Stories of vampires date back thousands of years. Our modern concept stems from Bram Stoker’s quirky classic Dracula and Hollywood’s Bela Lugosi—the romantic, sexually charged, blood-sucking outcast with a fatal susceptibility to sunlight and an abhorrence of garlic and crosses. In contrast, vampires of folklore cut a pathetic figure and were also known as the undead. In searching for some underlying truth in vampire stories, researchers have speculated that the tales may have been inspired by real people who suffered from a rare blood disease, porphyria. And in seeking treatments for this disorder, scientists have stumbled on a new way to attack other, more common serious ills.

Porphyria is actually a collection of related diseases in which pigments called porphyrins accumulate in the skin, bones and teeth. Many porphyrins are benign in the dark but are transformed by sunlight into caustic, flesh-eating toxins. Without treatment, the worst forms of the disease (such as congenital erythropoietic porphyria) can be grotesque, ultimately exacting the kind of hideous disfigurement one might expect of the undead. The victims’ ears and nose get eaten away. Their lips

By Nick Lane
Molecular Mechanisms

THE SUBSTANCES AT THE HEART of porphyria and photodynamic therapy are among the oldest and most important of all biological molecules, because they orchestrate the two most critical energy-generating processes of life: photosynthesis and oxygen respiration. Porphyrins make up a large family of closely related compounds, a colorful set of evolutionary variations on a theme. All porphyrins have in common a flat ring (composed of carbon and nitrogen) with a central hole, which provides space for a metal ion such as iron or magnesium to bind to it. When aligned correctly in the grip of the porphyrin rings, these metal atoms catalyze the most fundamental energy-generating processes in biology. Chlorophyll, the plant pigment that absorbs the energy of sunlight in photosynthesis, is a porphyrin, as is heme, which is at the heart of the oxygen-transporter protein hemoglobin and of many enzymes vital for life, including cytochrome oxidase (which generates energy by transferring electrons to oxygen in a critical step of cellular respiration).

Porphyria arises because of a flaw in the body’s heme-making machinery. The body produces heme and other porphyrins in a series of eight coordinated stages, each catalyzed by a separate enzyme. Iron is added at the end to make heme. In porphyria, one of the steps does not occur, leading to a backlog of the intermediate compounds produced earlier in the sequence. The body has not evolved to dispose of these intermediates efficiently, so it dumps them, often in the skin. The intermediates do not damage the skin directly, but many of them cause trouble indirectly. Metal-free porphyrins (as well as metalloporphyrins containing metals that do not interact with the porphyrin ring) can become excited when they absorb light at certain wavelengths; their electrons jump into higher-energy orbitals. The molecules can then transmit their excitation to other molecules having the right kind of bonds, especially oxygen, to produce reactive singlet oxygen and other highly reactive and destructive molecules known as free radicals. Metal-free porphyrins, in other words, are not the agents, but rather the brokers, of destruction. They catalyze the production of toxic forms of oxygen.

Photosensitive reactions are not necessarily harmful. Their beneficial effects have been known since ancient times. In particular, some seeds and fruits contain photosensitive chemicals (photosensitizers) called psoralens, which indirectly led scientists to experiment with porphyrins. Psoralens have been used to treat skin conditions in Egypt and India for several thousand years. They were first incorporated into modern medicine by Egyptian dermatologist Abdel Monem El Mofty of Cairo University, just over 50 years ago, when he began treating patients with vitiligo (a disease that leaves irregular patches of skin without pigment) and, later, those with psoriasis using purified psoralens and sunlight. When activated by light, psoralens react with DNA in proliferating cells to kill them.

Two American dermatologists, Aaron B. Lerner of Yale University and Thomas B. Fitzpatrick of Harvard University, were struck by the potential of psoralens. In the 1960s they showed that psoralens are activated by ultraviolet (UVA) rays, and the researchers later refined psoralen therapy using an ultraviolet lamp similar to those used in solariums today. Their method became known as PUVA (short for psoralen with UVA) and is now one of the most effective treatments for psoriasis and other skin conditions.

A Way to Kill Cancer Cells?
IN THE EARLY 1970S the success of PUVA impressed Thomas J. Dougherty of the Roswell Park Cancer Institute in Buffalo, N.Y., leading him to wonder if a variant of it could be effective against cancer. Activated psoralens can kill rogue cells to settle inflammation, but in comparison with porphyrins they are not potent photosensitizers. If psoralens could kill individual cells, could porphyrins perhaps devour whole tumors?
His idea was the beginning of true photodynamic therapy, in which photosensitizers catalyze the production of oxygen free radicals. It was built on earlier work, which revealed two medically useful properties of the porphyrins: they accumulate selectively in cancer cells and are activated by red light, which penetrates more deeply into biological tissues than do shorter wavelengths, such as blue light or UVA.

Dougherty injected a mixture of porphyrins into the bloodstream of mice with mammary tumors. He then waited a couple of days for the porphyrins to build up in the tumors before shining red light on them. His early setup was primitive and passed the light from an old slide projector through a 35mm slide colored red. His results were nonetheless spectacular. The light activated the porphyrins within the tumor, which transferred their energy to oxygen in cells to damage the surrounding tissues. In almost every case, the tumors blackened and died after the light treatment. There were no signs of recurrence.

Dougherty and his colleagues published their data in 1975 in the Journal of the National Cancer Institute, with the brave title “Photoradiation Therapy II: Cure of Animal Tumors with Hematoporphyrin and Light.” Over the next few years they refined their technique by using a low-power laser to focus red light onto the tumors. They went on to treat more than 100 patients in this way, including people with cancers of the breast, lung, prostate and skin. Their outcomes were gratifying, with a complete or partial response in 111 of 113 tumors.

Sadly, though, cancer is not so easily beaten. As more physicians started trying their hand with PDT, some serious drawbacks began to emerge. The affinity of porphyrins for tumors turned out to be a bit of an illusion—porphyrins are taken up by rapidly proliferating tissue, including the skin, leading to photosensitivity. Although Dougherty’s original patients were no doubt careful to avoid the sun, nearly 40 percent of them reported burns and skin rashes in the weeks after PDT.

Potency was another issue. The early porphyrin preparations were mixtures, and they were seldom strong enough to kill the entire tumor. Some porphyrins are not efficient at passing energy to oxygen; others are activated only by light that cannot penetrate more than a few millimeters into the tumor. Some biological pigments normally present in tissues, such as hemoglobin and melanin, also absorb light and in doing so can prevent a porphyrin from being activated. Even the porphyrin itself can cause this problem if it accumulates to such high levels that it absorbs all the light in the superficial layers of the tumor, thus preventing penetration into the deeper layers.

Many of these difficulties could not be resolved without the help of specialists from other disciplines. Chemists were needed to create new, synthetic porphyrins, ones that had greater selectivity for tumors and greater potency and that would be activated by wavelengths of light able to reach farther into tissues and tumors. (For each porphyrin, light activation and absorption occur only at particular wavelengths, so the trick is to design a porphyrin that has its absorption maximum at a wavelength that penetrates into biological tissues.) Physicists were needed to design sources that could produce light of particular wavelengths to activate the new porphyrins or that could be attached to fine endoscopes and catheters or even implanted in tissues. Pharmacologists were needed to devise ways of reducing the time that porphyrins spent circulating in the bloodstream, thereby restricting photosensitive side effects. Finally, clinicians were needed to design trials that could prove an effect and determine the best treatment regimens.

The ideal drug would be not only potent and highly selective for tumors but also broken down quickly into harmless compounds and excreted from the body. The first commercial preparation, porfimer sodium (Photofrin), was approved by the U.S. Food and Drug Administration for the treatment of various cancers. Although it has been helpful against certain cancers (in-
DOCTORS WHO ADMINISTER photodynamic therapy deliver photosensitive chemicals called porphyrins intravenously. These chemicals then collect in rapidly proliferating cells and, when exposed to light, initiate a cascade of molecular reactions that can destroy those cells and the tissues they compose. Some targets for the therapy include abnormal blood vessels in the retinas of people with age-related macular degeneration (the leading cause of adult blindness), cancerous tumors and atherosclerotic plaques in coronary arteries.

... AT THE MOLECULAR LEVEL

1. A porphyrin absorbs light, becoming activated.
2. The activated porphyrin passes this light energy to oxygen molecules, converting them to singlet oxygen.
3. Singlet oxygen reacts with other substances in cells to produce destructive oxygen free radicals; then cells die.

... IN THE EYE

1. To treat macular degeneration, a porphyrin (green) is injected into a patient’s arm. It takes just 15 minutes for the porphyrin to accumulate in abnormal blood vessels under the macula, which is the central part of the retina and responsible for color vision.
2. A red laser light activates the porphyrin, which leads to the destruction of the vascular tissue.
3. After therapy halts damage to the retina, the treated vascular tissue is reabsorbed by the body, and the overlying photoreceptors may settle back into place. Because vessel growth could recur, the patient may require several additional treatments.
... DEEP IN THE BODY

Even long wavelengths of visible light cannot penetrate very far into tissue, so photodynamic therapies for diseased tissue deep within the body require an internal light-delivery system.

1 Here, in an experimental therapy, an optical fiber has been threaded into an artery in which a porphyrin has accumulated in atherosclerotic plaques.

2 The fiber produces red light, activating the porphyrin.

3 Over the course of a few days, the porphyrin destroys unwanted plaques.

Including esophageal, bladder, head and neck, and skin cancers (and some stages of lung cancer), it has not been the breakthrough that had been hoped for and cannot yet be considered an integral part of cancer therapy. Surprisingly, though, the first photosensitizing drug to fulfill most of the stringent criteria for potency and efficacy without causing photosensitivity, verteporfin (Visudyne), was approved in April 2000 by the FDA not to treat cancer at all but to prevent blindness. As the theories converged with reality, researchers came to realize that PDT can do far more than destroy tumors.

Battling Blindness

One thing it could do, for instance, was combat age-related macular degeneration (AMD), the most common cause of legal blindness in our maturing Western population [see “The Challenge of Macular Degeneration,” by Hui Sun and Jeremy Nathans; SCIENTIFIC AMERICAN, October 2001]. Most people who acquire AMD have a benign form and do not lose their sight, but about a tenth have a much more aggressive type called wet AMD. In this case, abnormal, leaking blood vessels, like miniature knots of varicose veins, grow underneath the retina and ultimately damage the sharp central vision required for reading and driving. As the disease progresses, central vision is obliterated, making it impossible to recognize people’s faces or the details of objects.

Most attempts to hinder this grimly inexorable process have failed. Dietary antioxidants may be able to delay the onset of the disorder but have little effect on the progression of established disease. Until recently, the only treatment proved to slow the progression of wet AMD was a technique called laser photocoagulation. The procedure involves applying a thermal laser to the blood vessels to fuse them and thus halt their growth. Unfortunately, the laser also burns the normal retina and so destroys a small region to prevent later loss of vision in the rest of the eye. Whether this is worth it depends on the area of the retina that needs to be treated. For most people diagnosed with wet AMD, the area is located below the critical central part of vision or is already too large to benefit from laser coagulation.

Against this depressing backdrop, researchers at Harvard and at the biotechnology firm QLT, Inc., in Vancouver, B.C., reasoned that PDT might halt the growth of these blood vessels and delay or even prevent blindness. If porphyrins could accumulate in any rapidly proliferating tissue—the very problem in cancer—then perhaps they could also accumulate in the blood vessels growing under the retina. Verteporfin, a novel synthetic porphyrin, seemed promising because it had a good track record in preclinical, animal studies at QLT and at the University of British Columbia in the late 1980s and early 1990s.

Verteporfin accumulates in abnormal retinal vessels remarkably quickly: within 15 minutes of injection into an arm vein. When activated by red laser light, verteporfin seals off the vessels, sparing the overlying retina. Any blood vessels that grow back can be nipped in the bud by further treatments. Two major clinical trials, headed by Neil M. Bressler of the Wilmer Eye Institute at Johns Hopkins University, confirmed that PDT...
Coronary angioplasty is a minimally invasive procedure for treating arteries affected by atherosclerosis. It uses a tiny balloon to open arteries, so that atherosclerotic plaques do not occlude the entire vessel. Photoangioplasty could sidestep many of the problems of conventional angioplasty, notably the restenosis (re-narrowing) of treated arteries. The procedure involves injecting a porphyrin into the bloodstream, waiting for it to build up in the damaged arterial walls and then illuminating the artery from the inside, using a tiny light source attached to the end of a catheter. The light activates the porphyrins in the plaques, destroying the abnormal tissues while sparing the normal walls of the artery. The results of a small human trial testing the safety of the synthetic porphyrin motexafin lutetium were presented in March 2002 by Jeffrey J. Popma of Brigham and Women's Hospital at the annual meeting of the American College of Cardiology. Although it is still early in the testing process, the findings fuel hopes for the future: the procedure was safe, and its success at preventing restenosis increased as the dosage increased.

Accumulation of porphyrins in active and proliferating cells raises the possibility of treating other conditions in which abnormal cell activation or proliferation plays a role—among them, infectious diseases. Attempts to treat infections with the pigments had long been frustrated by a limited effect on gram-negative bacteria, which have a complex cell wall that obstructs the uptake of porphyrins into these organisms. One solution, developed by Michael R. Hamblin and his colleagues at Harvard, involved attaching a polymer—usually polylysine, a repetitive chain of the amino acid lysine—to the porphyrin. The polymer disrupts the lipid structure of the bacterial cell wall, enabling the porphyrins to gain entry to the cell. Once inside, they can be activated by light to kill the bacteria. In recent studies of animals with oral infections and infected wounds, the altered porphyrin showed potent antimicrobial activity against a broad spectrum of gram-negative and gram-positive bacteria. As antibiotic resistance becomes more intractable, targeted antimicrobial PDT could become a useful weapon in the medical arsenal. Several other, related photodynamic methods hinge on the finding that activated immune cells take up greater amounts of photosensitizing drugs than do quiescent immune cells and red blood cells, sparing the quiet cells from irreversible damage. In most infections, nobody would wish to destroy activated immune cells; they are, after all, responsible for the body’s riposte to the infection. In these cases, targeting immune cells would be equivalent to “friendly fire” and would give the infection free rein to pillage the body.

In AIDS, however, the reverse is true. The AIDS virus, HIV, infects the immune cells themselves. Targeting infected immune cells would then be more like eliminating double agents. In the laboratory, HIV-infected immune cells take up porphyrins, thereby becoming vulnerable to light treatment. In patients, the light could be applied either by withdrawing blood, illuminating it and transfusing it back into the body (extracorporeal phototherapy) or by shining red light onto the skin, in what is called transdermal phototherapy. In the transdermal approach, light would eliminate activated immune cells in the circulation as...
they passed through the skin. Whether the technique will be potent enough to eliminate diseased immune cells in HIV-infected patients remains an open question.

Autoimmune diseases, rejection of organ transplants, and leukemias are also all linked by the common thread of activated and proliferating immune cells. In autoimmune diseases, components of our own body erroneously activate immune cells. These activated clones then proliferate in an effort to destroy the perceived threat—say, the myelin sheath in multiple sclerosis or the collagen in rheumatoid arthritis. When organs are implanted, activated immune cells may multiply to reject the foreign tissue—the transplanted organ or even the body tissues of the new host, in the case of bone marrow transplants. In leukemia, immune cells and their precursors in the bone marrow produce large numbers of nonfunctional cells. In each instance, PDT could potentially eliminate the unwanted immune cells, while preserving the quiescent cells, to maintain a normal immune response to infection. As in HIV infection, the procedure might work either extracorporeally or transdermally. Much of this research is in late-stage preclinical or early clinical trials. For all the cleverness in exploring possible medical applications, though, we can only hope that more extensive clinical studies will bear fruit.

### Photodynamic Therapies

THE LIGHT-ACTIVATED drugs listed below are a sampling of those on the market or in development.

<table>
<thead>
<tr>
<th>DRUG</th>
<th>TARGET</th>
<th>MAKER</th>
<th>STATUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Levulan [5-aminolevulinic acid]</td>
<td>Acne and actinic keratosis [a precancerous skin disorder], Barrett’s esophagus [a precancerous condition]</td>
<td>DUSA PHARMACEUTICALS Toronto</td>
<td>On the market for actinic keratosis; Phase II trials (relatively small studies in humans) have been completed for Barrett’s esophagus</td>
</tr>
<tr>
<td>Photofrin [porfimer sodium]</td>
<td>Cancers of the esophagus and lung, high-grade dysplasia from Barrett’s esophagus</td>
<td>AXCAN SCANDIPHARM Birmingham, Ala.</td>
<td>On the market for esophageal cancer and nonsmall cell lung cancer; awaiting FDA decision on high-grade dysplasia</td>
</tr>
<tr>
<td>Visudyne [verteporfin]</td>
<td>Age-related macular degeneration, pathologic myopia and ocular histoplasmosis (eye disorders)</td>
<td>OLT, INC., and NOVARTIS OPHTHALMICS Vancouver, B.C., and Duluth, Ga.</td>
<td>On the market</td>
</tr>
<tr>
<td>Metvix [methylaminolevulnic acid]</td>
<td>Actinic keratosis, basal cell skin cancer and squamous cell skin cancer</td>
<td>PHOTOCURE Oslo, Norway</td>
<td>Awaiting final FDA approval for actinic keratosis; in Phase III trials (large studies of efficacy) for skin cancers</td>
</tr>
<tr>
<td>PhotoPoint SnET2 [tin ethyl etiopurpurin]</td>
<td>Age-related macular degeneration</td>
<td>MIRAVANT MEDICAL TECHNOLOGIES Santa Barbara, Calif.</td>
<td>Phase III trials have been completed</td>
</tr>
<tr>
<td>verteporfin</td>
<td>Basal cell cancer, androgenetic alopecia (male pattern baldness) and prostatic hyperplasia (enlarged prostate)</td>
<td>OLT, INC.</td>
<td>In Phase III trials for basal cell cancer; as OLT0074, in Phase I trials (tests of safety in small numbers of patients) for other conditions</td>
</tr>
<tr>
<td>PhotoPoint MV9411 [contains indium]</td>
<td>Plaque psoriasis</td>
<td>MIRAVANT MEDICAL TECHNOLOGIES</td>
<td>In Phase II trials</td>
</tr>
<tr>
<td>Antrin [motexafin lutetium]</td>
<td>Diseased arteries</td>
<td>PHARMACYCLICS Sunnyvale, Calif.</td>
<td>Phase II trials for peripheral artery disease and Phase I trials for coronary artery disease have been completed</td>
</tr>
<tr>
<td>Lutrin [motexafin lutetium]</td>
<td>Cancerous tumors</td>
<td>PHARMACYCLICS</td>
<td>In Phase I trials for prostate cancer and cervical intraepithelial neoplasia</td>
</tr>
</tbody>
</table>

**Photodynamic Treatments**


An overview of the nature of and treatments for porphyria can be found at [www.sciam.com/explore_directory.cfm](http://www.sciam.com/explore_directory.cfm)