several centuries. In the East, spruce and northern pine would decline in the southern parts of their ranges and expand northward. New England coniferous forests would be replaced by hardwoods, especially by oak. Southern pines might shift into the hardwood forests of eastern Pennsylvania and New Jersey. In the Southeast, some 18 tree species may become locally extinct, with forest lands being taken over by scrub or savanna.

In the West, the situation is not as bleak, as Douglas fir, ponderosa pine, and western hemlock can disperse upslope into the Rockies. In California and Oregon, the populations of Douglas fir would shrink in the lowlands and be replaced by western pine. If regional drought persisted and fires increased, total forested area in the West could be dramatically reduced and some species would go locally extinct.

Moreover, these projections are just for the lower 48 states, but as EPA points out, forest effects should be far more pronounced in Canada and Alaska, where the climate warming is expected to be greater. Large boreal forests "could be at significant risk," EPA notes.

All of these studies focus on the dominant canopy trees, for which there are adequate data. But as Davis and Zabinski point out, what happens to them will reverberate throughout the ecosystem. Each of the plants in the forest has its own physiologic tolerances and will respond differently to rising temperatures and changing rainfall patterns. They will scatter, perhaps in different directions, at their own speed. That means, for one thing, that forests as we know them will not simply be duplicated in the north; instead, new plant communities will emerge.

And, says Davis, if the situation looks grim for dominant canopy trees with abundant seed, it could be far worse for understory plants. Many woodland herbs, like *Trillium,* ladies's slippers, and trout lilies, produce few seeds and depend on the wind to scatter them. She calls their chance of dispersing to favorable habitats "disappearingly small."

To save many of these species, Davis and Zabinski call for recreating entire forest ecosystems, including dominant trees, understory plants, and important animals, "on a large scale" in northern locations. They also call for setting up new refuges at all latitudes and for bringing wild plants into cultivation to preserve genetic diversity. EPA, too, envisions "massive reforestation" in the North and perhaps the introduction of subtropical species in the Southeast to replace the forests that have disappeared.

■ **Leslie Roberts**

Detecting Mutations in Human Genes

New mutation detection methods are giving a boost to efforts to assess the risks of human exposures to environmental chemicals and radiation

For the first time, researchers are developing the ability to detect even the smallest mutations caused in human genes by chemicals or radiation. And not just in cells grown in Petri dishes. "You can do molecular analysis of mutations from real, walking people," says Jane Cole of the University of Sussex in Brighton, England.

In the past, only genetic damage sufficient to cause visible chromosomal abnormalities could be spotted in the cells of individuals who had been exposed to some environmental contaminant. Many mutagens do not produce visible chromosome damage, however, and the gene damage they cause therefore goes unnoticed in the older assays.

The new techniques are capable of picking up changes in a single base pair, promising more accurate assessment of the biological consequences of exposure to mutagenic agents, which have been linked to an increased risk of cancer and birth defects. Eventually, the methods may be able to tell not just whether an individual has incurred excess gene mutations, but also what the guilty agent was.

The most common method being used to detect human gene mutations is a T cell assay developed a few years ago by Richard Albertini of the University of Vermont in Burlington and, independently, by Alec Morley of Flinders Medical Centre in Bedford Park, South Australia. The procedure uses the HPRT (hypoxanthine-quanine phosphoribosyltransferase) gene as a mutation indicator, primarily because there is a simple selection method that can distinguish those cells in which mutations have inactivated the HPRT gene from those in which the gene is functional. The T cells themselves can be readily obtained from a sample of an individual's blood.

The assay determines the frequency of T cells that carry HPRT-inactivating mutations. For a normal adult, this is about five T cells in every million, Albertini says. Several groups have recently shown that the mutant frequency goes up in people who have been exposed to a variety of environmental mutagens. These include, for example, the chemotherapeutic drugs used to treat cancer patients and cigarette smoke. "Anyone who smokes has twice as many mutants in their blood as a nonsmoker," Cole declares.

Ionizing radiation also increases the frequency of T cells with HPRT mutations, and its effects can be very long-lived. Masayuki Hakoda of the Radiation Effects Research Foundation in Hiroshima, Japan, and his colleagues have shown that survivors of the 1945 atom bomb blasts in Japan still have higher than normal mutant frequencies more than 40 years after their radiation exposures.

" . . . in bacterial and mammalian systems each agent gives its own fingerprint of changes."

The effects of radiation can be seen even with very low dose exposures. For example, Karen Messing and her colleagues at the University of Quebec and Montreal in Montreal have looked at the T cell mutant frequencies in nuclear medicine and x-ray technicians. "We found a dose-related increase in mutant levels that was surprising because these people were exposed to very low doses," Messing says.

Most of these early studies were aimed at establishing the ability of the T cell assay to detect the HPRT mutations caused by environmental or occupational exposures. With that ability now documented, the researchers are moving ahead to characterize the biochemical nature of the mutations produced by the various agents.

This involves cloning individual mutant T cells and analyzing their HPRT genes to identify the particular sequence changes they have undergone. The sequence studies may provide a better understanding of the mechanisms of mutagenesis in humans. They may also provide information about whether a given mutagen leaves discernible "fingerprints" on the HPRT gene that can be used for identification purposes. "We hope to find a correlation between the agent and the

type of mutations it causes," Albertini explains.

There are reasons to expect that such correlations will be found. Early work on bacteria by Seymour Benzer of the California Institute of Technology in Pasadena and Jeffrey Miller of the University of California at Los Angeles indicated that mutagens produce their own characteristic spectra of gene changes. More recently, Barry Glickman and his colleagues at York University in Toronto have shown that this is true in mammalian, as well as in bacterial, cells.

Over the past few years, the York group has determined the nucleotide sequences of more than 1200 gene mutants that were induced by a variety of agents, including chemicals and radiation. "The bottom line is that in bacterial and mammalian systems each agent gives its own fingerprint of changes. In other words, when we see the changes we know what the agent was," Glickman says.

The bulk of the York group's work has been performed with bacterial and Chinese hamster cells that were exposed to the mutagens in laboratory dishes rather than in whole animals. If mutagens act in a comparable way in the cells of living human beings, then researchers may be able to identify an individual's environmental exposures by examining the spectrum of mutational changes in his or her HPRT gene.

Last year, in an effort to facilitate the characterization of human HPRT mutations, the principal researchers who are doing the work established an "HPRT Mutational Spectra Repository." The repository will collect data on HPRT mutations, and on other gene mutations when they become available, together with information on the types of exposures experienced by the individuals in whom the mutations were found. Establishing mutational spectra for the many different mutagens will nevertheless be, at best, a Herculean task if researchers have to analyze the HPRT changes in all the mutant T cell clones found in individuals exposed to the various agents.

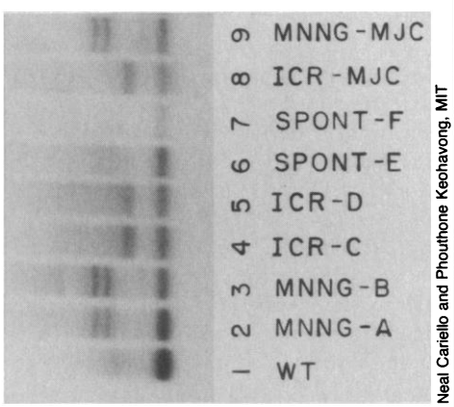
Meanwhile, William Thilly and his colleagues at the Massachusetts Institute of Technology have been exploring an approach to mutation detection that may be capable of giving mutational spectra directly, without the need for doing cell by cell DNA characterization. The approach was admittedly speculative. "Our laboratory could have fallen flat on its face, and we knew it, " Thilly says. Nevertheless, within the past year, the group's work has begun to show signs of success, although the method they are developing has not yet been applied to direct detection of mutations in living people.

A key element in the MIT workers' approach was their adaptation of a method for separating DNA molecules with different sequences that was originally developed by Stuart Fischer and Leonard Lerman of the State University of New York at Albany. In this method, called "gradient denaturing gel electrophoresis," double-stranded DNA that is migrating on an electrophoresis gel is submitted to a gradient of increasing concentrations of a chemical that causes the two DNA strands to separate.

The separation occurs at a specific concentration, which depends on the DNA sequence. Strand separation brings the migration of a DNA molecule to a halt. Each mutated and wild-type DNA in a sample prepared from human cells will therefore form a distinct band on the gel. "You can see them there as plain as a hand in front of your face," Thilly says.

The advantage of this approach is that the spectrum of DNA changes induced by a particular mutagen can be visualized without having to clone and characterize individual genes. Sequencing of the mutated genes can be readily achieved, however, if the investigator wants to define the exact changes induced by a mutagen. Mutant DNA bands can be simply sliced out of the separation gel, amplified by the polymerase chain reaction, and their nucleotide sequences determined.

According to Thilly, his group's method has another advantage in that it allows the determination of the mutational changes



Neal Carriello and Phouthone Keohavong, MIT

Chemically induced mutational spectra. *Treatment of human cells with two different types of mutagenic chemicals, designated MNNG and ICR, produced characteristic mutations in the HPRT gene that can be visualized by gradient denaturing gel electrophoresis. The band pattern of the wild-type (WT) gene is in the bottom lane. The DNA from the MMNG-treated cells (lanes 2, 3, and 9) shows two new bands, as does the DNA from the ICR-treated cells (lanes 4, 5, and 8), but the ICR pattern is different from the MNNG pattern. No mutations occurred spontaneously in untreated cells (lanes 6 and 7).*

that occur spontaneously in a person's cells as well as of the gene changes that might be induced by an environmental mutagen. This helps to correct for individual variations in the ability to repair damaged genes that could otherwise complicate interpretation of the spectra obtained. Without a spontaneous mutational spectrum as a point of reference, a person with poor DNA repair might erroneously appear to have had a heavy environmental exposure to a mutagen.

The MIT workers have recently shown that their DNA separation method works for samples obtained from human cells that were exposed to various mutagens in culture. DNA from treated cells shows mutant bands in the HPRT gene that were not present in DNA from control cells. Moreover, different mutagens produce their own characteristic pattern of mutant bands, a result indicating that they are producing distinguishable mutational spectra.

The MIT workers are just about to begin using their method to look at mutational spectra in suitable gene sequences in DNA from T cells obtained from human volunteers. "If our methods are to be any good, they will have to work in the real human community," Thilly notes.

Not all the mutation detection methods now being explored use the HPRT gene as a mutation indicator. For example, red blood cells ordinarily carry on their surfaces a protein called glycophorin A. Richard Langlois, Ronald Jensen, and their colleagues at the Lawrence Livermore National Laboratory in Livermore, California, have developed a method for determining the frequency of red blood cells that have lost the protein as a result of mutations in the glycophorin A genes.

In findings similar to those obtained with the HPRT gene, the Livermore group has shown that the frequency of mutant red blood cells is increased in atom bomb survivors and in individuals who have had cancer chemotherapy. However, the red cell method lacks the ready ability to characterize the mutations underlying the protein defect. As red blood cells mature they lose their genetic material. It may be possible, however, to trace the glycophorin A gene mutations in the bone marrow cells from which red blood cells develop.

In any event, researchers are developing tools that allow them to identify people who have incurred gene mutations as a result of environmental or occupational exposures to mutagens. The presence of such mutations does not necessarily mean that an individual will develop cancer or have a defective child, but their detection would at least sound a warning of potential danger.

■ JEAN L. MARX