Tracking Exposure to Toxic Substances

New ways to measure human exposure to carcinogens and mutagens provide a foundation for more precise assessment of risks

Low concentrations of potential carcinogens and mutagens that have become chemically linked to the DNA in human chromosomes can now be detected by a new set of analytical techniques. It may therefore be possible to measure with certainty the exposure to these chemicals and, through long-term studies, to identify agents that are true health hazards to humans. Eventually, it may also be possible to identify groups and even individuals who may be at the greatest risk of cancer because of occupation, life-style, or inherent susceptibility. These new assays, for detecting the carcinogen- or mutagen-DNA adducts were described at a recent conference at the National Institute of Environmental Health Sciences (NIEHS).*

The study of DNA adducts in animals has been very fruitful, says Curtis Harris of the National Cancer Institute (NCI): "We have more than 15 years of accumulated data correlating DNA damage with the administration of carcinogens and mutagens in animal studies. These data show that the formation of a DNA adduct is a crucial step in the development of carcinogenesis and mutagenesis. In the past 5 years, the scientific community has obtained similar information from studies of cultured human cells. These studies show that the major DNA adducts formed in human cells and in animals are identical. The logical sequence of events now is to look for adducts in humans."

The presence of specific DNA adducts in cells provides incontrovertible evidence that the individual from whom the cells came has been exposed to that chemical. The number of adducts may prove to be related to the extent of exposure, so that there is hope that quantitative monitoring of exposure can be achieved. The principal uncertainty about the adducts is their persistence once they have been formed. Studies in animals are equivocal, says Gerald Wogan of the Massachusetts Institute of Technology. In some cases, most of the adducts are "repaired" fairly quickly, while in other cases they persist for a long time. It is not yet clear, furthermore, whether cancer appears in the animals with high DNA repair rates or in those in which the adducts persist. These are topics that need to be thoroughly studied in humans.

If these uncertainties can be clarified and resolved, says Wogan, this type of biological monitoring will be valuable because it "takes into account absorption by all routes, integrates exposure from all sources, and therefore can be used as a basis for the estimate of risk from multiple chemicals." Most important, he adds, "the data obtained are directly related to adverse effects" and thus provide a better estimate of risk than does monitoring average levels of chemicals in the environment.

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Investigators have developed four new techniques for detecting exceptionally small numbers of adducts in human cells. They are based on specific monoclonal and polyclonal antibodies, fluorescence spectrophotometry, thin-layer chromatography, and gas chromatography. Of the four, the antibody techniques are most widely used, while the last two are potentially the most sensitive.

The binding of antibody to adduct is most commonly monitored with the enzyme-linked immunosorbent assay (ELISA). Regina Santella of Columbia University reported that with this technique she can detect one adduct in 10^8 nucleotides—about 100 adducts per mammalian cell. One such antibody described by Santella was useful for the detection of the carcinogen benzo[*a*]pyrene (BAP). Because BAP is commonly found in mixtures of carcinogens, such as those in cigarette smoke, most investigators assume that if BAP adducts are present, others will also be present.

Using the antibody technique, Santella, I. B. Weinstein, and Frederica Perera of Columbia, together with Miriam Poirier and Stuart Yuspa of the National Cancer Institute, observed measurable concentrations of DNA adducts in lung and tumor tissue from 4 of 14 lung cancer patients studied. Harris and co-workers have used similar antibodies to study about 150 individuals who smoke or who work in metal foundries or the roofing industry. He reported that BAP adducts are present in about 25 percent of blood samples from these individuals.

Perhaps the most detailed results with antibodies to DNA adducts were reported by Poirier. She and her colleagues studied cancer patients receiving the antitumor drug *cis*-diamminedichloroplatinum(II) or *cis*-DDP, which binds to cellular DNA and disrupts normal replicative functions. The primary advantage of studying *cis*-DDP in this context is that the precise amount of exposure to the drug is known, as well as the route and timing of that exposure.

Poirier's group assayed 138 blood samples from 54 individuals. The 18 samples from controls did not show *cis*-DDP adducts, she reported, but 44 of 120 samples from patients receiving chemotherapy for testicular and ovarian cancer did. Among the positive samples, the amounts of adduct were proportional to the amount of drug received by the patients, and adducts accumulated with increasing cycles of drug administration.

Interestingly, the remission rate among individuals who did not form measurable amounts of adduct was only half as high as the rate among those who did, suggesting a correlation between adduct formation and a positive response to therapy. This correlation is being studied further.

The second technique, synchronous fluorescence spectrophotometry, was developed by Tuan Vo-Dinh and Roger Rahn of Oak Ridge National Laboratory and applied to the study of DNA adducts by Harris and Kirsi Vahakangas of NCI. Aromatic hydrocarbons such as BAP are fluorescent when excited by light of appropriate wavelengths. In this second technique, the spectrum is scanned so that there is a defined difference between the wavelength of the exciting light and the wavelength monitored at the detector; this permits identification of individual fluorescing species. Vahakangas and Harris can now detect one adduct per 10^7 nucleotides, but Harris predicts that refinements will improve the sensitivity to about one adduct per 10^9 nucleotides.

Vahakangas reported that she and her co-workers used both the fluorescence

*"DNA Adducts: Dosimeters to Monitor Human Exposure to Environmental Mutagens and Carcinogens" was held 24 to 26 September 1984 at the National Institute of Environmental Health Sciences in Research Triangle Park, North Carolina.

technique and immunoassay on blood samples from 76 individuals who work near coke ovens and that about two-thirds of the samples showed detectable levels of adducts. Workers from an aluminum plant, in contrast, had a much smaller percentage of adducts. She also reported that blood samples from smokers, exsmokers, and nonsmokers had different levels of adducts.

The two newest techniques for identifying DNA adducts are also the most broad-based and potentially the most sensitive tests. In each case, the DNA is first separated into individual nucleotides and nucleotide adducts, and then both species are labeled to permit detection of the adduct when it is separated from the bulk of the DNA.

Kurt Randerath and his colleagues at Baylor College of Medicine use an enzyme to attach a ^{32}P-labeled phosphate group to each mononucleotide and adduct. The nucleotides can then be separated by conventional thin-layer chromatography; the adduct will show up as an unusual spot on the chromatogram. Because ^{32}P has a very high specific activity, the assay is very sensitive. Randerath has observed one adduct in 10^{10} normal nucleotides—the equivalent of one adduct per mammalian cell.

Another advantage of the ^{32}P assay is that it is not necessary to know the identity of the carcinogen. Any adduct that is present will show up on the chromatogram. The technique should even reveal nucleotides that have been altered by other carcinogenic and mutagenic processes, such as ultraviolet light and x-irradiation. The assay also requires less DNA than do other tests, and thus might be useful for studying DNA adducts in sperm, where less DNA is available.

Randerath reported that his group has used the assay on placental tissue and white blood cells from about 20 humans. The results are preliminary, he said, but they could readily distinguish between smokers and nonsmokers by the presence of adducts.

A related technique has been developed by Roger Giese and his colleagues at Northeastern University in Boston. Giese uses polyhalogenated aromatic compounds, such as pentafluorobenzoyl chloride, to label individual nucleotides. These "electrophores" have a strong affinity for free electrons, so that they are ionized with virtually 100 percent efficiency in an electron-capture detector or in the sample chamber of a mass spectrometer equipped with negative-ion chemical ionization.

Electrophore-labeled adducts can thus be separated from labeled nucleotides in a gas chromatograph and detected by either technique with a sensitivity of about one adduct per 10^8 nucleotides. This assay is still in an early stage of development, and Giese has not yet tested it on samples from humans.

With all four techniques, most investigators have looked for adducts in DNA from intact cells. But such adducts could also be present in body fluids. When DNA adducts are excised from DNA during the repair process, for example, the adducts may be excreted in urine. Herman Autrip of the Fibiger Institute in Copenhagen reported that he used synchronous fluorescence spectrophotometry to identify adducts of the carcinogen aflatoxin in urine. In this case, the concentration of adducts should be proportional to DNA repair activity, which in turn should reflect exposure.

Autrip and Johnstone Wakhisi of the University of Nairobi used the technique on urine samples collected in Kenya. The highest prevalence of adducts was observed in the Murang'a district (8.4 percent) and rural Nairobi (9.4 percent), areas where aflatoxin contamination of food had been suspected. No positive samples were observed in urban Nairobi, where the residents were more likely to eat packaged food. A seasonal variation in exposure was also observed in the Murang'a district, with the highest level of adducts (11.5 percent) observed in the summer, January to March, when old food supplies are being used up, and the lowest rate (3.1 percent) in July to September, when the residents are still eating fresh food.

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Now that sensitive methods for detecting DNA adducts are available, the major question is whether adducts in humans, once formed, persist for extended periods. Most of the evidence obtained from animals indicates that the concentrations of adducts are proportional to recent exposure, but the question of persistence is more complicated.

Frederick Beland of the National Center for Toxicological Research in Jefferson, Arkansas, reported that repair of damaged DNA in laboratory animals occurs at two different rates on different parts of the genome. Most DNA adducts, he finds, are repaired relatively rapidly, but there are specific regions in the DNA where repair is much slower and adducts persist for extended periods. Similarly, Marshall Anderson of NIEHS reported that he observed persistence of DNA adducts in animal cell types with slow turnover rates, such as brain and lung tissues. These results suggest that there may be relatively high levels of adducts reflecting recent exposure superimposed on a lower background level reflecting long-term exposure.

The preliminary results in humans seem to confirm this. Poirier observed that the concentration of *cis*-DDP adducts increased with increased administration of the drug and that the adducts persist for at least the 28-day period between chemotherapy cycles. Similarly, the level of BAP adducts in exsmokers was higher than levels in nonsmokers. The key point, most investigators agreed, is that the new assays make it possible to determine whether the adducts persist for long periods. "For the first time," says Frederick de Serres of NIEHS, "we have a tool to study these problems in humans."

An intriguing problem is suggested by the fact that investigators are observing large numbers of adducts per cell. Human genetic machinery is generally viewed as being fragile and subject to damage by even small perturbations. The presence of such large numbers of adducts suggests either that the DNA repair process is remarkably efficient at repairing damage before it can cause ill effects or that the cell can tolerate a large number of perturbations without hazard. "It must be remembered," concludes Harris, "that the formation of a DNA adduct is a necessary, but not a sufficient, step for carcinogenesis. There are other steps subsequent to formation of a DNA adduct that must occur before a tumor results, including promotion of tumor formation by other chemicals." It is presumably the need for these subsequent steps that explains, for example, why only one of 12 individuals who smoke two packs of cigarettes per day develop lung cancer.

"When we find these adducts," Harris continues, "we know that the individual has been exposed to the chemical and is at an increased risk of cancer, but we don't yet know how much that risk has been increased. The only way to answer that question is to follow populations with different levels of adducts for many years to see what happens to them. This will help us learn not only the risks from chemicals, but also the importance of the roles played by other factors."

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