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MEDICAL PROGRESS

CHEMICAL CARCINOGENESIS

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THE perception of cancer as a disease primarily related to the environment has been progressively strengthened since the publication of a Medical Progress article on chemical carcinogenesis in 1971.[1] By the mid-1960s, the view of the cause and pathogenesis of cancer had already begun to change radically from that of previous decades, during which no clear perception of the possible role of the environment in the genesis of most of the major forms of cancer was evident. Although the relation of cancer to environmental hazards in the work place and to a few unusual cultural patterns around the world was established, the examples were considered exceptional.

The general acceptance of cigarette smoking as a major factor in the development of lung cancer and the increasing number of examples of major shifts in the organ or tissue distribution of primary cancers with migration of distinctive ethnic groups from one geographic location to another were important in changing the whole perception of the genesis of cancer.[2,3]

This radical change has encouraged increasing attention to the nature of the environmental influences. The available epidemiologic evidence indicates the likelihood of several (if not many) components in this environmental outlook. Diet, carcinogenic chemicals, radiation, and viruses are among the major factors that appear to be involved. Of these, chemicals as carcinogens have been receiving increasing attention as having the greatest role in the genesis of cancer. How justified is this perception of the causation of cancer?

About one third of the cancer in North America and Europe is related to the use of cigarettes or other tobacco products. There is incontrovertible evidence that chemicals related to the work place or occupation are responsible for a segment of cancer in human beings (Table 1). To these must be added a growing list of naturally occurring chemicals and of drugs used in medicine. All these known agents, together with ultraviolet light as the single most common cause of cancer, can easily account for over 50 per cent of the cases

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...e 1. Chemicals Known or Strongly Suspected to be Carcinogenic for Human Beings.

	Site of Cancer
...nal Contacts	
	Bladder
	Skin and ...
	Bronchus, pleura, and peritoneum
	Marrow
	Bronchus
	Respiratory tract
	Prostate
	Bronchus
	Bronchus
	Bronchus and ...
	Skin and lungs
	Nasal sinuses
	Liver
...	
... epithelium	Bladder
...	Hematopoietic tissue
... steroids	Liver
	Skin
...rhazine	Bladder
...ulbesterol	Vagina
...pressive drugs	Lymphoid tissue
... (azathioprine)	Renal pelvis
...ins	Liver
...moke	Bronchus, mouth, pharynx, larynx, esophagus, bladder, and pancreas
...ntraceptives	Liver
... and ...	Mouth

...the United States, Canada, and several other ...tern countries.

...hat about the remaining cancers — those in the ... breast, uterus, hematopoietic-lymphoid system, prost... and other sites, including a substantial ...portion of those in the urinary bladder and pan... Can... be closely related to the chemical ...amination of the air, water, and food that has ...occurring in the industrialized countries?[4,5] No ...sive answer is available. Evidence can be offered ...or of such a view or against it.

On the positive side are the following considera...: the known relation, mentioned above, between many different types of cancer and exposure to chem... in tobacco and in the work place; the wide... presence of chemical mutagens and potential ...rcinogens in the environment in industrialized ...ntries, as monitored by short-term in vitro tests in ...okaryotes or eukaryotes; the observations that some religious groups in Utah and elsewhere, who have different patterns of tobacco and alcohol use and different codes of behavior, appear to have a lower incidence of some forms of cancer in the respiratory, gastrointestinal, and genitourinary systems[6]; the increasing evidence that cancers of many systems, resembling cancers in human beings, can be induced by chemical carcinogens in one or more laboratory animals; and the observations that cancers of the esophagus or liver have an unusually high incidence in some geographic locations in China, and that domestic animals in close contact with human beings in these locations frequently have similar cancers in the same anatomic sites.[7-10]

On the negative side, no clear-cut clinical experience or epidemiologic evidence has been presented to ...riminate environmental chemicals as causative agents for the wide variety of cancers seen clinically. According to German[11] and Cairns,[12] patients with xeroderma pigmentosum, who have a defect in their ...blasts in the repair of DNA damage by some chemicals as well as by ultraviolet light, have no elevated risk of cancer in organs or tissues other than the skin. If the general environmental exposure to chemicals were effective in the causation of cancer, such patients could be expected to have elevated incidences of cancer not related to ultraviolet light. In some cancers such as leukemia and cancers of the stomach and cervix, there were decreases in age-adjusted incidence and in mortality from 1969 to 1971 and from 19... to 1976 among white persons in the United St..., while there were only small increases in most of the other tumors, as compared with cancers of the lung and with melanoma.[13] No increase comparable to that of lung cancer has been seen in the past two decades in the patterns of cancer incidence in the United States.

None of the above considerations are by any means conclusive. Most of the arguments on either side have enough contingencies to leave the question in major doubt. The close monitoring of cancer occurrence over the next two decades may allow a more definitive position to be taken. In the meantime, a greater understanding of how chemicals are involved in cancer development would seem important in helping us to arrive at a conclusive position.

In addition, the large increase in the use of chemical carcinogens in the development of new experimental models for the study of the possible cause, development (pathogenesis), or treatment of cancers in specific organ or tissue sites in human beings further emphasizes the importance of the study of chemical carcinogenesis. Such studies should generate a much more critical understanding of the relative roles of exogenous and endogenous factors, including diet and hormones, in cancer development and behavior.

The orientation in this presentation will be mechanistic, not descriptive, and will attempt to relate fundamental concepts and understanding derived from experimentation in animals and from other sources to the causation, prevention, and (to some degree) therapy of cancer in human beings. For this reason, little coverage will be given to the many different types of chemical carcinogens themselves or to their distribution.[14]

Cancer as a Chronic Multistage Process

It is now appreciated that the development of cancer in human beings in various sites takes many years and that progressive tissue and cellular changes

can be seen during the long so-called latent period. Often, new cell populations appear that probably represent stages or steps in the cellular evolution from normal cells through initiated, preneoplastic, and premalignant cells to highly malignant neoplastic cells.[15-17] Research in laboratory animals has concentrated heavily on the early events and has suggested the existence of common patterns of early preneoplastic changes in several organ systems and in several species.[17] Research in human beings has concentrated largely on the later events and has confirmed the occurrence of atypical hyperplasias, dysplasia, and carcinoma in situ as probable precancerous steps in several organs late in the process.[17-19] However, the critical properties of each different cell population that relate to the possible roles of these cells in cancer development and to the manner in which the two aspects of carcinogenesis (the experimental and the human) fit together into some biologically meaningful pattern remain as challenges.[17]

The conceptual advances made in the late 1930s and 1940s concerning the first two early steps in chemical carcinogenesis, initiation and promotion, are well known.[15-17,20] However, their mechanistic bases are only now unfolding, and these will be topics for review. The basic validity of these concepts for cancer development in human beings has been established in a few instances and as such is reassuring both for the physician and for the scientist. For example, a relatively brief exposure to diethylstilbestrol during pregnancy may be associated with the development of vaginal neoplasia some 15 to 25 years later in the exposed person's daughters.[21] Parenthetically, it should be mentioned that it is not known whether diethylstilbestrol acts in this situation as a chemical carcinogen or whether the effect is predominantly hormonal. Again, a limited exposure for only a few months to a known chemical hazard, such as vinyl chloride, may lead many years later to the appearance of angiosarcoma of the liver.[22] Nonneoplastic cellular and tissue changes are seen during the apparent latent period.

Initiators and Initiation

Initiators

Until fairly recently, one of the most puzzling and confusing aspects of chemical carcinogenesis was the diversity in the chemical structure of carcinogens. This problem has now been largely resolved by the discovery of metabolic conversion of many carcinogens to highly reactive metabolites.[20] The best-known form of reactive moieties, generated from several different types of carcinogens, is the "electrophilic reactant," which is a positively charged molecule that reacts well with sites of electron densities in many different cellular components, including DNA, RNA, protein, glutathione, and probably also polysaccharides.[20] A minority of chemical carcinogens, such as some nitrosamides (e.g., alkyl nitrosourea), bis(chloromethyl) ether, and nitrogen mustard, are active by themselves and do not seem to require any metabolic conversion to more reactive metabolites.

Activation and Metabolism of Procarcinogens

The first major type of metabolic activation discovered was the conversion of an aromatic amine, 2-acetylaminofluorene, by *N*-hydroxylation to an N–OH derivative.[20] This type of metabolic product is seen with several aromatic amines, including the human bladder carcinogen 2-naphthylamine (*β*-naphthylamine).

More recently, much effort has been spent on the clarification of the activation of benzo(*a*)pyrene and the many other polycyclic aromatic hydrocarbons. Many of them are easily generated by burning (pyrolysis), are widely distributed in our environment, and are thought to be partly responsible for the carcinogenicity of coal-tar products, oils, and tars. These compounds undergo epoxidation to form reactive epoxides, some forms of which are considered to be ultimate carcinogens.[23] In the case of benzo(*a*)pyrene (and probably other polycyclic aromatic hydrocarbons), the initial site of epoxidation may undergo hydration to form a dihydrodiol in a reaction catalyzed by epoxide hydrolase. This inactive derivative, in turn, can be converted to another epoxide at a second site to form a dihydrodiol epoxide. These are considered to be the most likely ultimate carcinogens for at least some polycyclic aromatic hydrocarbons. This series of reactions for benzo(*a*)pyrene is illustrated in Figure 1. Another potentially important carcinogen for human beings that is subject to activation through epoxidation is aflatoxin B_1.[20] This substance undergoes oxidation at its 2,3-position, and this derivative, the 2,3-oxide, appears to be one form of ultimate carcinogen of this mycotoxin. Vinyl chloride is also activated through epoxidation.[20]

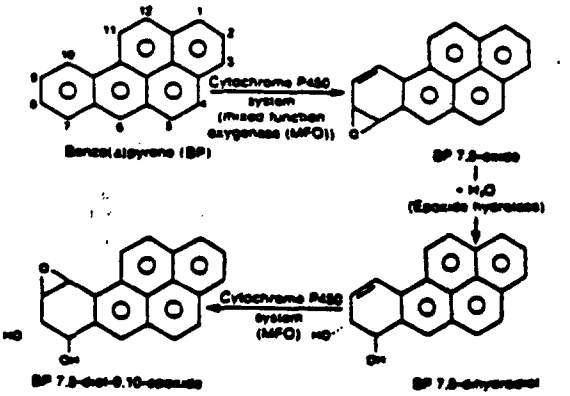

Figure 1. Current View of the Activation of Benzo(*a*)pyrene (BP) to Benzo(*a*)pyrene 7,8-Dihydrodiol,9,10 Epoxide through the Mixed-Function Oxygenase (MFO) System (Cytochrome P-450 System) and Epoxide Hydrolase.

Other types of metabolic conversions are also considered important in carcinogenesis. Nitrosamines are converted to highly reactive alkylating moieties, probably through oxidation. Aromatic or heterocyclic nitro-compounds, such as nitrofurans and 4-nitroquinoline-*N*-oxide, probably undergo initial reduction to hydroxy-amino-derivatives. In the case of 4-nitroquinoline-*N*-oxide, this form is considered to be an ultimate carcinogen.

For the majority of known conversions, the system most active in the cell is the "mixed-function oxygenase" system, consisting of several cytochromes P-450, reduced nicotinamide-adenine dinucleotide phosphate cytochrome reductase, and lipid.[24] This inducible system is located predominantly in the microsomal fraction of the cell (endoplasmic reticulum), although recent work increasingly points to a second comparable system in the nucleus.[25] In addition to these oxidative systems, reducing systems also exist for some procarcinogens, such as the aromatic or heterocyclic nitro-compounds. Diaphorase or other reduced nicotinamide-adenine dinucleotide phosphate or reduced nicotinamide-adenine dinucleotide reductases are widely distributed among different cells and are effective in reducing the nitro-group to the hydroxy-amino-derivative.

The liver is by far the most active and most versatile organ in the metabolism of procarcinogens and of xenobiotic agents generally. It and other organs have an ability to detoxify potential carcinogens as well as activate them, and the ultimate fate of a chemical depends largely on the balance between activation and inactivation — a balance that is easily modulated in major ways by drugs and other chemicals, age, nutrition, and hormones, as well as genetics.[26-31] For example, the carcinogenicity of several aromatic amines for the liver can be completely prevented by simultaneous exposure to phenobarbital or 3-methylcholanthrene — agents that induce many liver enzymes.[32,33] This phenomenon of resistance of the induced liver to some carcinogens may be of great practical importance to human beings. Virtually all people in the Western world have levels of several xenobiotic agents, such as chlorophenothane, dieldrin, aldrin, polychlorinated biphenyls, and dioxins, in their adipose and other tissues. These agents as a group are effective enzyme inducers in the liver. In addition, many drugs induce various microsomal or other enzymes in the liver or other tissues. Are these inducers making tissues more or less susceptible to the acute toxic or carcinogenic effects of other environmental agents?

The organ or tissue distribution of the carcinogen-metabolizing enzymes may be involved in the organotropism of carcinogens. Since there is an almost certain requirement for activation, and since the metabolic patterns affecting carcinogens and other xenobiotic agents are so variable from cell to cell or from organ to organ, the absolute activities of the various enzymes and especially their relative balance may have key roles in determining which organ will be a target for a particular carcinogen. An interesting example of modulation at this level is the liver-kidney axis with dimethylnitrosamine. This potent carcinogen normally induces liver cancer when taken through the gastrointestinal tract. However, if the animal is placed on a low-protein diet, the hepatic activation and metabolism fall off. This pattern allows more of the carcinogen to be available for action on the kidney and changes the dominant cancer pattern from the liver to the kidney.[34]

Clearly, the pathologic consequences of exposure to any potentially toxic xenobiotic are related not only to the pathways available for its metabolism but also to the physiologic state at the time of exposure. Thus, the presence of a known mutagen or carcinogen in the environment is by no means synonymous with a mutagenic or carcinogenic response by the exposed person.

Possible Molecular Targets for Ultimate Carcinogens

The molecular targets for the electrophilic reactants are many. To date, the bulk of the effort has been concentrated on DNA, with some interest in RNA and protein. In principle, many other molecular targets exist at the macromolecular and "micromolecular" levels. Because of the ease of relating changes in DNA to a permanent change in the biologic behavior of cells, DNA has received the lion's share of attention. Since the first discovery of the chemical nature of an adduct of a carcinogen with nucleic acids in a target organ in vivo,[35] there has been an increasing interest in establishing the chemical nature and site of interaction with several different carcinogens.[36] As shown in Figure 2, virtually every potential site for interaction with DNA bases (as well as with phosphates) reacts with one or more carcinogens. In attempts to narrow down the possibilities for relevance to cancer, sites that are more likely to be involved in hydrogen bonding between bases (purines and pyrimidines) in DNA, and thus to be involved in possible miscoding, are often considered most relevant. This presupposes that the major or only biochemical lesion in DNA that is relevant to cancer initiation is a miscoding lesion. This judgment may not be justified, since the evidence is circumstantial and inconclusive. Conceivably, other types of alterations in DNA besides miscoding, such as recombinations and gaps, may be important to the early events in cancer development.[12,36-39] In fact, Cairns has suggested that mutagenesis may be of only minor importance in the initial events in chemical carcinogenesis, and that genetic transposition including relatively large regions of the genome may be more relevant to this process.[12] The application of this newer technology to appropriate "clean" cell populations during the development of cancer is to be awaited with interest.

Another important aspect is the site of interaction of the ultimate carcinogen within the long DNA molecules. With several different approaches, such as

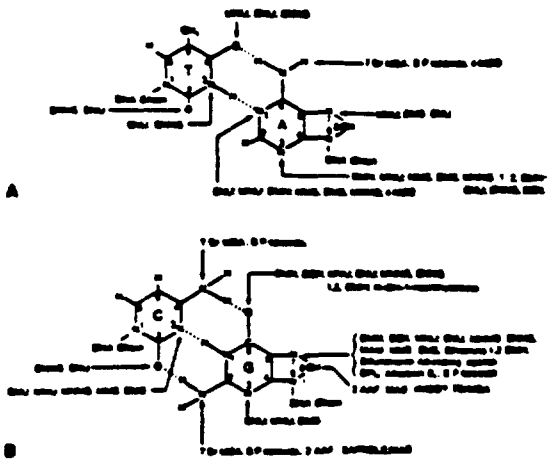

Figure 2. Diagrammatic Representation of Sites of Interactions of Activated Forms of Carcinogens with the Four Bases of DNA.

T denotes thymine; A, adenine; C, cytosine; G, guanine; MNU, *N*-methyl-*N*-nitrosourea; ENU, *N*-ethyl-*N*-nitrosourea; ENNG, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine; 7 Br MBA, 7-bromothyl-benzo(*a*)anthracene; BP epoxide, benzo(a)pyrene 7,8-dihydrodiol,0,10 epoxide; 4NQO, 4-nitroquinoline-*N*-oxide; DMS, dimethylsulfate; DMN, dimethylnitrosamine; MMS, methyl methanesulfonate; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; 1,2,DMH, 1,2-dimethylhydrazine; DEN, diethylnitrosamine; N-OH-1-naphthylamine, *N*-hydroxy-1-naphthylamine; 2 AAF, 2-acetylaminofluorene; MAM, methylazoxymethanol; EMS, ethyl methane sulfonate; BPL, *β*-propiolactone; and MAB, 4-methylaminoazobenzene.

studies of susceptibility to nucleases, separation of transcriptionally active from inactive DNA, or isolation of linker and nucleosome regions in chromatin, some of the interactions of carcinogens with DNA have been found to be nonrandom. However, no single region of high affinity has been found.[36] The use of DNA cloning for specific genes in biologically well-defined preneoplastic and early neoplastic populations should be profitable.

DNA Repair

In view of the essentially irreversible nature of initiation with chemicals and the apparent focal nature of the initiation process, major emphasis at the molecular level is given to DNA as a probable target in initiation. However, the evidence is largely circumstantial.

The bulk of evidence comes from studies in patients with xeroderma pigmentosum, who have a high incidence of skin cancers. The etiologic agent is ultraviolet light, and the vast majority of patients have a defect in their ability to repair the damage inflicted on DNA by this form of radiation. Such patients can be protected from skin cancer by careful avoidance of exposure to ultraviolet light.[40] Fibroblast cultures from these patients have increased sensitivity not only to ultraviolet light but also to some chemical carcinogens and mutagens.[41] Other diseases (e.g., ataxia-telangiectasia and Fanconi's anemia) also involve deficiencies in DNA repair as well as an increased risk of neoplasia.[42]

Experimentally, there is a correlation under some conditions between the formation of specific DNA adducts (such as O^6-methylguanine in the brain), persistence of this biochemical lesion, and the ultimate appearance of gliomas.[43] Other studies indicate that the genesis and persistence of O^6-methylguanine may be important but is insufficient to account for cancer production with some nitrosamines or nitrosamides[44] or 1,2-dimethylhydrazine.[45]

The major protection against cancer may reside in the efficiency with which we repair DNA that has been damaged by mutagens and carcinogens. As pointed out by German[11] and by Cairns,[13] since fibroblasts from patients with xeroderma pigmentosum show a defect in the repair of DNA damage by some chemicals as well as by ultraviolet light, such patients should have an elevated risk for cancer in organs or tissues other than the skin if chemical carcinogens are important in the genesis of many forms of cancer. Yet the available data show no apparent increase in the risk of cancers other than those of the skin in patients with xeroderma pigmentosum.[13] Patients with Bloom's syndrome do have an elevated risk. Such observations are interpreted as evidence against a role for the many environmental chemical hazards in the causation of human cancers generally, except in well-established instances such as respiratory-tract cancer in smokers and in persons with occupational exposures.[12] An alternative hypothesis must be entertained: that the role of repair may be quite different in the dynamics of cancer induction by chemicals, as compared with induction by ultraviolet light.

The past 10 years have seen a large expansion in studies of DNA repair, largely in vitro. These studies should lead to a much clearer delineation of the types of repair that may occur in carcinogenesis. What does happen remains poorly understood. Processes of repair by base excision, by "long-patch versus short-patch" removal, by possible recombination or other forms of post-replication repair, by removal of inter-strand cross-links or other more complex forms of damage, and by other mechanisms are slowly being studied in many different systems in vitro and to a much lesser degree in vivo.[36,41] The enzymology, although difficult, is also slowly becoming clear. In the case of methylating carcinogens, an interesting recent finding is the discovery of an enzyme in bacteria that removes the methyl group from the O^6 position of guanine in DNA by transmethylation with its transfer to a sulfur-containing moiety in protein.[46] This raises the question of whether some of the enzymes involved in repair of DNA damage perform physiologic functions other than repair that subserve normal cellular requirements.

Obvious failings in many of the attempts to correlate patterns of DNA repair with carcinogenicity include the wide gap between the chemistry and the bi-

...gy and the primitive state of the art of identifying ... quantitating discrete biologic steps in the carci...genic process.

...rcinogen Metabolism in Human Tissues

...gratifying aspect of recent studies of the early ...mical and biochemical events in carcinogenesis is ... overall similarity in patterns of activation and ...ype of adducts seen in tissues from human beings ... those from laboratory animals. Studies with ... nitrosamines, benzo(*a*)pyrene, aflatoxin B_1, and ... carcinogens[42-43] have shown that human tissues ... general resemble one or more animal tissues in ... patterns of activation and in the type of carcino...n DNA adducts formed.

...interesting development that may help to clarify ... aspects of this important phase of activation is ... use of radioimmunoassays for specific adducts ... DNA, especially with highly sensitive new ap...roaches to radioimmunoassay.[44-46] Such detection of ... quantities of carcinogen-induced adducts in DNA opens up the possibility of studying exposure ... to carcinogens. This technique, coupled with ... medical examination and appropriate epi...logic studies, might well offer new approaches to ... ultimate problem of risk assessment. Although ... too early to discuss such possibilities except in ... terms, adduct formation is an index of expo...not initiation, and persistence of a chemical le... DNA is by no means synonymous with cancer development. As discussed below, initiation of the carcinogenic process is not an inevitable consequence of adduct formation.

...an cells resisted in vitro transformation with ...als for several years. However, the past three ... have seen positive results in some human-cell ...[47-61]

...iation

...the classical system of skin carcinogenesis in ... and rabbits, a single or brief exposure to a carcinogen induces some change in the tissues, called initiation, such that focal proliferations called papillomas can be made to appear by application of croton oil or other agents called promoting agents, which by themselves are noncarcinogenic or only weakly carcinogenic. More recently, this phenomenon was also found in several other organs, such as the liver, brain, mammary gland, colon, and urinary bladder.[16,17] Such focal proliferations (papillomas, polyps, or hyperplastic nodules), in turn, are sites for further evolution to neoplasia. The term neoplastic refers to a cell that can proliferate without the need for an added or known stimulus for growth — i.e., a cell that has acquired some degree of autonomy. Initiation at the skin, liver, and mammary gland, and probably at most sites, is generally permanent. This suggests that the very early carcinogen-induced altered cells are not recognized by the host in a manner that leads to their destruction, and it raises serious doubts about the validity of the concept of immune surveillance at an early stage in carcinogenesis,[62] as has been proposed for host control of cancer development.[63]

These observations are most easily interpreted as reflecting some permanent change in the DNA in the rare cell affected during initiation in vivo. Whether the change is a mutation in a structural or regulatory segment of DNA[64-66] or whether it involves more complex rearrangements of larger segments of DNA, as in transpositions, remains unknown. Pertinent are the observations on transformation initiated with x-rays or with polycyclic aromatic hydrocarbons, in which some change or changes other than a mutation in a rare cell appear to be involved as an early step in initiation in vitro with a highly selected cell type.[67,68]

One cannot equate activation of carcinogens leading to DNA-adduct formation with initiation. For example, many carcinogens, such as benzo(*a*)pyrene and 7,12-dimethylbenz(*a*)anthracene, can be activated by the liver to form adducts without initiating or inducing liver cancer in adult animals. However, if coupled with one round of cell proliferation, many can initiate[69-72] and induce liver cancer.[73] In vitro, with different cell systems, cell proliferation early in the carcinogenic process is required for transformation with x-rays,[74] some viruses,[75] and some chemicals.[76] Thus, as shown in Figure 3, initiation is at least a two-step process: a biochemical step that is reparable, followed by a round of cell proliferation that "fixes" some change so as to make it essentially permanent. The cell proliferation can be generated by some primary mitogenic stimulus, such as partial hepatectomy, by a chemical mitogen,[69] or as a response to cell death.[77] The mechanistic role of cell proliferation in initiation is not understood. A popular hypothesis involves DNA synthesis or replication as somehow fixing damage in a daughter strand.

The probable requirement for cell proliferation for initiation has important implications for cancer development in many sites. In the nonproliferating tissues, such as the pancreas, salivary gland, liver, kidney, urinary bladder, and brain, an important rate-limiting step for beginning the carcinogenic process may be cell proliferation. Thus, concomitant cell ne-

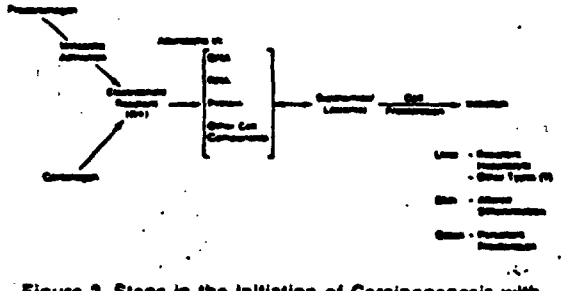

Figure 3. Steps in the Initiation of Carcinogenesis with Chemicals.

The listings under "Initiation" refer to some properties either used in the next phase, promotion (liver), or associated with the initiated tissue (skin or colon).

crosis induced by viruses, toxic agents, parasites, or dietary deficiencies, followed by cell regeneration, could be a major determinant of cancer initiation in many sites. In addition, the presence of many proliferating cells is probably one basis for the susceptibility of the fetus and neonate to many chemical carcinogens[78] and could account for the peak in cancer incidence in the first decade of life. The human fetus, unlike the rodent fetus, acquires the capability of activation of some carcinogens early in development[79,80] and thus may be at greater risk than some laboratory animals for cancer development with chemicals.

A most important question about initiation concerns the essential biologic nature of the initiated cells. What properties have they acquired that allows them, as a group, to be precursors for the ultimate development of cancer? The available evidence is against any conclusion that the initiated cells have acquired any autonomy of growth.[15,17]

In most systems, the properties critical to initiation remain unknown. In two continuously proliferating tissues, the skin[81] and the colon,[82] an early property of carcinogen-altered cells may be some disturbance in programming or control, such that the cells do not show the normal progression of differentiated properties. In the liver, the cell with acquired resistance to the inhibitory effects of carcinogens on cell proliferation has been shown to be one type of initiated cell.[17,83]

Although a majority of chemical carcinogens fall well within the current paradigm in which initiating effects are related to some form of DNA damage, there are known carcinogens that appear to be exceptions. A growing list of hypolipidemic agents[84] and several pesticides, herbicides, and other xenobiotics[85] have not been shown to generate mutagenicity or other DNA-damaging effects. Is this merely a reflection of deficiencies in our technology, or are there pathways to cancer that do not involve DNA damage of exogenous origin as essential early steps in the process?

Promoters and Promotion

The term "promotion" is often used for the process whereby neoplastic development, tumor formation, or cancer development is accelerated or encouraged in a tissue that has been exposed to an initiating dose or doses of a carcinogen.

Promoters

Early in the recent history of chemical carcinogenesis it was found that a noncarcinogen, croton oil, could stimulate tumor formation in the skin after a brief initial exposure to a carcinogen. This naturally encouraged a chemical "attack" on croton oil. Hecker[86] and Van Duuren[87] discovered that esters of the diterpene phorbol, isolated from croton oil, have potent promoting effects on mouse skin. Many biochemical, metabolic, and biologic effects are induced in many different normal cells by the phorbol esters and related compounds; in the vast majority of cases, the intensity of the particular effect closely parallels the efficacy of the compounds as promoters of skin papillomas in mice after exposure to a single dose of dimethylbenzanthracene or a similar carcinogen.[88,89] These effects include changes in morphology, microtubule polymerization, cell proliferation, enzyme induction, polyamine synthesis, phospholipid synthesis, membrane structure and function, ATPase, release of prostaglandins, inhibition and sometimes stimulation of differentiation, and many others in a wide variety of normal cells and cell lines. Many of the effects are considered pleiotropic.

Recently, with the use of a less active phorbol ester, ^{3}H-phorbol dibutyrate, membranes of fibroblast cultures and mouse epidermal cells were found to contain a high-affinity receptor for many phorbol esters, including one of the most active, 12-*O*-tetradecanoylphorbol-13-acetate.[90,91] The "natural" metabolites for the receptors have not been identified. Given the wide diversity and large number of effects seen in treated cells, it may be that more than one type of receptor is involved.

At present it is impossible to relate the findings in vitro to chemical carcinogenesis in vivo. Given the array of phenomena induced by the phorbol esters, how does one select relevance to promotion?

A major problem concerns the matching of the target cells in vivo and in vitro. In the intact animal, an active promoter does not induce focal proliferations (such as papillomas) in the normal skin, but does so only after initiation. However, many skin promoters do induce general hyperplasia of the epidermis in animals in which initiation has not taken place. Studies in several laboratories have shown that a general stimulation of cell proliferation is insufficient for the selective or differential stimulation of initiated skin to form papillomas.[91]

Promotion

A question that requires an early answer is whether the differential effect of a promoting environment on initiated tissues is directed primarily to the initiated cells or to the surrounding cells.[92] There is considerable circumstantial evidence to suggest that the first major phenomenon in promotion is the selection of an appropriately altered cell to produce a focal proliferation, and that promoting environments create differential effects on the initiated cells and on the surrounding cells.[17,93,92] Cancer frequently arises in an atrophic tissue or organ, not a hypertrophic one. Is this the consequence of a change in the local environment, favoring growth of the rare initiated cell?

As observed in vivo in experimental models in common use today, cell proliferation is an essential phenomenon in promotion. It is therefore to be anticipated that many biochemical changes that are seen in the different phases of the cell cycle,[75] such as an increase in ornithine decarboxylase activity, will be seen in promotion. Determining whether one or more of such changes in enzymes, the cell membrane, DNA organization, or other factors will have a special role in pro-

...n over and above that related to the cell cycle ...mains an interesting challenge..

...is a growing belief that one of the major fac... determining cancer development is the pres... and intensity of promoting environments.[96] In ...ition to some specific agents such as phenobarbi... butylated hydroxytoluene, and polychlorinated ...nyls for the liver,[95] prolactin for the mammary gland,[96] and bile acids for the colon,[97,98] diet has been ...und to influence carcinogenesis in several sys...ems.[3] However, the specific roles of individual die... components and of their balances remain to be ...tinguished in most instances.

...here is also a suspicion on the part of some inves...gators that tissues or organs may create a physio...logic promoting or selecting environment. The most ...ompelling evidence relates to carcinogenesis in the ...ma[7, 99] and the urinary tract.[101,102] In each of these locations, diversion of the normal movement of con...tents has been found to lead to a decreased incidence of neoplasia with known carcinogens.

There are several examples in which synergism or ...romotion may be operating in human beings. For ex...ample, exposure to asbestos, nickel, or uranium may ...e associated with only a relatively low risk of lung cancer. However, when coupled with cigarette smok...ing, the risk becomes extremely large — much larger ...han that associated with smoking alone.

...ose-sparing Steps

Progression

...ocal proliferative lesions resulting from a promot...ing environment, such as papillomas, nodules, or pol...yps, undergo a number of further changes before ma...lignant behavior is expressed.

...n many organs, including the bronchial tree in the ...moker one consistently sees several types of putative ...precancerous lesions, such as atypical hyperplasias, ...ysplasias, and carcinoma in situ.[18,19] How these le...sions relate to each other and to altered biologic be...havior of cells transformed in vitro (e.g., atypical growth, growth in soft agar, or tumorigenicity) is not ...lear and remains a potentially fruitful area for study.

An important property of many of these changes is ...eversibility. There is considerable evidence in exper...imental models and in human beings that some focal proliferative lesions, such as hyperplastic nodules in ...he liver (and their probable human equivalent, liver "adenomas," which are seen rarely in women using oral contraceptives), polyps in the colon, and papillomas in the skin, can undergo regression or remodeling with normal differentiation.[17,103] This area is becoming of particular interest in view of the reported efficacy of vitamin A analogues, such as *cis*-retinoic acid, in preventing or delaying cancer development initiated with chemical carcinogens[104] in several sites, such as the bronchus and the urinary bladder.

Transfection

An interesting recent development is the induction of transformation by the transfer of DNA or chromatin to susceptible cell lines. DNA from several types of cells transformed in vitro or in vivo with different carcinogens was shown to induce transformation.[105,106] Of great interest is the observation that DNA from normal cells was also effective.[106] These new approaches ...o chemical carcinogenesis open up many areas of exploration, including the question of whether the information contributions of the DNA are positive (i.e., they code for an identifiable component that is important in cell transformation) or negative (i.e., they disorganize the host genome in an appropriate manner).

Diet and Carcinogenesis

The composition of the diet may influence the incidence of cancer in some sites, such as the colon, breast, and endometrium.[107-109] The mechanism or mechanisms through which diets exert their influences are not well understood. In some instances, carcinogens occur in the food as natural substances, as contaminants (e.g., aflatoxins), or as products of food-preparation methods (e.g., pyrolysis). Dietary alterations also modulate the activation of carcinogens.

In other instances, micronutrients have a role in the endogenous formation of carcinogens. The past 10 years or so have seen a revival of the concept of the endogenous formation of carcinogens. This hypothesis was proposed in the 1930s in relation to cholesterol or other sterols, but it was progressively abandoned as the evidence failed to materialize. The resurrection of this idea assumes a new form in the genesis of nitroso-compounds from nitrite and secondary or tertiary amines.[110] Dietary amines or drugs such as oxytetracycline of chlorpromazine can react with nitrous acid, generated in the stomach from nitrite, to form nitroso-compounds. Some of these substances are carcinogenic in laboratory animals. The formation of such nitroso-compounds can be effectively inhibited by dietary ascorbic acid[111] and by vitamin E.

This general model is being explored in colon cancer in human beings.[112] The hypothesis states that carcinogens can be generated in the feces and that the levels of the carcinogens, as measured by mutagenic action, are influenced by the dietary composition, including vitamins C and E and fiber content.

Chemotherapy and Carcinogenesis

Some of the most effective chemotherapeutic agents for cancer are carcinogenic. For the treatment of cancer in patients above 50 or 60 years old, the importance of the risk of carcinogenicity is clearly minimal, given the long latent period for cancer development. However, in children and young adults, the development of second cancers years after the effective treatment of the primary tumor is now becoming a recognizable problem.[113,114] Over 25 drugs used in chemotherapy have been found to be carcinogenic in animals, and some have also been implicated in human beings.

The magnitude of the risk associated with the many treatment regimens is not fully known. However, for

some patients the risk of second primary tumors varies from 7 to 12 per cent.[115] These figures include some risk factors aside from the therapy, that are possibly genetic and favor second or multiple primary tumors. However, there is an additional risk from the chemotherapy itself. This risk will increase in volume as current types of chemotherapy for cancer become more successful. However, as mechanisms by which chemicals induce cancer and kill cancer cells are clarified, ways will be found to prevent the carcinogenic effects without interfering with the drugs' efficacy in treating cancer.

A common problem in cancer chemotherapy is the appearance of resistant cancer cells. Since resistant cells are also produced by carcinogens during carcinogenesis, and since such resistance constitutes one form of initiation,[17,83] it is conceivable that the induction of resistance to therapy and that of cancer are related.

New Bioassays for Carcinogens and Promoters

The basic knowledge that has been generated during the past 20 years or so has spawned a large array of short-term tests (mostly in vitro) for potential carcinogens, and more recently for promoters.[116] These tests generally fall into two broad groups: those that use some correlate of DNA damage or chromosomal damage as end points and those that use cell transformation as the end point.[116-118] The majority require the inclusion of an appropriate activation system.

This development is an essential requirement for rapid monitoring of our environment. In addition, these tests are being used increasingly as rapid assays for some more basic aspects of chemical carcinogenesis, such as patterns of activation in tissues and the endogenous production of carcinogens or mutagens, to name but two of many examples.

Conclusions

The past decade has produced new insights into the need for metabolic activation, the nature of the active molecules, the enzymology of activation, and the array of products generated, including major ultimate carcinogens. The interactions of such derivatives with DNA and other cell constituents and the possible toxicities and other effects on cells are becoming clearer. There is a general belief that DNA damage is involved in cancer initiation in most instances. The basic studies have generated over 100 short-term assays for possible carcinogens, with many of the assays reflecting damage to DNA or chromosomes.

The importance of the modulation of metabolic activation by enzyme induction with hormones, diet, drugs, and chemicals (including many environmental contaminants such as pesticides, herbicides, and polychlorinated biphenyls) is appreciated. How this ubiquitous exposure may influence cancer development in human beings is not known.

The repair of lesions in DNA is receiving increasing emphasis. The majority of such studies are in relatively simple in vitro systems and are generating insights into what may happen. The study of what does happen remains a major challenge. It may be aided by the development of ultrasensitive enzymatic radioimmunoassays for carcinogen-DNA adducts.

There is an increasing realization that many post-initiation phenomena, including promotion, are of major importance in cancer development. Alterations in diet, hormones, drugs, and xenobiotic agents have major influences through effects on promotion and other later steps. The reversibility of many of the precancerous steps is being studied with vitamin A and its analogues and with other dietary components.

Despite the major advances, the study of the later phases of chemical carcinogenesis lags far behind that of the early biochemical events. A nagging uncertainty in this field in general concerns the role of chemical carcinogens of either exogenous or endogenous origin in the causation and pathogenesis of some of the major forms of cancer. There is a general agreement that cigarette smoking and ultraviolet light are two major factors in the genesis of several important types of cancers. There is also incontrovertible evidence that many chemicals to which persons are exposed in the work place or its environs cause cancer in several organ systems. However, the relative quantitative importance of these considerations in the majority of patients with cancer not related to smoking is unclear. The resolution of this problem, although no doubt difficult, will almost certainly have a major impact on our approaches to cancer prevention in coming decades.

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react... the personnel and work stress. ...characteristics and perceived social ...integrated these effects. Job dissatisfa... was found to be an intermediary phase in the ...development of stress at work. The applied theor...al model was found feasible for explaining ...relationships between the concepts ...on the basis of the acquired results.
—Condensed from text

365/81 The Uses and Limits of Standard Exercise Tests. L.B. McGuire. Arch. Intern. Med. 141: 229-232, Feb. 1981. 37 refs.

Standard exercise electrocardiography to detect coronary artery disease involves limitations of accuracy in a population of apparently healthy persons, to the extent that its value for counseling such individuals is doubtful. Among subjects being examined for chest pain, however, the accuracy of the exercise ECG in predicting coronary obstructive disease is better. In addition, the presence of more severe coronary obstructive disease tends to be associated with more distinctly abnormal tests at low levels of exercise. This tendency of association between marked ST-segment displacement at low exertion levels and more severe obstructive disease adds a measure of prognostic value to the standard exercise ECG in persons with chest pain or after myocardial infarction. The addition of either isotopic cardiac imaging or coronary arteriography to exercise ECG will be appropriate for situations in which either the ECG is known to be nonspecific or inadequate exercise is achieved.
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366/81 Therapeutics of Hypertension in the Work Environment. G.T. McInnes and E.B. Macdonald. J. Soc. Occup. Med. 31: 31-37, Jan. 1981. 12 refs.

Essential hypertension is a major health problem among people at work. Current therapy is discussed and the potential role of the occupational health worker explained.
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367/81 Relative Importance of Cigarette Smoking in Occupational Lung Disease. P.C. Elmes. Brit. J. Ind. Med. 38: 1-13, Feb. 1981. 30 refs.

Since 1900 respiratory disease has remained a constant serious cause of chronic ill health and premature death in Britain. The falling importance of tuberculosis and pneumonia has been off-set by the rise in lung cancer. Bronchitis morbidity and mortality have fallen only slightly since 1935. To produce any real improvement in the future existing information as to cause must be studied. The relative contribution of occupational exposure is compared with the importance of cigarette smoking. Relevant information is scanty and has been produced to emphasise the existence of occupational diseases rather than assess their importance to the community as a whole. In Britain the evidence is that within the coal mining and iron and steel industries conditions are now such that dust exposure contributes little to the morbidity or mortality compared with the workers' smoking habits.

364/81 Ergonomics: The Mechanics of Man 2 — The Physiology of Work. C. Hayne. Occup. Health 33: 18-24, Jan. 1981. 10 refs.

Fatigue, though better understood today than ever before, is still too complex to be measured by any direct method. When considering the demands of work in relation to energy expenditure and fatigue, it is necessary to be aware of the many elements, both physiological and psychological, associated with human performance. The solution to fatigue may not always be in longer work pauses but in the redesign of activities to produce greater job enrichment and satisfaction.
—Condensed from text

363/81 Occupationally Induced Stress, Strain and Peak Loads as Related to Age. J. Ilmarinen and T. Rutenfranz. Scand. J. Work Environ. Health 6: 274-282, Dec. 1980. 25 refs.

From six different types of work 120 men, aged 25-60 yr, were classified according to the Ergonomic Job Description Questionnaire into four groups representing specific work content (producing forces, coordinating motor and sensory functions, converting information into reactions, and producing information). Work stress was assessed with measurements of oxygen consumption ($\dot{V}O_2$) and with the registration of the minutes spent at different stress levels. Relative aerobic strain (RAS) was defined as the $\dot{V}O_2$ during work as the percentage of $\dot{V}O_2max$. Strain was measured with continuous recordings of heart rate during the workday. Peak loads were estimated according to the relative number of minutes above the heart rate levels of 130, 150 and 170 beats/min. The $\dot{V}O_2$ during work was virtually identical in the age groups < 35, 35-50 and > 50 yr. The RAS tended to increase with age due to the decrease in $\dot{V}O_2max$. The strain remained practically the same in all the age groups in the group "coordinating motor and sensory functions" strain tended to increase with age. Peak loads over 150 beats/min were not met for the older subjects. The results suggest that stress and strain during work remain practically the same as age increases. The RAS, however, tends to increase with age within groups doing mainly physical work and with exposures to peak loads.
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