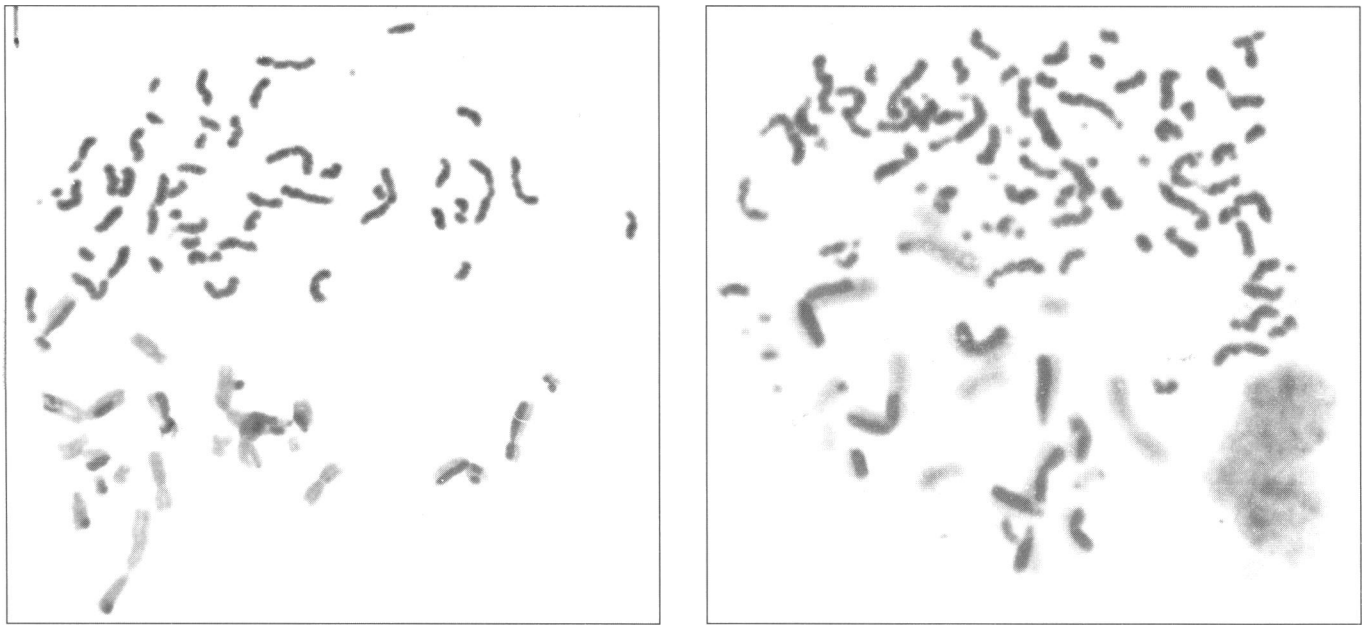

FIGURE 5. Prematurely condensed chromosomes obtained from fusion between a human interphase T-cell and a Chinese hamster ovary mitotic cell. A differential staining technique makes the prematurely condensed chromosomes dark and the mitotic chromosomes light. (*A*) Prematurely condensed chromosomes from control human T-cells; (*B*) prematurely condensed chromosomes from human T-cells irradiated with 8 Gy of X-rays.

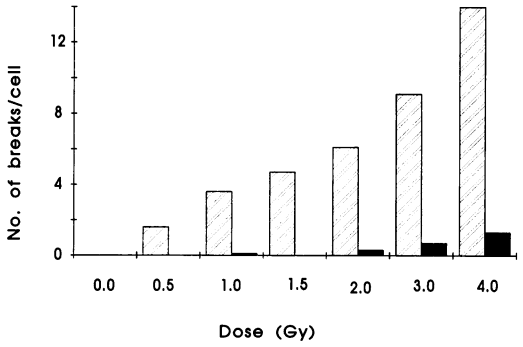

FIGURE 6. Induction of chromosomal breaks in human lymphocytes after irradiation with X-rays *in vitro*. The breaks were measured by the prematurely condensed chromosome method in nondividing cells 1 hr after irradiation (hatched bars) and in metaphase preparations of stimulated, dividing cells 48 hr after irradiation (solid bars).

classical detection of chromosomal breaks in metaphase preparations is demonstrated in Figure 6. In this experiment, human lymphocytes were irradiated with various doses of X-rays, and chromosomal breaks were scored using the PCC method 1 hr after irradiation and as chromosomal fragments in stimulated irradiated cells 48 hr after irradiation. As can be concluded from the data shown in Figure 6, the PCC method is about 10 times more sensitive than the classical method of scoring chromosomal fragments.

The PCC technique is also useful for monitoring responses to therapeutic treatment of malignant tumors (*21*). In principle, it will even be possible to study partial body irradiation due to occupational and/or accidental exposures. The cytogenetic damage in the fraction of exposed cells present among a huge number of normal cells can be detected with PCC. This was shown in a reconstruction experiment in which human lymphocytes were irradiated with X-rays (8 Gy) and mixed with a specified amount of un-irradiated cells (Table 3). Subsequently, the human cells were fused with mitotic Chinese hamster cells, and chromosomal fragments (PCC) were scored in individual cells; thus the fraction of damaged/undamaged cells in the mixed population can be accurately calculated. The PCC method is sensitive enough to detect the presence of only 3% unirradiated cells in a population of 97% irradiated cells (Table 3). This suggests that the extent of partial body irradiation *in vivo* could be accurately determined by the PCC method.

Table 3. Chromosomal damage in human lymphocytes after 8 Gy of X-rays as measured by premature chromosome condensation.

Damaged cells (initial), %	No. of cells scored	No. of cells damaged	Damaged cells (measured), %
0	156	0	0.0
10	100	13	13.0
30	91	26	28.6
50	46	18	39.1
70	55	41	74.5
80	56	46	82.1
90	57	51	89.5
95	48	45	93.8
97	52	51	98.1
99	45	45	100.0
100	79	79	100.0

Molecular Dosimetry of DNA Lesions

Approaches for using DNA lesions as a basis for elucidating dose–response relationships in carcinogen-exposed humans and animals have been developed through molecular dosimetry. In comparison with approaches based on external exposure, molecular dosimetry offers the distinct advantage of being capable of integrating dose-dependent differences in absorption, distribution, biotransformation, and DNA repair, allowing the determination of exposures to carcinogens more accurately and over a wide range of doses (*22*). Molecular dosimetry *in vivo*, especially in humans, is complicated by a number of factors, the major one being that DNA lesions are formed in relatively small quantities, at an upper limit of about 100 fmole/μg DNA [< 3 DNA lesions per 10^5 "normal" DNA nucleotides (*23–25*)]; in humans, often levels down to 3 DNA lesions per 10^7 "normal" nucleotides are often common.

Samples of human target tissues cannot be collected routinely, and surrogates (like DNA from nucleated blood cells) must be used. As long as there is a parallel between the level of DNA lesions in target and surrogate tissues, the latter can be used as a measure of the former. Another possible surrogate as an indicator of DNA damage may be protein damage: use of hemoglobin adducts as a dose monitor for alkylating and arylating genotoxic agents has been developed successfully by the group of Ehrenberg [(*26*) for further references, see Ehrenberg (*27*) and Neumann (*28*)]. The existence of a linear relationship between the levels of DNA lesions and of the carcinogen in the environment or absorbed into the body will, of course, facilitate such a comparison. Such parallels and linear relationships have been observed in experimental animals and are indicated in man, but cannot be generalized (*29*).

Two techniques—immunoassays to measure the presence of specific DNA adducts and ^{32}P-postlabeling—have found general utility for detection of DNA lesions in both experimental animals and humans. In the immunological approach, monoclonal or polyclonal antibodies are raised against either carcinogen-modified DNA or carcinogen–nucleoside DNA lesions (adducts) coupled to protein carriers. The antibodies are then used to quantify specific adducts in the DNA of exposed cells. In the ^{32}P-postlabeling method, some DNA adducts can be detected at a level approaching one adduct in about 10^{10} "normal" nucleotides (at least 10–100 times more sensitive than comparable immunochemical techniques). A major problem with the ^{32}P-postlabeling method is, however, identification of the observed adducts. Adducts to hemoglobin and serum albumin are the only protein adducts so far used for molecular dosimetry in human samples. Protein adducts are not repaired and have a relatively slow turnover rate, which allows detection of accumulated damage. Furthermore, protein samples are generally available in much larger quantities than DNA samples *in vivo*, allowing a

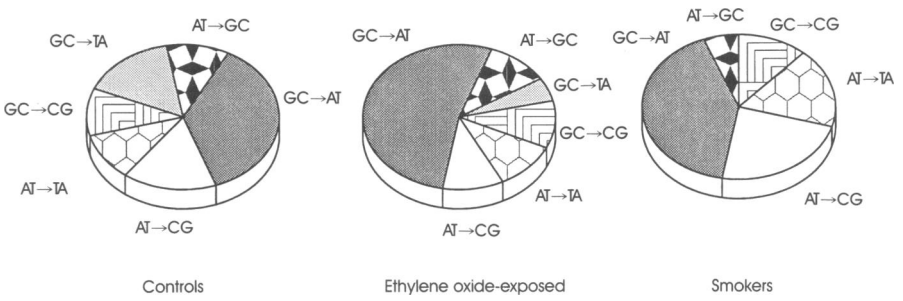

FIGURE 7. Induction of DNA alterations in the *hprt* gene of mutant human T-cell clones derived from blood. DNA base substitutions in mutant *hprt* clones of human T-cells from normal, healthy donors (controls), individuals occupationally exposed to varying doses of ethylene oxide, and from smokers.

more sensitive approach to molecular dosimetry [for reviews of DNA and protein adducts, see Lohman et al. (*24*) and Wogan (*29*)].

Gene Mutation and Spectral Analysis of DNA Alterations in Mutated Genes

Different genotoxic agents induce different DNA lesions in exposed cells, and the DNA alterations that result from those lesions vary with different agents. This spectrum of DNA alterations can be measured, for instance, in the endogenous *hprt* gene (located on the X-chromosome) of preselected mutant T-cells from the peripheral blood of exposed people.

Molecular analysis of DNA alterations in individual genes became possible after introduction of the polymerase chain reaction technique by Mullis et al. (*30*). Vrieling et al. (*31,32*) adapted this technique for the analysis of DNA alterations in the *hprt* gene of mammalian cells. For this purpose, cDNA derived from *hprt* mRNA from an expressed mutant gene is amplified and sequenced; by comparison with the known DNA sequence of the wild-type *hprt* gene, it is possible to determine which DNA alteration has been induced in the mutant gene. Figure 7 shows an example of the spectrum of DNA alterations found in *hprt* T-cell mutants isolated from the blood of normal, healthy people (background mutation spectrum; controls in Figure 7), in people occupationally exposed to high concentrations of ethylene oxide, and in smokers. Only the spectra of DNA base alterations are given; information can also be obtained on the occurrence of deletions, frameshifts, or base insertions in the *hprt* gene. The spectrum of DNA base alterations in the *hprt* gene already shows clearly that the background spectrum of DNA alterations is different from that of exposed people and that differences also occur depending on the agent. The spectrum of DNA alterations in mutant T-cells from smokers, for instance, is missing the class of GC to TA DNA base transversions, which is present in background mutations and to a lesser extent in mutated cells from people exposed to ethylene oxide (Fig. 7). The observation that the spectrum of mutations scored in the *hprt* gene of mammalian cells is unique for each genotoxic agent suggests that spectral analysis of DNA alterations, as measured in mutant T-cell clones, may be used in biomonitoring to indicate the kind of agents or mixtures of agents to which a population is exposed.

DNA Fingerprinting the Human Genome

Analysis of mutations or spectra of mutations in single endogenous genes in, for instance, human T-cell clones will not in most cases be sufficient for risk estimation, because the end point studied may not reflect the key event(s) leading to carcinogenesis. Alternative techniques are probably required to study un-selected mutation induction in target cells or to analyze mutations in key genes, such as oncogenes. It is expected that the field of DNA technology will provide the tools for such analyses. A preliminary approach is analysis of unselected mutations in the human genome using the DNA fingerprinting technique.

The human genome is estimated to contain several thousand micro- and minisatellite DNA sequences, which are spread more or less evenly over the chromosomes. Together, these sequences roughly compose about 1% of the total number of base pairs. It has been demonstrated that a fraction of these sequences [the so-called variable number of tandem repeats (VNTR) sequences] represents a relatively unstable part of the human genome during mitosis and/or meiosis (*33*). As a result, these sequences usually display extensive allelic variation in the human population. In view of their high copy number and their allelic variability, VNTRs can also serve as a genetic marker of such instability in the total genome. By using core probes, different sets of micro- and minisatellites, each consisting of several hundred loci (including VNTR loci), can be detected by Southern blot hybridization analysis. This process is also known as DNA fingerprinting.

In order to resolve DNA restriction fragments containing micro- and minisatellite sequences, two electrophoretic methods can be employed. Southern blot hybridization analysis encompasses the one-dimensional resolution of about 20–30 relatively large fragments (2–20 kb), while two-dimensional DNA fingerprinting can resolve virtually all members of a particular set of micro- and minisatellites. Using these two DNA fingerprinting techniques, the genome can be scanned for variations in micro- and minisatellite DNA sequences. Using this approach, it is

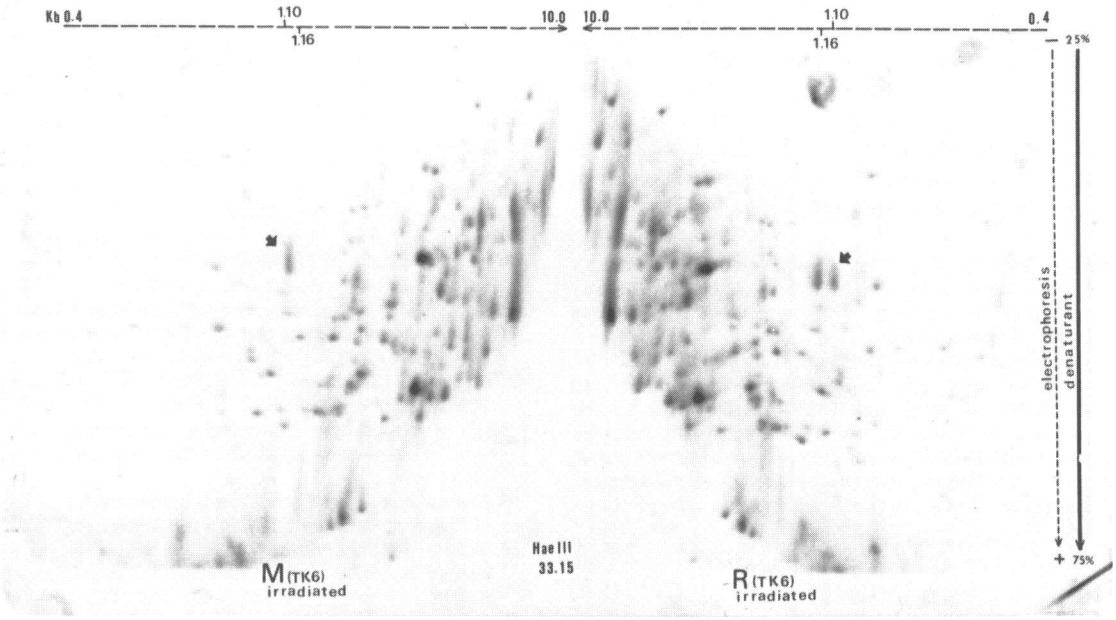

FIGURE 8. Two-dimensional DNA fingerprint of two clones (M and R) of the human lymphoblastoid TK6 cell line. Before isolation, the parent TK6 cells were irradiated with 0.54 Gy X-rays. A single mutant allele was found in clone R (arrow).

possible to measure human mutation rates at multiple sites dispersed through the genome (*34,35*).

In order to investigate the sensitivity of the DNA fingerprinting methods for the detection of DNA rearrangements and deletions, experiments were performed with the established human lymphoblastoid TK6 cell line. Independent TK6 clones were analyzed after X-irradiation in one- and two-dimensional gels (using the core probe 33.15 and *Hae*III as the restriction enzyme). After two-dimensional analysis of TK6 clones, an additional change was detected by comparing the DNA fingerprint of clones M and R; a single mutant allele (out of 550 analyzed) was observed (Fig. 8). Because few experiments have been done so far, however, this mutant allele may not have been induced by X-rays, but may due to the presence of mutant subpopulations in the stock culture. This problem is especially pertinent for the established lymphoblastoid cell line TK6, which has been cultured for many cell generations; it is, therefore, not surprising to detect the presence of subpopulations within the cell culture. The experiment demonstrated, however, that DNA alterations can be observed in unselected, randomly picked clones of cells of human origin.

Epidemiology and Risk Assessment

The main accomplishment of the past 20 years in the field of environmental and occupational monitoring of exposure to carcinogenic agents has been the development of methods for detecting qualitatively the genotoxic potential and, therefore, the possible carcinogenic potential of individual agents. The main deficiency has been that, neither in an absolute sense nor relatively in comparing chemicals, can a quantitative estimate be made of the carcinogenic potential of an agent for humans. Of course, a limited number of agents are known to be human carcinogens; however, this knowledge is far from sufficient for estimating the total carcinogenic potential of the universe of chemicals to which humans are exposed.

In human populations, the ad-hoc approach for determining the carcinogenic potency of a chemical will always fail, except in the rare cases in which a chemical is a carcinogen at any concentration, if the selection criteria for considering the chemical are based on nothing else but relative genotoxic potential, the amount and effectiveness of exposure, and mechanism of action. For a limited group of chemicals—the monofunctional alkylating agents—enough knowledge seems to be available to allow determination of relative genotoxic potential, to measure exposure to individual chemicals in a population, and, at least for directly acting agents, to allow a quantitative risk assessment. The best example so far is that developed by Ehrenberg: exposure of a human population to ethylene oxide using the radiation dose equivalent approach [for review, see Wright et al. (*36*)].

Because of the complexity of the situation, there is an increasing need in epidemiology for reliable, sensitive techniques to monitor exposure of human populations to mixtures of potential genotoxic agents. Although significant progress has been made in detecting DNA damage, DNA alterations, and mutations *in vivo*, the methods are often still too cumbersome to be applied to large populations and are restricted to a limited group of chemicals.

The striking organ and tissue specificity of known carcinogens in both experimental animals and humans should command considerable attention in the coming

years. The hypothesis that key DNA lesions will determine whether a chemical is an organ- or tissue-specific carcinogen may still be valid. Unfortunately, however, there is evidence that such key lesions may be different for different chemicals. So, more knowledge must be collected about the mechanism of carcinogenesis, beyond the formation of DNA lesions, before a rational approach can be evolved to identify specific or groups of key DNA lesions.

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