entering the human environment. Any further improvement at the screening level would entail an expenditure of effort out of all proportion to the value of the additional information that might be gained." The committee further acknowledged "that in this rapidly developing area the state of knowledge is such that, because each test is directed at a limited aspect of the hereditary process, equivalent evidence derived from other test systems with different genetic endpoints could be accepted as an alternative to part of the basic package. However, the onus would have to be placed on the applicant to prove that the evidence produced was at least as good as would be expected from the tests recommended above." The committee also recommended a number of suplementary tests, the results of which might help to eliminate the findings in the basic screening. Such additional evidence could be important in a risk-benefit analysis.

In conclusion, the relatively new subject of genetic toxicology, based on a wealth of genetic and molecular biological knowledge has clearly had a large influence on toxicological thinking and practice. As the results from consistent mutagenicity testing accumulate, further advances in the understanding of the significance to human welfare of chemicals with genotoxic properties must occur in the immediate future. In the meantime, the "state of the art" is such that legislative action based on mutagenicity tests alone should be regarded as premature.

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CARCINOGENICITY OF ENVIRONMENTAL CHEMICALS

by
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Since the pioneering work of the E.C. Miller, P.N. Magee and others (1-4), the binding of chemicals or their metabolites to DNA, subsequent repair processes and cell transformation are generally accepted as important steps in the induction of tumours by carcinogens (3-7). However, the relative importance of the various steps involved in carcinogenesis is more debatable. Some researchers stress the role of chemical binding to DNA; others claim that the subsequent reactions in cells are more essential and both endogenous and exogenous factors may profoundly affect chemical carcinogenesis through cocarcinogenesis (8-11). To distinguish the lesions caused by carcinogens to genetic material from ordinary toxic (terminal) reaction, Saffiotti (12) suggests that the effects of carcinogens on potential later tumorigenesis be called self-replicating effects.

The purpose of the present review is to summarize the various steps that may be involved in chemical carcinogenesis. Some attention will be given to the various extrapolations involved in the evaluation of carcinogenic risks to humans from exposure to chemicals.

Classification of Chemical Carcinogens

Evidence obtained during the last 15 years indicates that most chemicals pose a carcinogenic risk only through their metabolic activation to reactive metabolites by various enzyme systems during drug biotransformation (3,5,7-9). The chemicals thus activated are called precarcinogens (procarcinogens), pointing to the importance of metabolic activation in their carcinogenicity (8,13). Most of the known chemical carcinogens belong to this group. Some chemicals known to be carcinogenic may, however, have reactive groups in their initial structures, for which reason they have been called "direct" carcinogens. Less is known about the mechanisms of carcinogenicity of various metals and metalloids or fibres, but heavy metals may act as carcinogens due to their capability to disturb the fidelity of DNA synthesis (14).

SECTION I

Carcinogenesis is often divided into two phases. In the initiation phase, the carcinogens are able to render cells into a form (or forms) that can be transformed into proliferative cancer cells by various mechanisms of the promotion phase. The dose of carcinogens in the initiation phase is probably the most important factor in determining the carcinogenic response, whereas the mechanisms in the promotion phase may regulate the length of the latency period (15,16).

Activation and Inactivation of Chemical Carcinogens in Drug Biotransformation

Xenobiotics are metabolized by various enzymatic systems which are mainly located in the endoplasmic reticulum or cytosol of most or all cells (17,18). The most active tissue in drug biotransformation is the liver, which probably contains more than 80% of the total body activity.

The most important enzyme system participating in the metabolism of xenobiotics, including chemical carcinogens, is the cytochrome P-450 containing mono-oxygenase; its main activity is located in the endoplasmic reticulum but, to a lesser extent, also in nuclear and outer mitochondrial membranes (17-19). In addition, xenobiotics may be oxidized or reduced by various flavoprotein enzymes. Aldehydes can be metabolized by aldehyde dehydrogenases present in the endoplasmic reticulum, mitochondria and cytosol and probably also by aldehyde oxidase. Amines are metabolized by various amine oxidases. Certain azo-compounds and nitro-compounds may be reduced by cytosolic or microsomal reductases that may thus participate in the metabolic activation in their chemical carcinogenicity (17,18,20).

In the typical sequence of drug biotransformation (Table 1), drugs are first oxidized, mainly by the mono-oxygenase system. Then they may be conjugated to various organic or inorganic acids, a step which considerably increases both the molecular size of the metabolized drugs and also their hydrophilism (17,21-23). In some cases, the hydrolytic division of the conjugated residue from the conjugated carcinogen may result in metabolic activation of carcinogens. Hydrolytic enzymes participating in this type of metabolic activation are located in the endoplasmic reticulum, cytosol or lysosomes. The lysosomal enzymes, with a low pH

Table 1. Pathways of drug biotransformation

Enzyme activities	Location
Oxidative or reductive	Mono-oxygenase (mainly microsomal):
	- aliphatic and aromatic hydroxylations (epoxidation)
	- N-, S- or O-dealkylation
	- N-, S-oxidation (also by amine oxidase of microsomes)
	- dehalogenation (possibly also by flavoprotein enzymes of microsomes)
	- desulfuration
	- deamination
	Other oxidative enzyme activities (cytoplasmic or mitochondrial): alcohol, aldehyde, purine or amine oxidation
	Reductive enzymes (microsomal or cytoplasmic):
	- azo- or nitro-reduction
	- quinone reduction
Conjugation enzymes	Microsomal:
	- glucuronic acid conjugation
	- epoxide hydration[a]
	- methylation
	Cytosolic or mitochondrial:
	- sulfate conjugation
	- amino acid conjugation
	- acylconjugation
	- glutathione conjugation
	- epoxide hydration[a]
Hydrolytic enzymes	Microsomal Cytosolic Lysosomal

[a] As a matter of convenience, epoxide hydratase has been classified under conjugation enzymes.

optimum (24), may be important, for example, in urine where the enzymes such as β-glucuronidase may liberate carcinogens from glucuronic acid conjugates.

Microsomal Cytochrome P-450 Linked Mono-oxygenase Complex in Chemical Carcinogenesis

The microsomal mono-oxygenase system may be characterized as an NADPH-dependent microsomal respiratory chain organized in the membrane of the endoplasmic reticulum. Full activity of the mono-oxygenase system requires a flavoprotein enzyme called NADPH-cytochrome P-450 reductase, a cytochrome P-450 molecule and phosphatidylcholine. NADH-cytochrome b_5 reductase or other microsomal flavoprotein enzymes may also deliver electrons to the cytochrome P-450-substrate-molecular oxygen ternary complex (23).

A large functional and structural heterogenicity in the cytochrome P-450 molecules is associated with the mono-oxygenase system. The chromophore has a molecular weight of approximately 50 000-55 000 daltons, and one molecule of haem is bound to its glycoprotein apoprotein. Its function as the mono-oxygenase system of a huge variety of organic lipophilic compounds is related to a wide range of substrate specificity. Both the mono-oxygenase systems of humans and laboratory animals can be induced by the administration of various organic chemicals, and the induction has been found to change the metabolic fate of the chemical administered and also of other chemicals. As shown with various polycyclic hydrocarbons, drug hydroxylation by the mono-oxygenase system may also have a very clear stereo-selectivity (18,23).

Mono-oxygenase reactions are probably the most important steps in the generation of reactive intermediates from various chemical carcinogens. These intermediates, such as epoxides of polycyclic aromatic hydrocarbons or benzene (8), are highly electrophilic in nature and may attack various nucleophilic sites in proteins, RNA or DNA and also in glutathione molecules (3,5,8,25).

Such covalent binding of the metabolites of chemical carcinogens is a necessary but not an ultimate phenomenon in chemical carcinogenesis (3,5,8). In some cases, the reactive intermediates may have a free radical nature,

such as nitroso compounds (4). Such radicals may also be formed from chemical carcinogens by various microsomal or cytoplasmic reductases or oxidases (4,20,22).

The reactive intermediates formed from mono-oxygenase reactions of the carcinogens usually have other competing metabolic pathways apart from the binding to cellular macro-molecules. Epoxides may rearrange non-enzymatically to phenols or they may be further metabolized by various conjugation reactions. In some cases, the epoxides may even be reduced to the parent compound (26).

Conjugation Reactions in Chemical Carcinogenesis

When compared to the mono-oxygenase reactions, the step of drug conjugation in drug biotransformation usually leads to detoxification of the compound. The conjugated xenobiotics have increased molecular size and hydrophilism. As a result, they are less prone to metabolic treatments and, consequently, are usually excreted from the body.

Rarely, the conjugation phase may, however, lead to the formation of reactive intermediates from chemical carcinogens. In the metabolic activation of benzo(a)pyrene (a potent skin and lung carcinogen) to 7,8-dihydrodiol (its ultimate carcinogenic metabolite), the parent compound 9,10-oxide is epoxidated first at the 7,8-position. In the next step, it is hydrated by epoxide hydratase to 7,8-dihydrodiol that may then be metabolized further to the 9,10-oxide derivative (25,26). Another example is the participation of sulfate conjugation in the metabolic activation of acetylaminofluorene, a potent liver carcinogen (8,27). In the first step, the nitrogen acetylated in the compound is hydroxylated to N-hydroxyderivative. The hydroxy group may then be conjugated to sulfate conjugate, which is the active ultimate carcinogen of the compound in the rat liver. Glucuronidation at the amino nitrogen or at the aminohydroxyl oxygen may also be the ultimate step of metabolic activation of acetylaminofluorene in other laboratory animals (27).

A novel observation that certain hydrolytic enzymes may hydrolyze reactive forms of compounds from conjugated chemical carcinogens has raised the suspicion that

conjugated forms of chemical carcinogens could also be regarded as modes of metabolite transport and not as detoxified compounds (28).

Species and Tissue Variation in Metabolic Activation

One pertinent feature of the activities of drug-metabolizing enzymes is that their activities are modified by exposure to chemicals. This phenomenon may be regarded as a moderately selective induction of the biotransformation of the administered chemical. At least three different sets of induction of drug biotransformation exist: treatment with phenobarbital, polycyclic aromatic hydrocarbons or steroids. Polychlorinated hydrocarbons may have inductive properties of more than one type (23).

Genetic factors may be the most important single factors regulating the metabolic pathways of polycyclic aromatic hydrocarbons and possibly also those of other chemicals (29). In mice, the development of tumours, toxicity and other adverse effects caused by polycyclic aromatic hydrocarbons has been related to polycyclic aromatic hydrocarbons induced by their own metabolism.

Other factors that cause variation in the metabolism of chemical carcinogens are the differing metabolic pathways of drug metabolism in different organs (8) and the varying inducibility in them (23,30). This aspect clearly stresses the importance of the route of administration in drug biotransformation. Nutritional compounds are absorbed in the gastrointestinal tract, where they are partially metabolized and then transported mainly to the liver. In industrial and often in environmental exposures, the typical route of absorption is the respiratory tract, from which a lesser amount is initially transported to the liver.

Age, sex and hormonal factors may also cause variability in the metabolic treatment of chemical carcinogens (8). Particularly important is the poor inducibility of the drug conjugation enzymes as compared to that of drug hydroxylation enzymes in the foetus (31), leading to a potential generation of hazards in utero exposure.

Various nutritional and other environmental factors may profoundly affect the enzymes of drug activation (8,32,33). Particularly effective in this respect are polychlorinated biphenyls and TCDD. Disease states may also alter any of the various aspects of pharmacokinetics of chemical carcinogenesis in the body (8).

Binding of Chemical Carcinogens to DNA and Other Cellular Macro-molecules

Chemical carcinogens are thought to be mutagenic to cells through their (covalent) binding to DNA bases or phosphate diesters. In addition, noncovalent binding, such as interchelation, may also be possible (6,11). The binding may occur with purine or pyrimidine bases of DNA. The preferential sites seem to be guanine and adenine nitrogen or oxygen groups (11). The binding of the chemical carcinogens is thought to cause mutations due to the binding per se, erroneous DNA-repairing processes or DNA replication errors (11,34). Though the direct relation of DNA altered by the carcinogen binding and the genesis of carcinomas in the target tissues may be currently supported by circumstantial evidence, a good correlation has been found in some cases between the binding to DNA bases and the mutagenic and carcinogenic property (6,7).

An important aspect in chemical carcinogenesis is probably that most chemical carcinogens are toxic to various cell functions. Like many metabolically activated cytotoxic compounds, they attack various nucleophilic sites of proteins and ribonucleic acids, including the histone and nonhistone proteins associated with DNA (11,34). After administration of a chemical carcinogen, morphological changes can also be found in various subcellular structures (35).

DNA Repair Processes after Administration of Chemical Carcinogens

Cells have mechanisms that are able to repair damaged DNA. The repair process consists of the sequential activity of enzymes recognizing the damage, excising the damaged nucleotides (exonucleases and endonucleases) and synthesizing and attaching the new nucleotides to the DNA strand under repair (36,37). At least three different repair systems function in mammalian cells:

photo-activation repair for correction of ultraviolet damage, excision damage repair functioning throughout the cell cycle, and post-replication repair (36,37). Strong epidemiological evidence for the involvement of deficiency of DNA repair in ultraviolet light-induced tumours has been obtained from patients having Xeroderma pigmentosus, Ataxia teleangiectasia or Fanconi's anaemia (37). Patterson (37) suggests that DNA repair processes may affect the dose-effect relationships in chemical carcinogenesis because repair processes are able to correct DNA damage caused by chemical carcinogens.

Cell Transformation in Chemical Carcinogenesis

In the multistage process from cancer initiation, through cancer promotion to cancer development, new cell populations are formed from the mutated cells capable of dividing (38). The development of cancer from these new cell populations may also be a multistep process with precancerous cell populations as intermediates. The new cell populations may differ from parent cells in their biochemical and physiological parameters. In tissue culture, these cells may be distinguished because they grow without contact inhibition. Based on the difference in the growth of the parent and transformed cells, cell transformation tests have been used as short-term tests for evaluating the possible carcinogenicity of compounds (6,39). However, the various mechanisms behind the process of cell transformation are still poorly known.

Cocarcinogenesis and Inhibition of Chemical Carcinogenesis

In a broad sense, cocarcinogenesis in chemical carcinogenesis may be defined as the favour of carcinogenic response to endogenous or exogenous factors (9), some of which are listed in Table 2. Cocarcinogens form a miscellaneous group to which any endogenous or exogenous agents or states are attributed, including physiological or pathological conditions that favour the generation of cancer in the body. Consequently, factors that favour the uptake of a carcinogen by the target tissue or its metabolism through pathways which increase the concentration of reactive intermediates in the target tissue belong to this group. Many nutritional and lifestyle factors, such as alcohol or tobacco smoking, can be defined as cocarcinogens (8,9).

Table 2. Selected cocarcinogenic factors

Pharmacokinetic factors:
- factors favouring uptake to target tissues
- modifiers of drug metabolism
- increased generation of reactive intermediates (due to induction or due to inhibition of competing detoxification)
Nutrition (also acting through pharmacokinetics)
Hormonal status
Viruses (possibly also other micro-organisms)
Immunological factors
Promoters

During the cell transformation process, developing malignant cell populations differ immunologically from their parent cells. Consequently, immunological surveillance of tumour growth has been anticipated. Some evidence for the cocarcinogenicity of immunosuppression has been reported in humans (40).

Hormones have been found to be related to the development of many tumours. Progesterone has been shown to be cocarcinogenic after administration of 7,12-dimethyl benzo(a)anthracene, and estrogen may be able to abolish the effect of progesterone. As recently suggested, steroid hormones may affect tumour development through a mechanism similar to that of promoters (41).

Viruses and chemical carcinogens have been found to cooperate in carcinogenesis in some strains of animals, but they may be inhibitory to each other's carcinogenesis in other strains (9). Although derepression of viral genome has been suggested as the cause of carcinogenesis

after administration of carcinogens, this factor does not seem to be the case at least in some experimental conditions (9).

Promoters of chemical carcinogenesis belong to a special group of cocarcinogenic compounds. Given shortly after administration of a chemical carcinogen, they decrease considerably tumorigenic latency, increase the incidence of tumours and decrease the latency period (exposure to observation time) (9,41,42). Promoters such as phorbol esters, croton oil and some steroid hormones may have effects on various cellular events, such as protein, lipid, DNA and RNA synthesis. In general, the effect of promoters seems to be related to increased synthesis of various macro-molecules, probably favouring cell transformation (9,42).

The group of inhibitors of chemical carcinogenesis is evidently as miscellaneous as that of the cocarcinogens (Table 3). As mentioned previously, changes of metabolism of chemical carcinogens may decrease the generation of reactive intermediates from the compound or increase their detoxification. In some cases, viruses or hormones may act as inhibitors of chemical carcinogenesis (8,9,43). Vitamin A derivatives seem to be very effective inhibitors

Table 3. Inhibitors of chemical carcinogenesis

Pharmacokinetic factors:
- changed tissue distribution or decreased uptake to target tissues
- decrease in metabolic activation or increase in detoxification
Hormones
Viruses
Immunological factors
Vitamin A derivatives (inhibition of promotion?)

of chemical carcinogenesis. Their mechanism of action has been suggested to be opposite that of the promoters (44).

Evaluation of the Carcinogenicity of Chemicals

More than 60 000 chemicals are in common use, only a fraction of which has been tested for mutagenicity or carcinogenicity. An additional 1000 chemicals are introduced annually into common use (45).

Two different approaches are used to evaluate the carcinogenicity of chemicals. In the first approach, the carcinogenicity of the compound must be proven by epidemiological and experimental data. This approach, which is used by the IARC monographs, lists the evidence that must be indicated before a compound is regarded as a human carcinogen (46). Using a similar data base, the other approach evaluates whether or not the data obtained definitely prove that a compound does not pose a carcinogenic hazard (6,7,10).

The evaluation of the carcinogenicity of a compound has two distinct steps. The first one is the qualitative evaluation of a chemical, i.e. should a chemical be regarded as a carcinogen. The second one deals with the quantitative aspects of carcinogenic risks caused by a chemical known to be carcinogenic (7,10).

Qualitative Aspect of Carcinogenicity

Epidemiological studies are the only way to obtain information on the carcinogenicity of a compound to humans. Human exposures are, however, typically the mixed results of various chemicals, and relevant, accurate monitoring of exposure, even in prospective cohort studies, may often be economically impracticable. In case-referent studies, the estimation of exposure may only be a rough estimate in most cases. The latency period of carcinomas after exposure to chemical carcinogens may be long (in humans, 5-40 years from the initial exposure to the observance of the disease). Consequently, information from epidemiological studies may be inconclusive and, in the case of positive results, the information will always be obtained late in respect to intervention.

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Testing of carcinogenicity of a compound with animal experiments will always be laborious and expensive. Due to interspecies and other variations in the steps of chemical carcinogenesis, the relevant studies should include two species of animals, using both sexes and adequate controls. The number of animals should be sufficient to provide an adequate resolving power to detect the carcinogenic effect. The treatment and observations should be extended through most of the lifespan of the animals at a dose range including one level most likely to yield the maximum expression of the carcinogenic potential (7,47). Positive results in one species are, with due regard to interspecies differences, also hazardous to humans (7,10).

To date, the number of various short-term mutagenicity tests is considerable (cf. 6, 7, 10, 39). The tests use, for example, various bacteria and yeast, and in some of them, a post-mitochondrial supernatant of liver has been added in order to detect the carcinogenicity of compounds activated to reactive intermediates. Mutation tests also include the Drosophila mutations test. DNA repair tests have used labelled thymidine to detect correction of damaged DNA. Methods based on cell transformation in mammalian cell cultures use, for example, hamster embryo cells and mouse embryo fibroblast.

The value of short-term tests in the assessment of carcinogenic risks to humans has been questioned. However, in a recent international evaluation of various short-term tests in which a blind analysis of 42 chemicals was performed in 65 laboratories, several of the tests were found to correlate well with the carcinogenic potential of the compounds, but none of the tests could be recommended as a superior single test for the assessment of carcinogenicity[a].

The huge and proliferating number of chemicals mandates that priority be given to certain chemicals in respect to testing, even for mutagenicity. Such decisions can be made, for example, according to the chemical structure of

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[a] Unedited transcript of the public meeting on the international programme on the evaluation of short-term tests for carcinogenicity, Bethesda, 3 December 1979.

the compound. For example, hydrazines, N-nitroso groups or polycyclic hydrocarbons with "bay region" should be considered suspicious and should be further evaluated. In addition, many rather nonspecific short-term methods, such as the degranulation test, activation of the biphenyl hydroxylase test and nuclear enlargement tests, may be considered at the start of drug evaluation. Such tests are, however, nonspecific and insensitive (7).

Conclusions on the carcinogenicity of a compound may be based on epidemiological studies, bioassays in animals or both. The other types of evidence may be regarded suggestive in respect to carcinogenic hazard and, in the absence of positive epidemiological or bioassay data, such evidence should deserve further study. Negative data from mutagenicity tests, structural analysis or from studies on the metabolic pathways do not override positive animal tests (7,10,39,47).

Quantitative Estimation of Risk

The quantification of the risk posed by a carcinogenic compound to humans involves extrapolations such as those from high to low doses in a single biological system. In the absence of relevant conclusive data, except possibly in carcinogenesis induced by radiation, numerous models have been developed for use in the extrapolations. Such models have been based on the assumptions of the mechanisms behind chemical carcinogenesis (one-hit, multi-hit or Armitage-Doll multistage model) or on the models used in toxicity testing. In the latter case, additional safety factors have been included (for further references, see ref. 7). For practical reasons, linear dose-effect models seem justified. In the case when the "real" curve would be sigmoidal, a safety factor would be created with use of the linear model at low, but not at high, doses. The mathematical procedures *per se* are intended to provide upper limit estimates of risk from a statistical point of view (see refs. 7,48). However, due to biological variations, the risk estimates obtained do not necessarily involve upper limit estimations for humans. The latency period has in some cases been used for estimating quantitative risk at low doses. The idea that lower exposure levels could lengthen the latency period has, however, been questioned (49).

Another set of necessary extrapolations is the correlation between humans and other animals. Even if the qualitative decisions from laboratory animal data are conclusive in

respect to humans, the quantification of the human risk on the basis of laboratory animal data is less certain. Many biological variabilities may affect conversion factors. When evaluating the human risk in exposure, all ages, disease conditions, transplacental exposure and any special susceptibility states should be considered (7). Changes in experimental conditions may cause a wide variation in response in one strain of animal. Studies on the state of metabolic activation in human samples have shown a variability of one hundred times (50), and a similar variability in human response may be assumed at any other step of chemical carcinogenesis.

Sources of Human Exposure to Environmental Carcinogens

The often-made suggestion that chemicals may be responsible for 80-90% of human cancers has become a highly controversial and, to some extent, emotional issue among the various theories of chemical carcinogenesis (6,7,51-56). Some investigators state that most of the common cancers in humans are caused by factors referred to as lifestyle or even diet (52-55), and occupational factors, despite high levels of exposure in some cases, are less important. Other investigators suggest that occupational factors may contribute to etiology in more than 30% of adult human cancers (51).

All people are probably exposed to some extent to a multiple set of chemicals, some of which are mutagenic and others carcinogenic, during their lives (45,52-58). However, from a standpoint of epidemiological studies and also the prevention of chemical carcinogenesis, occupational exposures seem relevant (6,7,56). The exposure levels in certain occupations often far exceed those of the population in general, and occupational exposure to all synthetic chemicals and to purified or raffinated natural compounds occurs. In addition, evaluation of the levels of exposure, when combined with epidemiological studies, may give guidelines on the risks of the exposure of the population in general (6,56). Two or perhaps three types of exposure can be compared to occupational exposure: namely, exposure to certain medicines such as those used for treatment of malignancies having a known carcinogenic potential (46,59) and exposure of people to carcinogenic compounds in various catastrophic conditions. The factors of lifestyle with known carcinogenic potential (such as tobacco smoking or

alcohol drinking) are also among the single factors that have been used and may be used in order to obtain information on human carcinogenicity (52-57,59), and their regulation in respect to prevention seems possible, at least in certain cases (60).

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