Perspective

Too Many Rodent Carcinogens: Mitogenesis Increases Mutagenesis

BRUCE N. AMES* AND LOIS SWIRSKY GOLD

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.

The human diet consists of thousands of natural pesticides (chemicals that plants produce to defend themselves) (*4*); we calculate that 99.99% (by weight) of the pesticides in our diet are natural. Of the natural pesticides that have been tested in at least one rodent species, about half (27/52) are rodent carcinogens. These 27

The authors are in the Division of Biochemistry and Molecular Biology, Barker Hall, University of California, and the Lawrence Berkeley Laboratory, Berkeley, CA 94720.

*To whom correspondence should be addressed.

occur commonly in plant foods (*10*). We estimate that the average intake of these pesticides is about 1500 mg per person per day (*4*). By comparison, the average intake per day of residues of 100 synthetic pesticides is 0.09 mg per person per day (*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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8. Current epidemiologic data point to these risk factors for human cancer: cigarette smoking (which is responsible for 30% of cancer deaths), dietary imbalances, hormones, viruses, and occupation. "[T]he age-adjusted mortality rate for all cancers combined except lung cancer has been declining since 1950 for all individual age groups except 85 and above" [National Cancer Institute, *1987 Annual Cancer Statistics Review Including Cancer Trends: 1950–1985*, NIH Publication 88-2789 (National Institutes of Health, Bethesda, MD, 1988), p. II.3]. Although incidence rates for some cancers have been rising, trends in recorded incidence rates may be biased by improved registration and diagnosis. Even if particular cancers can be shown to be increasing (for example, non-Hodgkins lymphoma and melanoma) or decreasing (for example, stomach, cervical, and rectal cancer), establishing causes remains difficult because of the many changing aspects of our life-style. Life expectancy continues to increase every year.
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10. A search in foods for the presence of just these 27 natural pesticide rodent carcinogens indicates that they occur naturally in the following (those at levels over 10 ppm of a single carcinogen are listed in italics): *anise, apple*, banana, *basil*, broccoli, *Brussels sprouts, cabbage*, cantaloupe, *caraway, carrot, caulflower, celery, cherry*, cinnamon, cloves, cocoa, *coffee (brewed), comfrey tea, dill, eggplant, endive, fennel, grapefruit juice, grape, honey*, honeydew melon, *horseradish*, kale, *lettuce, mace, mango, mushroom, mustard (brown) nutmeg, orange juice, parsley, parsnip*, peach, *pear, pepper (black)*, pineapple, *plum, potato*, radish, raspberry, *rosemary, sage, sesame seeds (heated)*, strawberry, *tarragon, thyme*, and turnip (*4*). Particular natural pesticides that are carcinogenic in rodents can be bred out of crops if studies of mechanism indicate that they may be significant hazards to humans.

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