Mitogenesis Is Only One Factor in Carcinogenesis

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ABOUT A DECADE AGO BRUCE AMES DEVELOPED A DATABASE indicating that a large number of carcinogens are mutagenic in bacteria (*1*). This led him to conclude that "carcinogens are mutagens" and he mounted a vigorous campaign to alert all of us to the dire health hazards of synthetic chemicals, even warning us that children peacefully asleep at night were at great risk because of trace amounts of mutagenic flame retardants in their pajamas (*1*). However, a recent perspective by Ames and Gold (*2*) in *Science,* written in support of a paper by Cohen and Ellwein in the same issue (*3*), expounds the opposite idea that synthetic chemicals pose a negligible cancer risk to humans. Furthermore, the induction of increased cell division (mitogenesis) has been presented as the major rate-limiting factor in carcinogenesis. Ames has argued that environmental policies and regulatory guidelines should follow this new dictum (*2, 4*).

Multistage, multifactor carcinogenesis. Unfortunately, the mitogenesis theory does not incorporate something that Peyton Rous, Isaac Berenblum, Jacob Furth, Leslie Foulds, and others discovered and made obvious at least 40 to 50 years ago. That is the multistage/multifactor principle in cancer causation, in which cancers arise by a stepwise evolution involving progressive genetic changes, cell proliferation, and clonal expansion (*5*). Indeed, there is now direct evidence that human tumors display progressive changes in their DNA such as activating mutations in proto-oncogenes and inactivating mutations in putative growth suppressor genes, as well as gross chromosomal aberrations (*5, 6*). These data provide convincing evidence that mutations play a prominent role in the origin of human cancers. Moreover, it seems likely that the process also involves aberrations at the epigenetic level in gene expression and differentiation (*5*). Thus, there are multiple, rate-limiting events in the conversion of normal cells to fully malignant cancer cells. In this sense there are multiple and diverse types of causative factors (both exogenous and endogenous) that act in a cumulative manner to influence the incidence of specific human cancers.

In several organ systems at least three qualitatively distinct phases have been defined in the carcinogenic process: initiation, promotion, and progression (*5*). There is clear evidence indicating that the phorbol ester tumor promoters, phenobarbital, and tetrachlorodibenzodioxin (TCDD) do not simply act as indirect mutagens. To exert their optimal carcinogenic effect, these compounds must be applied *after* the initiator and their early effects are often reversible. Furthermore, the action of certain tumor promoters does not appear to be simply due to the induction of hyperplasia (*5*). In their Perspective Ames and Gold state that our understanding of tumor promotion and mitogenesis is fuzzy. However, there has been exciting progress in our understanding of the relationships between carcinogenesis and growth factors, receptors, phosphoinositide metabolism, protein kinases, transcription factors, and cell cycle control mechanisms (*5, 7*). Obviously, there are still major gaps in our knowledge, but this is also true with respect to our understanding of mechanisms of mutagenesis and DNA repair, particularly in mammalian cells. Furthermore, there is growing evidence that DNA-damaging agents (at noncytotoxic doses), tumor promoters, and growth factors can induce somewhat similar patterns of gene expression (*8*), which may be relevant to their combined effects.

I agree with the suggestion (*2*) that certain DNA-damaging agents might produce a high tumor yield because they induce both mutations and cell replication (or tumor promoter–like effects). Several years ago we provided evidence that genotoxic carcinogens can mimic some of the effects of the phorbol ester tumor promoters (*9*). This does not, however, provide assurance that such agents are hazardous only at high doses, since at low doses they could still act as initiators in tissues in which cell proliferation might not be rate-limiting (for example, the fetus or the adult bone marrow), in individuals who have increased levels of endogenous growth-promoting agents (such as hormones or growth factors), or in individuals who are also exposed to exogenous agents that stimulate cell proliferation. In addition, it is difficult in the absence of further information to predict the sensitivity of humans to the tumor-promoting, mitogenic, or cytotoxic effects of a novel compound. Thus, risk extrapolation under conditions in which individuals are exposed to multiple factors (which is the real world), and in heterogeneous populations, is much more complicated than envisioned by Ames and Gold.

Cell replication and mutagenesis. The theory that mitogenesis is the major rate-limiting factor in carcinogenesis requires that cell replication per se be highly hazardous because of the inherent danger of spontaneous mutations (*2*). However, extensive cell proliferation driven by normal endogenous agents is usually not carcinogenic. For example, extensive proliferation occurs during normal fetal and embryonic development, as well as in the continuous renewal in the adult of the entire skin, gastrointestinal epithelium, and bone marrow. Yet, skin cancer (in the absence of solar radiation), cancer of the small intestine, and hematopoetic neoplasms are relatively rare in the U.S. population, when compared to the incidence of breast, prostate, or colon cancer. With respect to breast cancer, it has been emphasized that excess estrogen could lead to increased proliferation of the mammary epithelium (*10*). Even under such conditions, however, the total mass of proliferating epithelial cells would constitute a small fraction of the total mass of proliferating cells normally present in the skin, small intestine, or bone marrow. Thus excessive cell proliferation per se is probably not the exclusive causative factor in human breast cancer.

During evolution, long-lived multicellular organisms must have developed defense mechanisms to protect them against the carcinogenic and other deleterious effects of spontaneous mutations. Otherwise all of us would be one large tumor mass rather than 5- to 6-feet-tall adults made up of over 10^{13} cells, many of which continue to replicate each day. I recognize, of course, that replication coupled to terminal differentiation is a protective mechanism. Protagonists of the theory that cell replication leads to cancer do not deal with this aspect or explain how this barrier might be broken during tumor development. I believe that this is one of the roles (but not the only role) of carcinogenic agents.

Natural versus synthetic carcinogenesis. Ames and Gold (*2*) emphasize that the human diet contains high levels of numerous naturally occurring toxins, and conclude that synthetic pesticides add only a trivial risk to this existing burden. Rodent diets are also loaded with many of the same naturally occurring toxins, even though the diets of mice and rats do not contain some of the exotic and rarely used spices mentioned by Ames and Gold. Thus, a commonly used rodent pellet diet contains corn, wheat, soybean, alfalfa, and milk, among other ingredients (*11*). Nevertheless, several

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compounds such as aflatoxin, TCDD, and dibromochloropropane (DBCP) added to the diet of mice or rats markedly increase tumor incidence, even when they are tested at very low levels. It is apparent, therefore, that in several cases the host is more sensitive to certain synthetic compounds than to the background level of natural pesticides, in terms of cancer risk. I see no reason to assume otherwise with respect to humans, unless there is specific evidence to the contrary for the compound in question. Furthermore, Ames and Gold admit that despite the vast number and prevalence of naturally occurring toxins there is little evidence, with the exception of aflatoxin, that they pose major carcinogenic risks to humans. They state, "Indeed a diet rich in fruit and vegetables is, if anything, associated with low cancer rates" (*2*). Various mechanisms might be invoked to explain this apparent discrepancy (such as natural selection or anticarcinogens in our diet), but the true reason is not known.

Spontaneous mutations. Ames and Gold (*2*) suggest that there is a high frequency of "spontaneous" or "background" DNA damage and repair in normal mammalian cells, on the basis of estimates of the frequency of depurination and oxidized bases in DNA, which appear to be as high as 1 in 10^4 nucleotides. I would emphasize, however, that this high level of spontaneous DNA damage is not usually associated with a high rate of mutation or carcinogenesis. However, we know that the production of much lower levels of DNA adducts ($\sim 1/10^5$ to $1/10^6$ nucleotides) by noncytotoxic doses of certain chemicals (benzo[*a*]pyrene and aromatic amines) is highly mutagenic and carcinogenic (*5*). I must conclude, therefore, that either the estimates of background DNA damage are too high or that the former types of DNA lesions do not have the same deleterious biologic effects as those produced by certain exogenous carcinogens (because of differences in DNA repair or disruption of normal base pairing, for example). We cannot conclude, therefore, that endogenous damage to DNA is equivalent to exogenous damage with respect to cancer risk. Moreover, I know of no direct evidence that the former type of DNA damage is actually carcinogenic.

Validity of rodent bioassays. The article by Ames and Gold (*2*), and a supporting editorial in *Science* by Abelson (*12*), imply that the standard rodent bioassays for carcinogens are highly misleading with respect to the human situation. They do not, however, provide direct evidence of such discrepancies. In fact, there is considerable evidence to the contrary. Thus, when adequately tested, virtually all of the specific chemicals known to be carcinogenic in humans are also positive in the rodent bioassays, and sometimes even at comparable doses and with similar organ specificity (*13*). Furthermore, the rodent bioassays have frequently revealed carcinogens that were subsequently found to cause cancer in humans (*13*). It is true that there are also a large number of chemicals that are carcinogenic in rodents that are not known to cause cancer in humans, but most of these have not been adequately evaluated in humans, because of their recent discovery or the relative insensitivity of epidemiologic studies. Recent epidemiologic studies (*13*) indicate that some of these compounds, including some major synthetic pesticides, may also be carcinogenic to humans (*13*).

Ames and Gold fault the rodent bioassays mainly because they believe that the positive results obtained are due to the use of excessive doses that exert cytoxic effects (*2*). Others, however, have emphasized that more than 90% of the carcinogenic effects seen in rodent studies conducted by the National Toxicology Program were also observed in the low dose groups (*13, 14*). Furthermore, contrary to the statement by Ames and Gold, carcinogenic effects in rodents are often not accompanied by obvious target organ toxicity (*14*).

Of course, no single laboratory assay will reliably predict the carcinogenic effects of a given compound in humans or its relative potency, in view of the complexity of the carcinogenic process and possible interspecies variations. Each case must be considered with respect to the data that are available from various sources. This is the standard practice now used by the major U.S. and international agencies that are charged with this responsibility (*13*). If rodent bioassays were to be discarded, what assay (or assays) could we use to evaluate the potential health hazards of a novel compound? It is ironic that Ames himself has made extensive use of the rodent bioassay data to develop a set of indices (called "HERP") of the relative carcinogenic hazards of compounds to humans (*2*). If the current rodent bioassay data are inherently flawed, how can the HERP indices be used for relative risk extrapolations with respect to natural versus synthetic pesticides or other compounds?

Future directions. Fortunately, Ames and Gold (*2*) conclude their article by emphasizing the need for more mechanistic studies, in view of major gaps in our knowledge of the process of cancer causation and the need to develop more mechanism-based methods for detecting potential human carcinogens. I and many other colleagues in carcinogenesis research heartily agree and are working toward these goals (*5*). I would hope, therefore, that until such knowledge and new methods are available, public policy in this vital area of human health will not be influenced by ad hoc assumptions and an oversimplication of the carcinogenic process.

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