CANCER RESEARCH

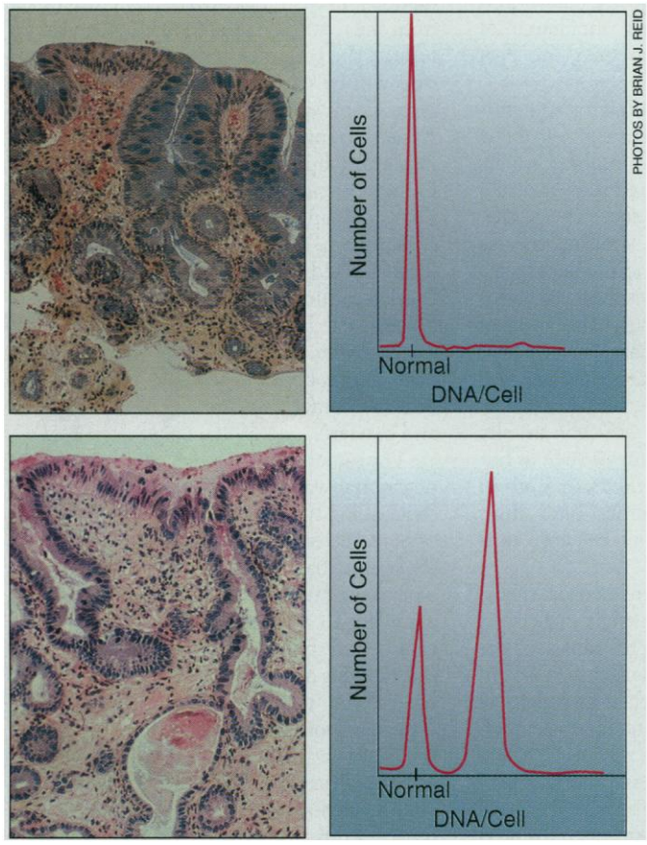
A New Test Gives Early Warning of a Growing Killer

None of the many faces of cancer is pretty, but oncologists are beginning to see one particularly nasty visage with alarming frequency. In the United States and parts of Europe, adenocarcinoma of the esophagus and its junction with the stomach is the most rapidly increasing cancer. Its incidence tripled between 1976 and 1990 in the United States, where it now afflicts about 10,000 people per year. To make matters worse, the cancer is extremely aggressive, killing two out of every three victims within 1 year and more than 90% within 5 years. "It's still a rare cancer," says cancer epidemiologist William Blot of the National Cancer Institute (NCI) in Bethesda, Maryland, "but the worry is that if it keeps on [rising], it's going to become a real public health problem."

The news is not all grim, however. Even as investigators struggle to understand the reasons behind the soaring incidence, a research team has developed what may be a significant new weapon—an early warning system that detects the adenocarcinoma's precancerous stages. Last week, Brian J. Reid of the University of Washington Medical Center in Seattle announced that he and his team have developed a test for cellular abnormalities that foreshadow the condition. Reid, who reported on the test at the General Motors Cancer Research Foundation annual conference, held 14 and 15 June at NCI, says early detection and treatment will enable 80% of esophageal adenocarcinoma patients to live 5 years or more—a sharp turnaround from the current mortality rates.

That prospect has researchers in the field excited. "It's a very important finding," says Mark Groudine, a radiation oncologist and molecular biologist at the Fred Hutchinson Cancer Center in Seattle. "The new test allows you to identify the disease at a very early stage, increasing the probability that a patient will survive." And as the excitement over the possibility of early diagnosis spreads, researchers are continuing to hunt for the reasons why the disease seems to be spreading—and interest has focused on diet and on certain medicines, such as the calcium blockers used to treat high blood pressure.

When esophageal adenocarcinoma does strike, it predominantly affects middle-aged and older white men. The first symptom is chronic heartburn caused by esophageal reflux, in which the stomach contents well up into the esophagus. Of patients who have this condition, one in 10 develops Barrett's esophagus, in which the disc-shaped epithelial cells that line the esophagus are replaced by columnar cells that secrete large amounts of mucus, presumably to protect the tissues from the harsh stomach acid. Within 10 years, one in 10 Barrett's esophagus patients progresses to the full-blown adenocarcinoma. One possible explanation, says Reid, is that "the chronic reflux of acid into the esophagus injures the cells, [triggering] an increase in their rate of division, so that they become more susceptible to environmental toxins that cause mutations."



First alert. A new test for adenocarcinoma highlights cells in the esophagus with excess DNA. Unlike patients with normal amounts of DNA in their cells *(top)*, those with abnormal amounts *(bottom)* have a 50% chance of developing the cancer within 3 years.

Reid and his colleagues developed that hypothesis during a study begun in 1983 that detailed the cellular changes that mark the progression of the disease from a precancerous to a cancerous condition. By examining esophageal biopsies periodically collected from 500 Barrett's esophagus patients, Reid's team found that some of the first events in the progression to adenocarcinoma are mutations in *p53*, a tumor suppressor gene whose protein product stops abnormal cells from dividing.

Defects in *p53* are thought to play a key role in as many as 50% of all human cancers (*Science*, 10 September 1993, p. 1385), and the changes in *p53* help propel cells down the road to esophageal cancer as well, says Reid. Over several years, large numbers of abnormal—but not yet cancerous—esophageal cells appear. Some of these cells lack fragments of chromosomes 5 and 9, while the nuclei of others contain huge masses of extra DNA, a phenomenon dubbed aneuploidy. In about 50% of these aneuploidy cases, the study showed, the abnormal cells became malignant in 18 months to 7 years (average 3 years). At the moment, Reid says, it is unclear whether or not the other 50% will also develop malignancies, possibly at a slower rate.

The discovery that aneuploidy played such a prominent role inspired the Reid team to develop a means to test for it, reasoning that it could be used as an early warning signal. To conduct the test, biopsy cells are stained with a dye that makes the DNA fluoresce under laser light. More DNA means a brighter glow, which is detected by an instrument called a flow cytometer. The machine can then calculate the proportion of cells with abnormally large amounts of DNA. "The beauty of the test," says Reid, "is that it is easily automated and can be done using equipment found in most major hospitals." Not only that, it seems to work well: The patients who tested negative in Reid's study remained cancer-free for at least 3 years. In contrast, half of the patients who tested positive developed cancer over the same time period.

For patients, the early warning means much improved odds of survival should they develop cancer. The only treatment available for the adenocarcinoma is surgical removal of the esophagus, an operation that claims the lives of about 10% of those who undergo it. For that reason, says Reid, the majority of patients who test positive opt for twice-yearly biopsies, delaying surgery until

the very first sign of cancer. But that still gives the patient a jump on the cancer, and analysis of survival data for 46 patients who have undergone surgery after first being alerted by the test indicates that 80% will live at least 5 years.

An even higher survival rate, of course, would follow from cancer prevention. With that end in mind, cancer epidemiologists are trying to pinpoint the risk factors associated with esophageal adenocarcinoma. Some are known already, such as smoking and diets low in fruits and vegetables and high in fats. But, says epidemiologist Thomas Vaughan of the Fred Hutchinson Cancer Center, "all the known risk factors account—roughly—for only half of the cases." Those risk factors also fail to explain the soaring incidence of esophageal adenocarcinoma over the past decade, he says.

Vaughan, in collaboration with epidemiologists from NCI, Yale University in New Haven, Connecticut, and Columbia University in New York City, started a 3-year-long study in 1992 intended to solve the riddle. He and his colleagues are scrutinizing the diets, degrees of obesity, and medical treatments of 700 patients with esophageal adenocarcinoma, 700 patients with other types of stomach and esophageal cancers, and 700 healthy people. For instance, the study will pay close attention to whether or not the adenocarcinoma patients ate abnormally large amounts of processed meats such as hot dogs, says Vaughan. These foods are high in chemicals called nitrosamines (and their precursors), which are known to cause cancer in lab animals.

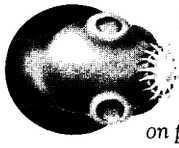
Other prime suspects are certain medicines that have been increasingly prescribed since the 1970s. According to Vaughan, the study consortium is particularly interested in two classes of drugs. One class includes the H_2-blockers that are used to treat stomach ulcers and—ironically—esophageal reflux. These drugs, which suppress production of stomach acid, may promote bacterial growth in the stomach, and certain stomach bacteria produce nitrosamines. The other class of drugs, which includes calcium blockers that are used to treat hypertension and asthma and certain anti-depressant drugs, triggers excessive esophageal reflux as a side effect.

Besides identifying and eliminating risk factors, cancer researchers have one more lead to follow. Occasionally, Barrett's esophagus—the precancerous condition—spontaneously reverts to normal. "It's the strangest thing we've ever seen," says Reid, "We would like to identify the cause and be able to do that on a regular basis." And that's a goal the current work might bring a step or two closer—an achievement that, if the incidence of cancer of the esophagus keeps growing at its current rate, could be very valuable indeed.

–Rachel Nowak

PARASITOLOGY

Genome Initiatives Tackle Developing World's Big Killers



For more News, Policy Forums, Perspectives, and Articles on parasitology, see special section starting on page 1857.

Despite the hundreds of millions of dollars that are now being poured into the Human Genome Project, our own genome will be far from the first to be sequenced in its entirety. In fact, the genomes of many simpler organisms will be sequenced long before our own genetic blueprint is deciphered—and included among their number will be a range of important human pathogens and parasites. Earlier this month, for instance, the genomics company Collaborative Research Inc. of Waltham, Massachusetts, announced that it had started a 6-month project to sequence the 1.8 million bases of DNA carried by *Helicobacter pylori*, a bacterium that is believed to induce stomach ulcers and cancer.

The same company is nearly halfway through sequencing the genome of *Mycobacterium leprae*, the leprosy bacterium—a project that should be completed within 2 years. And these bacterial projects are just the tip of the iceberg: A flurry of genome projects for human pathogens are now gearing up, including several targeting protozoan and worm parasites responsible for diseases that are major killers in tropical regions. Most of these projects are hampered by the lack of funding that seems to beset research on these diseases, but their proponents argue that they offer direct benefits to human health that are well worth additional investment.

VISUALS UNLIMITED

Leprosy bacterium. Half of *Mycobacterium leprae*'s genome has been sequenced.

For protozoa and worms, which possess genomes several times larger than those of bacteria, it's not yet practical to begin whole-genome sequencing. So, taking a cue from the Human Genome Project, researchers are trying to assemble genome maps and selectively sequence genes that are expressed in the organism. They hope to identify genes influencing metabolic pathways that could be drug targets or genes that encode antigens that could be built into vaccines.

Since so far only a few tens of genes have been identified in most parasites, many researchers believe the projects currently under way will revolutionize the field. "[We're] going to be able to generate information orders of magnitude faster," says molecular geneticist James Ajioka, who heads a Cambridge University lab that is starting to map the genomes of *Leishmania*, the African sleeping sickness agent *Trypanosoma brucei*, and *Toxoplasma gondii*, a protozoan that can cause fatal encephalitis in immunosuppressed people.

As ever with tropical parasitology, however, money is a problem. Although diseases such as malaria are responsible for more than 1 million deaths every year, they are not a high priority for funding agencies in the developed north (see p. 1857). Nevertheless, a few funders are starting to take an interest in parasite genomes. Last summer Britain's Wellcome Trust launched a 3-year, $1-million effort to produce a physical genome map of the malaria parasite, *Plasmodium falciparum*, involving labs in Australia, Britain, and the United States. And the leading international parasitology agency, the Geneva-based Special Program for Research and Training in Tropical Diseases (TDR)—which is run by three United Nations agencies—will soon start funding genome projects for five other major disease organisms: the protozoans that cause leishmaniasis, African sleeping sickness, and Chagas' disease; the nematode worms responsible for lymphatic filariasis; and the liver flukes that cause schistosomiasis.

Unlike the human genome effort, these projects will involve developing countries. TDR is actively encouraging the formation of lab networks involving developing-world researchers—a policy that will help bring these scientists into the molecular biology mainstream. "Having a genome effort...