Cell Proliferation: Concluding Remarks
by I. Bernard Weinstein

In beginning this overview, I want to acknowledge the fact that, even though I disagree with most of his conclusions, Bruce Ames has played an important role in further stimulating interest in cell proliferation in carcinogenesis. This meeting has provided a balanced discussion of the current state of knowledge in this field. It has also highlighted the complexities of cell proliferation, the need for further mechanistic studies, and the desirability of developing new methods that can be used to precisely quantitate the various parameters related to cell proliferation in the intact organism.

At this meeting and in a recent paper (1), Ames has put forth several postulates that I believe represent an oversimplification of the multistage carcinogenic process. These postulates include the following: a) the aging process per se is a major factor in human cancer causation; b) oxidative damage due to endogenous factors plays a major role in human cancer causation; c) mitogenesis (induced cell division) plays a major role in carcinogenesis by increasing mutagenesis; d) the standard National Toxicology Program (NTP) rodent bioassay frequently yields unreliable results with respect to predicting human carcinogens; and e) synthetic pesticides and certain other synthetic chemicals pose lesser carcinogenic risks than nature's pesticides and other naturally occurring chemicals in various food stuffs. Elsewhere, I have reviewed evidence that calls into question these conclusions (2,3). Some of this evidence was discussed at this meeting and will be briefly reviewed here. Some of Ames' postulates may apply in specific cases. I do not believe, however, that there is sufficient evidence at the present time to use these postulates as a general basis for discarding current methods of carcinogen detection and risk extrapolation or for altering current guidelines for regulating potential carcinogens.

With respect to Ames' criticism of the standard NTP rodent carcinogen bioassay, we have heard evidence from several speakers that in most cases the positive results obtained in this bioassay cannot be attributed to nonspecific toxicity or excessive cell proliferation as a consequence of high doses of the test agent (3). Furthermore, various experimental studies indicate that there is no simple correlation between cell proliferation or hyperplasia and carcinogenicity. At the same time, cell proliferation is obviously necessary for mutagenesis and tumorigenesis, and in some situations it may be one of the rate-limiting events. I doubt, however, that sustained cell proliferation is in itself sufficient to drive the carcinogenic process. We have heard examples in which high doses of an agent might produce a type of cellular toxicity that does not occur at low doses, for example, the production of crystalluria in the bladder by saccharine or the precipitation of α1-globulin in the kidney by certain organic solvents. These examples are instructive, but they do not invalidate all of the rodent bioassays. Indeed, it should be emphasized that rodent bioassays have predicted several known human carcinogens, and we should not ignore the evidence that chemical carcinogens play an important role in the causation of human cancer. Thus, chemicals in cigarette smoke cause about one-third of all the cancers in the United States, and over 50 specific chemicals or industrial processes are known to be carcinogenic in humans. It appears that dietary factors also play a major role, but the precise chemicals in our diet that are involved and the mechanisms by which they act are, in general, not known.

During this meeting, several investigators discussed evidence that the birth, growth, and death of cells are complex phenomena. It is not sufficient, therefore, to speak only of cell proliferation or mitogenesis. De novo cell replication can be induced by various agonists, both endogenous and exogenous. Carcinogenicity is not simply a regenerative response to cell toxicity. Furthermore, the fate of newly synthesized cells is a function of numerous subsequent events, which include further exponential division, terminal differentiation, programmed cell death (apoptosis), and tissue necrosis. There is no assurance, therefore, that mitogenic stimuli will lead to mutation and clonal expansion. We need much more information on the possible effects of and mechanisms by which various xenobiotic chemicals...
influence cell cycling (see below), cell replication, differentiation, and apoptosis.

It seems likely that certain xenobiotics might produce their carcinogenic effects by disturbing specific steps in signal transduction pathways and the control of gene expression, thereby enhancing cell proliferation and clonal expansion. Thus, the potent tumor-promoting role of the phorbol esters and certain related compounds is due to their ability to bind to and activate protein kinase C (PKC), an enzyme that plays a key role in signal transduction (2,4). It will be of interest, therefore, to determine whether other non-genotoxic carcinogens (i.e., agents that do not directly damage DNA) might exert their effects through growth-factor receptors, phospholipases, G proteins, specific protein kinases, or transcription factors. Insights into these mechanisms would provide a more scientific basis for risk extrapolation between species and tissues and from high doses to low doses.

I want to emphasize that it appears that genotoxic agents, by inducing complex cellular responses to DNA damage, can also perturb cellular pathways of signal transduction and gene expression (2). Thus, the effects of genotoxic agents on cell proliferation may not simply reflect nonspecific toxicity that occurs only at high doses of the agent. The phenomenon of inducible responses to DNA damage complicates risk extrapolation. The dose-response curve for the mutagenic effects of a given genotoxic agent may not be the same as the dose-response curve for the inducible effects produced by the same agent. The dose-response curve for carcinogenicity might, therefore, be a complex function of both types of effects. This curve could in turn be a function of numerous other variables (i.e., the target tissue, the species and age of the host, and parallel exposure to other agents). The mutational spectra in the p53 gene discussed at this meeting provide an exciting example of how mutations in the DNA of tumor cells might provide a fingerprint of the original causative agent and/or the original mutagenic event(s) involved in carcinogenesis. It will be of interest, therefore, to examine the mutational spectra of altered genes in tumors produced by nongenotoxic agents or by putative endogenous factors such as hormones or reactive forms of oxygen. These mutational spectra, when compared to those produced by known genotoxic agents, may provide clues to the underlying mechanisms.

At this meeting, several investigators discussed recent exciting findings on the factors that control the cell cycle and of check points that monitor the fidelity of each phase of the cell cycle. It seems likely that certain xenobiotic agents might enhance the carcinogenic process by affecting specific cyclins, the cdk protein kinases activated by these cyclins, or various protein kinases and protein phosphatases that also regulate these events. Furthermore, mutations in the related genes during the course of multistage carcinogenesis could abrogate normal check-point control mechanisms or influence the fidelity of DNA and chromosome replication, thus contributing to genomic instability. In this regard, it was of interest to learn that mutations in the p53 gene appear to abrogate a check-point related to DNA damage. Our laboratory recently found that a cyclin D gene, Prad 1, is amplified and overexpressed in about 25% of human esophageal cancers (5). Abnormalities in this gene have also been seen in other types of human tumors (5,6). Thus the cyclin-ckd kinase system may represent an important set of cellular targets in multistage carcinogenesis.

Several speakers discussed interesting theoretical models of multistage carcinogenesis. My own view is that such models are highly useful in the design of new experiments. Since, however, each of these models makes several important assumptions, I believe that at the present time none of these models can be used to do quantitative risk assessment with confidence or precision. A major challenge for future research is to develop experimental methods for identifying and quantitating each of the individual steps in the multistage process so that risk assessment calculations can be done with greater confidence.

In conclusion, I want to list what I think are certain key questions and new frontiers in the field of environmental carcinogenesis. The first is that we need much more basic research on the cellular and molecular mechanisms that control cell proliferation, the cell cycle, and cell death. Closely related to these subjects is the need to expand our knowledge on mechanisms of signal transduction and the control of gene expression. Second, I would encourage intensive research on the possibility that certain xenobiotics exert their carcinogenic or other toxic effects on cells not by damaging DNA but by targeting specific cellular molecules involved in signal transduction, gene expression, and cell cycle control. Third, we need to develop convenient biomarkers that can be used to score for perturbations in the latter events, in cells in culture and in rodents that are exposed to putative carcinogens. It is hoped that some of these biomarkers can be used in humans as an extension of current molecular epidemiology studies because at the present time these studies are restricted mainly to studying genotoxic-type events. Fourth, we need to expand our research into the roles of dietary and hormonal factors in human cancer causation. It is possible that these factors act through both genotoxic and nongenotoxic mechanisms. In recent studies (4), we have obtained evidence that a high-fat diet might enhance colon carcinogenesis by leading to the formation in the lumen of the colon diacylglycerol, an activator of PKC. Other mechanisms should also be investigated, using both experimental models and human studies. Finally, the emerging interest in intervention approaches, including dietary modifications and chemoprevention, holds great promise in terms of cancer prevention (4). These approaches should also help validate (or invalidate) some of the predictions made from experimental and epidemiologic studies, thus improving the science of risk assessment.
On behalf of all the conference participants, I want to thank the organizers and sponsors for providing an informative and stimulating conference on a fundamental area of biology that is highly relevant to the field of environmental health science. I am certain that the discussions we have had will catalyze advances in this important field of public health.

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