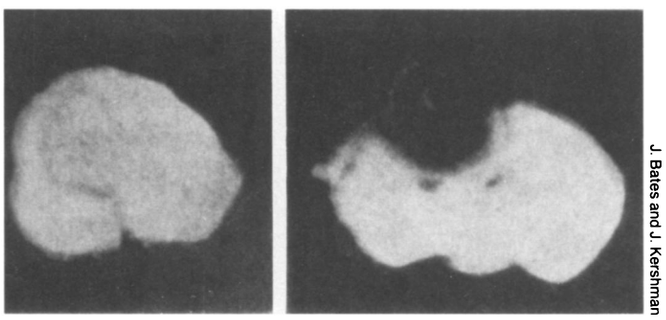
Shedding Light on Cancer

Doctors fight malignancies with photoactive dyes

By INGRID WICKELGREN

A doctor injects dye into a cancer patient. Blood vessels ferry the dye to almost all tissues, but it favors tumors, congregating in cancer cells or in vessels feeding the malignant mass. The dye does no harm (or good) until the physician supplies it with power in the form of light from a fiber slithered through a pore in the patient's body. Thus energized, the dye creates toxic molecules that kill the tissues they inhabit.

Propelled by a doctor's needle and a medical laser, researchers are adding color and light to the art and science of cancer treatment. About 20 years after its birth, the dye-and-light treatment remains experimental, but some researchers now see it as one of the most promising future weapons against cancer.



Unstained and stained (blue in original 1940s photos) mouse brain tissue illustrates Nile Blue's preference for these induced tumors. Diffuse area shows staining of infiltrating tumor cells.

J. Bates and J. Kershman

The potential of photodynamic therapy lies in what researchers call its "double selectivity." "The dye itself [should be] local to the tumor, and the light, depending on where you shine it, gives another degree of selectivity," says Chi-Wei Lin, director of the Urology Research Laboratory at Massachusetts General Hospital in Boston. Furthermore, "because [photodynamic therapy] is different from radiation and different from traditional therapeutic drugs, it can be used in addition to them without additional toxicities," says Allan Oseroff, associate professor of dermatology at New England Medical Center in Boston.

A German scientist coined the term "photodynamic effect" in 1900 to describe what happened in an experiment in which he killed paramecia, large single-celled organisms, with a dye and light. But no one tried using photosensitive agents to treat malignancies until the mid-1960s. Now, after years of effort to develop an effective approach, the first generation of such photodynamic agents is undergoing Phase III clinical trials, the last stage before possible approval by the Food and Drug Administration. Researchers are conducting these trials at more than 40 centers in the United States and Canada, and other scientists are trying to develop more effective agents.

Today's standard cancer therapies require heavy doses of radiation, debilitating drugs or often-disfiguring surgery to halt the uncontrolled growth within. For many cancers, says Oseroff, the existing treatments "are all different and they're all lousy, so you pick the one with the least side effects."

It's not that doctors lack the technology for phototherapy. Lasers with adjustable wavelengths are available and different types of optical fibers exist to deliver the light. Doctors can treat skin with a lens tip on the fiber to project a uniform circle of light, the esophagus or bronchus with a diffuser to distribute light laterally, or the whole bladder with a bulb for an even sphere of light.

Optical fibers can reach almost any part of the body without major surgery to treat a spectrum of cancers. (The technique works only with solid tumors.) Semi-hollow organs such as the bladder or bronchus are easiest reached, "but the brain is a wonderful candidate," Oseroff says. Because fibers as slender as a tenth of a millimeter can deliver enough light, a physician can reach deep into the brain through a hole in the patient's skull without doing a lot of damage.

But scientists still must struggle with chemistry, to find a light-reacting dye that will selectively travel to and affect tumors in humans without harming normal tissue. The search for such dyes mirrors scientists' ongoing quest for substances that discriminate between healthy and cancerous tissues.

Most of the dye testing – with mixed results – has focused on the photoactive dye developed in 1966. This dye, known initially as hematoporphyrin derivative (Hpd) and in purified form as Photofrin II, has been used to treat about 5,000 patients worldwide for many kinds of solid tumors, including those of the skin, lung, bladder, eye, head, neck and esophagus.

Photofrin II bears negative charge and is derived from a class of nitrogen-containing compounds known as porphyrins. This varied group not only includes dyes, but a molecule that carries oxygen in mammalian blood and another that plays an important role in plant photosynthesis.

Photofrin II seems to eliminate or shrink tumors in many cases, but reliable survival statistics are not yet available. Phase I and II trials began in 1978 under the direction of Thomas J. Dougherty of Roswell Park Memorial Institute in Buffalo, N.Y., to determine drug safety and dosage. However, these early trials used small numbers of patients and the larger-scale, Phase III trials, which also compare the therapy to FDA-approved treatments, began only about three years ago.

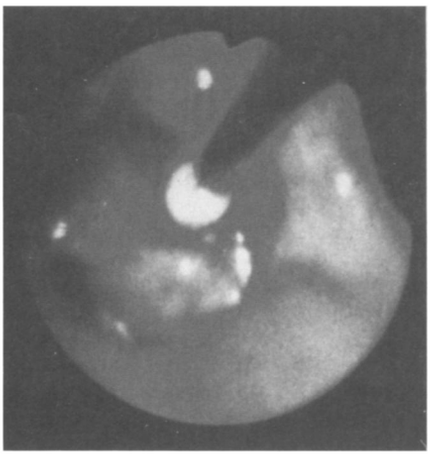
Australian researchers recently reported some discouraging results. Using Hpd and light to treat six patients with multiple skin cancers, they found that most of the 53 tumors initially disappeared – but then came back six months later. They describe their findings in the December PLASTIC AND RECONSTRUCTIVE SURGERY.

A patient undergoing photodynamic therapy will typically get 2 milligrams of Photofrin II per kilogram of body weight injected over about 5 minutes and immediate protection from bright light. Usually two to three days later, a physician uses an argon-pumped dye laser to shine light on the patient's tumor and surrounding area. The 630-nanometer light, which appears red to the human eye, penetrates 3 to 10 millimeters into tissue. For tumors inside the body, doctors deliver the light via an optical fiber.

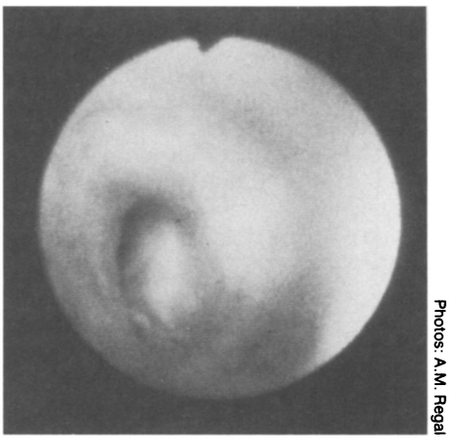
Although Photofrin II seems to work, it is not highly selective for tumor cells and often damages surrounding tissue, Oseroff says. Cancerous tissue takes up two to three times as much dye as normal tissue. Most tissues clear Photofrin II within several hours, but cancerous tumors, as well as liver, spleen, kidney and skin, retain the compound for days.

Because the skin holds Photofrin II, all patients injected with the dye must stay clear of bright light, especially sunlight, for up to 12 weeks after treatment. Patients may also experience eye photosensitivity, nausea, a metallic taste, liver toxicity and, in the case of brain and spinal cord tumors, excessive cerebral fluids. More serious hazards can occur when vital structures are treated. For example, one patient's carotid artery ruptured during photodynamic therapy for a tumor embedded in the artery.

Within the last few years, in an effort to find better, more selective photodynamic agents, researchers in several U.S. labs have begun studying new dyes, including a class of positively charged dyes. Although never tested in humans, positive dyes appear very selective for skin tumors in mice, and preliminary studies indicate some positive dyes clear from the noncancerous skin cells within about 24 hours.



A tumor blocks a human bronchus (left), leaving only a small airway (black area). Three days after physicians treat the patient with Photofrin II and light using a tumor-embedded fiber (dark "V" in left photo), the dead tumor is removed and the bronchus is clear (below).



Photos: A.M. Regal

Positive dyes may be more selective because they concentrate inside tumor cells. Although scientists used to think negative agents acted on the cells, they now believe these dyes "don't get into the cells, but block the blood supply to the tumors," says Louis Cincotta of the nonprofit Rowland Institute for Science in Cambridge, Mass.

Scientists theorize that electrical forces draw the positively charged dyes into tumor cells and into the mitochondria, the "power plants" inside cells. All cells tend to attract positive molecules because the inside of cells houses excess negative charge. But a tumor cell harbors even more negative charge. "In the majority of the malignant cells we've looked at so far, that part of the process that makes it malignant appears to change the magnitude of the cell's membrane potentials so they ingest much more, two to 10 times more, of the [positively charged] compound," Oseroff explains.

Malignant cells also tend to hold on to positive dyes longer than normal cells do. Normal cells typically retain a positive dye for an hour at most, but a tumor cell holds that dye for six hours to three days, Oseroff says. "The treatment is based on the fact that there's a higher initial uptake of the compound [by tumor cells] and there's a much slower release of it [from tumor cells]," he says. "So between those two factors, we find up to 50 times more compound in the tumor than in any other tissue."

If such findings hold true in humans, treatment with a positive dye would leave a patient's skin much less light-sensitive than does Photofrin II. In addition, greater dye-selectivity might allow doctors to treat very small tumors scattered over a wide area by illuminating whole areas of tissue with light, an advantage in treating certain types of bladder cancer. Further, some positively charged dyes may work in low-oxygen environments, where porphyrins will not, says Irene Kochevar, a biochemist at Massachusetts General Hospital.

Perhaps the most effective photodynamic therapy, Oseroff suggests, would team positive with negative agents to target both tumor cells and their blood supply. Oseroff sees phototherapy going the way of chemotherapy, which uses "combinations of agents rather than one alone." But theories of how the positive dyes concentrate in animal and human tumors have not been verified, and Mass. General's Lin warns that researchers must prove the agents work individually before combining them.

The first work on positive dyes was inspired in the early 1980s by research showing that tumor cells selectively retained a positively charged dye, Rhodamine 123. This work, headed by Lan Bo Chen at the Dana-Farber Cancer Institute in Boston, did not involve light. But it set Oseroff, then at Mass. General, and later Cincotta and co-worker James W. Foley, "on the track to using [positive dyes in] photodynamic therapy," Cincotta recalls. "We thought there might be something behind the fact that they are positively charged."

In 1984, Cincotta and Foley, working with Oseroff, began "investigating different [molecular] structures" of dyes in hopes of finding a good light-sensitive, tumor-targeting agent. Their most successful creation was a cyanine derivative called EDKC, which, Oseroff found, localized in cultured tumor cells and reacted with light to kill them. The EDKC work, published in the PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES in 1986, led to a patent issued to both Massachusetts General Hospital and the Rowland Institute of Technology, for the idea of using positive dyes in photodynamic therapy.

After completing the work on EDKC,

Cincotta and Foley began developing their own dyes. Their search of the scientific literature found papers published in the 1940s with "dramatic" photos of mouse brain tumors, selectively stained by a dye called Nile Blue. This earlier work, led by Margaret Lewis at the Wistar Institute of Anatomy and Biology in Philadelphia, was complemented by work of British scientists who independently found similar results after giving mice Nile Blue in a trial-and-error search for chemotherapeutic drugs. Nile Blue, by itself, failed as a chemotherapy. "In those days, they didn't think much about shining light [on molecules], so the work was just dropped," Foley says. Then three years ago, Cincotta and Foley picked it up again. By altering the structure of Nile Blue to make it phototoxic, the two researchers synthesized dozens of derivatives of the compound for future animal studies.

The first animal results come from Oseroff. Over the last 2½ years, he has tested more than 100 different positive dyes, and has found that EDKC and light destroy human-derived skin tumors in mice without visibly (to the eye or microscope) damaging surrounding skin. He also reports that a dye named Victoria Blue, when combined with light, eliminated more than 80 percent of skin tumors in 50 mice, with only minimal damage to normal skin. Recently, Oseroff tested a Nile Blue derivative that he says produced even better results in mice than Victoria Blue. He declines to discuss the details until his findings have been reviewed by other scientists.

Although Oseroff's results appear promising, his mice are not perfect models for human cancer treatment. Whether and how a dye concentrates in a tumor "depends on the properties of the tumor itself," Lin says. "So [a good] model must mimic the human tumor." And skin tumors may react differently to the dyes than other types of cancer, researchers note.

To be sure the compounds will work, "I think you have to do a number of animal studies [using different animals, cancers and dyes] and do them a number of times," Foley says. Adds Cincotta, "On the surface, it looks like [positive dyes] have these desirable properties. Researchers have achieved good results in cell culture and scattered results in mice studies, but it's premature to consider [these agents] great or not great."

In addition to positive agents, a number of new negatively charged dyes "have been introduced and show good localization in tumor cells," says Kochevar. Dougherty is now studying compounds that absorb 800-nanometer infrared light (just outside the visible range), which he says will penetrate tissues deeper than the 630-nanometer light absorbed by Photofrin II.

Heat seems to improve photodynamic therapy's effectiveness. Under conditions where dye-and-light combination kills very few tumor cells, applying 42.5°C heat for 30 minutes produces "between 20- and 200-fold extra killing," Oseroff says. He finds up to 99 percent tumor killing in cell cultures with his heat-dye method, which he uses with a number of dyes including some that cause little toxicity.

Lin says the last three years have seen an explosion of investigations of new dyes. "You turn around and see a new dye," he says. No one knows yet what compound will ultimately prove best. "People are exploring all the things you have to explore including toxicity and selectivity of the dye," Kochevar says. "Even though the work has been going on for a while, it's still in the early stages."

In the future, photodynamic therapy not only may give patients a less painful choice of cancer treatment, but could fill the gap where no safe, effective treatment exists today. Essential organs just can't be removed; nor can surgeons excise hundreds of tiny tumors. Sometimes, more radiation and/or chemotherapy would kill a patient, or a cancer has become resistant to anticancer drugs.

"We really believe this photodynamic therapy has promise," Foley says, but he emphasizes that its promise is not yet fulfilled. "One of the things we're very cautious about is that we're working in a very sensitive area. We don't want to get people's hopes too high."

Agreed, says Oseroff: "There are still no magic bullets." □

News of the week continued from p. 23

Catalytic antibodies do greasy work

Two years ago, researchers first reported getting antibodies to act like enzymes, proteins that catalyze specific chemical reactions. Since then, scientists have designed many catalytic antibodies, known also as abzymes. Each is tailor-made to do a specific chemical job, such as slicing one molecule in two or splicing two molecules into one. Now a team of six scientists has coaxed abzymes into doing something new – work in a greasy, organic environment that normally would deactivate them. The team describes its strategy in the Dec. 21 JOURNAL OF THE AMERICAN CHEMICAL SOCIETY.

Researchers predict that catalytic antibodies will become versatile tools for such jobs as purifying drugs, cutting and splicing proteins and destroying viruses. To date, abzyme research largely has been limited to reactions that occur in aqueous solutions. But the ability to use abzymes in organic solvents could make them handy for a variety of reactions that occur only in organic solutions, says Richard J. Massey, a coauthor of the report and vice president of IGEN, Inc., a biotechnology firm in Rockville, Md.

The scientists first load the antibodies into the watery interiors of greasy, microscopic spheres, called reverse micelles, that dissolve in organic solvents. The solvent used here is isooctane. A reactant chemical, dissolved in the isooctane, penetrates into the reverse micelles, where the abzymes catalyze a reaction, in this case cleaving the phenylacetate molecules. "This gets across the concept that we think will be important, namely, the ability of an antibody to work in an organic solvent," Massey says.

Catalytic antibodies and other enzymes speed the transformation of a chemical reactant into a product. Presumably, they achieve this by binding to the reactant and encouraging the formation of what chemists call the reaction's "transition state," a short-lived, intermediate chemical structure that, once formed, quickly yields the product molecule(s). Enzymes speed reactions by getting reactants into their transition states faster. To make an abzyme that specifically cleaves phenylacetate, the researchers obtained antibodies that bind to another longer-lived molecule that is similar in form to phenylacetate's fleeting transition-state.

Since all antibodies are structurally similar, the scientists expect that their success at getting the phenylacetate-cleaving abzyme to work in a reverse micelle will extend to other abzymes. And since the researchers can use well-established methods for obtaining antibodies to a variety of transition states, they expect the technology to have many applications. One possible use is to purify drugs that emerge from their chemical syntheses in two mirror-image forms, only one of which is active. By binding to and breaking up only the inactive form, abzymes might help drug companies purify their products, Massey says.

The researchers admit that hurdles remain ahead. Most abzymes made so far work at a snail's pace compared with the enzymes found inside cells. Also, most existing abzymes cut molecules in two. Peter G. Schultz of the University of California, Berkeley, an author of the recent paper and one of the earliest to study catalytic antibodies, says it is harder to make abzymes that splice together smaller molecules. Stephen J. Benkovic, a catalytic-antibody specialist at Pennsylvania State University, adds that it may be harder to get molecules that are "greasier" and even less water-soluble than phenylacetate to go inside the reverse micelles. – *I. Amato*