In this area, there is encouraging news. Last October, at the National Institutes of Health (NIH) Neural Prosthesis Conference, a team at another project, the Intraocular Prosthesis Project (IOP) at Johns Hopkins University Hospital in Baltimore, reported on work with three volunteers who had been blinded by retinitis pigmentosa. The retinas of the volunteers were stimulated in one of four quadrants with handheld electrodes, and the people saw phosphenes—points of light—in the corresponding quadrants of their visual fields. This indicated that ganglion cells were still passing signals—and spatially appropriate signals at that—to the optic nerve. "The ganglion cells are functioning in about 70% of their normal numbers in patients with outer-retinal degeneration," says Hopkins ophthalmologist Eugene de Juan.

That brings up the next problem: creating an implant that won't harm the retina. This tissue, says John Wyatt, an MIT electrical engineer and Rizzo's co-principal investigator, has the mechanical properties of "about one layer of sticky wet Kleenex." And that doesn't make things easy, as the implant's electrode array, made of polyimide plastic 50 microns thick, has razor-sharp edges. Before insertion, therefore, the array is coated in silicone and bent to match the retina's own curve, so that it rests on the surface without gouging.

In February and March, the researchers wired their prototype electrode array to a thin electric cable and placed it on the retinas of seven rabbits. A stimulus as low as 10 microamps per electrode—a level equal to that envisioned for a permanent, self-contained implant—generated electrical activity in the visual cortex, measured by scalp electrodes. The implants were removed after a few hours, but later this year the PRI group hopes to show that the prototype can be left inside a rabbit eye safely for days or weeks. The group will then graduate to tests in dogs, and—in 5 to 7 years, if all goes according to plan—to humans. The researchers are also testing alternative materials for building and encapsulating the implant, such as low-density plastics, that would be more resistant to corrosive saltwater in the body and would reduce the weight placed on the retina.

The PRI team is putting off the final problem—the electrical interface with the brain—until the device is ready to be implanted in humans. Wyatt explains that optimizing the frequency, timing, and spatial distribution of the electrodes' stimuli to produce useful images will require feedback from experimental subjects; in short, the researchers will have to ask people what they see.

Wyatt is cautiously optimistic that the groundwork laid by the present experiments will pay off. Although ganglion cells come in specialized varieties that respond to many different aspects of visual stimuli—including color, duration, and direction of movement—he says the experiments at Johns Hopkins make it reasonable to suppose that "if you stimulate the ganglion cells in an X pattern, you'll see an X." De Juan and his colleagues at Hopkins are exploring this further. The scientists are stimulating the retinas of their volunteers to determine how much current must be applied to make human ganglion cells fire and how closely together electrodes should be placed ro achieve maximum visual resolution.

Not everyone is sanguine about this approach. Schiller, for instance, thinks it's far too simplistic to expect that placing a relatively crude array on top of the highly differentiated ganglion cells will cause the cells to fire in an interpretable pattern—"especially when one appreciates that the retina itself is a very complex computer." Ronald Burde, an ophthalmologist at Albert Einstein College of Medicine in New York, adds that signal-blocking scar tissue is likely to develop wherever a permanent array touches the ganglion cells. "As a useful human tool a retinal implant is at least a half a century away, if not longer," Burde says.

Because of such doubts, the IOP team has not been able to secure—and the PRI researchers have not even applied for—large-scale government funding from sources such as NIH. As a result, all the retinal implant researchers are working with limited budgets drawn mostly from private backers, such as the Lions Club, the Research to Prevent Blindness Foundation, and the Seaver Institute of Los Angeles, which specializes in supporting selected high-risk scientific projects. And while all agree that success is a long shot, the implant developers insist that it's not quite as long as doubters suppose. Says Rizzo, "It's our job to do our work well and to show the skeptics that there's reason to reconsider."

–Wade Roush

TOXICOLOGY

Dioxin Receptor Knocked Out

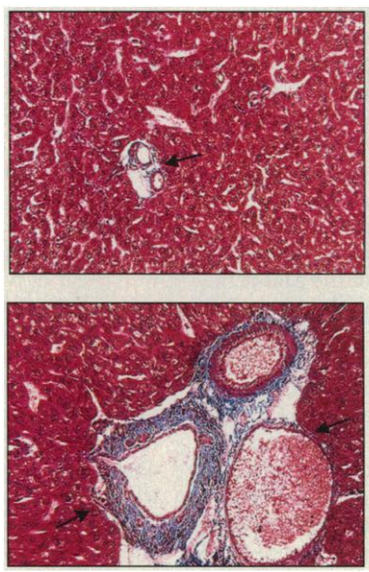
Everybody knows about the immune system for defending the body against infections. But what's less well known is that the body has another defense system: against toxic chemicals. A key feature of this chemical defense is a protein called the aryl hydrocarbon (Ah) receptor that helps detoxify a host of poisons, including polycyclic aromatic hydrocarbons, byproducts of combustion.

New results suggest that this defender protein plays a bigger role in the body than previously thought, perhaps by helping the liver and the immune system develop properly. Not only that, the findings appears to provide an insight into the workings of dioxin, a notorious pollutant that appears to exert its toxic effects, which include cancer and low sperm counts, by binding to the receptor. Indeed, any data shedding light on dioxin's modus operandi will come in handy at the Environmental Protection Agency (EPA), which is now re-evaluating dioxin's health risks to humans. "It's very exciting," says EPA's Linda Birnbaum, a dioxin toxicologist. "The mice will afford us a tool we haven't previously had."

The key to the new findings is a technique that is becoming increasingly important in molecular biology: the creation of knockout mice. On page 722, Frank Gonzalez, Pedro Fernandez-Salguero, and their colleagues at the National Cancer Institute report that they've produced mice lacking the Ah receptor by knocking out the gene encoding the receptor protein.

As soon as the first knockout mice were born, it became apparent that they were abnormal. About half the animals die within a week after birth, apparently succumbing to opportunistic infections that "they just don't have enough lymphocytes [a type of immune cell] to fight off," says Gonzalez. Those that do survive develop massive liver scars and only slowly build up near normal numbers of lymphocytes. Around 10 weeks of age, however, the animals again begin losing those cells and grow sickly, apparently from decreased immune function and liver problems. These effects suggest that the Ah receptor is vital to liver and immune system development and perhaps is "important in pathways crucial for survival," says Stanford University molecular pharmacologist James Whitlock.

Gonzalez suggests that the liver damage occurs because loss of the Ah re-



PHOTOS COURTESY OF JERROLD WARD

Knockout punch. Liver from mouse lacking the Ah receptor is heavily scarred *(bottom)*. A normal mouse liver is shown at top.

ceptor has deprived the animals of their normal defense against some as-yet-unidentified endogenous toxin. The lymphocyte problems are more difficult to explain, considering that the mouse thymus, where the cells begin to develop, appears to be healthy and to be producing adequate numbers of lymphocyte stem cells. "We don't know why the peripheral immune system is so depressed," says Fernandez-Salguero.

Still, says molecular biologist Chris Bradfield, who cloned the Ah receptor gene in 1992, the knockout mice appear "to favor a physiological role" for the Ah receptor in addition to its function in detoxification. Recent speculation along these lines has rested on circumstantial evidence. Researchers have found, for example, that the receptor is present in high levels in the fetal neural tube, which gives rise to the central nervous system, but is found in these tissues at much lower levels after birth. This suggests that it is needed for brain development, a hypothesis that may now be tested more directly in the knockouts.

But even though the Ah receptor may have several benefits, many researchers also think that the harmful effects of dioxin are triggered by its binding to the Ah receptor. Indeed, estimates of dioxin's health risk to humans are based on that assumption.

But just how risky dioxin is—and how it exerts its effects—is a matter of considerable debate. Not all researchers accept the current view that all of dioxin's harmful effects depend on it binding to the Ah receptor. The knockouts will enable researchers to determine whether some of dioxin's effects do in fact occur independently of the receptor. That information, in turn, might help pin down dioxin's risk to humans.

Much of the current concern about dioxin is based on developmental and reproductive effects seen in rodents. Extrapolating those results to humans would be reasonable if all the effects depend on the pollutant's binding to the Ah receptor, because the receptor is very similar in both humans and rodents. But if some of the effects in rodents occur independently of the receptor, then toxicologists would want to see if those alternate damage pathways are also present in humans. If they are absent, then it might be possible to downgrade dioxin's human risks.

Despite the potential of the knockout mice for such studies, Gonzalez cautions that experiments on the animals might be "very difficult" because of their sickly nature. But he is optimistic that this problem can be solved, perhaps by giving the animals an Ah gene attached to regulatory sequences that turn it on only in the liver. "If we can restore liver function," Gonzalez says, "we should be able to do long-term toxicity and carcinogenicity studies on these mice."

–Richard Stone

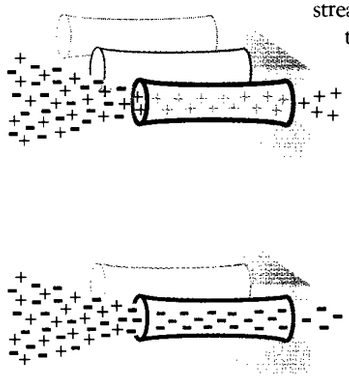
ILLUSTRATION: K. SUTLIFF

ELECTROCHEMISTRY

Throwing a Switch on a Nanoscale Sieve

Making things small is a big deal today. Scientists have built motors and gears no wider than a human hair, formed wires only a few atoms across, and carried out chemical reactions one molecule at a time. Now researchers at Colorado State University have created molecular sieves with pores as small as 1.6 nanometers (billionths of a meter) across that will let positive ions pass while blocking negative ions. Or vice versa. For, as electrochemist Charles Martin and his colleagues report on page 700, they've made the first artificial membrane whose selectivity can be changed as easily as flicking a switch.

The membrane can be adjusted to pass ions of either charge simply by changing its electric potential; the pore size can be modified as well. And these two attributes have other researchers eager to put the device to work. "It's a wonderful model for how ion-selective membranes work," says Henry White, a University of Utah electrochemist who studies ion transport through skin. Many biological membranes filter ions according to their electric charge, White notes, and "people want to know how ion transport is affected by the size and charge [of the pores in the membranes]." With the new membrane, they have the basis for an experimental model. And with further refinement, Martin suggests, the membrane could have industrial applications, separating molecules by size and charge.



Filter flip. With a negative electric potential *(shown as black)*, a gold-plated membrane lets through only positive ions. With a positive potential *(red)*, the situation is reversed.

Martin did not set out to invent a molecular sieve. His original plan, in 1990, was to make an array of nanoscopic electrodes that could, for example, detect trace levels of chemicals in solution. Martin and graduate student Vinod Menon began the construction project with commercially available filtration membranes: thin pieces of plastic riddled with tiny pores. By plating gold over the entire membrane, covering the two sides, and filling the pores, Menon created a structure of two gold layers connected by millions of tiny gold strands embedded in the membrane. Stripping off one of the gold layers left millions of gold disks scattered across the face of the membrane, each disk a viable nanoelectrode.

While building these nanoelectrodes, however, Martin realized that the same process might be used to produce a much more interesting device. By leaving both layers on and laying down less gold, he thought, it should be possible to create gold tubes through the pores instead of wires. The tubes maintained the membrane function, but the gold plating meant it would be possible to put an electrical charge on the tubes and control the types of ions they would let through. A positive charge would repel any positively charged ions but would allow a stream of negatively charged ions to pass through. A negative charge would reverse the effect and allow only positive ions to cross. The result: a switchable ion sieve.

There were membranes on the market that would pass either positive or negative ions, but none that could be switched back and forth without a complicated procedure to modify their chemical structure. Unfortunately, Martin recalls, "I didn't have enough person-power in the laboratory to start another new project, so I put it on the back burner."

The project moved to the front burner in 1994 when Matsuhiko Nishizawa, a postdoc from Japan, arrived in Martin's lab. It took Nishizawa a year of experimenting with temperature, pH, and other conditions of the gold-plating process, but when he was finished, he could not only create gold membranes but also control the tube width, ranging from an inner diameter of about 20 nanometers down to 1.6 nanometers.

The ability to switch ion selectivity is nice, Martin says, but the ability to make minuscule pores may prove more important. "These tubes have diameters approaching the size of molecules," he notes, "so maybe we can start thinking about filtering molecules on the basis of size." An industrial chemical process might, for example, require isolating a small, positively charged molecule from a solution containing both larger molecules and molecules with negative charges. A membrane with the correct pore size and charge would be able to physically filter larger molecules, and the membrane's charge would block those with negative charges. This could give the chemical industry a cheaper, one-step alternative to some of its multistep separation techniques. Martin says he has already started an experiment to test the nanoscheme. If it works, it would be no small achievement.

–Robert Pool