Markers of Exposure to Carcinogens

by Gerald N. Wogan*

Methods have been developed for the detection of exposure to carcinogens and other DNA damaging agents in experimental animals and man through the detection of carcinogens or their metabolic derivatives in body fluids, or through adducts bound covalently to DNA or hemoglobin. The successful use of urinary markers of genotoxic exposures has been reported with respect to nitrosopine as an indicator of exposure to N-nitroso compounds. The same approach has been used to detect AFB₁ and AFB₁-N'-Gua as markers of exposure to aflatoxin B₁; of 3-methyladenine produced as a result of exposure to methylating agents; and thymine glycol as an indicator of exposure to agents causing oxidative damage to DNA. Detection of adducts formed between genotoxic agents and hemoglobin has been reported in studies of populations occupationally exposed to ethylene oxide, in which 3-hydroxyhistidine and 3-hydroxyvaline have been measured, and in smokers, whose hemoglobin has been found to contain levels of 4-aminobiphenyl and 3-hydroxyvaline that were correlated with the frequency of cigarette smoking.

Detection of DNA adducts of genotoxic agents in the cells and tissues of exposed individuals has also been accomplished through the use of several types of analytical methods. Immunoassays and physicochemical methods have been applied to detect adducts formed through the major intermediate in the activation of benzo(a)pyrene, the 7,8-diol-9,10-epoxide (BPDE). This adduct has been found in the DNA of peripheral leukocytes of workers in foundries, aluminum manufacturing plants, roofers, and coke oven plants, and also in cigarette smokers. BPDE-DNA adducts have been detected by synchronous scanning fluorescence as well as by immunoassays conducted in ELISA or USERIA modes. The successful application of immunoassays to detect DNA adducts of cis-platinum in leukocytes of ovarian cancer patients receiving chemotherapy and O²-methyl guanine in the blood of populations at high risk for esophageal cancer has also been reported.

The method of ³²P-postlabeling for the detection of DNA adducts has confirmed their presence in placentae, peripheral leukocytes, and oral mucosal cells of tobacco smokers, as well as coke oven and foundry workers. Increased total levels of adducts were, in general, reflective of elevated levels of exposure.

Introduction

It is an honor to have been invited to participate in this symposium on “Scientific Advances in Environmental Health,” especially since its purpose is to recognize the many contributions of Dr. Norton Nelson to the development of the modern field of environmental health sciences and to the founding of the Institute of Environmental Medicine. Through his wisdom, insight, creativity, organizational genius and inexhaustible energy, he has played a major role in the initiation and development of many aspects of environmental health science throughout the international scientific community. The nature, scope, and direction of virtually every major component of current environmental health science research have been influenced directly or indirectly by Dr. Nelson’s activities. It is therefore entirely appropriate that this symposium has been organized in his honor, and it is a personal pleasure for me to have this opportunity to express my admiration, respect, and appreciation for his many contributions.

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In certain respects, the current status of the field of markers of carcinogen exposure represents a culmination of efforts to bring together current knowledge about mechanisms of carcinogenesis and mutagenesis and highly sophisticated analytical methods to detect molecular and cellular events that may be predictive of health risks. This is a very timely and important area of research endeavor, inasmuch as the process of risk assessment is finding increasing application by regulatory decision-making bodies in the process of formulating policies intended to minimize health risks resulting from exposure to hazardous substances. The process of risk assessment requires the use of factual data to define the health effects of exposure of individuals or of populations to such substances. By current definition, the process of risk assessment includes three elements: hazard identification, dose-response assessment, and risk characterization. Exposure assessment and epidemiology data are key components of the dose-response assessment, the objective of which is to define dose-incidence relationships for adverse health effects (such as cancer) in human populations. These components, together with animal bioassay data, appropriate extrapolations of information concerning dose-effects in animals, and interspecies differences
in response, effectively determine the quantitative features of the risk estimation and are therefore of critical importance.

Epidemiologic studies designed to evaluate the health significance of environmental chemicals, including carcinogens, are seriously compromised by the lack of quantitative exposure data for individuals in exposed populations. Monitoring data on levels of compounds in environmental media often represent the only information available, and average population exposure is therefore the only quantitative parameter that can be calculated. Biological monitoring, i.e., measurements that can be made on cells, tissues, or body fluids of exposed people, has the objective of defining "dose" (internal or biologically effective) on an individual basis. Information gained from such measurements has the potential for identifying potentially hazardous exposures before adverse health effects appear and also for establishing exposure limits for minimizing the likelihood of significant health risks. Because measurements are made on individuals, they provide an indication not only of exposure to a given substance, but also of the amount absorbed and metabolically transformed to activated derivatives, viz., the fraction bound to functionally important cellular sites. Biological monitoring data provide information complementary to that derived from analysis of environmental media, and because they can be interpreted in the context of known mechanisms of action, are more directly relevant to assessment of health risks. Exposure assessment strategies based on biological monitoring can therefore be designed to take into account exposures through multiple routes and integrate the consequences of intermittent as well as continuous exposures. They can also provide evidence of total risk from multiple sources and to multiple agents.

Certain attributes will be indispensable for a methodology to be adequate to provide accurate, quantitative measurements of exposure to environmental carcinogens and at the same time to fulfill the objective of providing early indication of long-term risk of cancer. Important among these attributes are the following: a) The analytical methods must be adequate to detect and quantify exposure to carcinogens/mutagens at ambient levels in the environment; b) they must be applicable to cells or body fluids that are readily accessible; c) measured values should be quantitatively related to exposure levels over a wide range; and d) they should integrate consequences of intermittent or continuous exposures to multiple agents. All of these attributes are applicable to accurate dosimetry of exposure alone. In order to be applicable in addition to the assessment of health risk, the methods should also be capable of detecting early biological effects predictive of long-term adverse health risks (i.e., cancer). The methods used in producing the information summarized in the following discussion are applicable to detection of exposure; at present, none of these can be considered adequately validated to provide direct evidence of cancer risk. Strategies for use of exposure information in the prospective assessment of genetic risk represent important future challenges, and their development and validation will require a great deal of additional research.

The subjects of biological monitoring and its applications in the field that has come to be known as molecular epidemiology have been the subjects of several recent comprehensive reviews. This discussion will emphasize detection methodology and will summarize currently available information on carcinogen exposure. A broader perspective on the field can be found in several more comprehensive reviews (1–8).

### Indicators of Genotoxic Exposures

Methods for detecting exposures to DNA damaging agents fall into two categorical types: measurements of levels of genotoxic chemicals, their metabolites and/or derivatives in cells, tissues, body fluids or excreta; and measurements of biological responses such as cytogenetic changes in exposed individuals. This discussion will emphasize recent developments in the former category, although important advances are being made in the development and application of biological indicators of genotoxic exposures as well. These include mutation markers based on altered phenotype [e.g., hypoxanthine phosphoribosyl transferase (HPRT) deficiency in peripheral leukocytes; altered gene product (e.g., mutant glycoporphin in erythrocyte membranes); gene inactivation (loss of specific mRNA); or altered DNA sequences (restriction fragment length polymorphism, or translocations involving oncogene sequences)]. Chromosomal markers that have been useful in detecting damage from genotoxic agents include structural aberrations, sister chromatid exchanges, and aneuploidy. The presence of micronuclei in circulating erythroid cells provides an additional index of genotoxicity.

Chemical dosimeters for several types of genotoxic exposures are also available for detection of carcinogen exposures. Major approaches depend on chemical or immunological analytical methods for the detection of carcinogens or their metabolic derivatives in blood or urine. Mutation assays in bacteria have also been used to detect the presence of mutagens in urine in relation to environmental exposures. A third avenue of investigation has been the detection of covalent adducts formed between carcinogens/mutagens and cellular macromolecules such as proteins (e.g., hemoglobin), or cellular DNA, or excreted in urine.

### Detection of Carcinogen Exposure in Man

Development of these detection methodologies has permitted studies designed to evaluate their adequacy for detecting exposure to carcinogens in man. Results of several such studies have been published, and Tables 1–5 summarize currently available data extracted from published reports dealing with various aspects of biomonitoring of exposure of humans to genotoxic agents of a vari-
Carcinogen exposure markers

Table 1. Urinary excretion of markers of genotoxic exposures.

| Urinary marker          | Exposure analysis | Exposure level | Excretion rate |
|-------------------------|-------------------|----------------|----------------|
| Nitrosamino acids       | Unknown           | High risk area (44) | 21.2 µg/day   |
|                         |                   | Low risk area (40) | 5.6 µg/day    |
| Nitrosoproline          | Cigarette smoke   | Smokers (13)     | 5.9 µg/day    |
|                         |                   | Nonsmokers (13)  | 3.6 µg/day    |
| Nitrosoproline          | Unexposed         | Nonsmokers (24)  | 3.3 µg/day    |
| AFB1                   | Diet              | Exposed subjects (20) | 0.1–10 µg/day, AFB1 equivalents |
| AFB1-N7-GuA            | Diet              | Low/high risk areas (983) | 12% positive |
| 3-Me-adenine           | None              | Unexposed subjects (9) | 4.5–16.1 µg/day |
| Thymine glycol         | None              | Unexposed subjects (9) | 0.39 nmole/kg/day |
| Thymidine glycol       |                   |                 | 0.10 nmole/kg/day |

*Numbers in parentheses are numbers of subjects.

Table 2. Hemoglobin adducts as markers of genotoxic exposure.

| Compound analyzed | Exposure source | Exposure level | Adduct level |
|-------------------|-----------------|----------------|--------------|
| N-3-(2-Hydroxyethyl)histidine | Ethylene oxide (occupational) | Exposed (5) | 0.5–13.5 nmole/gHb |
|                   | Control (2)     |               | 0.05 nmole/gHb |
| N-3-(2-Hydroxyethyl)histidine | Ethylene oxide (occupational) | Exposed (32) | 2.08 nmole/gHb |
|                   | Control (3)     |               | 1.59 nmole/gHb |
| N-3-(2-Hydroxyethyl)histidine | Ethylene oxide (occupational) | Exposed (7) | 0.68–6.0 nmole/gHb |
|                   | Control (3)     |               | 0.53–1.6 nmole/gHb |
| Hydroxyethylvaline  | Ethylene oxide (occupational) | Exposed (7) | 0.02–7.7 nmole/gHb |
|                   | Control (3)     |               | 0.03–0.93 nmole/gHb |
| Hydroxyethylvaline  | Cigarette smoke | Smokers (11)   | 389 pmole/gHb |
|                   |                  | Nonsmokers (9) | 58 pmole/gHb  |
| 4-Aminobiphenyl     | Cigarette smoke | Smokers (15)   | 154 pg/gHb    |
|                   |                  | Nonsmokers (26)| 28 pg/gHb     |

*Numbers in parentheses are numbers of subjects.

Table 3. Detection of BPDE adducts in DNA by immunoassay or chemical analysis.

| Type of assay* | DNA source | Exposure source | No. of subjects in which adducts were found | Adduct level |
|----------------|------------|-----------------|--------------------------------------------|--------------|
| ELISA          | Lung       | Tumor/nontumor  | 5/27                                       | 0.14–0.18 fmole/µg DNA |
| ELISA          | WBC        | Roofers         | 7/28                                       | 2–120 fmole/50 µg DNA |
|                | WBC        | Foundry workers | 7/20                                       | 37–47 fmole/50 µg DNA |
|                | WBC        | Controls (smokers) | 2/9                                       | Positive |
| SSFS           | WBC        | Aluminum workers| 1/30                                       | Positive for serum antibodies |
|                | WBC        | Controls        | 0/10                                       |             |
| USERIA         | WBC        | Coke oven workers| 18/27                                      | 0.4–34.3 fmole/µg DNA |
| SSFS           | WBC        | Coke oven workers| 31/41                                      | Positive     |
|                | WBC        | Coke oven workers| 11/41                                      | Positive for serum antibodies |
| USERIA         | WBC        | Coke oven workers| 13/38                                      | 0.1–13.7 fmole/µg DNA |
| SSFS           | WBC        | Coke oven workers| 4/38                                       | 0.38–2.2 fmole/µg DNA |
| ELISA          | WBC        | Coke oven workers| 22                                         | 1.2 fmole/µg DNA |
|                |            | (high, medium, low exposure) | 22                                         | 0.53 fmole/µg DNA |
|                |            | Controls        | 10                                         | 0.06 fmole/µg DNA |

*ELISA, enzyme-linked immunosorbent assay; SSFS, synchronous scanning fluorescence spectrophotometry; USERIA, ultrasensitive enzyme radio-immunoassay.
actions with proteins or nucleic acids was measured as an index of carcinogen exposure. Lu et al. (9) determined the daily excretion of nitrosamino acids in Chinese population groups residing in areas of high or low risk for cancer of the esophagus. This study was designed to gain evidence concerning a possible etiologic role for N-nitroso compounds, some of which are effective esophageal carcinogens in animals, as risk factors for man. The substantially higher values observed in persons in the high risk area indicate a higher level of exposure to these carcinogens during the study period. A similar approach was used by Hoffman and Brunemann (10) to define the potential of inhaled cigarette smoke for endogenous N-nitrosation of amines. The data revealed not only the existence of a substantial background of endogenous nitrosation in nonsmoking controls, but also a significant increase in smokers. Garland et al. (11) conducted an extensive investigation of individual and interindividual differences in the excretion of nitrosoproline in healthy volunteer subjects, all but two of whom were nonsmokers. As indicated in Table 1, the average value for these subjects is very close to that for the nonsmoking controls and residents of the low-risk areas in the earlier studies. In each of these studies, ingestion of ascorbic acid was shown to lower the excretion rates, as expected. The general inter-

laboratory agreement with respect to the values reported indicates the potential value of this measurement in detecting exposure to nitrosating agents in the environment.

Assessment of human exposure to aflatoxins through measurement of urinary excretion of the major DNA adduct has been reported in two studies. Groopman et al. (12) used the technique of immunoaffinity purification coupled with HPLC detection to identify the guanine adduct of aflatoxin B1 in the urine of persons residing in a commune in Guangxi Province of China, where independent evidence indicated that the dietary content of the carcinogen was high. The analytical method was adequately sensitive to detect the presence of the adduct in persons exposed at high levels, and also to quantify the excretion of aflatoxin M1, a metabolite, as well as unmetabolized carcinogen. Aflatoxin exposure was also measured by urinary excretion of the guanine adduct in populations living in areas with different liver cancer incidence in Kenya by Autrup et al. (13). The presence of the adduct was detected by HPLC used in combination with synchronous scanning fluorescence spectrophotometry, and it was detected in 12% of a large series (983) of samples collected over a period of years in different areas of the country. Both analytical methods require sophisticated analytical

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**Table 4. Detection of O4-methyl guanine and cis-platinum DNA adducts by immunoassay.**

| Compound analyzed | Source of DNA | Adduct level |
|-------------------|---------------|--------------|
| O4-MeG            | Esophagus/stomach tumor/nontumor (27/37) | 25–100 fmole/μg DNA |
|                   | European controls (5/12) | 25–45 fmole/μg DNA |
| cis-DDP-DNA       | WBC Infused patient | 5.6 fmole pGpG/μg DNA |
|                   |                 | 1.9 fmole pApG/μg DNA |
|                   |                 | 1.1 fmole (GMP)2/μg DNA |
|                   |                 | 0.06 fmole GMP/μg DNA |
| cis-DDP-DNA       | WBC Ovarian cancer patients (55) | CR 212 amole/μg DNA |
|                   |                 | PR 193 amole/μg DNA |
|                   |                 | NR 62 amole/μg DNA |

*a* Samples positive by radioimmunoassay/total samples.

*b* Peak levels: CR, complete response; PR, partial response; NR, no response to therapy.

**Table 5. Detection of aromatic DNA adducts by 32P-postlabeling.**

| Source of exposure | DNA analyzed | Subjects | Observations |
|--------------------|--------------|----------|--------------|
| Cigarette smoke    | Placenta     | Smokers (16/17) | Adduct 1 1.4×10⁸ N (P1) |
|                    |              | Nonsmokers (3/14) | 2.0×10⁸ N (ELISA) |
| Cigarette smoke    | Bronchus, larynx | Smokers (2) | Adduct 1 1.7–2.9 × 10⁵ N |
|                    |              | Nonsmokers (2/8) | (0.10–0.18 fmole/µg DNA) |
| Betel chewing      | Oral mucosa  | Exposed (69) | Adduct 1.8–14% of total |
| Tobacco chewing    |              |           | Levels 10⁵ N–10⁻⁷ N |
| Inverted smoking   |              |           |               |
| Cigarette smoke    | Oral mucosa  | Smokers (11/11) | Adducts 0.1–210 amole/µg DNA |
| Foundry workers    | WBC          | Exposed (10/10) | Adducts 0.4–1.7 amole/µg DNA |
|                    |              | Controls (5/10) | Total adducts 0.2–11.6×10⁶ N |
| Wood smoke         | Placenta, WBC| Exposed: Placenta (4) | Total adducts 12×10⁶ N |
|                    |              | WBC (8) | Not detected |
|                    |              | Control: Placenta (5) | 12×10⁶ N |
|                    |              | WBC (8) | Not detected |
instrumentation and are not yet applicable for routine monitoring.

Two additional approaches based on analysis of urinary components are being developed as potential monitors for exposure to genotoxic exposures. Shuker et al. (14) have explored the measurement of 3-methyl adenine in urine as an indicator of exposure to methylating agents. As indicated in Table 1, they have developed a GC-MS method using single ion monitoring capable of detecting the methylated base in nominally unexposed individuals. Further validation of the method will be required to determine its usefulness as an exposure monitor. Cathcart et al. (15) used an HPLC assay for determining levels of free thymine glycol and thymidine glycol in urine, as a noninvasive assay for oxidative DNA damage, since these compounds are products of DNA damage caused by ionizing radiation and other oxidative mutagens. While they were able to determine excretion rates for both compounds in healthy individuals, the method is very labor intensive and time consuming, and in its present form has found only limited application.

Hemoglobin Adducts
A summary of representative data on measurements of hemoglobin adducts derived from exposure to carcinogens is presented in Table 2. Most of the work done to date has concerned the measurement of N-3-(2-hydroxyethylehistidine (HEH) or hydroxyethylvaline (HV) as monitors of exposure to ethylene oxide. This methodology, in which the alkylated derivative was measured by GC-MS, was developed by Ehrenberg and his colleagues, and was used in the study of occupationally exposed workers (16). Average data shown in Table 2 for exposed subjects and unexposed controls indicate the sensitivity of the method for detecting exposure. Other findings in this study indicated the general agreement of the data obtained in man with those predicted from earlier studies in the mouse and also demonstrated the superiority of the method for providing a cumulative measure of exposure as compared to point monitoring of air levels.

The same methodology was subsequently applied by Van Sittert et al. (17) in a study of workers in an ethylene oxide manufacturing plant, in which cytogenetic and immunologic endpoints were made simultaneously in the same individuals. These investigators reported a substantially higher background of HEH in their control subjects and reported no significant difference between exposed and unexposed persons. Farmer et al. (18) compared levels of HEH, as determined by GC-MS on protein hydrolysates in which the adduct was concentrated by ion exchange chromatography, with levels of HV determined by GC-MS analysis of hemoglobin subjected to Edman degradation before analysis. They found that the two methods of analysis gave consistent results, especially at high levels, and also that higher levels of background alkylation (of unknown origin) were obtained in measurements of HEH than with HV, suggesting that the latter assay would show greater sensitivity in monitoring exposure to ethylene oxide. This method was subsequently applied in a study of cigarette smokers and nonsmokers (19). The results demonstrated an elevation of HV levels in smokers that was quantitatively compatible with measured levels of ethylene in the smoke to which they were exposed.

Bryant et al. (20) have developed a method for the analysis of 4-aminobiphenyl covalently bound as the sulfenic acid amide to the 93-beta cysteine of human hemoglobin. The method involves hydrolysis of the hemoglobin followed by GC-MS determination of the parent amine after derivatization. Application of the method to smokers and nonsmokers revealed consistently elevated levels in smokers and also a detectable background of the adduct in nonsmokers of undetermined origin. Collectively, these results indicate the applicability of analysis of hemoglobin adducts as monitors of exposure to carcinogens of different structural types and mode of action.

Detection of BPDE Adducts in DNA by Immunoassay or Chemical Analysis
Several studies have been conducted to date that were designed to determine exposure to the ubiquitous polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) through the detection of derivatives covalently bound to DNA. Immunoassays and physicochemical methods have been applied to detect derivatives formed through the major intermediate in the activation pathway, the benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE). Immunoassays have been applied in two modes, enzyme-linked immunosorbent assay, (ELISA) and ultrasensitive enzyme radioimmunoassay (USERIA), both employing polyclonal antisera that recognize BPDE-DNA adducts, with various levels of cross-reactivity with structurally related congeners. In an early pilot study in humans, Perera et al. (21) demonstrated that the ELISA assay was sufficiently sensitive to detect adducts in the DNA extracted from lung tumors as well as from nontumor tissue in lung cancer patients, but the small number of subjects studied precluded conclusions concerning exposure history.

Shamsuddin et al. (22) employed ELISA and USERIA assays to investigate the levels of BPDE-DNA adducts in the white blood cells (WBC) of roofers and foundry workers, in view of their exposure to high levels of BaP. Adducts were detected in a significant proportion of exposed individuals and also in 2/9 control subjects, both of whom were cigarette smokers. The technique of synchronous scanning fluorimetric spectrophotometry (SSFS) was applied in the analysis of DNA of WBC collected from workers in an aluminum plant for the presence of BPDE-DNA adducts (23). The limit of detection of this method as applied is about one adduct in 107 nucleotides. One sample of WBC DNA from the series of 30 exposed subjects showed the presence of a detectable level of BPDE.

Harris et al. (24) analyzed the DNA of WBC of coke oven workers by USERIA and by SSFS to determine the frequency and levels of BPDE adducts as markers of ex-
posure, as these workers are known to be exposed to high levels of BaP and are also at an elevated risk of lung cancer. Approximately two-thirds of the workers had detectable levels of BPDE-DNA adducts as determined by immunoassay, and an even larger proportion showed spectral evidence of the presence of BPDE by the SSFS assay. Antibodies to the DNA adducts were also detected in the serum of 27% of the workers. Coke oven workers were also the subjects of a study by Haugen et al. (25), who sought to evaluate the genotoxic effects of their occupational exposures through a study of BPDE-DNA adducts with simultaneous measurements of urinary excretion of PAH metabolites and air monitoring. As in the earlier study, DNA adducts were measured by USERIA and SSFS, and the sera were examined for the presence of anti-DNA adduct antibodies. The results of the study were in close agreement with those obtained earlier by Harris et al. (24) in all respects. Perera et al. (26) have recently applied ELISA in the study of PAH adducts in the DNA of WBC collected from Finnish foundry workers classified as having high, medium or low exposure, as well as unexposed control subjects. Mean levels of DNA adducts increased with exposure, and there was a highly significant difference between the control and pooled exposure group means.

Immunobassay techniques have also been used to detect adducts of DNA-damaging agents other than BPDE. Radioimmunobssay of O\(^{6}\)-methyldeoxyguanine was performed by Wild et al. (27) on human esophageal and cardiac stomach mucosal DNA from tissue samples obtained during surgery in Linxian County, China, an area of high risk for both esophageal and stomach cancer. Similar analyses were conducted on samples collected from hospitals in Europe. With this methodology, O\(^{6}\)-MeG was detected in about two-thirds of samples from the high risk area, as compared to 5/12 from the control area. Adduct levels were also higher in samples from people living in the high risk areas. DNA adducts induced by the antinecancer drug cis-diamminechloroplatinum (II) were detected in the WBC of cancer patients treated with the drug through application of ELISA methodology (28). The immunobssay procedure used four antisera capable of detecting different adducts, including intrasrand cross-links on pGpG sequences (which proved to be the major adduct); intrasrand cross-links on pApG sequences; inter- or intrastrand cross-links on two guanines separated by one or more bases; or monofunctional adducts bound to guanine. Results indicated that the susceptibility of WBC to adduct formation can show strong individually determined differences.

Reed et al. (29) also used ELISA methodology to study adducts derived from cis-platinum in WBC of ovarian cancer patients being treated with the drug. Values for median adduct levels were grouped by complete response (CR), partial response (PR), and no response (NR), and statistical analysis of the data showed that higher level of adduct formation correlated with clinical responsiveness to the drug.

These data show that adducts formed in DNA of accessible cells of people exposed to DNA damaging agents of a variety of types can be detected, and in many instances quantified, by currently available immunological and/or chemical methods. Application of these methodologies in well-designed longitudinal studies in man will permit further evaluations of their validity and limitations in actual use.

Detection of Aromatic DNA Adducts by \(^{32}\)P-Postlabeling

The postlabeling procedure developed by Randerath and his collaborators has been extensively applied to studies of DNA adduct formation in a variety of experimental systems, and the ability of the methodology to detect adducts of a large number of carcinogens (about 50) has been reported. The procedure has recently been extended to studies in humans, with results that can be summarized as follows. Everson et al. (30) investigated the presence of DNA adducts in human term placentas by both ELISA and by the \(^{32}\)P-postlabeling assay. The immunoassay revealed a small but insignificant increase in BPDE adduct levels in placentas from smokers compared to nonsmokers. However, the postlabeling assay detected a number of adduct types, the major one being strongly related to maternal smoking during pregnancy. Subsequently, Randerath et al. (31) compared adduct levels in human bronchus and larynx from smokers with those produced in mouse skin treated with cigarette tar. The human tissues were found to contain detectable levels of adducts, one of which (designated adduct 1) was identical to a major and persistent adduct formed in mouse skin. Paini and Stich (32) used the postlabeling assay to investigate DNA adducts in exfoliated mucosal cells collected from the oral cavity of three groups at high risk for oral cancer. Five chromatically distinct adducts were found in both the high risk groups and nonsmoking controls. Individual adducts were detected in 30 to 90% of the samples, and no adducts were found in high risk groups that did not also appear in control groups. Thus, although the method was successful in detecting adducts derived from unknown sources, it did not differentiate between exposed and nonexposed populations.

Using the same approach, Chacko and Gupta (33) analyzed the DNA from oral mucosal cells of cigarette smokers and nonsmokers to determine whether smoking-related adducts could be identified. Two chromatographically distinct adducts of unknown identity were found in smokers but not in nonsmokers. In addition, the levels of these major and several minor adducts were substantially higher in smokers. Phillips et al. (34) analyzed DNA from normal human bone marrow mononuclear and non-mononuclear cells for the presence of aromatic adducts. Ten out of 10 individuals showed the presence of adducts, which were not present in fetal bone marrow, but were present at lower levels in the DNA of peripheral WBC. Their data suggested that the adducts resulted from environmental exposure to unidentified genotoxic agents. Phillips et al. (35) also employed the postlabeling assay to analyze WBC DNA from foundry workers for the pres-
ence of adducts that might reflect differing levels of exposure to BaP. Adducts were found in 10/10 workers at levels detectable by the analytical method, but none of these had the chromatographic properties characteristic of adducts derived from BaP. Adducts were also identified in nonexposed control subjects. The results indicated significant interindividual differences in DNA binding among people receiving similar levels of exposure.

Reddy et al. (36) investigated WBC and placental DNA for the possible presence of adducts derived from exposure of pregnant women to residential wood combustion smoke. Detectable levels of unidentified adducts were found in all placetas of exposed and nonexposed women; none of the nine adducts found was present in WBC DNA of any of the subjects. The results suggest that residential wood combustion smoke did not elicit aromatic DNA adducts at detectable levels but that placental DNA contained detectable levels of adducts of unknown identity or origin. Collectively, these results demonstrate the capability of the postlabeling method to detect DNA damage arising from a variety of environmental exposures. However, additional experience with its applications in human studies will be required to establish fully the pertinent analytical characteristics of the method.

**DNA Adducts and Hemoglobin Adducts as Measures of Exposure and Susceptibility**

The information summarized previously provides valuable evidence concerning some features of currently available methodologies for detecting and quantifying covalent adducts to DNA and hemoglobin in human material. The data show that detecting exposure to carcinogens of a variety of chemical types is feasible using methods currently available or being developed. Substantial additional technological refinement and validation will be required before any of the current methods will be routinely applicable in large-scale epidemiologic studies. However, it seems likely that many of them will eventually come into broader application. In interpreting the data generated by their application, it is important to take into account all pertinent information concerning the parameters being measured. Some of the important points in this context can be summarized as follows.

Concerning the use of DNA adduct levels as measure of exposure and susceptibility, virtually all of the available evidence comes from experimental systems, especially experiments in which adduct formation in carcinogen-treated animals were studied in relation to tumor formation in response to the treatment. Most carcinogens have been shown to form complex spectra of DNA adducts, and qualitatively similar adduct profiles can be formed in sensitive and resistant species/strains/tissues. Adduct persistence may or may not be related to susceptibility and/or target tissue specificity. In target tissues, maximum total adduct levels usually reflect carcinogen potency and dose, and maximum total adduct levels are linearly related to dose over a wide range. Many factors have been identified that can affect the levels of DNA adducts observed in cells of treated animals at any given time. The exposure-sampling interval can markedly affect observed levels, as determined by the pharmacokinetic properties of carcinogens of specific chemical types. An important component of this process is the capability for metabolic activation, which can be affected by a variety of genetic as well as environmental factors (e.g., inducers or inhibitors). Exposure to protective agents such as antioxidants or other dietary ingredients can also exert marked effects. DNA repair capacity, as determined by genetic factors, as well as kinetics (e.g., saturability), or modulating factors is also an important determinant of adduct stability. Collectively, these factors indicate the complexity of the problem of meaningful interpretation of DNA adduct levels as quantitative measures of exposure.

In relation to the use of DNA adduct levels as indicators of long-term risk, the situation is further complicated by additional factors. Generally, the target tissue at risk to a given genotoxic exposure is unknown, and reliance must be placed upon measurements of adduct levels in surrogate cells such as peripheral white blood cells. The validity of the DNA analyzed for target cell DNA is difficult to assess. Extrapolation of DNA adduct levels as indicators of cancer risk necessarily entails simplifying assumptions about the multistage nature of the cancer process, which cannot be evaluated with current information. In addition, point measurements of DNA adduct levels may or may not provide evidence of multiple and variable exposures.

Protein adducts have no putative mechanistic role in carcinogenesis and are primarily regarded only as measures of exposure. Evidence from experimental animals and from human studies supporting the interpretation of protein adduct levels as exposure monitors can be summarized as follows. Measurement of hemoglobin adducts has certain obvious technical advantages, inasmuch as the protein can readily be obtained in abundant quantities. Sensitive and specific methods have been developed for two classes of carcinogens, and additional ones are in the process of development. Carcinogens of diverse chemical structures have been shown to bind to hemoglobin in vivo. Adducts are stable over the erythrocyte lifespan, thereby giving an integrated measure of multiple exposures over a substantial time period. Levels of hemoglobin adducts have been shown to be linearly related to dose for at least seven carcinogens of different types. Importantly, hemoglobin adduct levels have been shown to be related, within a factor of two, to DNA adduct levels in target tissues for three agents: ethylene oxide, trans-4-dimethylaminostilbene, and 2-acetylaminofluorene. Hemoglobin adducts therefore provide very useful complementary data to DNA adduct levels as dosimeters of carcinogen exposure.

This information, derived from applications of current methodologies for detecting and quantifying levels of DNA and hemoglobin adducts in human samples indicates that adequate levels of sensitivity have been at-
tained for several classes of DNA damaging agents to make possible the detection of adducts resulting from ambient levels of exposure. Specificity for the detection of compounds of known structure is also a common feature of most of the methods, and some are suited to detect total DNA damage from multiple sources. With respect to practicability, certain of the methods (e.g., immunoassays) are applicable to large numbers of samples, whereas others (e.g., postlabeling, thymine glycol analysis) are technically more complex and time consuming. Validation will be required to avoid systematic errors in measurement before the methods are applicable to large scale epidemiological studies. Methodological attributes such as accuracy, reproducibility (intra- and interlaboratory), and applicability to stored samples will need to be determined by appropriately designed collaborative studies among qualified laboratories. Additional factors such as variations of observed values due to age, sex, race, etc., as well as effects of possible interfering factors (e.g., diet, smoking, alcohol, etc.) will require definition through properly designed human studies of limited scope in order to validate the approach for epidemiological surveys.

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