Measuring mutagens and carcinogens in the body

Setting limits for chemical exposure in the workplace has always been a complicated and controversial procedure, mainly because it is difficult to extrapolate from animal exposure studies and airborne chemical levels to adverse human health effects. To solve this problem, and to better study how chemicals cause cancer, researchers have developed new techniques enabling them to determine how much of a given chemical actually gets into the human body and damages key biological molecules.

This new detection technology, says a spokesman for the Occupational Health and Safety Administration (OSHA), "will provide the type of evidence that will help in rule making and will put regulations on firm scientific footing." The spokesman adds that the technology should be "one of the most promising developments in monitoring exposure to chemicals in the workplace and assessing their potential health hazard."

Randerath: Careful isolation of chemically modified DNA.

While air filters and chemical-sensitive badges can detect surface contamination by a particular chemical, finding chemically modified biological molecules is incontrovertible proof that a person was not only exposed to the chemical but also that it was incorporated into the person's system. "Now, we can begin answering some key questions on how chemicals in the environment cause genetic lesions, mutations and cancer," says Frederick J. de Serres, associate director for genetics at the National Institute of Environmental Health Sciences (Research Triangle Park, N. C.).

In fact, OSHA has already made use of these new detection techniques in setting guidelines for ethylene oxide exposure. The oxide, used as a sterilant for surgical equipment, came under fire in 1983 when investigators found that it caused tumors in laboratory animals. "The exposure limits we set," says the OSHA spokesman, "were based in part on data from the new technology."

The technology was designed to measure exceedingly low levels of compounds that have become linked chemically to DNA or proteins. Some techniques use monoclonal antibodies to find the chemically modified biological molecules, known as adducts of DNA or protein. Others combine classical chemical techniques with highly sensitive analytical methods, such as gas chromatography combined with mass spectrometry or fluorescence spectrophotometry, to gain the same end.

Sensitivity. But while the techniques may differ in method and in the adducts they can detect, all are capable of finding less that one adduct in 10 million unmodified molecules. Says Regina P. Santella, assistant professor of public health at Columbia University (New York City), "We're talking about sensitivity down in the range at which the toxic effects of a chemical are first seen."

Of all the chemicals in existence, both natural and man-made, the most dangerous are the very few that are able to enter a cell and react with the individual nucleotide bases that make up DNA. Researchers studying cancer-causing processes in laboratory animals and cultured human cells have determined that the process of entry and reaction is a crucial step in the development of cancer and mutations. But extending this process from animals and human cells to live humans has been a problem. "We've had no way of proving that such reactions occurred in humans," says Kurt Randerath, professor of pharmacology at Baylor College of Medicine (Houston). Thus, Randerath notes, it has been difficult "to prove that certain substances cause cancer and birth defects."

Cigarette smoke is a substance that has eluded positive linkage with cancer. Hundreds, if not thousands, of studies have documented that cigarette smoking causes cancer in laboratory animals, and population studies indicate the same holds true in humans. The tobacco industry, however, claims steadfastly that cigarette smoke has never been documented to produce the biochemical damage in humans that leads to cancer.

Proof? But now Santella, Randerath and other researchers have found adducts of DNA and benzopyrene—a major suspected carcinogen in cigarette smoke—in tumors taken from human patients with lung cancer. Such adducts were absent in lung tissue taken from nonsmokers. "I would say," says one researcher, "this is pretty good evidence that a compound in cigarette smoke is causing these tumors."

Randerath has also been studying the interaction of DNA with various chemicals important to the chemical process industry, such as aromatic amines, azo compounds, heterocyclic drug intermediates and nitroaromatic compounds. While his data are not yet complete, Randerath says that only a few of the substances tested so far form adducts with human DNA. However, he notes, those compounds that do form adducts "will probably prove toxic."

'Evidence to help rule making and to put regulations on firm scientific footing'

To measure DNA adducts in human samples, Randerath isolates DNA from white blood cells exposed to a suspected carcinogen, breaks the DNA into individual nucleotide bases, and uses the enzyme polynucleotide kinase to label both normal and modified bases (the adducts) with radioactive phosphate. Next, he separates the adducts by thin-layer chromatography—the adducts migrate differently on the chromatogram than unmodified bases—and uses autoradiography to measure the adducts relative to normal bases. "This method is sensitive and fairly easy to perform," he says. "But, more important, you don't need to know beforehand what compound the DNA has reacted with."

However, those assays that use mon-

oclonal antibodies to detect DNA adducts work only if the adduct is known beforehand; then an antibody specific to that adduct can be prepared. This limits use of these assays, even though they are just as sensitive and are easier to use than those that rely on chromatographic separations. Santella, for example, prepared synthetic adducts by reacting benzopyrene with DNA. "We knew the structure of the adduct and knew how to make it," she explains. "Without that type of information, antibody methods aren't practical."

One way to increase the practicality of antibody assays, though, would be to use chromatographic techniques to isolate adducts. Once isolated, the structure of the adduct could be determined—and thus the identity of the chemical that attacked the DNA—and a synthetic adduct could be prepared. This adduct could then be used to make an antibody. Many investigators are planning to use this production method, Randerath says, because monoclonal assays would be "more suitable for routine monitoring."

Another approach to such routine monitoring is to bypass the DNA completely and, instead, look at blood proteins. "We know that many carcinogens react with proteins, too," says Santella, "and it's a lot easier and more practical to get hemoglobin from red blood cells than DNA from white blood cells."

Adduct stability. Moreover, protein monitoring would alleviate one concern that researchers have about the stability of DNA adducts. Such adducts are repaired by the body to some extent, although the degree of repair depends on the particular adduct, where on the DNA molecule it is located, and on a person's individual metabolism. Proteins in red blood cells, on the other hand, are not repaired by the body, but exist untouched for the lifetime of the blood cell, about four months. Says Santella: "Looking at hemoglobin adducts could give us an accurate picture of total exposure over several months time."

The techniques for detecting very small numbers of adducts are well established. What remains before these techniques become standard in monitoring and regulatory activities is for researchers to demonstrate their utility on a large number of common workplace chemicals. This, in the estimate of those working in the field, is beginning; within a year or two, a sufficient data base should be accumulated to judge the technique's relative merits. □

JOSEPH ALPER in Washington, with B. J. Spalding

Close to FDA's finish line: Five AIDS-linked tests

Heckler: No. 1 HHS health priority.

Less than 10 months after National Cancer Institute scientist Robert Gallo discovered a virus, human T-cell lymphotrophic virus type III (HTLV-III), believed to cause acquired immune deficiency syndrome (AIDS), five companies have unveiled tests that detect antibodies to the virus in human blood. Food and Drug Administration (FDA) approval of one or more of the tests, delayed for several weeks, is imminent.

When approval comes, the race will be on among five key contenders for an estimated $175 million worldwide market—$70 million in the U. S.—for blood and plasma screening. The outcome should rest on several factors: the accuracy of the firms' tests; the firms' starting position in the blood banks, plasma houses and clinics that make up the market for the tests; and the approval date for each test. Awaiting the starting signal are Abbott Laboratories (North Chicago), Electro-Nucleonics (Columbia, Md.) and three joint ventures: Litton Bionetics (Kensington, Md.) with Johnson & Johnson's Ortho Diagnostics (Raritan, N. J.); Genentech (San Francisco) with Baxter-Travenol (Deerfield, Ill.); and Biotech Research Laboratories (Rockville, Md.) with Du Pont (Wilmington, Del.).

Two more. Lagging behind these companies by at least several months are two other firms that are developing tests based on independently discovered AIDS-associated viruses: Genetic Systems (Seattle), which has a marketing agreement with American Hospital Supply (Evanston, Ill.); and a joint venture of Chiron Corp. (Emeryville, Calif.) and Pandex Laboratories (Mundelein, Ill.).

The five leading tests are enzyme-linked immunosorbent assays (ELISA), in which either beads or microtiter plate wells coated with inactivated parts of the Gallo virus will cause antibodies in the blood being tested to link to an enzyme that causes a color change. This is not unlike tests already used to detect hepatitis B antigen in a market already dominated by Abbott.

Because of its position in the market for hepatitis B tests, and because its AIDS test is nearly the same for lab technicians to run, Abbott will immediately grab 60% of the U. S. market, says analyst Peter F. Drake of Kidder Peabody & Co. (New York City). "It's going to be a very easy training" for those used to Abbott's hepatitis tests, says David A. Thompson, president of Abbott's Diagnostics Div., who expects his firm to take about 50% of the market.

At least one major market segment may already be sealed. The American Red Cross (ARC) will need an estimated 6 million tests/year for its blood banks. ARC has clinically tested all five kits and picked one based on its own data, says Dr. S. Gerald Sandler, ARC associate vice-president for medical operations. Although each of the companies procured its own samples for clinical tests, ARC used uniform samples.

Pending FDA approval, ARC won't say which test it has chosen. But other blood banks and the plasma industry are "up for grabs," says Kathryne Carr, assistant to the scientific director at Genetic Systems. She thinks Abbott "will get a good run for its money" in both areas. Apart from ARC, members of the American Assn. of Blood Banks will use 6 million tests/year, while the plasma industry, represented by the American Blood Resources Assn. (ABRA), will use 10 million tests/year.

Not far behind. ABRA Executive Director Robert W. Reilly says Litton/Ortho has wide blood bank contacts, while Travenol/Genentech and Electro-Nucleonics are both plugged into the plasma market. Nobody has the edge with free-standing clinical labs, he says. Du Pont claims an advantage with plasma houses, to whom it sells centrifuges. "I believe Abbott will probably be No. 1, but I firmly believe that we probably won't be very far behind," says David D. Mooberry, group vice-president of Du Pont's Biomedical Products Dept.

Kidder Peabody's Drake, though, predicts that because of the accuracy of its test, Electro-Nucleonics will take second place (*table, p. 26*). That firm, he says,