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*