Chemical carcinogenesis: Too many rodent carcinogens*

(tumor promotion/mutagenesis/mitogenesis/animal cancer tests)

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ABSTRACT   The administration of chemicals at the maximum tolerated dose (MTD) in standard animal cancer tests is postulated to increase cell division (mitogenesis), which in turn increases rates of mutagenesis and thus carcinogenesis. The animal data are consistent with this mechanism, because a high proportion—about half—of all chemicals tested (whether natural or synthetic) are indeed rodent carcinogens. We conclude that at the low doses of most human exposures, where cell killing does not occur, the hazards to humans of rodent carcinogens may be much lower than is commonly assumed.

In current strategies to prevent human cancer, chronic rodent bioassays are the major source of information used to predict the risk to humans from chemical exposures. This paper addresses the issue of the role of cell division (mitogenesis) in animal cancer tests and the implications of an improved theory of mechanisms of carcinogenesis for the assessment of cancer hazards to the general population. In animal tests done at the maximum tolerated dose (MTD), about half of the chemicals tested are rodent carcinogens (1–7). We argue that the explanation for a high percentage of chemicals being carcinogens at the MTD is that these high doses stimulate mitogenesis, which increases rates of mutagenesis and carcinogenesis. While chemicals selected for testing are primarily synthetic industrial compounds, the high positivity rate does not imply that synthetic chemicals are more likely to induce tumors in rodents than naturally occurring chemicals. [The chemicals in the human diet are nearly all natural (8).] To the extent that increases in tumor incidence in rodent studies are due to the secondary effects of administering high doses, then *any* chemical that increases mitogenesis (e.g., by cell killing) is a likely rodent carcinogen. The correct analysis to determine the proportion of rodent carcinogens among chemicals would require a comparison of a random group of synthetic chemicals with a random group of natural chemicals. This analysis has not been done. We have examined the available results from the limited number of natural chemicals tested and have found that about half are rodent carcinogens, just as for the synthetic chemicals (8).

The high proportion of carcinogens among chemicals tested at the MTD emphasizes the importance of understanding cancer mechanisms in order to determine the relevance of rodent cancer test results for humans. A list of rodent carcinogens is not enough. The main rule in toxicology is that "the dose makes the poison": at some level, every chemical becomes toxic, but there are safe levels below that. However, the precedent of radiation, which is both a mutagen and a carcinogen, gave credence to the idea that there could be effects of chemicals even at low doses. A scientific consensus evolved in the 1970s that we should treat carcinogens differently, that we should assume that even low doses might cause cancer, even though we lacked the methods for measuring

carcinogenic effects at low levels. This idea evolved because it was expected that (*i*) only a small proportion of chemicals would have carcinogenic potential, (*ii*) testing at a high dose would not produce a carcinogenic effect unique to the high dose, and (*iii*) chemical carcinogenesis would be explained by the mutagenic potential of chemicals. However, it seems time to take account of new information suggesting that all three assumptions are wrong.

Carcinogens Are Common in Rodent Tests

More than half of the chemicals tested to date in both rats and mice have been found to be carcinogens in chronic rodent bioassays at the high doses administered, the MTD (1–7). Synthetic industrial chemicals account for 350 (82%) of the 427 chemicals tested in both species; about half (212/350) were classified as rodent carcinogens (1–7). Even though the overwhelming weight and number of the chemicals humans eat are natural, only 77 natural chemicals have been tested in both rats and mice; again about half (37/77) are rodent carcinogens (1–6). The high proportion of positives is not due simply to selection of suspicious chemical structures. While some synthetic or natural chemicals were selected for testing precisely because of structure or mutagenicity, most were selected because they were widely used industrially—e.g., they were high-volume chemicals, pesticides, food additives, dyes, or drugs (2). The natural world of chemicals has never been looked at systematically. We explain below why the developing understanding of the mechanisms of carcinogenesis justifies the prediction that a high proportion of all chemicals, natural and synthetic, will prove to be carcinogenic to rodents if tested at the MTD. How to select the MTD is a process that has been changing (9–11).

A chemical is classified as to carcinogenicity in our analysis based on the author's positive evaluation in at least one adequate experiment (3–6) using the criteria given in ref. 8. Rodent carcinogens clearly are not all the same: some have been tested many times in several strains and species and others in only one experiment; some (e.g., safrole) are positive in two species and they or their metabolites are genotoxic in animals; some (e.g., D-limonene) are only positive at one site in one species and are not genotoxic.

Mechanism of Carcinogenesis

It is prudent to assume that if a chemical is a carcinogen in rats and mice at the MTD, it may well be a carcinogen in humans at doses close to the MTD. However, understanding the mechanism of carcinogenesis is critical to the attempt to predict risk to humans at low doses that are often hundreds of thousands of times below the dose at which an effect is observed in rodents. There are two major problems. (*i*) Within rodents, how can measurable carcinogenic effects at

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Abbreviation: MTD, maximum tolerated dose.
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dose rates near the MTD (i.e., at doses that may cause significant cell killing and mitogenesis) be used to estimate the effects *in rodents* of dose rates so much lower that they will cause little or no cell killing or, at any rate, will cause an amount that is well within the "normal" range of cell death and replacement? (*ii*) Between species, how can carcinogenic effects in a short-lived species such as the rat or mouse be used to estimate effects in a long-lived species such as the human? Cancer increases with about the fourth or fifth power of age in both short-lived rats and long-lived humans (12–15). In order to achieve a long life-span, humans have evolved many types of defenses that collectively ensure that they are orders of magnitude more resistant to spontaneous cancer at a particular age than rats (12–15). Thus, in both types of extrapolation there may be systematic factors that make the carcinogenic effects vastly less in humans than would be expected from simple extrapolation—so much so, indeed, that no quantitative extrapolation is likely to be possible in the near future from studies at or near the MTD in laboratory animals to the effects of low dose rates in humans (12, 13).

The Role of Mitogenesis. The study of the mechanisms of carcinogenesis is a rapidly developing field that can improve regulatory policy. Both DNA damage and mitogenesis are important aspects of carcinogenesis, and increasing either substantially can cause cancer (1, 16–21).

Endogenous rates of DNA damage are enormous. Mutagens are often thought to be only exogenous agents, but endogenous mutagens cause massive DNA damage (oxidative and other adducts) that can be converted to mutations during cell division. We estimate that the DNA hits per cell per day from endogenous oxidants are normally $\approx 10^5$ in the rat and $\approx 10^4$ in the human (15, 22, 23). These oxidative adducts are effectively but not perfectly repaired; the normal steady-state level of just 8-hydroxydeoxyguanosine (1 of about 20 known oxidative DNA adducts) in rat DNA has been measured as 1/130,000 bases or about 90,000 per cell (22, 24). We have argued that this oxidative DNA damage is a major contributor to aging and the degenerative diseases associated with aging such as cancer. Thus, any agent causing chronic mitogenesis can be indirectly mutagenic (and consequently carcinogenic) because it increases the probability of endogenous promutagenic DNA adducts being converted to mutations (Fig. 1). Furthermore, endogenous rates of DNA damage are so high that it may be difficult for exogenous mutagens to increase the total DNA damage significantly by low doses that do not increase mitogenesis.

Mitogenesis is itself mutagenic in numerous ways (Fig. 1). (*i*) A dividing cell is much more at risk for mutation than a quiescent cell (27). Cell division allows adducts to convert to mutations. The time interval for DNA repair during cell division is short, and adducts are converted to gaps during

replication. Endogenous or exogenous damage is therefore generally increased if cells are proliferating.

(*ii*) During cell division, single-stranded DNA is without base-pairing or histones and is thus more sensitive to damage than double-stranded DNA.

(*iii*) Cell division triggers mitotic recombination, gene conversion, and nondisjunction, which together seem orders of magnitude more effective than an independent second mutation (28–32) in converting a heterozygous recessive gene (e.g., a tumor-suppressor gene) to homo- or hemizygosity. Heterozygotes at the human HLA-A gene are spontaneously converted to homozygotes during cell division (33). The above mechanisms could account for gross chromosomal alterations that occur frequently in human tumors (34–40).

(*iv*) Cell division allows gene duplication, which can increase expression of oncogenes that are otherwise weakly expressed (41).

(*v*) Cell division can increase the expression of the *myc* and *fos* oncogenes (42).

(*vi*) Cell division allows 5-methyl-cytosine in DNA to be lost, which can result in dedifferentiation (43, 44), thus often causing further mitogenesis.

In support of high "spontaneous" mutation rates in dividing cells is the observation that background hypoxanthine phosphoribosyltransferase mutations that arise *in vivo* in human T lymphocytes arise preferentially in dividing T cells (45–48). The well-known mitotic instability of tumors (tumor progression) might be explained by the fact that cells in some tumors are proliferating constantly.

Suppression of intercellular communication causes mitogenesis. At near-toxic doses some chemicals interfere with cell–cell communication in quiescent tissues (e.g., the liver, the major target site for carcinogenesis in rodents), thereby causing mitogenesis and carcinogenesis (49–51). Trosko and his associates (49, 50) have proposed that suppression of gap junctional intercellular communication in contact-inhibited cells could lead to cell proliferation by cell death, cell removal, promoting chemicals, specific oncogenic products, growth factors, and hormones.

Mitogenesis from exogenous and endogenous factors can cause cancer. (*i*) Toxicity can cause injury to tissues, resulting in replacement cell division (52–56). In an experimental cancer model, the surgical removal of part of the liver causes neighboring cells to proliferate (52–54). The incidence of liver cancer is low in humans (but not in some strains of mice) unless the liver is chronically damaged. Viruses or alcohol excess, for example, cause damage to the liver, which is a risk factor for cancer. Salt excess is a major risk factor in human stomach cancer because it causes mitogenesis (57–64). Chronic toxicity can also cause an inflammatory reaction, since phagocytic cells unleash a barrage of oxidants in
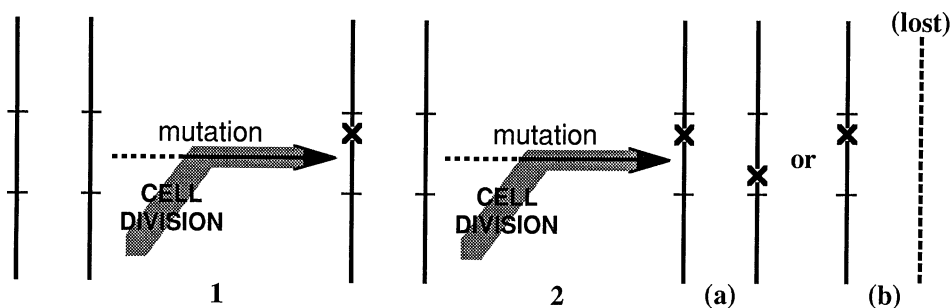
FIG. 1. Mitogenesis (induced cell division) is a major multiplier of endogenous (or exogenous) DNA damage leading to mutation. The pathway to inactivating (**X**) both copies of a recessive tumor-suppressor gene is shown (two vertical lines represent the pair of chromosomes carrying the genes). Cell division increases mutagenesis due to the following: DNA adducts converted to mutations before they are repaired (*1* and *2a*); mutations due to DNA replication (*1* and *2a*); vulnerability of replicating DNA to damage (*1* and *2a*). Mitotic recombination (*2a*), gene conversion (*2a*), and nondisjunction (*2b*) are more frequent, and the first two give rise to the same mutation on both chromosomes. This diagram does not attempt to deal with the complex mutational pathway to tumors (25, 26).

destroying dead cells at a wound. The oxidants produced are the same as in ionizing radiation, so chronic inflammation may be equivalent to irradiating the tissue (65). The oxidants produced as a result of inflammation may stimulate oncogenes and cell proliferation (66–69). Chronic irritation and inflammation cause cancer in animals (111). Chronic inflammation is, as expected, a risk factor for human cancer (70–72); the carcinogenic effects of asbestos (73) and the NO_x in cigarette smoke, for example, may be due primarily to inflammation, which increases both mitogenesis and mutagenesis.

(*ii*) Chronic infections from viruses, bacteria, schistosomes, and other organisms cause cell killing and consequent mitogenesis and can be risk factors for cancer. Two examples are the human hepatitis B virus, a major cause of liver cancer in the world (74, 75), and human papilloma virus 16, a major risk factor for cervical cancer whose main effect on cells is to increase proliferation (76). A study on transgenic mice that overproduce one protein of the hepatitis B virus, a surface antigen, results in cell turnover that causes all of the mice to develop hepatocellular carcinomas (21).

Human T-cell lymphotropic virus type I causes constitutive expression of the T-cell interleukin 2 receptor. This may commit the cell to unremitting *in vivo* cell division with an increased likelihood for the occurrence of critical mutations leading to T-cell leukemia/lymphoma (77, 78). Chronic *Helicobacter* (*Campylobacter*) infection is thought to be a risk factor for stomach cancer (79, 80) and chronic schistosome infection a risk factor for bladder and colorectal cancer (81).

(*iii*) Hormones can cause mitogenesis and are risk factors for breast and other human cancers (81, 82).

Thus, agents causing mitogenesis are proper carcinogens and are important in human cancer (81, 82). The classic tumor "promoters" such as phenobarbital and phorbol myristate acetate cause mitogenesis and are in fact complete carcinogens in animals when tested thoroughly (83). The cell division induced in the rat liver (a quiescent tissue) by certain mitogens (without cell killing) is less potentially carcinogenic than cell division induced by toxicity (cell killing and cell replacement) (84). A possible explanation for the lack of effect of the mitogens is the death of hyperplastic tissue after the mitogenic stimulus is removed (84). Some cells normally divide more than others, and presumably this is balanced by normal defenses. Thus, cells that normally proliferate (e.g., stem cells of the small intestine) are not necessarily more susceptible to tumorigenesis. In the small intestine and other epithelial tissue, the non-stem cells that are discarded account for most of the cell division. Chemicals that increase mitogenesis at low doses relative to toxicity may not be common, but are of particular interest to identify.

Animal Cancer Tests and Mitogenesis. *Mutagenicity.* Analyses of animal cancer tests to date indicate that a high proportion ($\approx$40%) of chemicals that are carcinogenic in chronic tests at the MTD are not mutagenic (in *Salmonella*) (2, 85, 86). Since mitogenesis itself can be indirectly mutagenic, nonmutagens at the MTD are likely to be acting by this mechanism. If the nonmutagens that are carcinogenic in rodents at the MTD cause cancer chiefly through cytotoxic mechanisms, and if this cytotoxicity exhibits a steep upward-curving (or a threshold) dose response, then for such chemicals the choice of the MTD is clearly critical for tumor induction. Other nonmutagenic carcinogens that are not active through cell killing but through mitogenesis from other causes have been discussed (18–20).

Genotoxic chemicals are even more effective at causing mitogenesis at high doses (by cell killing and cell replacement); since they also act as mutagens, they can give a multiplicative interaction not found at low doses. However, potent mutagens such as 2-acetylaminofluorene have been shown to induce liver tumors in mice at moderate doses (that

do not increase cell division) with only the endogenous mitogenesis that primarily occurs during development (20). In rodent cancer tests mutagens, in contrast to nonmutagens, are more likely to be carcinogenic (2, 85, 86), more likely to be positive in both rats and mice (2, 85, 86), more likely to be toxic at lower doses (2), and more likely to cause tumors at multiple sites (86). The mutagenicity of a chemical in *Salmonella* does not necessarily indicate the mechanism in a rodent. Of 340 chemicals tested both for carcinogenicity in rats and mice and for mutagenicity in *Salmonella*, 28% of the noncarcinogens are mutagens in *Salmonella* that presumably are not acting as mutagens in rodents (ref. 2; L.S.G., unpublished work). Even those mutagens that are carcinogens may not all be acting as genotoxins in animals, because of detoxification and other complexities. In a study of pairs of mutagenic isomers, one isomer a carcinogen and the other not, only the carcinogen was mitogenic: 1- vs. 2-nitropropane (112); 2,4- vs. 2,6-diaminotoluene (113).

Dose–response relation. Some evidence supports the idea that toxicity at or near the MTD induces mitogensis, but below a certain dose no mitogenic effect is observed. Therefore, if animal cancer tests are primarily measuring the effects of mitogenesis, then the dose response would be expected to curve steeply upward (1, 18–20, 87–89). When doses too low to produce much mitogenesis are used, and the cell-division rate reverts to something well within the normal range, no significant enhancement remains to multiply up any other effects of the chemical, leading to an upward-curving dose response for carcinogenicity, even for mutagens. This means that a 10-fold reduction in dose in a rodent experiment would produce much more than a 10-fold reduction in risk. This prediction is strongly confirmed by several recent analyses including the large-scale EDO1 study on the mutagen 2-acetylaminofluorene (20). A similar result is found in an extensive dose–response experiment with the well-characterized mutagens diethylnitrosamine and dimethylnitrosamine in rats (90–92). With diethylnitrosamine, at doses near the MTD the induced ethylated adducts show a linear dose response, and the induced mitogenesis shows a threshold; the tumors induced at doses near the MTD, however, show a clearly upward-curving dose response (88–92). A similar case is seen with the mutagen formaldehyde (18, 19, 89).

Mutagens, because they damage DNA, are very effective at killing cells (they have lower MTDs than nonmutagens on average) (2), and thus are also very effective at causing cell proliferation and inflammatory reactions. Thus, even though mutagens can have some effect at low doses in the absence of mitogenesis (18–20), carcinogenicity for both mutagens and nonmutagens at the MTD is primarily caused by mitogenesis. If a chemical is nonmutagenic and its carcinogenicity is due to cell proliferation that results from near-toxic doses, one might commonly expect a virtual threshold in the dose response (1, 18–20, 52–54). Mitogenesis in rodent carcinogenesis has not thus far been a focus of much experimental work; experimental evidence is discussed in refs. 18–20. An analysis of rodent target organ toxicity among 53 carcinogens did not indicate a major effect of toxicity on carcinogenesis at 2 years, but mitogenesis was not measured even though it can be high without histologically observed lesions (87).

Several analyses have examined the dose response in National Cancer Institute/National Toxicology Program bioassays. One analysis of the shape of the dose–response curves in 344 tests indicates that at the high doses used, a quadratic dose response is compatible with more of the data than a linear one for both mutagens and nonmutagens (D. Hoel and C. Portier, personal communication). Another analysis of 52 tests indicates that more than two-thirds of the carcinogenic effects would not have been detected if the high dose had been reduced from the estimated MTD to one-half the MTD (9). A third study showed that only 10% of the

Medical Sciences: Ames and Gold

Proc. Natl. Acad. Sci. USA 87 (1990) 7775

dose–response functions indicated a possible plateau (a leveling off of the dose response). For the compounds in which an apparent plateau was observed in one site, the result was generally not replicated in other target sites in the same experiment, in the other sex of the same species, or in other species (93). Our explanation for the observation that a plateau in the dose response is uncommon is that toxicity-induced mitogenesis is usually important (93).

Other work suggests that cell killing is also an important factor in radiation carcinogenesis (94, 95). In addition, low doses of radiation induce antioxidant defenses that protect against the mutagenic and toxic effects of larger doses of radiation or other oxidizing agents (96–100).

These considerations of mechanism suggest that at chronic doses close to the toxic dose, any chemical, whether synthetic or natural, and whether genotoxic or nongenotoxic, is a likely rodent and human carcinogen. Not all chemicals would be expected to be carcinogens at high doses; the MTD may not be reached (101) or the chemical may be toxic without causing cell killing or mitogenesis.

Human Cancer

The major preventable risk factors for cancer that have been identified thus far are tobacco, dietary imbalances (102–108), hormones (81, 82), infections (74–82), and high-dose exposures in an occupational setting (109, 110), as has been discussed extensively in the literature. What is chiefly needed is to take seriously the control of the major hazards that have been reliably identified, without diverting attention from these major causes by a succession of highly publicized scares about factors that may well be of little or no importance as causes of human disease. Moreover, we need to make progress toward the identification of at least a few more major causes and to understand better the hormonal determinants of breast cancer, the viral determinants of cervical cancer, and the dietary determinants of stomach and colon cancer. In this context, the most important contribution that animal studies can offer is insight into possible mechanisms (e.g., more studies on mitogenesis) and into the complex natural world in which we live and in which life expectancy is still increasing.

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