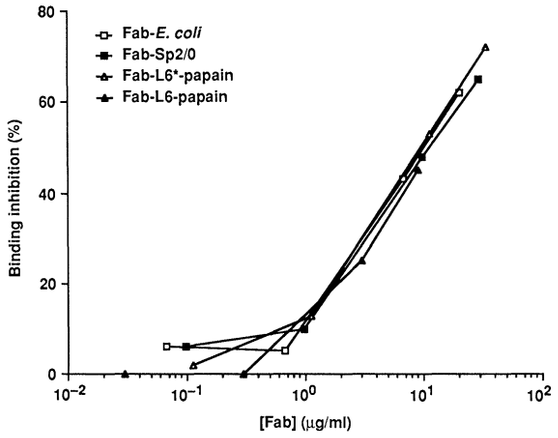

Fig. 3. Binding inhibition of bacterial Fab. Bacterial Fab, Sp2/0 Fab, and proteolytically produced chimeric L6 Fab (L6*) and mouse L6 Fab were used to inhibit FITC-labeled mouse L6 antibody binding to the surface of antigen-positive C3347 colon carcinoma cells.

bodies to protease cleavage will be obviated; a consistent, homogeneous preparation can be produced. Of additional interest is the relative ease with which the Fab cDNA genes can be modified before expression in bacteria. For example, modifications of the primary structure of either the Fd or κ chain (or both) that are useful for subsequent conjugation of imaging or therapeutic agents or fusion to other peptides (*16*) can be introduced by site-directed mutagenesis techniques. We found that *E. coli* can properly assemble a functional two-protein unit with a complex pattern of intra- and interchain disulfide linkages and that sufficient quantities of this material may be prepared for eventual use as a human diagnostic and therapeutic reagent.

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Technical Comments

Carcinogenic Risk Estimation

In their widely publicized and popularized article "Ranking possible carcinogenic hazard," Bruce N. Ames *et al.* (17 Apr. 1987, p. 271) conclude that "analysis on the levels of synthetic pollutants in drinking water and of synthetic pesticide residues in foods suggests that this pollution is likely to be a minimal carcinogenic hazard relative to the background of natural carcinogens" and thus that the "high costs of regulation" of such environmental carcinogens are unwarranted. These conclusions reflect both flawed science and public policy.

Although Ames *et al.* challenge the validity of animal carcinogenicity data for quantitative estimation of human risk, they nevertheless use such extrapolations, based on the percentage *H*uman *E*xposure dose/*R*odent *P*otency dose (HERP), for ranking carcinogenic hazards. Apart from the fact that HERP rankings are based on average population exposures excluding sensitive subgroups, such as pregnant women, the derived potencies of Ames *et al.*, doses inducing tumors in half the tumor-free animals, are misleading. Potencies for "synthetic pollutants," such as trichloroethylene, are derived from bioassays in which lowest doses are large fractions of the maximally tolerated dose (MTD), whereas potencies for more extensively studied "natural carcinogens," such as aflatoxins, are generally derived from titrated doses, orders of magnitude below the MTD. Since dose-response curves are usually flattened near the MTD (*1*), potencies derived from high-dose testing yield artificially low risk estimates; HERPs for "synthetic" carcinogens are thus substantially underestimated compared with many "natural carcinogens."

Compounding this misconception, Ames *et al.* maintain that carcinogenic dose-response curves rise more steeply than linear curves and that tumor incidences increase more rapidly than proportional to dose. At high doses, dose-response curves are usually less steep than linear curves (*1*), as also recognized elsewhere by Ames and his colleagues (*2*). Thus at MTD doses, large further dose increases may induce only small increases in tumor incidence, perhaps reflecting competition between transformation and cytotoxicity (*3*); linear extrapolations from high-dose tests thus underestimate low-dose risks.

For Ames *et al.*, the term "carcinogen" heterogeneously includes direct and indirect influences, including promoting and modifying factors and mutagens. Caloric intake is considered "the most striking rodent carcinogen." However, no correlations have been established between food intake and tumor incidence among animals eating ad libitum, despite wide variations in caloric intake and body weight (*4*), nor have correlations been established between obesity and most human cancers. In the statement by Ames *et al.*, "at the MTD a high percentage of all chemicals might be classified as 'carcinogens'," toxicity and carcinogenicity are confused. However, among some 150 industrial chemicals selected as likely carcinogens and tested neonatally at MTD levels, fewer than 10% were carcinogenic (*5*). Many highly toxic chemicals are noncarcinogenic, and carcinogen doses in excess of the MTD often inhibit tumor yields. While Ames *et al.* revive the discredited theory that chronic irritation causes cancer, most irritants are noncarcinogenic, and there is no correlation between nonspecific cell injury and carcinogenic potency (*6*).

Ames *et al.* classify ethanol as carcinogenic, "[one of the two] largest identified causes

of neoplastic death in the United States" along with tobacco; their HERP indices for a daily glass of wine and "average" occupational exposure to formaldehyde are similar. In four rodent tests cited by Ames *et al.*, alcohol was noncarcinogenic; in the fifth, an experiment with alcohol of undefined purity, carcinogenicity was "extremely low." While epidemiologic studies have incriminated alcohol—particularly in promoting or synergizing tobacco smoke, in upper digestive tract cancers, and also in inducing cirrhosis, a risk factor for liver cancer (*7*)—there is no evidence incriminating alcohol per se as a potent carcinogen for the general population, particularly nonsmokers. Although two cohort studies not cited by Ames *et al.* demonstrate weak associations between breast cancer and alcohol consumption (*8*), their significance is limited by minimal dose-response relationships, several contrary studies, and the contamination of alcoholic beverages with carcinogens including urethane, methylglyoxal, nitrosamines, and pesticide residues.

While diffusely defining carcinogens, Ames *et al.* artificially categorize them as "natural" or "industrial," saying that the former hazards should somehow limit concerns on the latter. However, dietary levels of "natural carcinogens" such as aflatoxins and dimethylnitrosamine are influenced by harvesting and storage technologies and nitrite additives, respectively. Moreover, predominant exposure to other "natural carcinogens" results from industrial activity; examples include asbestos, heavy metals, uranium, and formaldehyde. While emphasizing "natural carcinogens" and "nature's pesticides" in food as major carcinogenic exposures, Ames *et al.* ignore natural dietary anticarcinogens and antimutagens, such as porphyrins, phenolics, and retinoids (*9*). Although risks from aflatoxin and alcohol, described as two most important and potent carcinogens, depend on synergism with hepatitis B virus and tobacco smoke, respectively, risk estimates for most synthetic carcinogens are based on single-agent exposures only. While "natural carcinogens" have long played a role in human cancer, concerns must also focus on recent incremental effects of increased production of and exposure to nonsynthetic carcinogens, such as asbestos and heavy metals, and on the novel and escalating production and exposure to "synthetic carcinogens" (*10*). Although some petrochemicals have been proved to be carcinogenic, most have not been tested; moreover, much industrial data is at best suspect or unavailable (*11*).

The National Institute for Occupational Safety and Health estimates that 11 million workers are exposed to ten high volume industrial carcinogens (*12*). Up to tenfold increases in organ-specific cancer rates are reported among those who work with asbestos, uranium, and arsenic and in coke plants and among those exposed to specific petrochemicals and to some 20 less well-defined processes, such as dry cleaning, spray painting, and plumbing (*12*); excess childhood leukemia is also associated with parental occupational exposures to organic solvents and related chemicals (*13*). Just one of the few well-studied occupational carcinogens, asbestos, responsible for up to 10,000 annual cancer deaths (*14*), is second only to tobacco of all known causes of human cancer.

Growing evidence demonstrates that pervasive contamination of air, water, soil, and food with a wide range of industrial carcinogens, generally without public knowledge and consent, is important in causation of modern preventable cancer. Even if hazards posed by any industrial carcinogen are small, their cumulative, possibly synergistic, effects are likely substantial. Eating food contaminated with residues at maximum legal tolerances of only 28 of 53 known carcinogenic pesticides, excluding numerous other carcinogenic pesticides and incremental exposure in drinking water, is estimated to be potentially responsible for 1.5 million excess lifetime U.S. cancers (*15*). Trichloroethylene is a common contaminant of drinking water, generally resulting from improper disposal of industrial wastes; lifetime consumption levels of 250 parts per billion found in contaminated wells in Woburn, Massachusetts, together with other related carcinogens not considered by Ames, *et al.*, is associated with excess risks of cancer (*16*), childhood leukemia, perinatal deaths, and birth defects (*17*). Some 20 retrospective and case control studies have associated trihalomethane-contaminated water with gastrointestinal and urinary tract cancers (*18*). As only a few organic drinking water contaminants are characterized (*19*), and as inhalation and cutaneous exposures may be as important as ingestion (*16*), risk estimates, excluding possible interactive effects, are likely to be misleadingly low. Nevertheless, Ames *et al.* ignore these limitations and also the substantive epidemiologic data and assert that "the animal evidence provides no good reason to expect that chlorination of water or current levels of man-made pollution of water pose significant carcinogenic hazards," and that the risk from contaminated Woburn water is 1/10,000 that of a glass of wine.

Community air pollution from industrial emissions, and thus proximity of residence to certain industries, is a recognized cancer risk factor. Numerous studies, controlled or stratified for smoking, demonstrate associations between excess lung cancer rates and heavy metal and aromatic hydrocarbon emissions (*20*); exposure to benzo[*a*]pyrene, a conventional combustion index, increased lung cancer mortality by 5% per nanogram per cubic meter of air (*21*). Others estimate that "the proportion of lung cancer deaths in which air pollution is a factor is 21%" (*22*). Concerns have recently focused on defined industrial emissions, including arsenicals, benzene, chloroform, vinyl chloride, and acrylonitrile, which in both sexes are associated with excess overall and organ-specific, standardized community cancer rates; carcinogenic trace metals and volatile organic community air pollutants, have been incriminated in some 0.6 to 2.3 per 1000 excess lifetime cancers (*23*). Ames *et al.*, however, trivialize risks from "general outdoor air pollution."

Ames *et al.* state that cancer mortality rates "have mostly been steady for 50 years" apart from "lung cancer due to tobacco and melanoma due to ultraviolet light." This is based on analyses that exclude people over 65 and blacks of all ages (*24*) and which ignore the following: effects on mortality rates of the approximately 70% reduction in gastric and cervical cancer mortality since the 1940s which have been masked by increasing mortality from cancers at other sites; probability estimates that have projected marked increases in mortality rates for a wide range of malignancies for those born in 1985 compared with those born in 1975 (*25*); very recent increases in premenopausal breast cancer mortality (*26*); the role of nonsolar causes of melanoma (*26*); and the role of other major causes of lung cancer besides smoking (*27*). While smoking is a major cause of lung cancer, the importance of other causes is evidenced by increasing rates in highly urbanized and highly industrialized communities; disproportionately increasing rates for black males not attributable to smoking pattern differences; increasing rates in nonsmokers while rates for other tobacco-related cancers, such as those of the buccal cavity and pharynx, are declining; increasing rates in some groups of nonsmoking workers; increasing rates in women, greater than can be accounted for by increased smoking; and, increasing proportions of lung cancers that are adenocarcinomas, which are less closely associated with tobacco smoking (*12, 27*). Incidence rates, not considered by Ames *et al.* and which can "reveal changes in cancer occurrence that are not apparent in the mortality data" (*26*), from 1950 through 1985 increased overall by 37%; by 20% or over for pancreas cancer; by 51% for urinary bladder cancers; by over 100% for non-Hodgkins lymphoma, multiple myeloma, and malignant mela-

noma in both sexes; by 31% for female breast cancer; by 92% for testis cancer; by 67% for prostate cancer; and by 63% for colorectal and 142% for kidney cancers in males (*26, 28*).

Apart from fundamental problems inherent in Ames's views on carcinogenesis and his dismissal of concerns about industrial carcinogens as "chemophobia," positions editorially endorsed (*29*), his current views and recommendations contrast strikingly with those previously and strenuously propounded (*30*).

Besides proper concerns about naturally occurring carcinogens and tobacco, prudent policy must reflect overwhelming data on incremental exposure to industrial carcinogens and their association with increasing cancer rates, besides reproductive, neurotoxic, and other toxic effects (*31*). The existence of natural hazards clearly does not absolve industry and government from the responsibility for controlling industrial hazards. From public health, ethical, and policy perspectives, the important distinction is not between "natural" and "synthetic" carcinogens, but between preventable and nonpreventable cancers.

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29. P. H. Abelson, *Science* **237**, 473 (1987); further illustrations of the relationship between Ames and the editors of *Science* is provided in B. Ames, *ibid.* **226**, 1396 (1984).
30. In 1977, Blum and Ames warned that the synthetic carcinogenic pesticide ethylene dibromide (EDB) is a "potent carcinogen" whose structural similarity to the flame retardant tris is one of the reasons why the synthetic chemical tris "should not be used." They also pointed to "enormous possible [carcinogenic] risks" from using an untested chemical in [a fire retardant in] pajamas, predicted that a "steep increase in the human cancer rate from these suspect . . . chemicals may soon occur" "as the 20- to 30-year lag time of chemical carcinogenesis in humans is almost over," [A. Blum and B. Ames, *Science* **195**, 17 (1977)]. Blum and Ames also emphasized the need for high-dose testing in an effort to compensate for the "inherent statistical limitation in animal cancer tests" and expressed concerns about "the effects of the large-scale human exposure to the halogenated carcinogens [including] vinyl chloride, Strobane-toxaphene, aldrin-dieldrin, DDT, trichloroethylene . . . [and] heptachlor-chlordane." In 1979, Ames and his colleagues demonstrated that carcinogenesis dose-response curves usually rise less steeply than linear curves and criticized the view that many carcinogens have activity only at very high doses [W. Hooper, R. Harris, B. Ames, *ibid.* **203**, 602 (1979)]. Ames also stressed the need to establish "priorities for trying to minimize human exposure to these [synthetic] chemicals" [B. Ames, *ibid.* **204**, 587 (1979)]. Four years later, however, he reversed himself and concluded that cancer rates were not rising, that synthetic carcinogens posed only trivial risks, and that the real culprits were natural carcinogens and faulty life-styles, tobacco, and high-fat diets, citing Doll and Peto (*24*), who "guestimated" that diet, particularly fat, is incriminated in 35% of all cancer deaths, as the basis for this statement [B. Ames, *ibid.* **221**, 1256 (1983)]. Now, the carcinogenic hazards of high-fat diets are virtually dismissed in a few parenthetic words "[a possible risk factor in colon cancer . . .]," presumably reflecting Peto's recent reversal on the role of fat [R. Peto, *ibid.* **235**, 1562 (1987)], and in its place alcohol now emerges, alongside tobacco, as [one of the two] "largest indentified cause of neoplastic death in the United States."
31. S. S. Epstein, *Congr. Rec.* **133**, E3449 (1987).
32. We thank M. Jacobson, T. Mancuso, M. Schneiderman, and A. Upton for their helpful comments.

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Response: We agree with only the last two sentences of the letter of Epstein *et al.* Correcting each of their errors would require lengthy explanations and would duplicate previous detailed analyses (*1–3*), so here we cover only the main issues.

■ *Half the chemicals tested in animals are carcinogens.* Our exhaustive database of animal cancer tests listed 392 chemicals tested in *both* rats and mice at or near the maximum tolerated dose (MTD). Of these, 60% of the synthetic chemicals and 45% of the natural chemicals were carcinogens in at least one species (*1*). The finding that about half of tested chemicals are positive in rodents has been reported for many sets of data; we cited among others the studies of the National Toxicology Program (NTP). We concluded that the proportion of chemicals found to be carcinogens is strikingly high. Epstein *et al.* ignore our data and citations and cite the early Innes *et al.* study to support their conclusion that the proportion of carcinogens is low. This misrepresents the facts. The Innes tests (120 chemicals, not 150 as stated by Epstein *et al.*, 11 positive) used only one species and were much less thorough than modern tests: they therefore were less likely to detect a carcinogenic effect (*4*).

The proportion of carcinogens is about as high for natural chemicals as for industrial chemicals. Therefore, our diet is likely to be