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Endogenous genotoxic agents and processes as a basis of spontaneous carcinogenesis

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Summary

A list of endogenous DNA-damaging agents and processes is given. Endogenous *electrophiles* are found with the cosubstrates of physiological transfer reactions (S-adenosylmethionine for methylation, ATP for phosphorylation, NAD⁺ for ADP-ribosylation, acetyl CoA for acetylation). Aldehyde groups (glyceraldehyde-3-phosphate, formaldehyde, open forms of reducing sugars, degradation products of peroxidation) or alkylating degradation products derived from endogenous nitroso compounds represent additional possibilities. *Radical*-forming reactions include leakage of the superoxide anion radical from terminal cytochromes and redox cycles, hydroxyl radical formation by the Fenton reaction from endogenous hydrogen peroxide, and the formation of lipid peroxides. *Genetic instability* by spontaneous deaminations and depurinations as well as replicative instability by tautomer errors and in the presence of mutagenic metal ions represent a third important class of endogenous genotoxic processes. The postulated endogenous genotoxicity could form the mechanistic basis for what is called 'spontaneous' tumor incidence and explain the possibility of an increased tumor incidence after treatment of animals with non-genotoxic compounds exhibiting tumor-promoting activity only. Individual differences are expected to be seen also with endogenous DNA damage. The presence of *endogenous* DNA damage implies that *exogenous* DNA-carcinogen adducts give rise to an incremental damage which is expected to be proportional to the carcinogen dose at lowest levels. An increased tumor risk due to exposure to exogenous genotoxic carcinogens could therefore be assessed in terms of the background DNA damage, for instance in multiples of the mean level or of the interindividual variability in a population.

A large number of potent chemical carcinogens (aflatoxins, aromatic amines, polycyclic aromatic hydrocarbons, nitroso compounds) form chemically reactive (electrophilic) intermediates which

bind covalently to DNA of target cells. If these DNA-carcinogen adducts are not repaired before the DNA replicates, a mutation in a daughter strand can result. If this occurs in a critical gene, a heritable step towards malignant transformation of the cell could be taken. In an attempt to correlate carcinogenic potency with genotoxic potency (Lutz, 1979), a number of carcinogens were identified which did not bind covalently to

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DNA (Lutz, 1986a). The mechanism of tumor induction by these compounds could either rest upon a genotoxicity not based on DNA adduct formation, or upon non-genotoxic mechanisms of action, such as a more rapid expression of some endogenous DNA damage to tumor growth (Lutz, 1982; Lutz and Maier, 1988).

The idea that endogenous compounds might be responsible for what is called 'spontaneous' tumors emerged from the structural similarity of steroids with polynuclear aromatic hydrocarbons (Dannenbergh, 1958). Years later, the hypothesis of potential endogenous mutagens and carcinogens was elaborated in a more general way by Soloway and Lequesne (1980). They expanded the list with epoxide intermediates of aromatic amino acids, of squalene, and of various steroids, as well as with peroxides and hydroxyalkyl amines postulated as intermediates in the oxidative dealkylation of alkylamines. The authors concluded (without presenting quantitative data) that endogenous genotoxins might be of primary importance. An experimental approach to answer the question in a quantitative manner was not available at that time.

In this overview, a list of potential endogenous genotoxic agents and processes will be presented and some general consequences for toxicology will be discussed. Selected aspects will be discussed in other contributions to this special issue.

Endogenous genotoxicity

Electrophilic genotoxic agents

Most well-known potent chemical carcinogens pass through a stage of electrophilic reactivity during metabolism, and the carcinogenic potency of these compounds is related to the extent of covalent binding to DNA (adduct formation; Lutz, 1979). The electrophilic 'ultimate' forms react not only with DNA but with all nucleophilic centers available in a cell, the proportions being dependent on localization, concentration and nucleophilic strength of the reaction partners. The sulfhydryl group of cysteine, free or in a peptide configuration, is the most reactive cellular nucleophile, nucleic acids are much weaker. Water is also a weak nucleophile but because of the high concentration in a cell (about 50 M!) most electro-

TABLE 1
ENDOGENOUS ELECTROPHILIC COMPOUNDS

Class/Reaction	Example	Comment
Group-transferring coenzymes	S-Adenosylmethionine NAD ⁺ ATP, Acyl-P, Enol-P	Aberrant reactions with nucleophiles in the absence of substrate and transferase enzyme
Aldehyde (Ketone) (Schiff base formation + rearrangements)	Pyridoxalphosphate Formaldehyde Reducing sugars 4-Hydroxynonenal	Intermediate in glycolysis Demethylation of steroid and choline Ring-open forms From lipid peroxidation
Hydroxymethylamine Epoxide	From adrenaline Squalene-2,3-oxide Cholesterol-5,6-oxide Estroside-16 α ,17 α Ethylene oxide	Oxidative <i>N</i> -dealkylation For steroid biosynthesis Remarkably stable Remarkably stable Ethene from lipid peroxidation
Quinone	From catecholamine, estrogens	Reactive with DNA?
Enol ether Alkylating agent	Fecapentaenes From <i>N</i> -nitroso compounds	Fecal mutagens Nitrosation in stomach, in bacteria, macrophages
Aromatic amine	Kynurenine	Degradation of tryptophan; <i>N</i> -oxidation unlikely

philic molecules react to some extent with water, i.e., they are hydrolyzed. This also means that all physiological compounds which can be hydrolyzed, could, in principle, also react with nucleophilic centers in DNA and become genotoxic.

Group-transferring coenzymes. The first 4 examples given in Table 1 are all physiological group transfer reactions which are normally controlled by the presence of enzymes so that only the wanted substrates are used. In the absence of the enzyme and a suitable acceptor molecule, reactions with other nucleophiles can occur. The reactivity with weak nucleophiles such as water or DNA is slow, but these transfer processes are so general and ubiquitous that all cells might be affected to some extent. While the potential genotoxicity of S-adenosylmethionine has been described (Barrows and Magee, 1982; Naeslund et al., 1983; Lindahl, 1990, this issue, p. 305) I am not aware of experimental data with NAD⁺ or active phosphate groups.

Aldehydes. The reactivity of carbonyl groups of aldehydes with nucleophiles is well known to the chemist. This reactivity might be important because of the relatively high concentrations. However, with formaldehyde generated in rat liver by a first-order oxidation of methanol (Lutz, 1986b), we were not able to detect any increase in DNA-protein crosslinks in rat liver. Since endogenous formation of formaldehyde by oxidative demethylation during steroid biosynthesis will be much lower than from high-dose methanol oxidation, the putative DNA damage must be very small. Other aldehydes, such as open forms of reducing sugar molecules (Bucala et al., 1984; Lee and Cerami, 1987; Lee et al., this issue) or aldehydes derived from lipid peroxidation (Grafström et al., this issue, p. 175; Esterbauer et al., this issue, p. 223) might be more important.

Oxidative demethylation of *N*-methyl compounds proceeds via *hydroxymethylamine* intermediates. The methylene carbon atom between the nitrogen and the oxygen is electrophilic and a reaction with a cellular nucleophile can result. This reaction is reversible, however, and it is unlikely that large amounts could be present on DNA.

Epoxides. The endogenous epoxides appear to fall into 2 categories. Those which have been investigated in vivo (cholesterol-5,6-oxide, estroside) were chemically inert with nucleic acids (Caviezel, 1984) and not mutagenic (Glatt et al., 1983). Potentially more reactive epoxides which could be formed as intermediates of aromatic hydroxylations (catecholamine biosynthesis) have not been isolated so far. The role of epoxides as endogenous genotoxic agents is therefore uncertain.

Quinones are known to react readily with glutathione and other thiols. Reactivity with DNA appears to be much slower. The synthetic estrogenic carcinogen diethylstilbestrol, for instance, forms quinones in vivo, but it is still not known to what extent estrogenic vs. genotoxic activity contributes to the tumor induction in male hamster kidney. We have not been able to detect DNA adducts in this target organ after administration of radiolabeled diethylstilbestrol (Lutz et al., 1982). However, with the more sensitive postlabeling technique, adduct formation has been reported (Liehr et al., this issue, p. 269).

Enol ethers. The mutagenic activity detected recently in human fecal extracts has been attributed to the presence of enol ethers (conjugates of polyunsaturated alkenes with glycerol; Gupta et al., 1984). The presence of 5 conjugated double bonds has been seen only in samples from humans and pigs. The source of this unsaturated alkenyl chain is not known.

Bile acids have for a much longer time been discussed as endogenous factors in the etiology of colon cancer. Most data available indicate an epigenetic rather than a genotoxic mode of action, however (Hill, this issue, p. 313).

Diazonium ions can be derived from *N*-nitroso compounds. The nitrosation of precursors can occur in the stomach under acidic conditions or can be catalyzed by bacteria (Bartsch et al., this issue, p. 255). In addition, nitroso compounds have been shown to be generated in activated macrophages during oxidative breakdown of arginine (Miwa et al., 1987). Depending on the nature of the nitrosated precursor (Shephard et al., 1987), nitroso compounds decompose spontaneously to electro-

TABLE 2
RADICAL-FORMING COMPOUNDS

Class	Example	Comment
Molecular oxygen	Superoxide anion radical	Leakage from cytochromes Redox cycling quinone-hydroquinone
Unsaturated lipids	Hydroxyl radical Peroxides, hydroperoxides	From hydrogen peroxide Endogenous in prostaglandin synthesis
Quinone	Semiquinone radical	Endogenous: coenzyme Q

philic species (e.g., primary amino group; Maier et al., this issue, p. 193) or they can require metabolic activation. These variables determine whether a specific tissue is affected or not.

Aromatic amines are well-known carcinogens for the human bladder. This structural element is also seen in degradation products of tryptophan, the kynurenines. Unlike with standard aromatic

amine carcinogens, these tryptophan catabolites are not monosubstituted but they do contain other substituents for further metabolism. Oxidation at the nitrogen (which is required for enzymatic activation to a mutagen) is therefore probably only a minor pathway.

In summary, a large number of endogenous compounds are electrophilic or can form electrophilic intermediates. The above list is not nearly

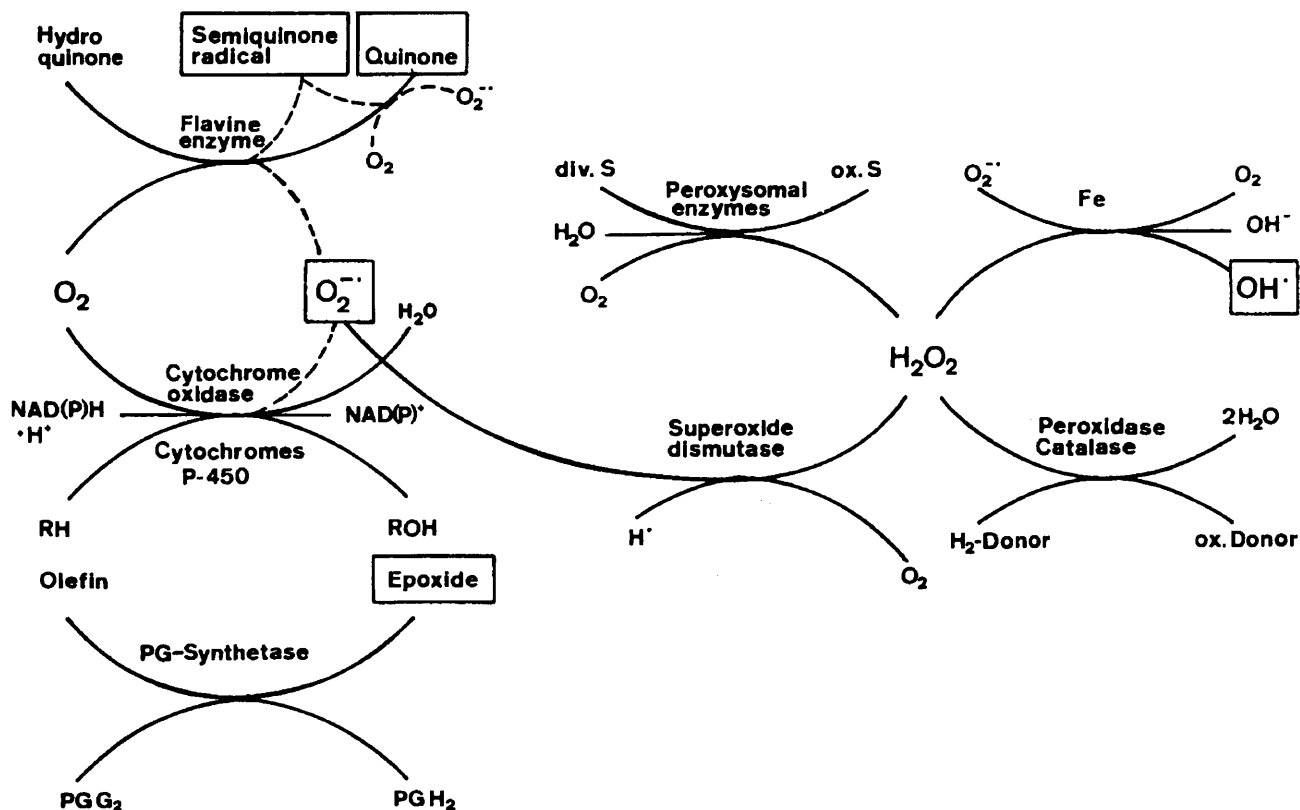


Fig. 1. Scheme of enzymatic pathways involved in the generation and inactivation of oxygen radicals. Formation of electrophilic metabolites in coupled reactions. Genotoxic compounds in boxes. S, RH: substrates; PG: prostaglandin.

complete. The analysis of a number of natural amino acids for mutagenicity in the Ames test has revealed, for instance, that conditions can be found for many amino acids to become mutagenic for *Salmonella* (Glatt et al., this issue, p. 235).

Radicals

Two major types of reactions leading to oxygen-centered free radicals, reduction of molecular oxygen and lipid peroxidation, have recently been reviewed by Marnett (1987). A third class, the formation of semiquinone radicals, has been added in Table 2 for the sake of completeness.

Peroxidation of lipids is not only due to autoxidation but is also a physiological process, for instance in prostaglandin synthesis. As a side reaction of the reduction of the hydroperoxide prostaglandin G_2 to the hydroxide prostaglandin H_2 , chemically reactive epoxides can be formed with appropriate substrates (Fig. 1, bottom left). A very general process leads to the very reactive and dangerous hydroxyl radical $OH\cdot$ from the superoxide anion radical $O_2^{\cdot-}$. This oxygen species is formed as an intermediate by 1-electron reduction of molecular oxygen. It is an unavoidable leakage product of the reduction of oxygen to water by the terminal cytochromes. This process is fundamental for the energy production by aerobic organisms (Adelman et al., 1988). In addition, the respiratory burst of macrophages is an example where the generation of oxidative equivalents to destroy foreign material is a desired process. In general, however, the protection of aerobic organisms by appropriate enzymatic detoxification is obligatory for survival. It involves dismutation of 2 superoxide molecules to hydrogen peroxide and molecular oxygen by superoxide dismutase. Hydrogen peroxide is not very reactive by itself. The problem is the Fenton reaction to the hydroxyl radical which is catalyzed by iron or copper ions in the presence of a reducing agent such as the superoxide anion (Fig. 1, right-hand side).

Additional pathways leading to superoxide anions and to hydrogen peroxide are shown in the left and middle top part of Fig. 1. (i) A number of peroxisomal enzymes oxidize substrates with concomitant production of hydrogen peroxide. This aspect led to the hypothesis that the hepatocarcinogenic activity of peroxisome stimulators

(hypolipidemic agents like clofibrate, plasticizers of the di(2-ethylhexyl)phthalate type) is due to indirect DNA damage by uncontrolled hydroxyl radicals. (ii) The redox cycling of quinones/hydroquinones can involve the 1-electron step to the semiquinone radical. The unpaired electron of the semiquinone can be transferred to molecular oxygen.

The processes summarized in Fig. 1 are of a very fundamental nature and it is not surprising that oxygen-related processes have been investigated for a long time in chemical carcinogenesis and aging. It has to be kept in mind, however, that highly efficient protective mechanisms have evolved in aerobic organisms. Furthermore, I am not aware of any report where DNA damage has been demonstrated unequivocally in a target cell population after treatment of animals with a carcinogen postulated to act by indirect, oxygen radical-related DNA damage. On the other hand, the urinary excretion of astonishingly high levels of thymidine glycol, thymine glycol, and 8-hydroxy-deoxyguanosine (8-OH-dG) indicates that oxidation of DNA is an unavoidable, permanent process. The steady-state level of oxidized nucleotides in DNA depends upon the rate of repair of the lesion involved. High levels of 8-OH-dG (but not of thymidine glycol; Hegi et al., this issue, p. 325) are found in control rat liver DNA. The consequences of these lesions for carcinogenesis are not known. It is difficult, therefore, to predict the importance of the above-mentioned processes.

Genetic instability

Spontaneous mutagenesis in microorganisms has been of interest for many decades and the genetic approach has provided valuable insight into the types of mutations occurring, such as replication errors, recombination errors, and repair errors (review by Sargentini and Smith, 1985).

The genetic material cannot therefore be regarded as a stable entity, even in the absence of reactive compounds. The best known examples of instabilities are compiled in Table 3. Loss of the guanine or adenine base from DNA is judged to be in the order of 20000 per mammalian cell per 24 h (Lindahl and Nyberg, 1972). Deaminations occur much less frequently, in the order of 10 times per cell per 24 h. Thanks to highly efficient

TABLE 3
GENETIC INSTABILITY

Class	Example	Comment
Chemical instability	Depurination Deamination	Much less frequent, but note: from 5-methylcytosine to thymine!
Replicative instability	Tautomer errors 'Mutagenic' metal ions	Some essential!
Postreplicative instability	Sister-chromatid exchange	

repair (Lindahl et al., this issue, p. 305), the steady-state level of damage appears to be reduced to about 1 error per cell.

It is important to note that the deamination of 5-methylcytosine (m^5C) leads to thymine, i.e., to a normal DNA base (Ehrlich et al., this issue, p. 277). The T-G mispair can be repaired in the correct way because of the opposite m^5C , but this is possible only as long as the DNA is in a double-stranded conformation. Once the 2 strands separate for replication, this neighborhood information is no longer available and an adenine will be placed opposite the wrong thymine base. This type of mutation might be important for the regulation of gene expression because the formation of m^5C is known to be used for regulatory purposes.

DNA replication, in its own right, is not a perfect process. Estimations of the number of mismatches per generation in eukaryotic cells span 3 orders of magnitude, probably because the fidelity is dependent on the specific cell type and the conditions of the assay (Loeb et al., this issue, p. 297). It is therefore difficult at the present time to predict the relative importance of these sources of DNA damage in vivo.

In addition to these changes at the nucleotide level, a number of rearrangements of the genetic material appear to happen frequently and be required for some specific tasks (e.g., structural variability of antibodies). Close to the idea of genetic damage is the phenomenon of sister-chromatid exchange. Although the consequences of this process in terms of mutations are not known it is a

sign of genetic instability with the inherent possibility of structural changes.

The above-mentioned list should convincingly show that DNA damage is not avoidable and that exogenous genotoxic agents merely increase the DNA damage and the probability for a cell to accumulate all changes required for cancerous transformation within the life span of the organism.

DNA adducts and carcinogenesis

Experimental approaches

Attempts to treat animals with radiolabeled physiological compounds (estrone, estradiol, formaldehyde generated by methanol) and identify DNA adducts or DNA-protein crosslinks have all produced negative results (Caviezel et al., 1984; Lutz, 1986b). Only with the introduction of the postlabeling method for nucleotide-carcinogen adducts by Randerath et al. (1981) did new possibilities open. Radiolabeled spots indicating modified nucleotides were found not only after treatment with carcinogens but also in untreated animals.

DNA adducts and tumor incidence

With an exogenous carcinogen, the quantitative relationship between steady-state DNA adduct level and tumor incidence has been investigated with aflatoxin B₁ (Buss and Lutz, 1988). It was shown that an adduct level of 1 per 10⁶ nucleotides is formed in liver DNA of male F344 rats upon chronic administration of aflatoxin B₁ which would induce about 50% tumor incidence at 2 years. The DNA damage seen with postlabeling in *untreated* rats (so-called I-compounds) has been shown to increase with age and achieves levels in the order of 1 per 10⁷ nucleotides in the liver at the age of 10 months (Randerath et al., 1988). This is about one tenth of the aflatoxin adduct level that would result in a 50% tumor incidence. Since the reported spontaneous liver tumor incidence in male F344 rats is only 1–2% it appears that the 2 types of DNA modifications do not have the same effect in hepatocellular carcinogenesis (Table 4).

In humans, a similar comparison was attempted by correlating lung cancer incidence with DNA adduct levels determined in bronchial biop-

TABLE 4
CORRELATION OF DNA ADDUCT LEVELS WITH TUMOR INCIDENCE

Agent(s)	Organ	Species	Adducts/10 ⁹ nucleotides	Cumulative incidence
Smoking	Bronchus	Human	100 ^a	10-20% ^b
'Endogenous' (non-smoker)	Bronchus	Human	35 ^a	1-2% ^b
Aflatoxin B ₁	Liver	Male rat	850 ^c	≈ 50%
Endogenous	Liver	Male rat	≈ 100 ^d	1-2%

^a Only large, lipophilic adducts (Stich and Dunn, 1988).

^b 0-74 years, average smoker vs. non-smoker.

^c At 2 μg/kg/d (approx. TD₅₀) (Buss and Lutz, 1988).

^d Randerath et al., 1988.

sies of smokers and non-smokers (Stich and Dunn, 1988). The mean level of adducts determined in smokers was only a factor 3 higher than in non-smokers. The tumor incidence is higher by a factor 10, however (Table 4). This quadratic relationship between DNA damage and tumor incidence finds an interesting counterpart in a correlation of lung cancer incidence with the number of cigarettes smoked per day. Here again, a quadratic fitting was significantly better than a linear one (Zeise et al., 1987).

Two shortcomings of the above analysis must be mentioned. (i) It is not possible in humans to separate truly endogenous from unavoidable DNA damage, for instance by dietary carcinogens (especially aromatic amines and polynuclear aromatic hydrocarbons from heat processing). (ii) The method used for the determination of the adducts in the human DNA samples was appropriate only for large, lipophilic adducts.

I-compounds: DNA damage or differentiation signal?

The most recent data indicate that some ³²P spots indicative of DNA adducts decrease in the course of treatments which would ultimately lead to liver tumors (Randerath et al., this issue, p. 245). Therefore, it appears as if not all postlabeling spots have the meaning of a DNA damage. If we take into account that individual I-spots can represent as little as a few adducts per cellular genome it is tempting to postulate that this could also be a physiological postreplicational modification of the DNA, possibly for regulating gene

expression. This hypothesis has been excluded by Randerath et al. (1988) on the basis of experiments with partial hepatectomy in rats. One week after a two-third removal of the liver, the level of m⁵C was back to the old value whereas the level of I-compounds was still down at about 46%, in agreement with the dilution factor due to newly synthesized DNA. Still, it has to be considered that the new parts of the liver, although regenerated with respect to number and size, have not yet reached their final status of differentiation and might not have restored the old level of I-compounds.

In summary, the postlabeling method as an extremely sensitive tool to detect altered DNA nucleotides has enabled us to detect what might be endogenous DNA damage. At the same time, a number of I-compounds decreased together with cancer formation. It will be most challenging to investigate the chemical nature of these adducts, regardless of whether they represent damage or signal, or both.

Toxicological consequences of endogenous DNA damage

Dose-response relationship for exogenous carcinogens

If we accept the presence of endogenous DNA damage, the damage exerted by exogenous carcinogens merely gives rise to an increment which results in an exposure-related additional risk of tumor formation. At very low dose levels, where all processes follow first-order kinetics, the

additional damage is expected to be proportional to the dose (Lutz, 1987). In view of the analysis discussed above it is doubtful whether this proportionality also holds between DNA damage and tumor incidence. Linear extrapolation of a tumor incidence can only be postulated on the assumption that the DNA damage produced by the exogenous carcinogen affects only 1 step in the process of carcinogenesis. The presence of endogenous DNA damage does not, therefore, help to solve the problem of low-dose risk estimation.

Exposure to exogenous carcinogens will produce additional DNA damage. This increment can now be expressed in terms of an average endogenous DNA damage (Fig. 2A). One could, for instance, estimate the dose of the exogenous carcinogen which would double the average background level of DNA adducts.

Individual variability

Individuals differ with respect to the activity of activating and inactivating enzymes responsible for the formation and detoxification of electrophilic intermediates (Autrup et al., 1986). A similar variability is expected to govern the reactions of endogenous genotoxic agents with DNA and the repair of the lesions. It is therefore expected that individuals will show different levels of endogenous DNA damage and be at different risk of spontaneous tumor formation. The damage exerted by a given dose of an exogenous carcinogen could therefore be expressed as an increase in multiples of the variability (expressed for instance as a standard deviation) within the population (Fig. 2B).

It is not known whether individuals with a high level of endogenous DNA damage would also be at a higher risk for the conversion of exogenous carcinogens to DNA adducts, and vice versa. If it can be shown that this is the case, an individual starting with a high level of endogenous DNA damage would generate adducts from exposure to an exogenous genotoxin at a steeper slope than a low-risk individual (Fig. 2C). Under such a condition, high-risk individuals might have to be warned more urgently than low-risk individuals not to engage in activities which might be associated with additional exposure to genotoxic carcinogens (smoking, workplace, hobbies).

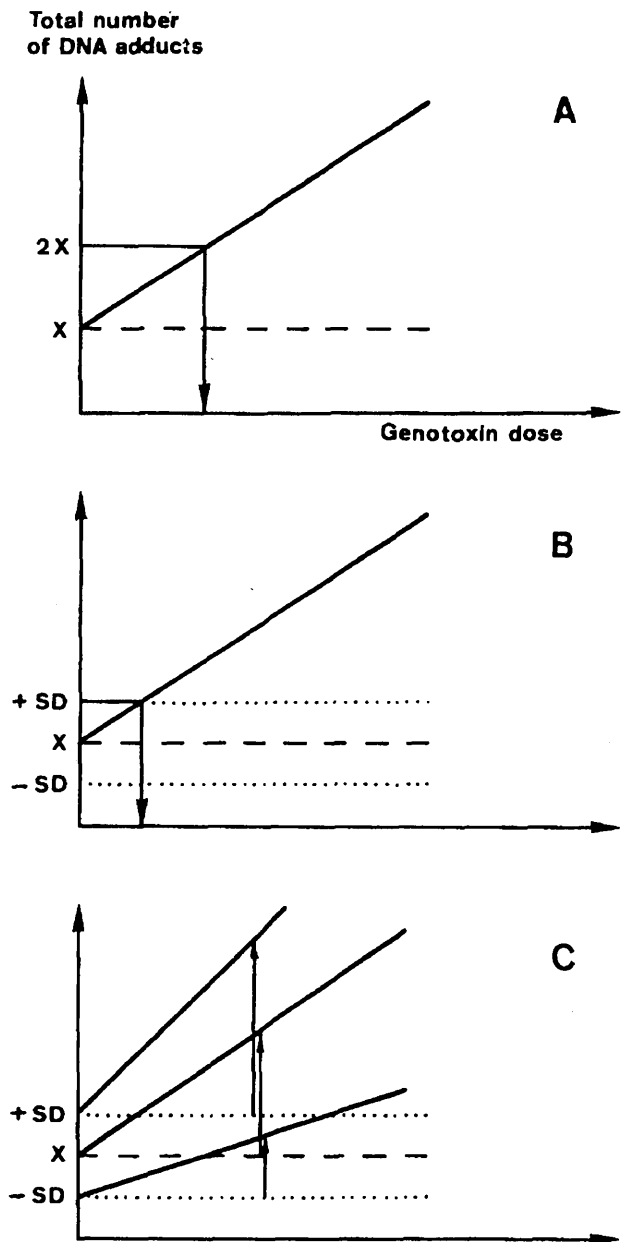


Fig. 2. Level of DNA adducts as a function of exogenous genotoxin dose. x , mean level of endogenous DNA adducts in a population. SD, standard deviation, denoting the individual variability within a population.

Conclusions

The new possibilities discussed above to value the effects of exposure to an exogenous DNA-damaging agent could form an interesting alternative to the absolute model used so far. In absolute terms, an acceptable daily intake of a carcinogen is often estimated on the basis of a rather arbitrary level of 1 additional tumor induced per

10^6 – 10^7 persons exposed. With endogenous DNA damage being present as a background, the additional risk from exogenous increments could be rated in relation to this unavoidable aspect of carcinogenesis.

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