Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis

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A CLARIFICATION OF THE MECHANISM OF CARCINOGENESIS is developing at a rapid rate. This new understanding undermines many assumptions of current regulatory policy toward rodent carcinogens and necessitates rethinking the utility and meaning of routine animal cancer tests. At a recent watershed meeting on carcinogenesis, much evidence was presented suggesting that mitogenesis (induced cell division) plays a dominant role in carcinogenesis (*1*). The work of Cohen and Ellwein in this issue (*2*) is illustrative. Our own rethinking of mechanism was prompted by our findings that: (i) spontaneous DNA damage caused by endogenous oxidants is remarkably frequent (*3*) and (ii) in chronic testing at the maximum tolerated dose (MTD), more than half of all chemicals tested (both natural and synthetic) are carcinogens in rodents, and a high percentage of these carcinogens are not mutagens (*4*).

Mitogenesis increases mutagenesis. Many "promoters" of carcinogenesis have been described and have been thought to increase mitogenesis or selective growth of preneoplastic cells, or both. The concept of promotion, however, has been fuzzy compared to the clearer understanding of the role of mutagenesis in carcinogenesis. The idea that mitogenesis increases mutagenesis helps to explain promotion and other aspects of carcinogenesis (*2, 5*).

A dividing cell is much more at risk of mutating than a quiescent cell (*4*). Mutagens are often thought to be only exogenous agents, but endogenous mutagens cause massive DNA damage (by formation of oxidative and other adducts) that can be converted to stable mutations during cell division. We estimate that the DNA hits per cell per day from endogenous oxidants are normally $\sim 10^5$ in the rat and $\sim 10^4$ in the human (*3*). This promutagenic damage is effectively but not perfectly repaired; for example, the normal steady-state level of 8-hydroxydeoxyguanosine (1 of about 20 known oxidative DNA adducts) in rat DNA has been measured as 1 per 130,000 bases, or about ~~47,000~~ per cell (*3*). We have argued that this oxidative DNA damage is a major contributor to aging and to 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 converting endogenous DNA damage into mutations. Nongenotoxic agents [for example, saccharin (*2*)] can be carcinogens at high doses just by causing chronic mitogenesis and inflammation, and the dose response would be expected to show a threshold. Genotoxic chemicals [for example, *N*-2-fluorenylacetamide (2-AAF) (*2*)] are even more effective than nongenotoxic chemicals at causing mitogenesis at high doses (as a result of cell killing and cell replacement). Since genotoxic chemicals also act as mutagens, they can produce a multiplicative interaction not found at low doses, leading to an upward curving dose response for carcinogenicity. Furthermore, endogenous rates of DNA damage are so high that it may be difficult for exogenous mutagens to increase them significantly at low doses that do not increase mitogenesis. Therefore, mitogenesis, which can be increased by high doses of chemicals, is indirectly mutagenic, and seems to explain much of carcinogenesis (*1, 4, 5*). Nevertheless, the potent mutagen 2-AAF (*3*) induces liver tumors at moderate doses in the presence of only background rates of mitogenesis. Detailed studies of mechanism, particularly in the case of apparent exceptions, are critically important.

Causes of human cancer. Henderson and co-workers (*6*), and others (*4*), have discussed the importance of chronic mitogenesis for many, if not most, of the known causes of human cancer, for example, the importance of hormones in breast cancer, hepatitis B (*7*) or C viruses or alcohol in liver cancer, high salt or *Helicobacter* (*Campylobacter*) infection in stomach cancer, papilloma virus in cervical cancer, asbestos or tobacco smoke in lung cancer, and excess animal fat and low calcium in colon cancer. For chemical carcinogens associated with occupational cancer, worker exposure has been primarily at high, near-toxic doses that might be expected to induce mitogenesis.

Epidemiologists are frequently discovering clues about the causes of human cancer, and their hypotheses are then refined by animal and metabolic studies. During the next decade, it appears likely that this approach will lead to an understanding of the causes of the major human cancers (*8*). Cancer clusters in small areas are expected to be common by chance alone, and epidemiology lacks the power to establish causality in these cases (*9*). It is important to show that pollution exposure that purportedly causes a cancer cluster is significantly higher than the background of exposures to naturally occurring rodent carcinogens (*4*).

Causes of cancer in animal tests. Animal cancer tests are conducted at near toxic doses (the maximum tolerated dose, MTD) of the test chemical, for long periods of time, which can cause chronic mitogenesis (*1*). Chronic dosing at the MTD can be thought of as a chronic wounding, which is known to be both a promoter of carcinogenesis in animals and a risk factor for cancer in humans. Thus, a high percentage of all chemicals might be expected to be carcinogenic at chronic, near toxic doses and this is exactly what is found. About half of all chemicals tested chronically at the MTD are carcinogens (*4*).

Synthetic chemicals account for 82% (350/427) of the chemicals adequately tested in both rats and mice (*4*). Despite the fact that humans eat vastly more natural than synthetic chemicals, the world of natural chemicals has never been tested systematically. Of the natural chemicals tested, approximately half (37/77) are carcinogens, which is approximately the same as has been found for synthetic chemicals (212/350). It is unlikely that the high proportion of carcinogens in rodent studies is due simply to selection of suspicious chemical structures; most chemicals were selected because of their use as industrial compounds, pesticides, drugs, or food additives.

One major group of natural chemicals in the human diet are the chemicals that plants produce to defend themselves, the natural pesticides (4). We calculate that 99.99% (by weight) of the pesticides in our diet are natural. Few natural pesticides have been tested in at least one rodent species, and again about half

Erratum: This paragraph has been corrected back to our original from the garbled version printed.

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Aerratum: 90,000

(27/52) are rodent carcinogens. These 27 occur commonly in plant foods (10). The human diet contains thousands of natural pesticides and we estimate that the average intake is about 1500 mg per person per day (4). This compares to a total of 0.09 mg per person per day of residues of about 100 synthetic pesticides (4). In addition, of the mold toxins tested at the MTD (including aflatoxin) 11 out of 16 are rodent carcinogens.

The cooking of food produces thousands of pyrolysis products, and we estimate that dietary intake of these products is roughly 2000 mg per person per day. Few of these have been tested; for example, of 826 volatile chemicals that have been identified in roasted coffee, only 21 have been tested chronically, and 16 are rodent carcinogens; caffeic aid, a non-volatile carcinogen, is also present. A cup of coffee contains at least 10 mg (40 ppm) of rodent carcinogens (mostly caffeic acid, catechol, furfural, hydrogen peroxide, and hydroquinone) (*4*). Thus, very low exposures to pesticide residues or other synthetic chemicals should be compared to the enormous background of natural substances.

In the evolutionary war between plants and animals, animals have developed layers of general defenses, almost all inducible, against toxic chemicals (*4*). This means that humans are well buffered against toxicity at low doses from both man-made and natural chemicals. Given the high proportion of carcinogens among those natural chemicals tested, human exposure to rodent carcinogens is far more common than generally thought; however, at the low doses of most human exposures (where cell-killing and mitogenesis do not occur), the hazards may be much lower than is commonly assumed and often will be zero (*4*). Thus, without studies of the mechanism of carcinogenesis, the fact that a chemical is a carcinogen at the MTD in rodents provides no information about low-dose risk to humans.

Trade-offs. Pesticide residues (or water pollution) must be put in the context of the enormous background of natural substances, and there is no convincing evidence from either epidemiology or toxicology that they are of interest as causes of human cancer (*4, 9*). Minimizing pollution is a separate issue, and is clearly desirable for reasons other than effects on public health. Efforts to regulate synthetic pesticides or other synthetic chemicals at the parts per billion level because these chemicals are rodent carcinogens must include an understanding of the economic and health-related trade-offs. For example, synthetic pesticides have markedly lowered the cost of food from plant sources, thus encouraging increased consumption. Increased consumption of fruits and vegetables, along with decreased consumption of fat, may be the best way to lower risks of cancer and heart disease, other than giving up smoking. Also, some of the vitamins, antioxidants, and fiber found in many plant foods are anticarcinogenic.

The control of the major cancer risks that have been reliably identified should be a major focus, and attention should not be diverted from these major causes by a succession of highly publicized scares about low levels of synthetic chemicals that may be of little or no importance as causes of human disease. Moreover, we must increase research to identify more major cancer risks, and to better understand 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 carcinogenesis mechanisms and into the complex natural world in which we live.

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Carcinogens and Human Health: Part 1

Bruce N. Ames and Lois Swirsky Gold (Perspective, 31 Aug., p. 970) posit that most human exposure to synthetic chemicals that are rodent carcinogens poses little or no risk of cancer. They argue that the high doses used in rodent bioassays cause tumors largely by inducing cytotoxicity with resultant compensatory cell proliferation (mitogenesis) that converts DNA damage (mostly caused by endogenous compounds in food) into mutations. In their view, mitogenesis dominates the carcinogenic process with the result that thresholds exist for "nongenotoxic" rodent carcinogens, and the dose-response curve for genotoxic carcinogens is sublinear. They conclude that the current U.S. regulatory policy, which calls for controlling involuntary exposures to industrial chemicals and pesticides identified as carcinogenic in the laboratory, imposes unnecessary costs on society and conveys no benefit in terms of health protection. Thus they dismiss the potential risks from the more than 1 billion pounds of pesticides and related products produced annually in the United States and the estimated 22.5 billion pounds of toxic chemicals released or disposed of each year in this country (*1*).

These arguments are not new (*2*). Thus far, however, lengthy deliberations by U.S. and international health protection agencies, scientific advisory boards, and panels of experts have rejected proposals to relax standards for carcinogens and have supported the use of animal tests as predictors of effects in humans (*3*). U.S. agencies involved in risk assessment policy have adopted the general assumption of low-dose linearity for carcinogens—regardless of their presumed mechanism of action.

The rationale for these decisions is threefold: (i) the lack of adequate understanding of mechanisms by which carcinogens (especially those termed "nongenotoxic") exert their effect; (ii) the absence of an identifiable threshold or safe level of exposure for a diverse human population; and (iii) the desirability of preventing cancer through the use of testing in model systems, obviating the reliance on epidemiologic data in humans. This rationale remains valid in light of current knowledge.

First, a large body of data on chemical carcinogenesis and the molecular biology of cancer supports a far more intricate mechanistic explanation of tumor induction by both nongenotoxic and genotoxic carcinogens than one which is dominated by mitogenesis. Available rodent bioassay data do not show a consistent correlation between organ toxicity at the target site and carcinogenicity (*4*). Moreover, there are few cases of rodent carcinogens that are positive only at the high dose (*4–5*). In addition, not only does epidemiology fail to show a threshold at the lower bound of exposure to carcinogens in the workplace, but low-level community exposures to "occupational carcinogens" such as arsenic have resulted in increased incidence of cancer (*6*).

On another level, the multistage process of cancer development is known to involve both mutagenic and nonmutagenic mechanisms. These result in the induction of multiple direct and indirect genetic changes at target oncogenes or tumor suppressor genes as well as alterations in signal transduction pathways involved in growth control (*7*). There is no evidence that these molecular events occur only at high, toxic doses (*8*). Despite recent exciting advances in the molecular biology of cancer, many uncertainties remain.

In light of the uncertainty about mechanisms and human dose-response, the assumption of low-dose linearity for carcinogens continues to be a reasonable one (*9*). It is consistent with the fact that humans are exposed to multiple carcinogens, capable of additive and even multiplicative effects. It is also a prudent assumption given the striking interindividual variation in the biologic response to carcinogens. Recent studies show an impressive range of human response to xenobiotics in terms of the activation and detoxification of carcinogens, covalent binding to DNA, and DNA repair (*10*). Such findings argue against the concept of a single population threshold for a carcinogen.

The large and growing burden of cancer in the United States (now at 500,000 cancer deaths per year) vividly demonstrates the need for prevention. Prevention has always been the guiding principle in toxicology and public health policy and now merits increasing emphasis (*11*). Prevention means not only addressing those cancer risks already established as "major" contributors to the disease burden (such as smoking) and researching new potential "major" risks, as Ames and Gold suggest, but also reducing current involuntary exposures to identified industrial carcinogens (*12*). Indeed, on the basis of a highly simplified (called "HERP") system for ranking carcinogens developed by Ames and Gold, the estimated range of risk for "natural" and man-made carcinogens is comparable (13). While it is tempting to simplify the regulatory process for carcinogens, one can do so only by ignoring the complex biology and etiology of the disease itself.

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Response: Perera neither summarizes our views accurately nor acknowledges the evidence that contradicts the assumptions of the "toxic chemicals from industrial pollution" view of cancer causation and worst-case, low-dose risk assessment models (*1–3*).

Perera's statements about what we think is causing the enormous endogenous DNA-damage rate from oxidants are incorrect; it is from normal metabolism (*4*), not from "endogenous compounds in foods." This oxidative damage helps explain the epidemiological findings that lack of sufficient dietary antioxidants from fruits and vegetables appears to be a major contributor to various types of cancer, heart disease, cataracts, and other degenerative diseases that come with aging (*5*). Oxidants are also produced in large amounts during inflammation, and oxygen radicals are a stimulus for cell proliferation, that is, the wound-healing response. Antioxidants protect against all of these effects. We think that dietary imbalances such as folate (*6*) and antioxidant deficiencies are major contributors to human cancer.

The natural world makes up the vast bulk of chemicals that humans consume each day in both weight and number: ~1500 milligrams of natural plant pesticides and ~2000 milligrams of chemicals from cooking food, compared with 0.09 milligram of synthetic pesticide residues and a smaller amount of water pollutants (*2*). We have discussed why the toxicology of synthetic and natural chemicals is not fundamentally different (*3*). All chemicals are "toxic chemicals" at some dose, not just by-products of industry.

About half the *natural* chemicals tested chronically in rats and mice at the maximum tolerated dose (MTD) are carcinogens (*1, 3*). These tests on natural chemicals are the control for the high-dose toxicology in which a high percentage of all chemicals test positive (*3*). Since similar percentages of natural and synthetic chemicals are positive, one cannot just assume that every industrial pollutant is a potential time bomb, while every natural chemical is likely to be harmless. In roasted coffee, among 22 chemicals tested, 16 were rodent carcinogens. Thus one cup of coffee contains 10 milligrams of known rodent carcinogens, about equivalent in weight to the potentially carcinogenic synthetic pesticide residues one eats in a year (assuming half of the untested synthetic residue-weight will be carcinogenic in rodents) (*1*). There is every reason to expect that the thousand other chemicals in roasted coffee would produce a plethora of rodent carcinogens if tested at the MTD. This is also likely to be true of the many thousands of natural pesticides in plant foods (27/52 natural pesticides tested are rodent carcinogens) (*2*). Possible carcinogenic hazards from the few natural chemicals tested often rank much higher than those from pesticide residues or water pollution (*2, 7*). Thus there is no theoretical reason or convincing epidemiological evidence (*8*) that pesticide residues or water pollution are significant causes of cancer. Chemicals, whether natural or synthetic, are unlikely to be of importance at levels tens of thousands of times below the MTD. Occupational exposures, in contrast, can be very high and significant (*9*).

Citation of the paper by D. G. Hoel *et al.* (*10*) as evidence against a role for mitogenesis in carcinogenesis ignores the fact that cell proliferation was not measured. Moreover, assessing which lesions may actually represent a toxic response is largely subjective, particularly from routine histopathology done only at the end of an experiment. Another critical complexity is that mutation through mitogenesis is not of interest in cells that are discarded (for example, from epithelial tissues) or killed (from apoptosis). It is not toxicity that is important, but chronic mitogenesis in nondifferentiated cells that are not discarded; also, mitogenesis can occur without toxicity (*1*).

Perera indicates that the assumption of low-dose linearity for carcinogenesis is reasonable: we present numerous findings to the contrary (*1*). It is well documented by geneticists that cell division is an important factor in mutagenesis and can be of dominant importance for loss of heterozygosity through mitotic recombination or nondisjunction (*1*). Understanding the role of mitogenesis in mutagenesis and that of increased mitogenesis in tests at high doses helps one understand the upward-curving dose responses with diethylnitrosamine, formaldehyde, 2-acetylaminofluorene, and saccharin (*1, 11*). Such an understanding can also explain the result that half of the chemicals tested at the MTD are carcinogens and that about 40% of these are apparently not genotoxic (*1*). Several recent findings indicate an important role for mitogenesis. These include the experiments of M. L. Cunningham *et al.* (*12*) that compare carcinogenic and noncarcinogenic isomers of mutagenic compounds and show that mitogenesis is increased only in the carcinogenic isomer; the study of H. A. Dunsford *et al.* (*13*) of transgenic mice that overproduce one protein of the hepatitis B virus increases that cell turnover: all the mice develop hepatocellular carcinomas; the finding that caloric restriction in rodents lowers both rates of mitogenesis and spontaneous tumor rates (*14*); the role of mitogenesis in several types of human cancer; and more (*1*). DNA adducts are not the same as mutations, and a linear dose-response for adducts will not be a linear dose-response for mutagenesis or carcinogenesis when mitogenic effects are nonlinear. Carcinogenesis models that include the effects of mitogenesis make more biological sense than those that do not (*15*).

Perera discusses low levels of chemicals causing cancer, but chemicals are rarely tested at doses below the MTD and half the MTD. Moreover, about half of the positive sites in animal cancer tests are not statistically significant at half the MTD. With only two doses and a control in cancer tests, information about dose response shape is limited. Even at these high doses, however, a quadratic dose response is compatible with more of the data than a linear one for both mutagens and nonmutagens, and a plateau in the dose response (which could indicate a super carcinogen) is uncommon (*16, 17*).

Perera's evidence that a "low level of community exposure to 'occupational carcinogens' . . . resulted in increased incidence of cancer" is from a paper (*18*) whose authors examine "residence in areas with heavy levels of arsenic and cadmium." The study did not measure personal exposure, but levels in the soil; and after adjustment for smoking and occupation there was no statistically significant relative risk of lung cancer. In comparison, natural arsenic in U.S. water supplies may be the most important potential carcinogen in tap water (*19*). Both natural arsenic in water and natural radon in indoor air are present at high levels at some locations, and major efforts were put into miniscule amounts of industrial pollutants were long neglected, while.

We agree with Perera that cancer prevention is important, but we would put more effort into studying carcinogenesis mechanisms and dietary imbalances and into encouraging the public to eat more fruits and vegetables and less animal fat.

Perera suggests that current policy attempting to protect the public at 10^{-6} hypothetical, worst case risk (~380,000 times below the MTD of a rodent carcinogen) (*20*) from industrial pollution, while ignoring the natural world is prudent, whatever the cost. We believe this is neither scientifically sound nor useful; it confuses regulators and the public as to what is important and diverts resources from more important risks and is therefore counterproductive (*21*). Pollution control is desirable (*22*), but can-

cer risk estimates at miniscule doses should not be a surrogate for the environment.

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Carcinogens and Human Health: Part 2

Bruce N. Ames and Lois Swirsky Gold (Perspective, 31 Aug., p. 970) and Philip H. Abelson (Editorial, 21 Sept., p. 1357) question the use and validity of long-term laboratory animal studies to identify potentially hazardous compounds. These laboratory experiments, like other biological assays, are not perfect; yet in the absence of adequate epidemiologic data they are the best available methods for identifying and assessing potential human health risks (*1*).

All of the chemicals known to cause cancer in humans also cause cancer in laboratory animals (*2*). Eight of these chemicals were first shown to induce cancer in laboratory animals (*3*); subsequently, epidemiologic investigations showed that they also induced cancer in humans (*2, 4*). A more recent example, 1,3-butadiene, widely used in the rubber industry, was reported in 1983 to cause multiple cancers in mice at concentrations permitted in the workplace (*5*). Further studies showed that 1,3-butadiene caused cancers at low inhalation exposures; epidemiological studies now suggest that 1,3-butadiene is carcinogenic to humans (*6*).

There are two major sources of information from which to estimate the potential adverse effects of such substances on public health: controlled laboratory studies with experimental animals and in vitro systems; and human epidemiology studies, often based on workplace exposures or inferred from less easily controlled studies of the general population (7). Gathering data about humans can confirm past hazards but, unfortunately, this information comes too late to prevent the continuation of disease and is costly and difficult to obtain (*8*).

Accordingly, the United States, the Organization for Economic Cooperation and Development (OECD), the World Health Organization's International Program on Chemical Safety, the International Agency for Research on Cancer (IARC), the Japanese National Institute of Hygienic Sciences, and various industrial laboratories are conducting studies in animals to estimate the risks to humans from chemicals, as well as to provide the basis for risk management and the reduction of potential human health hazards.

The National Cancer Institute (NCI) and, subsequently, the National Toxicology Program have evaluated approximately 400 chemicals for their toxic and carcinogenic effects in laboratory animals (*9*). These toxicology studies are typically carried out in both sexes of two species of rodents divided randomly into sets of 50 to 60 animals per exposure and control groups; three exposure concentrations are gradated down from a top level selected to show some toxicity that should not compromise unduly the animal's well-being or growth and survival. The criteria for selecting substances for comprehensive evaluation have evolved over time. Initially, applied research focused on suspected carcinogens; more recently, chemicals produced in great volumes and to which many people are exposed have been added to the list.

The magnitude of the task of evaluating these chemicals is enormous. In 1990, the Chemical Abstracts Services catalogued the 10 millionth unique chemical in their computer collection. In 1984, the National Research Council of the National Academy of Sciences estimated that information about the potential effects on humans exists for only 20% of the thousands of common chemicals (*10*). The staff of the House Agriculture Subcommittee on Department Operations, Research, and Foreign Agriculture compared information available to the Environmental Protection Agency (EPA) with the data required by law for 1200 active pesticide ingredients and found that for 79 to 84% of registered and commercially used active ingredients oncogenicity studies were inadequate and for 90 to 93% mutagenicity studies were inadequate (*11*). In 1990 the OECD reported that about half of the highest production chemicals had not been subjected to adequate toxicologic evaluation (*12*).

At present, 54 chemicals, mixtures of chemicals, or occupational exposures are considered carcinogenic to humans (*2, 3*). Of the 34 identifiable chemicals or mixtures of chemicals that have been shown to cause cancer in humans, 31 also induce cancer in animals. The remaining three have not been adequately studied in experimental models.

NCI studies have implicated certain pesticides in human cancers (*13*), and epidemiologist R. Doll has stated that the eventual number of occupational carcinogens could be quite large (*14*).

Since one object of laboratory animal experiments is to study the toxic effects of a compound, the effect must be elicited for the experiments to have value: the amount of chemical administered must produce a response. The exposure level used in long-term carcinogenesis experiments, often called the maximum tolerated dose (MTD) (*15*), has been selected to produce some mild toxic effects but not to alter normal growth and development. Unless cancer is the lethal end point, life-spans also must be equivalent to controls.

A range of doses is used to compensate for the relatively small number of animals, generally 50 to 100, in a test group (*1, 15, 16*). The maximum doses are sometimes relatively high, but rarely massive. In many cases (for example, 1,3-butadiene, benzene, phenacetin, vinyl chloride, methylene chloride, and ethylene dibromide) studies were conducted at exposure levels near or below that to which humans are actually exposed.

One of the myths surrounding the animal bioassay is that using the MTD can result in unique carcinogenic effects that are not present at lower exposure concentrations. Chemicals that are carcinogenic only at the maximum dose studied are historically rare. In approximately 90% of the compounds studied, supporting evidence for carcinogenicity at the same target site is seen at lower doses.

Regarding toxicity and carcinogenicity, D. G. Hoel *et al.* (*17*) reported that in 73 of 127 positive sex-species-specific experiments carried out by the National Toxicology Program (NTP), there was a statistically significant increase in tumor incidences in both the low- and high-dose groups, and in another 42 there was a numerically elevated carcinogenic response in the low-dose group relative to that in controls. Only 3% of the chemicals were considered to be possibly "high-dose only" carcinogens. For example, when 1,3-butadiene was studied in mice at inhalation exposure levels of 625 and 1250 parts per million (ppm), it was found to be carcinogenic (*5*). It was still carcinogenic at concentrations as low as 6.25 ppm (*18*), an otherwise nontoxic level. Thus, to separate chemicals into different categories of mechanism of action for risk assessment purposes on the basis of "high-dose only" results is premature.

Further, the systemic toxic effects (such as hepatotoxicity) of chemicals, are often not correlated with the site(s) of carcinogenic action (*17*). For example, Monuron, a pesticide, produced liver degeneration and hepatocytomegaly in male mice (toxicity with cell death and no cancer), but no increase in liver tumors. Asbestos causes mesothelioma without evidence of asbestosis. This supports the view that cancer is not merely a consequence of toxicity (*19*).

The study period for carcinogenesis bioassays is typically 2 years, not the lifetime of the animal model, which is closer to 3 to 4 years. In other words, the cancers that appear are those of late middle age, not of the very old. This may be important when we consider that the age-adjusted incidences of human cancers are rising (*20*), about 1% in each of the last 15 years, even as the human

lifespan in the United States is increasing, which makes early identification of hazardous substances more urgent (*21*).

An issue that is widely discussed concerns the evidence necessary to decide that a chemical is carcinogenic in rodents. In a large series of statistical comparisons, some apparently significant differences between chemically exposed and control groups will occur by chance (*16*). The NTP estimates that the false positive rate associated with NTP rodent studies is at most only 7 to 8% (*22*). The false negative rate (those chemicals that exhibit no carcinogenicity during the period of the study but that would eventually be shown to be carcinogenic) is much more difficult to evaluate. Further, each sex of each species is considered a separate experiment and is reported separately by the NTP. This permits others, such as the EPA or the IARC, to evaluate and index independently carcinogenicity.

Studies of cell division and reduction-oxidation reactions as mechanisms in the development of cancer are contributing to our understanding of the carcinogenic processes and are the subject of intense scrutiny in laboratories around the world. However, metabolic effects that occur in living systems and the interaction of many genetic factors appear to be crucial in these processes as well (*23*).

We are, indeed, developing a "new toxicology" based on well-conducted rodent studies supplemented by relevant information on pharmacokinetics, and mechanistic studies involving oncogene activation or suppressor gene inactivation. Scientists, including those at the National Institute of Environmental Health Sciences and elsewhere, have identified what appears to be the same activated *Ki-ras* oncogene in mouse and human lung tumors (*24*). The *H-ras* gene has been shown to have the same amino acid sequence in both humans and rodents (*25*). Similarly, tumor suppressor genes have also been identified across species (*26*).

Recently, it has been said that naturally occurring substances have not been adequately tested in NTP protocols. In fact, 25 to 30% of the chemicals evaluated so far in the NTP program, such as benzene, asbestos, and formaldehyde, occur naturally. However, few of the many substances that occur in plants have been tested under current protocols. Moreover, humans are exposed to mixtures of these compounds that include natural anticarcinogens, antioxidants, and fiber (*27*). The NTP welcomes nominations of such compounds for toxicologic evaluation as well as suggestions for innovative test methodology.

Recent research has documented increases in cancer mortality in industrial countries over the past two decades, increases not linked to cigarette smoking, aging, or improved diagnoses. All forms of cancer except lung and stomach cancer increased from 1968 to 1987, mainly in persons over age 55 (*21, 28*). Cancer is a complex of more than 200 diseases with multiple causes, multiple stages, and long latencies. In sifting through probable causes of these cancer patterns in industrialized countries, the role of a number of variables must be carefully assessed, including those linked to industrial chemicals, altered food supply, and lifestyle practices. Given the complexity of these multiple concerns, toxicology studies that use animals as surrogates for humans shall continue to play a major role in resolving these puzzles for cancer and for a host of other diseases (*8, 28*).

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Response: Rall is defending the National Toxicology Program (NTP) Carcinogen Bioassay Program that he directed for many years. Our papers do not argue to discontinue the bioassay program, but rather point out that we know more now than we did when the program was started, that certain serious difficulties should be addressed, and that results from bioassays are being used inappropriately.

Much of Rall's letter discusses occupational exposures to chemicals, which can sometimes be at very high doses. One purpose of the bioassay program has been to test industrial chemicals that workers have been exposed to at high levels. We agree with Rall that it is important to identify chemical carcinogens in the workplace. We have discussed in our own work that permitted worker exposure levels (PEL) for some rodent carcinogens are too close to the doses that induce tumors in test animals (*1*). For high occupational exposures little extrapolation is required from the doses used in rodent bioassays, and therefore assumptions about extrapolation are less important. This contrasts with the large extrapolations from the low doses of human exposures from pesticide residues or water pollution. While more occupational chemical carcinogens are likely to be detected, it seems unlikely that they will contribute to more than a few percent of all human cancer.

To extrapolate from levels at the maximum tolerated dose (MTD) or one-half the MTD, where almost all cancer tests are done, to the low exposure levels for the general population, however, requires information on mechanism such as those S. M. Cohen and L. B. Ellwein have used in their analysis (*2*). The attempt to prevent cancer by regulating low levels of synthetic chemicals by "risk assessment" with the use of worst-case, 1-in-a-million maximum risk scenarios is not scientifically justified. On average, 1-in-a-million maximum risk from a linearized model is 380,000 times below the MTD used in rodent bioassays (*3*). It seems to us unlikely that ingestion of any chemical at that level is of interest. Testing chemicals for carcinogenicity at near-toxic doses in rodents does not provide enough information to predict the excess numbers of human cancers that might occur at low-dose exposures. In addition this cancer prevention strategy is enormously costly and could be counterproductive if it diverts resources from more important risks.

The current regulatory process does not take into account (*4*) (i) that the natural world of chemicals makes up the vast bulk of chemicals humans are exposed to; (ii) that the toxicology of synthetic and natural toxins is not fundamentally different; (iii) that about half of the *natural* chemicals tested chronically in rats and mice at the MTD are carcinogens; and (iv) that testing at the MTD can frequently cause chronic cell killing and consequent cell replacement (a risk factor for cancer that can be limited to high doses) and that ignoring this mitogenesis effect greatly exaggerates many low-dose risks.

Positive results are remarkably common in high-dose screening tests for carcinogens, clastogens (agents that break chromosomes), teratogens, and mutagens (*4*). About half the chemicals tested, whether natural or synthetic, are carcinogens in chronic, high-dose rodent tests. About half the chemicals tested as clastogens in tissue culture tests are positive. A high proportion of positives is also reported for rodent teratogenicity tests: 38% of the 2800 chemicals tested in laboratory animals "have been teratogenic" in the standard, high-dose protocol. It is therefore reasonable to assume that a sizable percentage of both synthetic and natural chemicals will be reproductive toxins at high doses. Mutagens may also be common: of 340 chemicals tested for carcinogenicity in both rats and mice and mutagenicity in *Salmonella*, 46% were mutagens and 70% were either mutagens or carcinogens or both. Mutagens were nearly twice as likely to be carcinogenic as nonmutagens. How much the high frequency of positive results is due to bias in selecting chemicals is not known. Even if selection bias doubled the percentage of positives, which we think is unlikely, the high proportion of positives would still mean that almost eveything natural we eat contains carcinogens, mutagens, teratogens, and clastogens. Thus, testing a random group of natural pesticides and pyrolysis products from cooking should be a high priority for these various tests so that an adequate comparison can be made to synthetic toxins. The NTP selection of chemicals to test has paid almost no attention to natural pesticides and pyrolysis products in our diet.

What chemicals should be tested in the bioassay, given that we are living in a sea of rodent carcinogens (as defined by high-dose tests), the vast proportion of which are likely to be natural? We need to take a broader view of the chemical world and try to identify the greatest potential carcinogenic hazards, whether natural or synthetic; only a tiny fraction of the chemicals humans are exposed to are ever going to be tested in rodent bioassays.

We have recently compared the possible hazards of some rodent carcinogens, using the ratios Human Exposure/Rodent Potency (HERP) and Permitted Exposure/Rodent Potency (PERP). One strategy for choosing chemicals to test is to prioritize chemicals according to how they might rank in terms of possible hazard *if* they were to be identified as rodent carcinogens. A useful first approximation is the analogous ratio of Human Exposure/Rodent Toxicity (HERT). HERT would use readily available LD_{50} values rather than the TD_{50} (carcinogenic potency) values used in HERP. LD_{50} is related to the MTD and the TD_{50} (*5*), and the ranking of human exposures on HERP and HERT will likely be similar. The number of people exposed is also relevant in attempting to prioritize systematically among chemicals. Chemicals with high HERT and population exposure could then be investigated in more detail as to mutagenicity, mitogenicity, pharmacokinetics, and so forth, as discussed by Cohen and Ellwein (*2*) and by Rall. Natural and synthetic chemicals could both be ranked, and if natural chemicals in foods such as chlorogenic acid in coffee, psoralens in celery, or indole carbinol in broccoli turned out to be important, they might be bred out or, for processed foods such as coffee, extracted.

There are alternative strategies to that of testing chemicals one by one that may lead to identifying more important risk factors for human cancer. If the NTP did a series of bioassays each with a particular vitamin or micronutrient deficiency in the rodent chow, we believe they could turn up a series of carcinogenic risks that are of major importance for people. In mice, a marginal folate deficiency is very effective at breaking chromosomes (*6*). More than 30% of the

U.S. population is marginally folate-deficient. There is also epidemiological evidence that folate deficiencies cause birth defects in humans. Accumulating epidemiological evidence indicates that vitamins E and C and betacarotene are major protective factors against both cancer and heart disease, yet a sizable percentage of the public is deficient in these antioxidants. Choline deficiency increases cancer rates in rats (7). In addition, calorie reduction dramatically lowers mitogenesis rates and spontaneous tumor rates in rodents. Protein reduction lowers spontaneous tumor rates in rats. Ad libitum feeding, which encourages overeating, is routinely done in bioassays; overeating increases spontaneous tumor rates, and a variation in food intake is important in tumor incidence (*8*). Human cancers can be due to a variety of factors, such as dietary imbalances, hormones, and chronic infections, that are not likely to be uncovered by screening chemicals in rodents, even if we knew which chemicals to test (*9*).

The NTP strategy to analyze mechanisms is a useful change. Increased mitogenesis rates are clearly important in mutagenesis, and we believe that also adding routine measurements of mitogenesis to the 13-week toxicology study and the 2-year bioassay would provide information that would improve dose setting, interpretation of experimental results, and risk assessment. Such information may help to distinguish among rodent carcinogens, for example, between butadiene and sodium saccharin, for which the risk at doses a hundred times below the MTD appears to be vastly different. The work of Cunningham *et al.* at the NTP is a good example of how mechanism studies help to differentiate among chemicals. Their experiments showed that with two pairs of mutagenic isomers (1- versus 2-nitropropane and 2.4- versus 2.6-diaminotoluene), one isomer a carcinogen and the other not, only the carcinogen was mitogenic (*10*). It may be that half the rodent carcinogens are not acting as genotoxins in vivo and that their risk at low doses is zero, but we should look for compounds like butadiene that may be carcinogens at doses as low as 100 times below the MTD (*4*). If there are super carcinogens (*5*), butadiene is a possible example. Butadiene and vinyl chloride are DNA cross-linking agents, and it would be of interest to see whether this property is important in unusual activity at low doses. Studies of mechanisms, including mitogenesis, should help to clarify this. It is clear that the mechanisms of action for all rodent carcinogens are not the same and that one cannot use a simple linearized risk assessment model for all of them.

Rall states that it is a "myth" that testing at the MTD can result in effects that are unique to the high dose and cites the analysis of Hoel *et al.* We think that it is not a myth and that there is accumulating evidence to support mitogenesis effects unique to high doses for particular chemicals analyzed, for example, formaldehyde, melamine, and saccharin. One-half the MTD (which is the "low" dose in a bioassay) is a high dose and can also result in mitogenesis. Our point is that rodent bioassays provide virtually no information about low doses because they are conducted at the MTD and one-half the MTD, both high and close to one another in comparison to low-dose human exposures. It is a rare chemical that is tested across a range of doses. With only two doses and a control in cancer tests, information about dose-response is limited. Even at these two high doses, 44% of the positive sites in NTP bioassays are statistically significant at the MTD, but not at one-half the MTD (among 365 positive sites analyzed in the Carcinogenic Potency Database). Because the NTP bioassays do not measure mitogenesis, Hoel *et al.* (*11*) used an indirect, but inadequate, method to examine the issue. We have discussed the details of this inadequacy (*4, 12*).

Rall cites a recent paper (*13*) that purports to show an overall increase in cancer mortality rates; however, eminent epidemiologists dispute the interpretation (*14*).

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Carcinogenesis Debate

In her News & Comment article discussing our papers on carcinogens (9 Nov., p. 743), Jean L. Marx says that our position is, "Below the toxic dose, carcinogenesis would not be a problem . . . because there would be no increased cell proliferation," that is, thresholds are the general case. That is not our view, as is clear from our papers. It is reasonable to assume that low levels of mutagens might add a small increment to our enormous endogenous level of DNA adducts coming from oxidant by-products of normal metabolism. However, the risk should be considerably lower than predicted by linear extrapolation from high dose tests because increases in mitogenesis can be unique to high doses and inducible general defense systems act as a buffer at low doses. The risk from nonmutagens at low doses may be zero (for example, in the case of saccharin). Our view, as can be seen in our papers, is not that mitogenesis is a single-factor explanation for carcinogenesis. Rather our view is that you cannot understand mutagenesis (and therefore carcinogenesis) without taking mitogenesis into account and that at high doses chronic mitogenesis can be the dominant factor. This is also the view of S. M. Cohen and L. B. Ellwein and is supported by their work (Articles, 31 Aug., p. 1007).

Numerous researchers have pointed out for years that chronic mitogenesis is important in carcinogenesis. Our theoretical point is that this is because of effects on mutagenesis. Loss of heterozygosity due to nondisjunction, gene conversion, and mitotic recombination occurring during cell division can be much more frequent than loss of heterozygosity due to an independent second mutation (*1*). Cell division is important in general for markedly increasing the probability of mutation and, for recessive genes, is likely to be of dominant importance after the occurrence of the first mutation.

Some of the other criticisms of our papers reported by Marx will be addressed in our responses to forthcoming letters in *Science*.

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(February 8, 1991)

Letters

Carcinogens and Human Health: Part 3

Bruce N. Ames and Lois Swirsky Gold (Perspective, 31 Aug., p. 970) and Philip H. Abelson (Editorial, 21 Sept., p. 1357) raise questions about the interpretation and application of cancer information in regulating chemicals. They seem to suggest that (i) the rodent bioassay is misleading, (ii) risk assessment is too cautious, and (iii) these factors distort the regulatory process, creating public anxiety about phantom hazards while real risks are ignored. These suggestions involve a mix of science and politics. We wish to respond, point out alternative scientific perspectives, and discuss the appropriateness of the Environmental Protection Agency's (EPA's) approach.

In their statements about rodent bioassays, Ames and Gold and Abelson take a few often cited examples and generalize to other bioassays in ways that are contradicted by much of the accumulated scientific evidence. First, carcinogenicity is not necessarily a consequence of high-dose toxicity. Many bioassays have shown either toxicity without carcinogenicity or carcinogenicity without toxicity. D. G. Hoel *et al.* (*1*) have analyzed results from National Toxicology Program bioassays of 99 chemicals, of which 53 were positive. For only seven could target organ toxicity be the cause of all observed carcinogenic effects. Second, carcinogenicity has generally been confirmed at less than maximally tolerated doses. Of the 99 chemicals in the analysis by Hoel *et al.*, just three caused cancer at the highest dose only. Third, rodent bioassays are indicative of human cancer risks. Allen *et al.* (*2*) have analyzed results of studies of 23 chemicals causing cancer in both rodents and humans. At high doses, rodent cancer incidences were good predictors of human cancer incidences. Because rodent carcinogenicity is not restricted to high doses, there is reason for concern about low-level human exposures.

Scientists in both industry and government have long recognized the need for careful interpretation of high-dose rodent bioassays, including consideration of supplemental information from other sources. They have improved the bioassay design to include, among other things, doses that do not cause substantial levels of toxicity. Rodent bioassays are critical in determining whether a chemical can cause cancer at some dose. Multiple-dose rodent bioassays are useful in distinguishing effects at high and low doses, as shown by the analysis of 2-acetylaminofluorene (2-AAF) by S. M. Cohen and L. B. Ellwein (Articles, 31 Aug., p. 1007).

The suggestion that risk assessment is too cautious, and that this caution no longer makes sense in view of the recent clarification of the mechanism of carcinogenesis in standard rodent bioassays, begs the question of whether a scientific consensus has emerged to support Ames's view of "the mechanism." Cancer comprises many diseases arising from a variety of mechanisms in rodents and humans, as Cohen and Ellwein illustrate with two mechanisms for mouse liver tumors induced by 2-AAF. High-dose toxicity is a mechanism for a few chemicals, but not the majority. For most chemicals, current data either support the likelihood of carcinogenic effects at low doses or are inadequate to rule them out.

The question is how to act when confronted with alternative risk projections that cannot be resolved with current data. EPA bases its risk assessments on health-conservative principles, properly so, because EPA has a responsibility to protect public health from the potentially damaging alternatives. Thus, when current data do not resolve the issue, EPA assessments employ the assumption basic to all toxicological evaluation that effects observed in animals may occur in humans and that effects observed at high doses may occur at low doses, albeit to a lesser extent.

That said, we point out that not all assumptions used in assessing risk are conservative in nature. For example, we generally have not studied potential synergistic interactions from exposures to multiple chemicals. We assume that risks are additive, although we know that for cases such as tobacco smoke and asbestos, the combined risk is much greater. As another example, there are almost no studies of cancer resulting from early life exposure. We assume that children are as sensitive as adults, althoug we know that for many pharmaceutical children are more sensitive than adults.

In response to the suggestions that thes factors distort the regulatory process, crea ing public anxiety about phantom hazarc while real risks are ignored, and that currer levels of synthetic chemicals are of littl importance compared to background leve of natural substances, we believe that sub stantially higher levels of synthetic chemical might be found in food, water, and air if th current system of regulatory limits were no in place. This system is mandated under number of laws enacted to reflect a long standing public demand for action on con trollable chemicals that present hazards t human health or the environment. To se the wisdom of this approach, one need onl look at countries that have not controlle environmental contamination. We are fa from convinced that Ames and Gold hav made a persuasive case for allowing unre stricted addition of pesticides to the foo supply.

At the same time, we agree with Ame and Gold that there are likely to be natura substances that warrant attention and test ing. In the meantime, EPA cannot ignore it responsibility to evaluate and contol syn thetic chemicals just because there may als exist natural risks that we cannot entirel eliminate. We note that the testing tha Ames advocates would involve the anima tests that Abelson characterizes as "an obso lescent relic of the ignorance of past dec ades," since no one, including Ames an Gold and Abelson, has yet devised an ac ceptable alternative.

Finally, EPA's current and evolving ap proach to risk assessment and risk manage ment is founded in scientific consensus o methods and peer review of practice. I provides a consistent and responsible way t evaluate scientific information and make in formed judgments in an area of science tha is relatively new and constantly changing. I allows the public to see what we are doing This provides an opportunity for scientifi scrutiny, which we welcome as a framewor for evaluation and improvement. In th meantime, we cannot and should not be to quick to abandon approaches that, despit certain limitations, have served the publi well.

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Response: The letter from scientists at the Environmental Protection Agency (EPA) raises many of the same issues about the use of bioassay data to estimate human risk that were discussed in earlier letters from Frederica Perera (21 Dec., p. 1644) and David P. Rall (4 Jan., p. 10) and in the article by Jean L. Marx (News & Comment, 9 Nov., p. 743). We have responded to these points in our comment on Marx's article (Letters, 14 Dec., p. 1498) and in our replies to Perera (Letters, 21 Dec., p. 1645) and to Rall (Letters, 4 Jan., p. 12), as well as in our earlier papers (*1*). For example, we have restated our view that mitogenesis markedly increases mutagenesis, that toxicity at high doses can cause mitogenesis, and that mitogenesis should not be ignored in models of carcinogenesis. We have explained that the analysis of D. G. Hoel *et al.* (*2*) cannot address the question of the role of mitogenesis in high dose animal cancer tests because mitogenesis was not measured. We have also suggested that research on mitogenesis be a high priority and that it can improve the regulatory process.

As evidence that "rodent bioassays are indicative of human cancer risks" the EPA letter discusses an analysis by B. C. Allen and colleagues (*3*) and concludes that "at high doses, rodent cancer incidences were good predictors of human cancer incidences." We disagree with this interpretation because the analysis of Allen *et al.* did not attempt to predict cancer incidences. Instead, it examined the rank order correlation between carcinogenic potencies estimated from animal bioassays and from epidemiological studies. Moreover, this analysis was based on 23 chemicals that caused tumors in *either* rodents or humans, not, as stated by the EPA letter, on chemicals that induced tumors in both rodents and humans; nine of the chemicals lacked sufficient evidence of carcinogenicity in either rodent tests or human epidemiological studies. The Allen paper was discussed by several toxicologist-sand statisticians, none of whom considered the work indicative of prediction of cancer *incidence* from animals to humans (*4*).

The EPA letter questions whether there is a scientific consensus to support the view that effects of mitogenesis at high doses can be unique to high doses. It also states that risk assessment is an "area of science that is relatively new and constantly changing . . ." and that current practice "provides an opportunity for scientific scrutiny, which we welcome as a framework for evaluation and improvement." Our papers should be seen within the context of that scientific scrutiny and evaluation. We recognize that current regulatory procedures are grounded in peer review of methods and practice. Our view is that the consensus that developed in the 1970s was based on assumptions that recent evidence suggests are wrong. The high proportion of carcinogens among chemicals tested at the maximum tolerated dose (MTD) emphasizes the importance of understanding cancer mechanisms in order to determine the relevance of rodent cancer test results for low-dose human exposures. A list of rodent carcinogens is not enough.

The EPA letter states that, when confronted with alternative risk projections that current data do not resolve, "EPA assessments employ the assumption basic to all toxicological evaluation that effects observed in animals may occur in humans and that effects observed at high doses may occur at low doses, albeit to a lesser extent." The main rule in toxicology, however, is that "the dose makes the poison": at some level, every chemical becomes toxic, but there are safe levels below that. A consensus developed in the 1970s that we should treat carcinogens differently, that we should assume that even very low doses might cause cancer; this consensus was based on the precedent of radiation, which is both a mutagen and a carcinogen; radiation gave credence to the idea that there could be effects of chemicals even at low doses although we lacked the methods for measuring such effects. This idea evolved because it was expected that (i) only a small proportion of chemicals would have carcinogenic potential and (ii) testing at high dose would not produce a carcinogenic effect unique to the high dose. In our papers and replies to letters in *Science*, we have discussed in detail the accumulating evidence from a variety of disciplines suggesting these assumptions are wrong and therefore that it is time to re-evaluate them.

The risk assessments on which regulations are based are not scientifically justified. Testing chemicals for carcinogenicity at near toxic doses in rodents does not provide enough information to predict the numbers of human cancers that might occur at low-dose exposures. We have discussed the importance of ranking possible carcinogenic hazards and the uncertainties in risk assessments (*5*). Therefore, the public might be better served if EPA were to present its risk assessments as comparisons to its estimates of risks from cups of coffee, beers, and so forth, given the enormous natural background of potential rodent carcinogens.

The EPA letter points out that not all assumptions used in their risk assessments are conservative, for example, the potentia synergistic interactions among chemicals We agree that some interactions can poten tiate carcinogenesis; however, interaction can also be inhibitory, and at low dose defenses in humans are usually inducible The main conservative assumption is tha the effects of mitogenesis at high doses ca be ignored in low dose extrapolations.

With respect to regulatory policy, th EPA letter states that if current regulator limits were not in place then higher levels o synthetic chemicals might be found in ai water, and food. Our papers do not argue t discontinue regulation nor, as EPA misrep resents us, to allow "unrestricted addition of pesticides to the food supply." Regula tion involves trade-offs, and the best scienc is necessary so that regulation does no become counterproductive. We have dis cussed these important trade-offs (*1*).

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Erratum: In the 21 December response by Bruce Ames and Lois S. Gold (Letters, p. 1645) to the letter Frederica P. Perera (p. 1644), the last sentence of t third paragraph in column three should have read, "Bo natural arsenic in water and natural radon in indoor are present at high levels at some locations and were lo neglected, while major efforts were put into minisc amounts of industrial pollutants." In the 4 Janu response by Bruce N. Ames and Lois S. Gold (Letters, 12) to the letter by David P. Rall (p. 10), reference should have read, "D. G. Hoel, J. K. Haseman, M. Hogan, J. Huff, E. E. McConnell, *Carcinogenesis* 9, 20 (1988)"; reference 14 should have read, "E. Marsh *Science* 250, 900 (1990); R. Doll, *Eur. J. Cancer* 500 (1990); C. Hill, E. Benhamm, F. Doyon, *Lan* 336, 1262 (1990); S. Freni, *ibid.*, p. 1263."

Mitogenesis Is Only One Factor in Carcinogenesis

I. BERNARD WEINSTEIN

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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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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