Carcinogenesis Mechanisms: The Debate Continues

I. B. Weinstein, in his Perspective of 25 January, "Mitogenesis is only one factor in carcinogenesis" (p. 387), misstates our view of carcinogenesis. Geneticists have long known, but Weinstein does not take into account, that cell division is critical for mutagenesis. If one accepts that mutagenesis is important for carcinogenesis, then mitogenesis *must* be important. The inactivation of tumor suppressor genes is also known to be important in carcinogenesis, and one function of tumor suppressor genes is to inhibit mitogenesis (*1*). Once the first copy of a tumor suppressor gene is mutated, the inactivation of the second copy (loss of heterozygosity) is more likely to be caused by mitotic recombination, gene conversion, and nondisjunction (all dependent on cell division), than by an independent second mutation (*2*). Thus loss of heterozygosity will be stimulated by increased mitogenesis. Mitogenesis increases the chance of every mutational step, but it is a much more important factor for tumor induction after the first mutation has occurred. This explains the temporal and synergistic relation of mutagenesis and mitogenesis (*2*). Naming this "initiation" and "promotion" confuses mechanistic issues.

The idea that "promoters" are not in themselves carcinogens is not credible on mechanistic or experimental grounds (*2*). Every classical "promoter" adequately tested is carcinogenic such as phorbol ester, phenobarbital, and catechol. The very word "promoter" confuses the issue, since mitogenesis may be increased by a high, but not a low dose. Mitogenesis would increase clonal expansion of dominant oncogenes and would cause loss of epigenetic modification through events such as mitotic recombination (*2*). Chronic mitogenesis itself can be a risk factor for cancer; theory predicts it, and a large literature supports it (*2, 3*). Of rodent carcinogens, 40% are not detectable mutagens and may not be carcinogens at low doses. They should be investigated to see if their carcinogenic effect results from inducing mitogenesis.

We and Weinstein agree "that certain DNA damaging agents might produce a high tumor yield because they induce both mutations and cell replication." Mitogenesis can often be the dominant factor in carcinogenesis at doses close to the maximum tolerated dose (MTD), even for mutagens. Mitogenesis can be caused by toxicity of chemicals at high dose (cell killing and subsequent replacement), by interference with cell-cell communication at high doses (*4*), by substances such as hormones binding to receptors that control cell division (*3*), by oxidants (the wound healing response), and by viruses (*2*). Increased mitogenesis in cells that are not discarded is the important factor, not toxicity, and effects will vary by tissue.

Weinstein dismisses the enormous DNA-damage rate from normal endogenous oxidants without good reasons. A normal rat cell has about 10^6 oxidative adducts at any one time, and this increases with age (*5*). Also about 10^5 new oxidative adducts per cell are formed every day, and most are repaired (*5*). These are the same adducts produced by radiation, an oxidative mutagen. We conclude that endogenous oxidative damage is a major factor in aging and the degenerative diseases of aging such as cancer. This high endogenous level of adducts reinforces evidence from epidemiology that deficiency of antioxidants (*6*) and mitogenesis (*2, 3*) are important risk factors for cancer.

Weinstein states that endogenous damage is unimportant because spontaneous tumor rates aren't high, yet in standard 2-year rodent bioassays about 40% of controls develop malignant tumors. It does not follow that endogenous adducts should be ignored because 10^5 to 10^4 adducts per cell of benzo[*a*]pyrene or of aromatic amines are associated with transformation. The proper assessment of the carcinogenic effect of a given level of adducts has not been done: it would require in vivo measurements of all adducts, mitogenesis, and tumor induction. Benzo[*a*]pyrene at doses close to the MTD could increase mitogenesis and give rise to a variety of mitogenic and mutagenic quinone oxidants (*7*) that would result in unmeasured oxidative adducts.

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As indicated by Weinstein, it is generally accepted that cancer arises from normal cells as the result of genetic alterations and that more than one genetic change is required for the formation of a malignancy. However, one should fully appreciate the relationship between genetic damage and cell proliferation in the context of our article (31 Aug., p. 1507).

Geneticists have known for decades that DNA does not replicate with 100% fidelity and that there is endogenous DNA damage. Thus, every time DNA replicates, there is a rare chance that a mistake might occur in a gene critical to the carcinogenic process. An agent can increase the likelihood of DNA damage by either directly altering the DNA (genotoxicity) or by increasing the number of times DNA replicates (cell proliferation).

Weinstein draws conclusions on the basis of a deterministic approach (if A takes place, then B results). Ours is a probabilistic perspective (if A takes place, then in a random, probabilistic fashion, B *may* result). Quantitative, probabilistic, and time-varying aspects of the critical variables in carcinogenesis, including direct genetic damage and cell proliferation, can explain the disparate observations of carcinogenesis in animal models (*1*) and in human epidemiologic studies (*2*), including the "multistage, multifactor nature of carcinogenesis" referred to by Weinstein. For example, he correctly states that there is active cell proliferation in embryonic and fetal tissues. However, as in the examples we described in our article, if the probability of unrepaired genetic damage occurring in a critical gene is exceedingly low (say, one per 10^6 cell divisions), and if at least two errors must occur in the same cell for it to become malignant (requiring an expected 10^{12} cell divisions), it is unlikely that a cancer will arise by the time of birth even in a rapidly proliferating tissue.

We also emphasized that the critical genetic damage must occur in a cell with the potential to divide and develop into a can-

cer, not in a differentiated cell destined to die and be replaced. In the skin model, proliferation of stem cells in the basal layer, in contrast to differentiated keratinocytes, is necessary for carcinoma development. Similarly, an adenomatous polyp of the human colon, a proliferation of stem cells, has the potential to develop into carcinoma, whereas a hyperplastic polyp, a proliferation of differentiated mucus-producing cells, does not.

Our focus on cell proliferation did not question the importance of rodent bioassays, but rather their interpretation for human risk assessment. Bioassays ought to be complemented with experimental information about genotoxicity, cell proliferation, and mechanism in the quantification of dose-response relationships. Short-term screens, whether for genetic damage or increased cell proliferation, are far from 100% predictive of carcinogenicity and, thus, are not a replacement for the long-term bioassay.

Unfortunately, there has been an uncritical acceptance of the notion that a positive result in a rodent bioassay automatically implies a carcinogenic risk for humans. While this may well be the case for genotoxic agents, for nongenotoxic agents there will be exceptions, especially if the proliferative response occurs only at high doses. For example, melamine, a nongenotoxic compound, produces bladder cancer in rodents by forming urinary calculi at high doses, but not at low doses. The Environmental Protection Agency has evaluated melamine on this basis (*3*). Melamine is an easily understood example of a chemical that is carcinogenic in animals but, because of mechanistic and dose-related considerations, is not likely to be carcinogenic in humans at the doses to which we are exposed. There are many other chemicals that fit into this category.

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Response: Ames and Gold have concluded that current policies to reduce nonoccupational exposures to industrial carcinogens are unjustified. We raise the following questions about their major arguments (italics).

1) *Are carcinogenic risks from low levels of synthetic chemicals negligible?*

While naturally occurring chemicals, including dietary fat, probably play important roles in influencing the incidence of certain forms of human cancer, the exact proportion of cancers that are due to "natural" versus synthetic carcinogens is not known. Moreover, one must be cautious about misclassifying as "natural" carcinogens that result, at least in part, from human activities (for example, cigarette smoke, nitrosamines in food, heterocyclic amines in cooked meat, and aflatoxin in grains.

What is negligible? Even the more conservative estimates suggest that 30,000 cancer deaths each year in the United States may be due to synthetic chemicals in the workplace and ambient environment (*1*). Preventive measures could reduce these unnecessary deaths. Surveillance of new products is required to assure that these numbers do not increase. In some cases, such as methylene chloride in paint strippers and pesticides used in the home, consumers are exposed to high levels of carcinogens. In addition, bioaccumulation of certain chemicals in water supplies, food sources, soil, and tissues can result in a long-term carcinogenic hazard to the general population and in permanent alterations of the biosphere.

2) *Is endogenous DNA damage the major contributor to human cancer?*

There is no direct evidence that oxidative damage to DNA (other than that associated with ionizing irradiation, which also causes DNA strand breaks), depurination, or other endogenous damage to DNA are carcinogenic. This is an interesting hypothesis but not a fact to be used in setting regulatory policies. On the other hand, there *is* convincing evidence that many exogenous agents (both genotoxic and nongenotoxic) can increase cancer incidence in experimental animals and humans.

3) *Is cell proliferation* per se *carcinogenic?*

It is obvious that cell proliferation is required for both point mutations and more complex genetic changes and is an essential component of multistage carcinogenesis. This does not mean that it is always the dominant rate-limiting factor. There is no consistent correlation between the intrinsic proliferative index of a tissue and cancer incidence in that tissue, in either laboratory animals or humans (*2*). Nor is there evidence that even well-studied experimental tumor promoters (di(2-ethylhexyl)phthalate, phenobarbital, dioxin) act simply by inducing sustained cell proliferation (*3*).

4) *Are rodent carcinogenicity data irrelevant to humans because they are derived from assays in which high, toxic, and mitogenic doses were used?*

Extensive analyses of the National Toxicology Program rodent carcinogen bioassay database indicate that there is *not* a consistent correlation between carcinogenicity and organ toxicity. Clinical chemistry data and histopathology also support this conclusion (*4*). Studies cited by Ames and Gold as evidence of the role of mitogenesis in carcinogenesis examined cell proliferation only on the ninth day after carcinogen treatment (*5*). They did not directly evaluate the relationship between cell proliferation and induction of cancer.

All of the known human carcinogens, when adequately tested, are carcinogenic in rodent bioassays. Rodent bioassays have predicted a number of human carcinogens. Recent epidemiologic studies suggest that this is also true with certain pesticides (such as dichlorvos) and with the industrial chemical 1,3-butadiene (*6*). Rodent bioassays are, therefore, extremely valuable in cancer prevention.

5) *Is the burden of naturally occurring carcinogens in food sources much greater than that contributed by contamination with synthetic chemicals?*

This argument is based mainly on the estimate by Ames and Gold that "99.99%" of dietary pesticides by *weight* are natural. Indeed, they have compiled voluminous lists of "nasty" substances in the natural environment. There are, however, no carcinogenicity and potency data for most of the compounds they list. An exception is caffeic acid, which is a major contributor to their estimate of 99.99%; however, its potency is several thousand times lower than that of synthetic pesticides such as mirex, DDT, and aldrin (*7*).

The use by Ames and Gold of a "HERP" (human exposure/rodent potency) index to compare "natural" to man-made risks is based on several unfounded assumptions about human exposure and extrapolations from rodent carcinogenicity data (*8*). Illogically, the index is based on the very same rodent bioassays they criticize as being largely irrelevant to humans.

6) *For chemical carcinogens associated with human cancer, has exposure been primarily at high near-toxic mitogenic doses and would low levels of exposure be below the threshold for carcinogenicity?*

Epidemiologic evidence previously cited by one of us (F.P.P.) (Letters, 21 Dec., p. 1644) as contradicting the "high dose only" theory of carcinogenesis (a case-control study carried out by researchers at the National Cancer Institute) was dismissed by Ames and Gold as not significant. These epidemiologic studies revealed, however, that after adjustment for smoking and occupation, there was a statistically significant increased risk of lung cancer in persons who had experienced residential exposure to

smelter emissions of arsenic decades earlier (*9*). Similarly, a subsequent case-control study (*10*) showed a relative risk of 2.0 for lung cancer among men who had lived near an arsenic-emitting smelter in Sweden which could not be explained by smoking habits or occupational background. Epidemiologic studies have also found associations between cancer and other nonoccupational exposures to carcinogens, including ambient air pollution, environmental tobacco smoke, and asbestos (*11*). Moreover, epidemiological studies do not suggest a threshold for carcinogens. On the contrary, an increasing risk with increasing exposure is generally seen [as, for example, with arsenic, asbestos, uranium mining, coke oven emissions, and cigarette smoking (*12, 13*)].

There are both theoretical and biological arguments for not assuming that thresholds exist for carcinogens (*14*). In actuality, dose-response curves are difficult to ascertain, especially at low levels of exposure. Furthermore, combined exposures may lead to cumulative or synergistic effects (*15*). Hence, U.S. regulatory agencies use linear, no-threshold models unless there is convincing scientific evidence that they are incorrect in individual cases.

Recent studies have revealed not only significant background levels of molecular damage from environmental carcinogens but also significant genotoxic and other biologic effects of low-level occupational and ambient exposures to carcinogens such as polycyclic aromatic hydrocarbons and ethylene oxide (*16, 17*). In the case of ethylene oxide, worker exposures were generally below the current occupational health standard (*17*).

7) *Is it true that current regulatory guidelines do not use a balanced approach?*

The depiction by Ames and Gold of a current national policy that "attempts to protect the public at 10^{-6} hypothetical, worst case risk . . . from industrial pollution . . . whatever the cost" is erroneous. Indeed, most major statutes explicitly require agencies to take the costs of regulation into account (*18*).

We have consistently argued for a balanced approach to the problem of human cancer prevention. Risks from both natural and synthetic carcinogens are of concern. The appropriate policy for natural carcinogens is to test suspect constituents and to advise and educate the public about dietary factors that may be either hazardous or protective. Indeed, the American Cancer Society, the National Cancer Institute, and other organizations are already doing this. The policy for synthetic carcinogens is testing and regulation of those that pose significant risks, with use of the most cost-effective measures to reduce human exposure. This, in fact, is also the current policy of U.S. regulatory agencies (*18*). Ignoring the potential health hazards of synthetic carcinogens is antithetical to current preventive public health policies in the United States and many other countries.

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