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Application of Molecular Biomarkers in Epidemiology

by Martyn T. Smith[1] and William A. Suk[2]

Introduction

The conference "Application of Molecular Biomarkers in Epidemiology," was held at the National Institute of Environmental Health Sciences, February 21-22, 1990. The primary objective of the conference was to provide an up-to-date review of some of the molecular biomarkers currently available in order to promote discussion between laboratory scientists and epidemiologists on the utility of these biomarkers. Biomarkers are indicators of molecular and cellular events in biological systems that may allow epidemiologists and other health professionals to better examine the relationships between environmental hazards and human health effects. Biomarkers fall into three basic categories: biomarkers of dose, effect, and susceptibility. Many laboratories are using molecular biology and sophisticated chemical techniques to develop such biomarkers, but their application in epidemiological studies has been quite limited so far. The current and future use of biomarkers in epidemiological studies at Superfund sites and in the workplace was discussed.

This conference report is formatted somewhat differently from the other reports of the Superfund Basic Research Program in its series of conferences in 1990. The biomarkers conference was different in its presentation in that the series of short talks by the participants were used as discussion points to be expanded at the various round tables. The conference consisted of six sessions of 15-min presentations and two round-table discussions. On the first day, there were two sessions on biomarkers of carcinogenesis and biomarkers of chemical exposure, which were followed by a round-table discussion on the usefulness of these biomarker methods in epidemiology. A further session of talks on correlation studies in animal models followed. On the second day, there were three sessions of 15-min talks followed by a 2 hr round-table discussion at the end of the day. The first two sessions described biomarkers of individual phenotypic variability and biomarkers of health effects other than cancer, and the last session focused on the current use of biomarkers in epidemiological studies. A summary of the conference program is provided in the Appendix.

Biomarkers of Carcinogenesis

The first session was chaired by M. T. Smith (University of California, Berkeley) and I. B. Weinstein (Columbia University). Smith began the session by recounting recent progress in our understanding of the cancer process and the implied lessons for biomarkers. Work by Vogelstein and others clearly indicates that genetic alterations occur throughout the cancer process. Moreover, these genetic changes are not only point mutations, but chromosome-wide events such as aneuploidy-induction and translocations. Screening chemicals for their ability to induce genetic damage should therefore involve looking at many different end points.

Molecular biology advances should allow investigators to evaluate chemicals much more rapidly than current cytogenetic approaches. W. Thilly (Massachusetts Institute of Technology) characterized an approach that involves the use of a high-fidelity polymerase chain reaction and denaturing gradient gel electrophoresis. This method allows his group to amplify specific segments of DNA (e.g. an exon or coding region of the gene coding for hypoxanthine-guanine phosphoribosyl transferase; *hprt*) and to study the spectrum of mutations produced by chemical exposure and by spontaneous/background events. Thilly described mutational spectra produced by various chemicals including hydrogen peroxide and spontaneous events, and showed how they differ markedly. He suggested that this rapid and simple methodology could be used on large numbers of people, individually and as pooled samples, to identify mutational spectra characteristic of particular chemical exposures. Thilly's approach of looking directly at the genotype differs from, but is complementary to, that taken by R. Albertini (University of Vermont), who addressed the selection of lymphocytes with a mutant phenotype resulting from changes in their *hprt* gene. These lymphocytes can then be analyzed for the nature of the mutations in *hprt*. Albertini recounted how deletions at least as large as 5 megabases can be tolerated at *hprt* locus (on the X-chromosome) and how different clones of lymphocytes can be produced by

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different types of mutation at *hprt*. Albertini's group has shown that the mutant frequency at *hprt* in newborns is much lower than in adults and increases with age. Moreover, *hprt* in lymphocytes is sensitive to smoking. Thus, epidemiological studies using *hprt* mutational analysis would have to control for both age and smoking.

Another gene that is used to analyze for genetic mutation in humans is the gene that codes for the glycophorin A protein on the surface of red blood cells. W. Bigbee (Lawrence Livermore National Laboratory) reported on the development of state-of-the-art flow cytometric methods to measure mutations in the glycophorin A gene. Because red blood cells do not contain DNA, the red mutational events must occur in the bone marrow precursor cells. Thus, glycophorin A is particularly suitable for screening human exposure to mutagenic chemicals that act on the bone marrow to potentially cause leukemia and other hematological disorders. This group recently published a new version of the glycophorin A assay that can be performed on commercially available flow cytometers.

Different methods of measuring micronuclei (small DNA-containing membrane-bound vesicles outside the main cell nucleus) that arise via chromosome lag or fragmentation in peripheral blood cells were reported on by J. T. MacGregor (SRI International). Micronuclei are potentially important for the detection of aneuploidogens and clastogens.

G. Lucier (National Institute of Environmental Health Sciences) reported how his group has compared different biomarkers *in vitro* including correlating DNA adduct formation with various genetic end points. One new assay of genetic damage they have helped develop measures nucleoid sedimentation and correlates closely with DNA strand breakage.

At the end of the session, Weinstein discussed the need for markers of epigenetic events in carcinogenesis as well as ones of genetic alterations. He reviewed the need for biomarkers of clonal cell proliferation and discussed the role of increased protein kinase C activity in promoting cancer. His group is studying the relationship between increased "phorbin" gene expression and protein kinase C activation. They plan to measure release of the phorbin gene product into the serum as a possible marker of this activation and clonal cell proliferation.

Biomarkers of Chemical Exposure

The second session began with B. Hammock (University of California, Davis) reviewing and evaluating immunological approaches to assess human exposure to toxic chemicals. He related how immunoassays could be used in practical as well as mechanistic studies, in environmental monitoring to study food residues, groundwater contamination, and spills at waste disposal sites. Biological monitoring could also be facilitated, as immunoassays for a number of protein and DNA adducts have been developed. Another potential use of antibodies is the cleanup of samples by immunoaffinity chromatography before more elaborate chemical analysis.

R. Haas (California Department of Health Services) expanded upon this dialogue, reporting on the production of antibodies to detect altered blood proteins for biological monitoring. He depicted attempts to synthesize hemoglobin adducts of styrene oxide and hydroquinone, and to generate antibodies to these modified blood proteins. The major obstacle to this procedure has been the need to overcome the extreme immunogenicity of hemoglobin, so that a minor modification by a particular environmental toxicant may not lead to production of antibodies against the adduct, only against hemoglobin. Both Hammock and Haas stressed the importance of hapten synthesis. Covering the functional group of importance during coupling to a carrier is a common cause of failure to produce the desired antibodies.

The formation of polycyclic hydrocarbon esters in human hemoglobin and their characterization by synchronous fluorescence spectrometry was reported on by P. Skipper (Massachusetts Institute of Technology). Using this state-of-the-art technique, his group found in human blood average levels of 3–10 pmole of benzo[a]pyrene/g globin, which was unaffected by smoking status. A new method for looking at cysteine residues or cysteinyl adducts of blood proteins was recounted by S. Rappaport (University of North Carolina, Chapel Hill). Raney nickel is used to cleave the C–S bond between the adducted chemical and the cysteine residue and to subsequently measure the released product. His group has thus measured adducts formed between styrene oxide and hemoglobin or albumin in the blood of styrene-exposed workers. Using data from both *in vitro* and *in vivo* experiments, Raney determined the amount of alkylation by styrene oxide. He calculated that the method he described could detect exposures between 0.4 and 4.0 ppm in workers and approximately 0.2–2.0 ppm in the general population, The method, which should be applicable to many other chemicals, is being refined to improve sensitivity.

DNA–protein crosslinks are a major form of genetic damage that can be readily analyzed. M. Costa (New York University) illustrated that in cells exposed to *cis*platinum or chromium VI, four major proteins were complexed to the DNA. Costa's group has unambiguously identified the protein crosslinked to the DNA as actin, based on proteolytic maps, reaction with an actin antibody, and alterations in restriction enzyme digestion of DNA containing the crosslinked protein. However, when cells are exposed to formaldehyde, histones, not actin, are complexed to the DNA. Thus, particular classes of chemicals may produce different protein–DNA crosslinks. In addition to being a promising biomarker of exposure, DNA–protein crosslinking is a major form of genetic damage and one that persists during cell proliferation. Costa suggested this approach could be used to identify exposure to particular groups of toxic chemicals and also delineated a new technology for measuring DNA–protein crosslinks that relies on SDS-PAGE gel electrophoresis and electroblotting.

Recounting his experience in measuring DNA adducts in human populations, K. Hemminki (University of Helsinki) described using the ^{32}P-postlabeling procedure and immunoassays to determine the formation of DNA adducts in Finnish foundry workers. Rather than absolute quantitation of polycyclic aromatic hydrocarbon (PAH) DNA adduct formation, he used a score of adduct formation to express his results. He gave the foundry workers a 2.1 adduct score. After vacation, they had a 1.0 adduct score, demonstrating that adduct levels were transient and stable for only approximately 1 month. Hemminki then compared PAH–DNA adducts in Polish foundry workers and in local and rural residents. The score in local residents was almost as high as in the foundry workers, both of which were significantly higher than in the rural area. This emphasizes the

importance of assessing background environmental exposure in any studies of worker populations. He further stated that quantitative aspects of ^{32}P-postlabeling were not sufficiently studied and described the difficulties in producing quantitative numbers with these methods. ^{32}P-postlabeling can best be used to assess relative exposures rather than absolute levels of exposure/dose.

Urinary metabolites can also be used to assess chemical exposures. While these may be transient in nature, it is important to note that a large majority of chemicals are converted mainly to urinary metabolites, which makes this relatively simple screening approach widely applicable.

A. Buckpitt (University of California, Davis) defined the potential for monitoring naphthalene exposure through urinary metabolites. Because the 1*S*,2*R* epoxide of naphthalene is the major binding species and pulmonary toxicant, Buckpitt related the importance of screening for the 1*S*, 2*R* mercapturic acid in urine when quantitating the biologically important dose of naphthalene. By comparing it to the 1*R*,2*S* levels, one can determine the ability of people to convert naphthalene to an active metabolite capable of causing injury to the lung.

Round-Table Discussion 1

The charge to the first round-table discussants was to evaluate whether the biomarkers described in the first two sessions (biomarkers of carcinogenesis and biomarkers of chemical exposure) meet the necessary epidemiological criteria. The round-table discussion clearly indicated the tremendous communication gap between many practicing epidemiologists and those laboratory scientists currently developing molecular biomarkers. The major points raised can be summarized as follows. Epidemiologists need well-characterized, simple, sensitive, and inexpensive ways of assessing exposure and health effects or predictors of health effects that can be applied to large populations. Laboratory scientists tend to want to be on the cutting edge of the development of new biomarkers and are continuously pushing forward using the latest techniques (and associated jargon) in developing such methods. Although laboratory scientists tend to be consistently dissatisfied with their present product, epidemiologists are willing to use these methods before all the problems associated with the methods are worked out.

Epidemiologists argue that the utility of the new technology has to be tested in the field, where populations are exposed to a variety of potentially hazardous substances. In their study of the associations between exposure to toxic chemicals and various disease end points, epidemiologists often find exposure assessment to be one of the main problems of epidemiological studies.

Epidemiologists are eager to try out new tools that may prove to be more sensitive and accurate in order to better understand the relationships between the processes leading from exposure to disease. This applies especially to environmental exposures that affect many people, yet the responses elicited by exposure are relatively low and homogeneously distributed so that new and more sensitive detection methods are sorely needed. Epidemiologists also pointed out that laboratory scientists need to be aware that the methods they develop can only be applied in accessible tissues and that this tissue may have to be frozen or preserved in some manner before analysis. Issues relating to stability of biomarkers during storage were therefore discussed, and laboratory scientists were encouraged to study this in more detail. R. Montesano (International Agency for Research on Cancer) raised the point that the prevalence of genetically susceptible subpopulations may significantly influence the range of background levels in the general population, and better characterization of these subgroups will be essential.

Correlation Studies in Animal Models

J. Swenberg (University of North Carolina, Chapel Hill) illustrated how his group has measured DNA adduct formation in tissues of rodents exposed to the carcinogens vinyl chloride and ethylene oxide. Ethylene oxide is a brain carcinogen in rodents and forms the most DNA adducts in this tissue, but it also produces considerable levels of DNA adducts in nontarget tissues. Studies in animal models can therefore be used to correlate a particular biomarker with subsequent carcinogenic outcome.

Comparing various biomarkers in rodent studies using benzene, butadiene, and other compounds, R. Henderson (Lovelace Inhalation Toxicology Research Institute), reported on how 82–91% of the metabolized dose of these chemicals was converted to urinary metabolites detectable for only 48 hr. She pointed out, however, that given this large percentage conversion rate, urinary metabolites should be an effective biomarker of continuous low-level exposures. Only 0.1–0.4% of the metabolized dose was converted to hemoglobin adducts, but such adducts do, of course, accumulate. She further warned of the dangers of using rat hemoglobin as a surrogate for human hemoglobin, as the former is more sensitive to alkylation. Mouse hemoglobin seems to be a far more appropriate animal model.

At the end of the first day, G. Wogan (Massachusetts Institute of Technology) reported on work in his laboratory that correlated the formation of DNA adducts in rat liver with the activation of *ras* oncogenes and the subsequent development of liver tumors. The analytical methods used to detect their presence at an early stage are readily applicable to human tissues and may therefore provide a means for detection of early premalignant changes in humans exposed to environmental carcinogens. Wogan has shown that chemicals representative of the generic class of genotoxic chemicals (i.e., chemicals that have the ability to damage DNA and cause mutations and cancer) have been shown to activate oncogenes by inducing similar mutations, and detection of these in preneoplastic cells may provide a sensitive means to detect hazardous environmental exposures.

Biomarkers of Individual Phenotypic Variability

The second day began with descriptions of several new ways of assessing differences in the ability of humans to metabolize various chemicals, either to toxic or nontoxic products. There are substantial differences in the levels of particular enzymes involved in the metabolism of chemicals in people. These differences seem to be related to both genetic and environmental influences. Especially interesting were the depictions of noninvasive methods allowing epidemiologists to characterize a given individual's ability to metabolize certain groups of chemicals. An example of this was the method related by M. A. Butler (National Center for Toxicologic Research), which requires humans to

drink coffee and then have their urine analyzed for the manner in which they metabolize caffeine. This has been found to be associated with their levels of cytochrome P-450 IA2, which demethylates caffeine at the 3-position to 1,7-dimethylxanthine. By determining the ratio of this 1,7-metabolite to others in the urine, it is possible to assess an individual's P-450 IA2 activity level. This is important because this P-450 enzyme catalyzes the first step of arylamine activation to metabolites that are carcinogenic in the bladder. Studies in Italian and Chinese populations have shown a bimodal distribution of activity with $<30\%$ of the populations having high P-450 IA2 activity. Studies are underway to determine if workers who developed bladder cancer from exposure to arylamines are rapid caffeine metabolizers with high P-450 IA2 activity.

F. P. Guengerich (Vanderbilt University) recounted how more than 20 cDNA sequences were known for human cytochrome P-450s, but three seem to predominate in their importance in the activation of procarcinogens. These were P-450 IA2, discussed above, which is also called $P\text{-}450_{PA}$ and activates phenacetin, arylamines, and food pyrolysis products; P-450 IIIA4, also called $P\text{-}450_{NF}$, which metabolizes nifedipine, aflatoxins, polycyclic dihydrodiols, and 6-aminochrysene; and P-450 IIE1, also called $P\text{-}450_{j}$, which metabolizes ethanol, benzene, alkylnitrosamines, and chlorinated hydrocarbons. These P-450s are inducible (e.g., IA2 by cigarette smoke, PAHs etc., and IIIA4 by barbiturates). Noninvasive markers of activity are available for IA2 (caffeine) and IIIA4 (nifedipine), and Guengerich's group is actively working on a marker for IIE1.

About 40% of the human population lack the μ class of glutathione transferases through gene deletion. Previous studies have shown a correlation between lack of glutathione transferase μ and lung cancer risk. J. Wiencke (University of California, San Francisco) discussed how his group demonstrated that lymphocytes of persons lacking μ transferase are much more susceptible to genetic damage from epoxides. He indicated, therefore, that lack of μ transferase may be an important susceptibility factor for genetic damage from environmental carcinogens.

Differences in the ability of humans to metabolize chemicals via acetylation are well known. W. Weber (University of Michigan) reported how there has recently been much progress in understanding the differences within the human population of the enzymes involved in these particular metabolic processes. There is promise that we will soon be able to use several noninvasive techniques to characterize the differences in human populations and their ability to metabolize chemicals.

Biomarkers of Health Effects Other Than Cancer

The fifth session opened with D. Katz (University of California, Davis), summarizing how computer-assisted sperm analysis can be used to assess male reproductive capability. Using this technique, his laboratory demonstrated that perchloroethylene exposure can cause subtle changes in sperm motility, which may lead to reduced fertility. Another researcher from the University of California, Davis, B. Lasley, is developing biomarkers of female reproductive capability based on nonradiometric assays for urinary metabolites of pituitary, ovarian, and placental hormones. Such assays could provide a rapid means of detecting early pregnancy loss and ovarian function in women exposed to toxic hazards. Much work needs to be done, however, to determine the endocrine profiles of reproductive abnormalities in normal women with these methods before the methods can be effectively used in epidemiological studies.

The need for additional, practical biomarkers to screen for birth defects was discussed by M. Khoury (Centers for Disease Control). Cytogenetic characterization of markers such as trisomy of chromosome 21 in Downs syndrome should become much easier with the application of chromosome-specific fluorescent hybridization probes. The application of molecular technologies such as the polymerase chain reaction should also make detection and characterization simpler because only a few cells are needed. In a series of population-based neurotoxicological studies, L. Costa (University of Washington) related how human lymphocytes express many of the neurochemical receptors found on nerve cells. This is an important research advance and presents a myriad of opportunities, empowering human lymphocytes' use as accessible surrogates for dopamine and other neurochemical receptors on nerve cells. Analysis of receptor levels/occupancy on lymphocytes may therefore provide peripheral markers of neurotoxic effects.

It was reported by C. Snyder (New York University) how different immune function assays can be used as indicators of chemically induced immune system damage. He also discussed some of their associated pitfalls. A. Brody (National Institute of Environmental Health Sciences) portrayed recent progress toward developing biomarkers of pulmonary injury, especially markers of exposure to particulate matter. The best marker of exposure to particles is the detection of the inhaled material at the sites where the earliest inflammatory lesions and subsequent disease develop. In animal studies it has been possible to determine the amount of particulates that deposit on the bifurcations of alveolar ducts. Whether such markers could be useful for human studies is difficult to predict. By using autoradiography it is also possible to measure cell proliferation in the lung and to predict which cell populations are the first to respond to particulates and noxious agents. For further information on biomarkers of pulmonary toxicity, readers are referred to the National Research Council monograph (*1*).

Liver damage and subsequent cell proliferation could be measured by release of certain liver-specific growth factors into the bloodstream. G. Michaelopoulos (Duke University Medical Center) delineated how, for example, hepatopoietin A is a 100,000 molecular weight liver cell mitogen and growth factor that is released into the blood during regenerative cell growth after liver damage. An ELISA assay has been developed to measure this factor in blood, which may be a highly sensitive biomarker of subtle damage and hepatic cell proliferation.

Use of Biomarkers in Epidemiology Studies

The final session of the conference concentrated on the current use of biomarkers in epidemiological studies. Case histories of the use of biomarkers such as protein adducts, DNA adducts, sister chromatid exchange (SCE), and micronuclei were recounted. S. Tannenbaum (Massachusetts Institute of Technology) began the session by characterizing the use of hemoglobin adducts to assess exposure to arylamines such as 4-aminobiphenyl (ABP). Working together with epidemiologists

from the National Cancer Institute and the University of Turin, Italy, his group has shown that ABP adduct levels are higher in smokers of black tobacco (as opposed to blonde tobacco), which is associated with a higher risk of bladder cancer. Moreover, in all groups, including nonsmokers, they found about a 50% higher adduct level in persons of the slow acetylator phenotype. The adduct level was therefore affected by the type of tobacco smoked and the individual's acetylator phenotype. F. Perera (Columbia University) presented a series of large ongoing epidemiological studies, the objective of which is to correlate various biomarkers of exposure, effect, and susceptibility in humans exposed to styrene, aminobiphenyl, ethylene oxide, and polycyclic aromatic hydrocarbons. Similarly, J. Yager (Electrical Power Research Institute) reported on a study performed on styrene-exposed workers in Washington State. Extensive industrial hygiene measurements of worker dosimetry were performed on this population so that correlations could be made between worker exposure and various biomarkers. The biomarkers studied included DNA adducts, hemoglobin adducts, and SCE and micronuclei formation in peripheral lymphocytes. Only SCE induction data were fully analyzed at the time of the meeting, and Yager delineated statistical approaches to determining the relative contributions of styrene exposure, cigarette smoking, age, and other factors. An approximate 10% chemically related increase in SCE levels can be measured by this approach with statistical significance in 20–40 individuals.

Round-Table Discussion 2

In the final 2 hr of the conference, a second round-table discussion was held. A group of eight epidemiologists and a group of laboratory scientists were assembled. Both groups were asked to respond to two questions: How can current biomarkers be used most effectively? What new types of biomarkers need to be developed?

M. Schenker (University of California, Davis) started the discussion by emphasizing the need for biomnarkers of exposure because of the importance of ascertaining exposures in epidemiological studies. L. Fine (National Institute of Occupational Safety and Health) followed with comments about how biomarkers of exposure could be used in intervention studies to test the effectiveness of particular control strategies. He then raised an ethical concern regarding the misuse of biomarkers of susceptibility, cautioning that these biomarkers could potentially and incorrectly serve as a screening mechanism to exclude groups of workers from the workplace. Fine concluded his remarks by pointing out that many occupational and environmental studies are now evaluating nonclassical exposures, such as those from electrical fields, and that biomarkers need to be developed that can evaluate these kinds of exposures.

C. Shy (University of North Carolina, Chapel Hill) focused the discussion on ways of evaluating the effectiveness of using biomarkers in field investigations. The feasibility of biomarkers as an epidemiological tool could only be determined, for example, when the following information is known: what types of samples need to be collected; how much of the samples can be stored; and how long the samples can be stored. Shy raised questions about study and research design that he felt would also have to be clarified, including determination of the size of the sample required in order to have sufficient power, whether serial sampling would be necessary, the associated costs of carrying out the studies, and the level of expertise required both in the field and in the laboratory. Finally, Shy emphasized the need to correlate biomarkers with the standard techniques currently used to evaluate exposures and the need for "gold standards" to assess their validity.

The importance of correlating the presence or level of a particular biomarker with a clinical outcome was highlighted by H. Checkoway (University of Washington). Through such correlation, the significance of detecting a change in a biomarker could be ascertained. His remarks were followed by J. Andrews (Agency for Toxic Substances and Disease Registry), who counseled investigators to use biomarkers in field studies so that the validity, sensitivity, and usefulness of the studies could be determined. A. Caporaso (National Cancer Institute) concurred that the use of biomarkers as measures of exposure, dose, or susceptibility must be validated in epidemiological studies and urged epidemiologists to work closely with laboratory scientists.

Addressing the use of a biomarker as a tool in epidemiological investigations, A. Wilcox (National Institute of Environmental Health Sciences) pointed out that epidemiologists need simple, well-characterized, repeatable standard assays that can be applied in studies of hundreds or thousands of people. Underscoring those remarks, S. Swan (California Department of Health Services) then drew the audience's attention to the potential problem of sample and selection bias that would arise if specially self-selected groups within the population were more likely to participate in studies where samples of urine or semen, for example, were collected on a regular basis. She also suggested conducting paired studies to compare the more traditional epidemiological measures with biomarkers currently being developed.

Perera concluded the discussion among the first group of scientists by suggesting that the most effective use of biomarkers would be to incorporate them in a series or battery of tests to be performed in a population-based study, and including both specific and generic types of markers. She also commented on the utility of comparing biomarkers in human and animal studies and how such correlative studies would provide opportunities to more fully validate promising biomarkers.

The second group of scientists assembled with Smith leading the discussion. He pointed out the importance of biomarkers of effect because of the latency period often involved with cancer and other forms of disease. He also raised the prospect of technology transfer of techniques currently being developed and suggested that the techniques could be transferred to large government or contract laboratories that could perform analyses on large numbers of samples. This would help bridge the gap between development laboratories and field epidemiologists. Finally, Smith brought up the ethical dilemma that will face scientists when studies are conducted involving individuals living near Superfund sites. What do the investigators tell the public? How do scientists disseminate information about the significance of the presence of biomarkers and how they correlate to risk of disease? How are the uncertainties about risk estimates explained?

W. Suk (National Institute of Environmental Health Sciences) framed his comments within the context of the goals of the Superfund Basic Research Program. He stressed the importance of the

need for epidemiologists and molecular biologists to work together in the development and validation of advanced techniques for the detection, assessment, and evaluation of the effects on human health of hazardous substances, especially at low levels of exposure. Furthermore, he explained that new and cogent methods to assess the risks to human health presented by hazardous substances need to be effectively dealt with if one is to take basic research and implement it practically and productively in the field. Suk then elaborated on Smith's comments about technology transfer and added that he viewed epidemiologists as instrumental in carrying out this transfer as biomarkers are moved from the laboratories to the field.

Emphasizing the necessity of developing predictive biomarkers and urging that the scientific community not settle for poorly characterized markers, C. Harris (National Cancer Institute) pointed out that such research efforts will require a large expenditure of funds. He called for continued investigation into molecular mechanisms in developing biomarkers of effect for cancer. It is his opinion that investigators should focus future efforts on tumor-suppressor genes, not activated oncogenes, and concentrate on developing technologies for markers of effect in this area. He was confident that these tools would be forthcoming but that they would involve sophisticated techniques and would be expensive. Harris ended with remarks about our growing understanding of genetic predisposition as a factor affecting an individual's risk of disease and reiterated earlier comments about the ethical issues that are raised surrounding potential worker discrimination issues.

The need to incorporate relevant biomarkers into monitoring and animal studies and to have these studies examine different end points to determine if the biomarkers are predictive in these models was the focus of Yager's comments. She also raised the question of how to determine when an assay is robust enough (i.e., predictable enough, sensitive enough, specific enough) to be used. Finally, she underscored the need to identify the sources of biological variability in the development and application of biomarkers.

The final remarks from the group came from Wogan, who reemphasized comments made earlier about the practicability and accessibility of the sample material. He added that application of biomarkers to broad segments of the population would be limited unless techniques developed in the laboratories could be translated into field procedures applied to readily accessible bodily fluids. Wogan then provided the group with a historical perspective by pointing out that the development of science has been driven in the past by the emergence of new and important technologies and that he foresaw an exciting period of cancer research ahead. Furthermore, as technologies acquired usefulness, the associated costs would decline and he was hopeful that the new biomarker technologies currently being developed could be made cost effective.

Summary

The principal conclusions and opportunities that can be drawn from this conference are as follows. The meeting demonstrated the large communication gap that still exists between most epidemiologists and laboratory scientists. This problem could be overcome if epidemiologists worked closely with laboratory scientists at the outset of any project so that a better understanding could be built between them. Epidemiologists need simple, well-characterized, reproducible assays that can be applied to hundreds or thousands of people. Most laboratory scientists have little interest in running large numbers of assays, but wish to continually refine their methods so that they stay on the "cutting edge" of basic research. This problem could be overcome if the new laboratory technology could be transferred to contract laboratories or small companies. Problems of technology transfer therefore need to be addressed. Current and new biomarkers need to be better validated in the field and by studying animal models. More information on the background expression of biomarkers in the general population is needed (i.e. what is the normal range?). Ethical issues, such as the possibility that biomarkers of susceptibility could be used to exclude people from the workplace, need to be addressed.

Appendix

Application of Molecular Biomarkers in Epidemiology: Program

Welcome and NIEHS view
D. Rall, NIEHS
Introduction to Meeting
M. Smith, University of California, Berkeley
Epidemiological criteria for the perfect biomarker
R. Neutra, NIEHS
IARC view of biomarkers
R. Montesano,

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hprt Mutations in T-lymphocytes
R. Albertini, University of Vermont
Glycophorin A assay
W. Bigbee, Lawrence Livermore National Laboratory
Micronuclei formation in human blood cells
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G. Lucier, NIEHS
Biomarkers of promotion
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B. Hammock, University of California, Davis
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R. Haas, California Department of Health Services
PAH esters in human hemoglobin
P. Skipper, Massachusetts Institute of Technology
Cysteinyl adducts in blood proteins
S. Rappaport, University of North Carolina, Chapel Hill
DNA-protein crosslinks as biomarkers of exposure
M. Costa, New York University
DNA adducts as biomarkers
K. Hemminki, University of Helsinki
Urinary metabolites
A. Buckpitt, University of California, Davis

Session III
Relationships between DNA adducts, cell proliferation, and carcinogenesis
J. Swenberg, University of North Carolina, Chapel Hill
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R. Henderson, Lovelace Inhalation Toxicology Research Institute
Correlations between DNA modifications, oncogene activation, and hepatocarcinogenesis

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G. Wogan, Massachusetts Institute of Technology
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F. P. Guengerich, Vanderbilt University
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M. A. Butler, National Center for Toxicological Research
Human glutathione transferase μ deficiency
J. Wiencke, University of California, San Francisco
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W. Weber, University of Michigan

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D. Katz, University of California, Davis
Assessment of female reproductive capability
B. Lasley, University of California, Davis
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M. Khoury, Centers for Disease Control
Peripheral markers of neurotoxicity
L. Costa, University of Washington
Immune function assays as indicators of toxicant exposure
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H. Checkoway, University of Washington
Aflatoxin
J. Groopman, Johns Hopkins University
Inherited predisposition and molecular dosimetry in cancer risk
C. Harris, National Cancer Institute

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ADDITIONAL READING

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