LETTERS

Agricultural Research System

According to the editorial page, "*Science* serves its readers as a forum for the presentation and discussion of important issues related to the advancement of science. . . ." In that context *Science* is to be congratulated for the article by Nicholas Wade in the 5 December 1975 issue (News and Comment, p. 959) concerning agricultural research. On the other hand, *Science* readers are entitled to more objectivity than is demonstrated in that article. The quotes attributed to me are in error as well as being misleading. Even more serious are the remarks concerning Assistant Secretary of Agriculture Robert W. Long. A similar article by the same author (News and Comment, 17 Jan. 1975, p. 150) drew comments from within the Department as well as outside, with assurances that Long is a capable administrator of agricultural research programs and is highly respected by the agricultural research community.

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I much regret that Lovvorn feels he has been misquoted. Misunderstandings are always possible, but I do not believe that the quotations attributed to him are in error.—NICHOLAS WADE

Carcinogenicity Tests

In his capacity as chairman of the AAAS Committee on Scientific Freedom and Responsibility, John Edsall is in a position to influence public policy; because of his stature as a distinguished researcher, he is in a position to influence scientific thought. He notes in his letter of 18 July 1975 (p. 174) the finding that some carcinogens are mutagenic in bacteria (*1*). This has been interpreted to mean that those carcinogens cause cancer by somatic mutation and has been taken by many as support for the venerable hypothesis that the malignant transformation of cells is a mutational event. In addition, the screening of compounds for their capacity to cause bacterial mutations has been adopted by a number of laboratories as a means of indicating carcinogenic potential. The implied relation between mutagenesis and carcinogenesis (*2*) still needs careful scrutiny with regard to its scientific validity and also because of its implications for public policy.

Unfortunately, an enormous variety of materials has been shown to be carcinogenic if applied persistently enough in the right places at the right times. A classic example is "solid state" carcinogenesis, in which a variety of inert, insoluble materials are carcinogenic if implanted under the skin in the form of continuous sheets, but not if implanted in the form of pellets (*3*). Excessive application of normal steroid hormones causes cancer, as does the simple transplantation of some endocrine organs into the spleen of the same animal (*4*). It is difficult to accept mutagenesis as the origin of these cancers.

A simple listing of agents as carcinogens has little meaning unless accompanied by a quantitative indication of carcinogenic potency and the conditions under which these were determined. The hazards involved in readily accepting a screen for carcinogenic hazard based on other biological effects are illustrated in a recent article by Wood *et al.* (*5*). Benzo[*a*]pyrene is a potent carcinogen and a widespread environmental pollutant. Its carcinogenic activity has been attributed to its metabolic products. The 4,5-oxide was highly mutagenic in bacterial tester strains, but benzo[*a*]pyrene itself and the 7,8- and 9,10-oxides had no significant mutagenic activity. However, only benzo[*a*]pyrene and the 7,8-oxide produced tumors, while the 4,5-oxide induced few, if any, tumors in mice [see note added in proof of (*5*)]. In other words, the screen missed the carcinogens and implicated the noncarcinogen.

Acceptance of screening for carcinogenicity by determining mutagenicity lends tacit support to the hypothesis that malignant transformation of cells is caused by somatic mutation. This hypothesis has been tested explicitly in several experiments and has been found wanting in each case. Transplantation into frog eggs of nuclei from frog carcinoma cells results in normal swimming tadpoles (*6*). This shows that the carcinoma nuclei had the normal genomic complement, capable of making every functional tissue of the tadpole. This is the same type of evidence which has served as the basis for general acceptance of the idea that cell and tissue differentiation are epigenetic phenomena (*7*). Heidelberger's group (*8*) has shown that the powerful carcinogenic hydrocarbon methylcholanthrene, at concentrations which cause no significant cell death, produces malignant transformation in 100 percent of the clones of mouse prostate cells in culture. This would certainly be an extraordinary outcome if the malignant transformation were caused by mutations, which occur at extremely low frequencies in clearly defined situations. Finally, Braun (*9*) has shown that single cells of the crown gall tumor of tobacco give rise to normal tobacco plants when grafted to cut stem ends of tobacco. They yield seeds which are generatively normal in every respect. While one may argue that plant tumors are not equivalent to animal tumors, they are certainly more closely related than are bacterial mutations.

There are other types of evidence which are inconsistent with assuming a genetic basis for the malignant transformation (*10*). Perhaps none of the tests can be considered conclusive by itself, but together they make a far weightier case *against* a mutational origin of the malignant transformation than does the evidence *for* such an origin. Unfortunately, there is no clearly established mechanism for obtaining a heritable change in cells other than a change in the genetic complement of the cell, although some have been considered (*11*). It is inescapable, however, that such a mechanism must exist to explain the stable differentiation of cells which have identical nuclear genomes (*7*).

I have no argument with the desirability of screening for mutagens in the environment by a simple and economical test. However, to use this kind of screening as a test for carcinogenicity is a bit like looking under the lamppost for the coin lost a block away because of the availability of light. For the present, we must still assume the hard and expensive task of looking for carcinogens by determining a compound's carcinogenic action because that is the only way we can know what we have found.

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For the last 10 years I have been involved in the development and validation of a rapid, sensitive, and economical test method (using *Salmonella* bacteria and mammalian microsomal enzymes) for de-

tecting environmental mutagens (*1–4*). One of the main results to come out of this work (in addition to the test system itself) is the fact that almost all carcinogens are mutagens. About 90 percent (156) of 174 carcinogens tested for mutagenicity were shown to be mutagens (*2*), and these 156 carcinogens cover a wide variety of classes of chemicals known to be carcinogenic. Also, almost all of the tested chemicals known to be carcinogenic in humans were shown to be mutagenic. These include β-naphthylamine, benzidine, cigarette smoke condensates, *bis*-chloromethyl ether, aflatoxin B_1, vinyl chloride, 4-aminobiphenyl, chlornaphazin, 4-nitrobiphenyl, and cyclophosphamide (*2*). We tested 109 "noncarcinogens" (including 46 common biochemicals, none of which were mutagens) and, despite the severe statistical limitations in defining "noncarcinogenicity" in tests using small numbers of animals, few of them showed any degree of mutagenicity (*2*). The test is highly selective in discriminating between carcinogens and closely related "noncarcinogenic" analogs.

Therefore, there is an extremely high probability that chemicals found to be mutagenic in the *Salmonella* test will turn out to be carcinogens. In fact, many chemicals in the environment which were found to be mutagenic subsequently were tested and found to be carcinogenic, among which are the major Japanese food additive AF-2 (now banned) (*5*) and the widely used industrial chemical and grain fumigant ethylene dibromide (*1, 2*). Two hair dye components, which we found to be mutagenic (*4*), have been shown to cause cell transformation and to break mammalian chromosomes (*6*) (thorough cancer tests are still under way). Fractions of cigarette smoke condensate first shown to be mutagenic (*7*) have also been shown to cause cell transformation (*8*).

It has been estimated that 80 percent of human cancer is attributable to environmental causes, yet only a small proportion of the chemicals to which humans are exposed are being tested (*9*) in the expensive ($100,000), long-term (2 to 3 years) cancer tests in rodents. A relatively high percentage of genetic abnormalities among human births may also be attributable to environmental causes, yet our knowledge of the mutagenicity of environmental chemicals in mammals is limited. We are living in a rapidly increasing flood of chemicals that have not been tested for mutagenicity or for carcinogenicity, from flame retardants in our children's pajamas to pesticides accumulating in our body fat. An explosive increase in the incidence of birth defects and human cancer may be the outcome if too many of the thousands of new chemicals to which humans are exposed turn out to be powerful mutagens and carcinogens. These two health problems appear to be closely intertwined, and a partial solution to both of them is likely to come from a much more rigorous effort to minimize exposure of humans to mutagenic substances and radiations.

Evidence that, with few exceptions, carcinogens are mutagens, has confirmed the importance of using the *Salmonella* mutagenicity test as a primary screening technique to pinpoint potentially dangerous chemicals, even in complex mixtures (*7*), and to *complement* the traditional long-term animal tests for carcinogenicity. The test is simple, costs a few hundred dollars per chemical, and can be used to screen thousands of chemicals a year. Fifty-three chemical and drug companies have already requested the tester strains, and many have started extensive screening programs involving thousands of chemicals. The director of the National Cancer Center Research Institute in Japan has indicated that a major program is planned in which the *Salmonella* mutagenicity test will be used for general screening of chemicals.

On the basis of our work, and the lines of evidence noted below, I find compelling the theory that chemical carcinogens cause cancer through damage to DNA (somatic mutation).

1) It is known that cell regulation can be easily altered by mutation and that a heritable change in cell regulation is a characteristic property of a cancer cell.

2) The theory is simple and accounts for what is known about the molecular biology of cancer (*10*) and chemical and radiation carcinogenesis.

3) Studies on the genetics of cancer (*11*) suggest this idea.

4) Human mutants exist who are extremely prone to cancer and who lack DNA repair systems (*12*).

5) There is a correlation between capacity for repair of DNA damage and the occurrence of organ specific cancer (*13*).

6) Active forms of many carcinogens are electrophiles capable of interacting with DNA (*14*).

7) Ninety percent of carcinogens are mutagens (*1–4*); it is our hypothesis that the aromatic part of aromatic carcinogens is involved with stacking interactions in DNA, causing frameshift mutations (*1–4*).

8) The well-known human carcinogen asbestos has recently been shown to be an efficient breaker of chromosomes (*15*).

By bringing up "solid state" carcinogenesis, where disks of almost any solid material are implanted under the skin of animals and some tumors result after an extended period, Rubin implies that everything is a carcinogen, when, in fact, relatively few chemicals are carcinogens (*9*). Solid state carcinogenesis may well be due to disruption of stem cell programs and, if so, is not at all inconsistent with somatic mutation theory (*10*)—it is due to a physical effect, is clearly different from chemical carcinogenesis, and has yet to be shown to be an important factor in human carcinogenesis (*16*). Rubin also states that a simple listing of carcinogens has no meaning without a quantitative indication of potency. Unfortunately, little data is available from animal studies on comparative carcinogenic potency of different chemicals, as dose-response curves and lifetime studies are rare. We have been quite frustrated by this, because our dose-response curves indicate a 10^6-fold range of mutagenic potency in the test, and we would very much like to calibrate our system against animal cancer data. In the few cases where quantitative animal cancer data are available, there is a rough quantitative correlation between carcinogenicity and mutagenicity (*17*).

Rubin refers to an article by Wood *et al.* (*18*) on the mutagenicity of benzo[*a*]pyrene derivatives. Wood *et al.* used my test system, but without mammalian liver microsomes, because they were studying active forms of benzo[*a*]pyrene. Thus, as expected, they did not find benzo[*a*]pyrene to be mutagenic. We showed previously that benzo[*a*]pyrene is a powerful mutagen in the presence of liver microsomes, which convert it to an active form (this conversion by microsomes is also necessary for carcinogenicity in the animal) and that it is inactive without the microsomes (*1, 2*). The subject of the epoxides is complicated (there is much activity and controversy in the field), as different epoxides are formed and degraded depending on the conditions of enzyme induction in the animals and on which tissue is used. The 4,5-oxide has been reported to be carcinogenic by a different group [see (*2*)], many of the epoxides are quite unstable, and the Wood group used our old tester strains, which were not nearly as sensitive as our newer strains for polycyclic hydrocarbons. In general, malignant transformation assays, the *Salmonella* test, and mammalian mutagenicity tests are showing good agreement (*19*). The carcinogenicity and mutagenicity results for the polycyclic hydrocarbons show an excellent correlation (*2*).

The frequency of cell transformation does not seem to be a strong argument against DNA being the critical target for the polycyclic hydrocarbons. In the transformation assays now being done regularly (with hamster, mouse, and rat cells) only a few percent of the cells treated are transformed with potent carcinogens, such as polycyclic hydrocarbons (*20*). Under these

Applications Invited for AAAS Congressional Science Fellowships

The American Association for the Advancement of Science invites applications for the fourth consecutive year of its Congressional Science and Engineering Fellow Program.

The Program selects postdoctoral level to midcareer scientists and engineers to spend one year on the staffs of individual congressmen, congressional committees, or with some other area of the Congress. In the past three years, 15 AAAS Fellows have been in positions in the House and Senate and the Office of Technology Assessment. Each Program year includes several AAAS Fellows as well as several other Fellows selected by six cooperating affiliated professional societies.

The award will be $16,000 for the period of one year, beginning 1 September 1976. An intensive two-week orientation is developed for the Fellows by the AAAS. Each Fellow's assignment is worked out by that individual with guidance by the AAAS.

Candidates may apply from any physical, biological or behavioral science or field of engineering. The term science is used broadly to include the system sciences, public health, and other technical professional areas. Candidates are required to be members of the AAAS or concurrently applying for membership.

Detailed information on the application procedure and other information about the program are available from **Dr. Richard A. Scribner, Director, AAAS Congressional Science Fellow Program, AAAS, 1776 Massachusetts Avenue, NW, Washington, D.C. 20036**. The deadline for application is 31 March 1976. Announcement of the awards will be made on about 1 May 1976.

conditions one sees cell killing, often very marked, and also a considerable percentage of cells have chromosome breakage and other chromosome abnormalities, such as aneuploidy (*21*). Specific chromosomal abnormalities (somatic mutation) caused by the chemical carcinogens are thought by some to be responsible for the expression of malignant transformation and carcinogenicity (*22*). The experiments mentioned by Rubin with the polycyclic hydrocarbons on the prostate cell line (not used much at present because of its aneuploidy and high spontaneous rate of transformation) have several complications that make it difficult to calculate the frequency of cell transformation: it is notoriously difficult to wash polycyclic hydrocarbons out of cells, and the transformants are expressed per clone rather than per cell (large numbers of cells, with a few foci, are present at the end of the experiment). Mutation in animal cells is not necessarily that rare, even when single genes are looked at: 3 to 6 percent of cells treated with a frameshift mutagen (which works at mutational hot spots) give rise to immunoglobulin mutants (*23*).

It is likely that mutation (initiation) is not the only cause of cancer and that a few environmental chemicals may well work through other mechanisms, (*10, 24*); however, there is not much evidence that such environmental chemicals (or even viruses) are contributing in a major way to human cancer. Rather, the evidence indicates that chemicals and radiations in the environment (cigarette smoke, ultraviolet light, nitrosamines, and so forth) damage DNA and that this damage, incurred throughout our lifetimes, is the initiator of most of human cancer. DNA damage is quite likely to be a major contributor to birth defects, aging (*25*), and heart disease (*26*) as well.

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The "Sunday Seminar"

We are a group of Americans who attended a symposium on nucleic acids sponsored jointly by the Soviet and American academies of science. The symposium was held in Moscow from 29 September through 4 October 1975. While in Moscow we visited the "Sunday Seminar," a weekly meeting of a group of scientists, most of whom have applied for emigration visas and as a result have lost their jobs. These people are not permitted to function as working scientists: they do not have access to laboratories, classrooms, scientific meetings, or even scientific libraries. The current head of the Sunday Seminar is Mark Azbel, in whose apartment the group meets. He is a theoretical solid state physicist of international reputation, who lost his positions at Moscow State University and at the Landau Institute of Theoretical Physics 3 years ago. He is now being threatened with arrest if these scientific seminars continue. Most of the scientists present the day we visited were physicists and mathematicians. The biologists included Edward Trifonov and his wife Helene Lebedeva; before losing their jobs, they worked, respectively, in the Kurchatov Institute of Atomic Energy and at the Institute of Genetics and Selection of Industrial Microorganisms. Both were involved in research on basic problems in biology of interest to our group and now face an uncertain future. During our visit to the Seminar, which lasted several hours, some of us presented summaries of our work as Trifonov translated. Even though most of the Soviet scientists were from different fields, they were grateful for this direct professional contact.

We urge all scientists who visit the Soviet Union to make an effort to contact these dispossessed colleagues and to visit the Seminar, if it still exists (*1*). External contact is of vital importance to these scientists, whose intellectual life has been severely curtailed by their government and who are permitted neither to work productively within their society, nor to leave it. This destructive treatment of individuals, whatever the pretext, is clearly in violation of the spirit of the Helsinki Accords and can only serve to impede future cooperation between American and Soviet scientists.

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Notes

1. The following was reported in the Washington *Post* (5 Jan. 1976, p. A7): "Soviet police told a weekly seminar of Jewish scientists today that the meetings must stop because of complaints from neighbors. . . ."