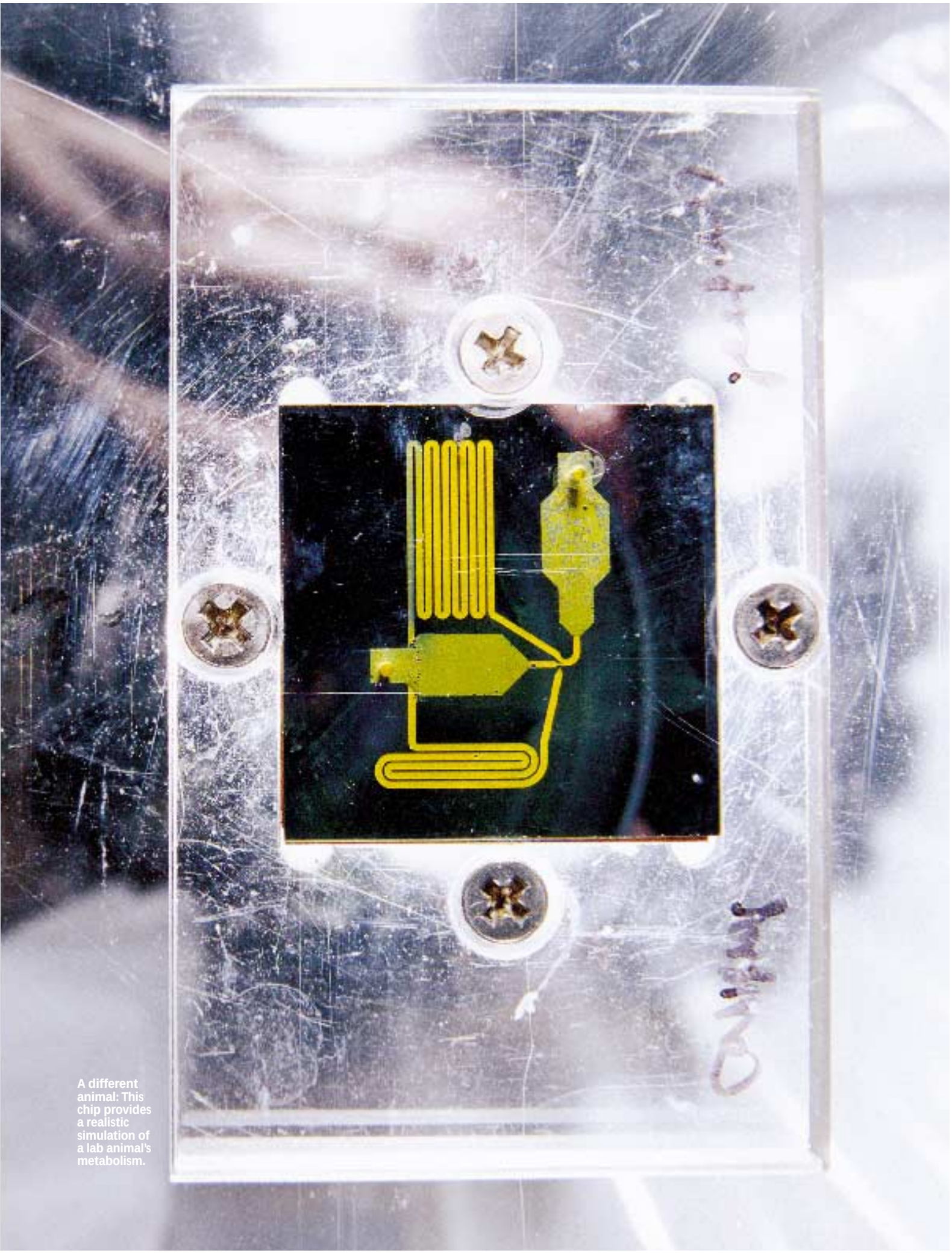
the silicon guinea pig

Pharmaceutical companies anxious to see if experimental drugs have toxic side effects may soon turn to a thumbnail-sized silicon chip, packed with live cells, that mimics the metabolism of a lab animal. Such "animal on a chip" devices could help to quickly and cheaply spot toxic compounds, sparing companies years and millions of dollars in the drug discovery process.

BY DAVID H. FREEDMAN | PHOTOGRAPHS BY DAVID BARRY



A different animal: This chip provides a realistic simulation of a lab animal's metabolism.

AT FIRST GLANCE, Michael Shuler's chip could pass for any small silicon slab pried out of a computer or cell phone. Which makes it seem all the more out of place on a bench top in the Cornell University researcher's lab, surrounded by petri dishes, beakers, and other bio-clutter and mounted in a plastic tray like a dissected mouse. The chip appears to be on some sort of life support, with pinkish fluid pumping into it through tubes. Shuler methodically points out the components of the chip with a pencil: here's the liver, the lungs are over here, this is fat. He then injects an experimental drug into the imitation blood coursing through these "organs" and "tissues"—actually tiny mazes of twisting pipes and chambers lined with living cells. The compound will react with other chemicals, accumulate in some of the organs, and pass quickly through others. After several hours, Shuler and his team will be closer to answering a key question: is the compound, when given to an actual human, likely to do more harm than good?

This so-called animal on a chip was designed to help overcome an enormous obstacle to discovering new drugs: there is currently no quick, reliable way to predict if an experimental compound will have toxic side effects—if it will make people sick instead of making them well. Testing in animals is the best drugmakers can do, but it is slow, expensive, often inaccurate, and objectionable to many. To minimize the number of animal tests, drug companies routinely screen drug candidates using cell cultures—essentially clumps of living human or animal cells growing in petri dishes or test tubes. The approach is relatively cheap and easy, but it gives only a hazy prediction of what will happen to a compound on the circuitous trip through the tissues and organs of an animal.

Shuler is among a handful of researchers who are developing more sophisticated cell cultures that simulate the body's complex organs and tissues. MIT tissue engineer Linda Griffith, for one, has built a chip that mimics some of the functions of a liver, while Shuichi Takayama, a biomedical engineer at the University of Michigan, has built one that imitates the behavior of the vasculatory system *(see "Other Animal-on-a-Chip Efforts," p. 67)*. But while such efforts have produced convincing analogues of parts of human or animal bodies, Shuler has gone a step further. Working with colleague Greg Baxter, who launched Beverly Hills, CA-based Hurel to commercialize the technology, Shuler has combined replicas of multiple animal organs on a single chip, creating a rough stand-in for an entire mammal. Other versions of Shuler's chips attempt to go even further, using human cells to more faithfully reproduce the effects of a compound in the body.

Drug companies are interested, and no wonder: they routinely make thousands, even tens of thousands, of compounds in hopes of finding one that is effective against a particular target. Chips such as Shuler and Baxter's could mean a cheap, fast, and accurate way to weed out compounds that would eventually prove toxic, saving companies years and millions of dollars on the development of worthless drugs. According to a recent study by Tufts University's Center for the Study of Drug Development, for each drug that reaches market, the drug industry spends an average of $467 million on human testing—the vast majority of the money going to drugs that fail, either because they aren't effective or because they prove toxic. If more failures could be identified before animal testing even began, companies could focus more of their time and money on the winners. "Everyone in the industry hopes to have surrogates for animals and humans when it comes to testing compounds," says Jack Reynolds, head of safety sciences for Pfizer, the world's largest pharmaceutical firm. "This is the sort of technology we'd want in our toolbox."

POISON PILLS

The toolboxes of drug developers are already stocked with a host of simple cell-culture tests aimed at quickly predicting which would-be drugs will have toxic side effects. The problem with these tests is that they're often *too* simple. A typical scenario: researchers squirt a solution containing an experimental medication into petri dishes where live cells harvested from a rat's lungs float in a nutrient-rich broth. If the cells die, the researchers table the compound and try another; if the cells survive, they begin the lengthy and expensive process of testing the compound on mice, rats, and other animals. But the compound's failure to kill the lung cells offers little insurance that it won't make people sick.

When a person takes a drug, its active ingredient goes on a wild ride to get to the target cells: it might be absorbed by the gut, broken down by enzymes in the liver, hoarded for weeks by fat cells, screened out by a brain membrane, and whirled through the whole ordeal over and over again by the blood. When that happens, an otherwise harmless compound can accumulate in a particular organ until it reaches toxic levels. Or it can be transformed into a different compound altogether, which itself is toxic. Pfizer's Reynolds estimates that, of drug candidates that end up proving unsafe, approximately 40 percent acquire their toxicity after being converted to other compounds in the body.

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One reason that conventional cell-culture tests often mislead researchers is that they don't present the complex brew of enzymes and other chemicals that a drug can encounter and react with in the various tissues of the body. And simple cell cultures don't reveal how much of a drug actually gets to different types of cells, in what form, and for how long. Indeed, nearly half of the drugs that seem safe in cell-culture testing prove toxic in animal tests; and even more fail when they encounter the complex tissues and organs of humans. Researchers hope, however, that cell cultures that better simulate the conditions in the body will do a far better job at spotting toxic drugs, reducing the reliance on animal and human testing. "The holy grail of the industry is to be able to predict toxicity from a cell culture," says Peter Lord, head of mechanistic toxicology in preclinical development at Johnson and Johnson Pharmaceutical Research and Development.

A cultured approach: Cornell University's Michael Shuler takes cell cultures to a new level.

TINY PLUMBING

Michael Shuler is a 57-year-old, lanky chemical engineering professor who has nurtured a side interest in biological processes since junior high school. By 1989 he had become interested in toxicity testing, and he had been pondering the unreliability of conventional cell cultures when an idea occurred to him: could you make a cell culture that replicates the journey through the various organs? He recognized it as a chemical engineering problem: glass chambers lined with different types of cells and hooked up via tubes to each other and to a pump that sent fluid through them would far more realistically simulate a body, and tests employing them might predict what happens in living animals much more accurately.

After several months, Shuler and students had constructed a bench-top conglomeration of cells and plumbing providing a crude working model of a set of mammalian organs. It sort of functioned, but Shuler knew there was a big problem with its fidelity: almost all of the chemistry in the body takes place in tissues packed with minute canals and chambers, where critical reactions hinge on the ability of various chemicals to concentrate in some places and diffuse in others, depending in part on the microscopic geography. Mixing everything up in big beakers would distort that delicate balance. Plus, at this size the system wouldn't be practical or cheap enough for large-scale testing.

Meanwhile, molecular biologist Greg Baxter had just joined Cornell's Nanobiotechnology Center as a research scientist. His specialty was microfluidics—essentially, microscopic plumbing on a chip. On his second day he buttonholed Shuler at his lab, wondering if he had any projects that could benefit from ultraminiaturization. Funny you should ask, said Shuler.

It took just two meetings to hammer out the basic chip design and a year to produce the first prototype. To build one of the devices, the researchers carve minute trenches that look like faint scratches into a thumbnail-sized silicon chip; these trenches serve as fluid-carrying pipes. Producing microfluidic features on chips for testing chemical reactions and imitating biological processes is not new. But by combining their skills in chemical engineering and microfabrication, Shuler and Baxter add a significant twist: they've engineered the sizes, lengths, and layout of all the trenches in an attempt to closely duplicate the fluid flows and chemical exposures that cells experience in real organs.

The trenches act as surrogate blood vessels, carrying chemicals within and between the chip's ersatz organs, which are themselves composed of trenches that are tightly spiraled or snaked into dense clots roughly half a centimeter wide. Thousands of living cells are fixed to the floor of each organ's trenches. A brick-sized external pump circulates a nutrient-rich fluid—a stand-in for blood—through the chip. When a test compound is added to the fluid, its silicon journey is roughly analogous to what it would undergo in a live mammal, thanks to 13 years of fiddling with each organ's size, pattern, and interconnects, and with the sizes and shapes of the various trenches. "We wanted the cells' environment to be as realistic as possible, from the delivery of nutrients and the removal of waste products to the mechanical stresses that it experiences," says Shuler.

OTHER ANIMAL-ON-A-CHIP EFFORTS

PROJECT LEADER	GROUP	TECHNOLOGY
Dawn Applegate	RegeneMed (San Diego, CA)	Chips lined with human liver tissue for drug screening
Linda Griffith	MIT (Cambridge, MA)	Liver on a chip for drug screening
Paul Kosnik	Tissue Genesis (Honolulu, HI)	Chips with vascular and ligament cells for developing tissue replacement
Shuichi Takayama	University of Michigan (Ann Arbor, MI)	Cell-culture chips with channels that mimic the vasculatory system
William Wang	Pharmacom (Iowa City, IA)	Drug-screening chips that will include cells from the brain and other organs

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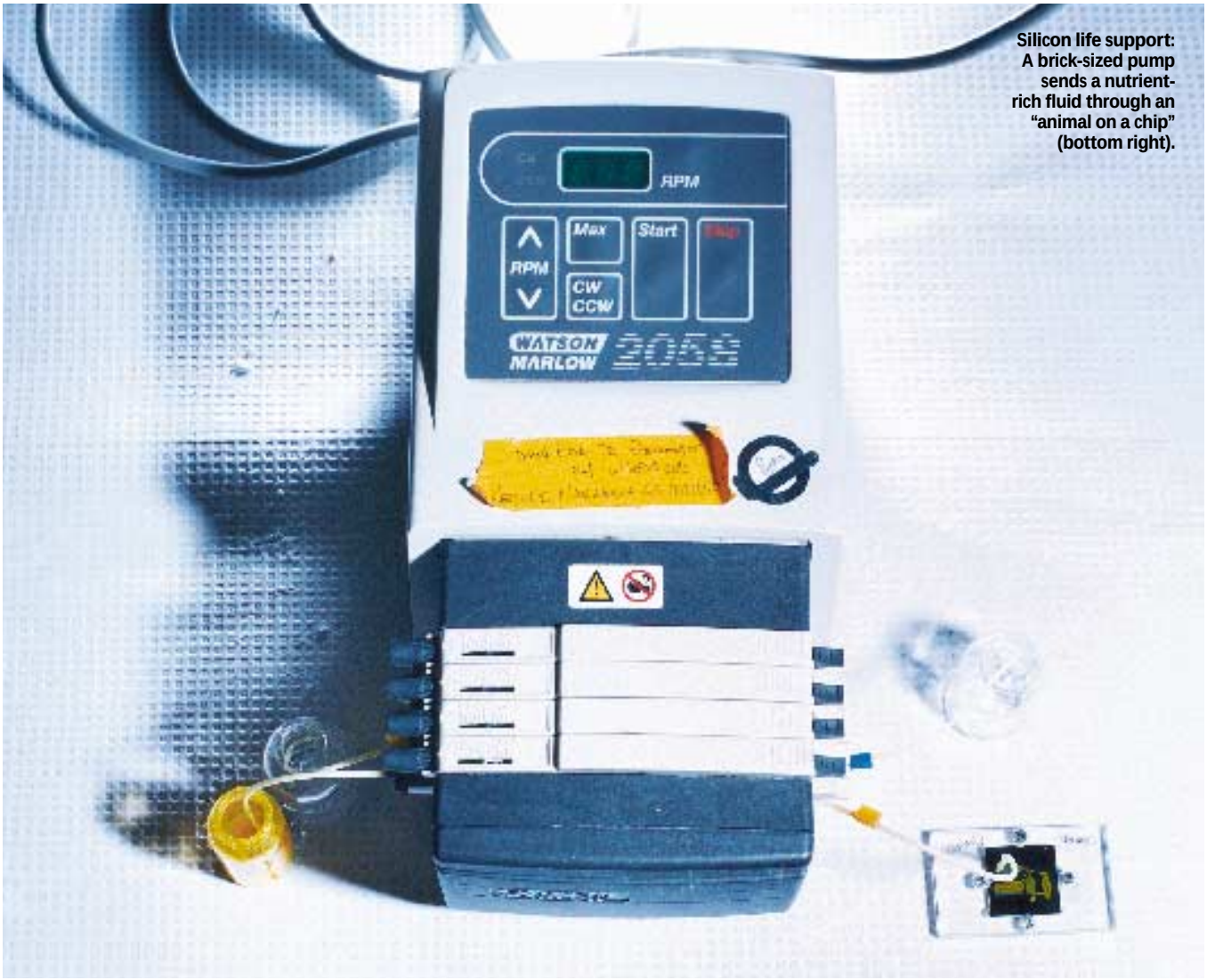
After a test compound has circulated through the chip for several hours, the cells in the chip are monitored, either with a microscope or via embedded sensors that can test for oxygen and other indicators. Do the cells absorb the compound? Does it sicken or kill them? As in an actual animal, each organ or tissue plays a specific role in the chip. The liver and gut break some compounds down into smaller molecules, for example, while the fat—jammed not only with cells, but also with a spongelike gel—often retains compounds, allowing them to leak out later. A "target" organ or tissue is usually included to demonstrate the ultimate effects of the compound; this might be a cancer tumor, or an especially vulnerable tissue, such as the lung's, or bone marrow.

The chips, of course, will have to be extensively tested before drug firms will use them widely. Still, early signs are encouraging. Shuler ran one experiment with naphthalene, a compound used in mothballs and pesticides. Excessive exposure causes lung damage, but you wouldn't know it from standard cell-culture tests. That's because the culprit isn't naphthalene itself but rather two chemicals produced by the liver when it breaks naphthalene down. If you knew that and splashed those by-products directly on lung cells in culture, you'd observe such a severe response that you'd conclude even slight exposure to naphthalene is extremely dangerous. But that's wrong, too; as it turns out, fat cells yank much of the toxic compounds out of the system. Shuler's chip convincingly mimics this chain of events, yielding a realistic measure of the damage.

Such precise simulation promises to help drug companies improve their screening of drug candidates—and waste less time and money on those that will ultimately fail animal tests. According to Baxter, the chips are ready for such an application right now, and six large companies are currently talking to Hurel about adopting the technology. Shuler, aided by a team of students and collaborators at Cornell and elsewhere, is working on further shrinking and automating the technology. The goal: a sheet-of-paper-sized bank of 96 chips that plugs into a robotic lab setup that very rapidly adds test drugs and monitors the results. The system could not only replace conventional cell cultures but also reduce a reliance on animal experiments, in which researchers must use a great number of animals to test dif-



Silicon life support: A brick-sized pump sends a nutrient-rich fluid through an "animal on a chip" (bottom right).

ferent doses of a drug, and must monitor those animals over time to pick up subtle side effects. "We're talking about running a test in one or two days that would take months with animals," says Shuler. Shuler projects a per-chip production price of about $50 complete with cells, compared to the hundreds or even thousands of dollars it takes to acquire and maintain a single lab animal.

KIND OF HUMAN

Chips that replicate the functioning of animals will likely be the first versions of the technology to make a commercial impact. But the hope is that once those prove to accurately predict the results of animal tests, human-on-a-chip versions will provide a good indication of how toxic a drug is likely to prove in human trials.

Animal testing plays that role now, but not very well. Four out of five drugs that make it through animal testing end up failing in human clinical trials, usually because of safety concerns. Part of the problem is that mice can't tell you they have headaches, blurred vision, or stomach cramps. But the larger issue is simply that animals' organs, and the processes that take place in them, are not identical to those of humans. No one knows how many drugs that would have been safe in humans were shelved because they sickened some animals. (Penicillin, for instance, is toxic to guinea pigs but fortunately was also tested on mice.)

Chips containing simulated human tissues and organs could also allow researchers to work out complicated multidrug schemes for treating various diseases without putting patients through agonizing rounds of trial and error. Shuler, for instance, is zeroing in on anticancer cocktails. He incorporates human cells from uterine or colon tumors in his chips, setting up a more realistic model of a particular type of cancer. He can then test the ability of various combinations of chemotherapy drugs to kill the cells without sickening the rest of the system. "To find good combination therapies, you need to run a lot of tests to determine the right doses and the order in which the drugs are given," he explains. "It's the sort of problem we can get our hands around with this technology."

Neither Baxter nor Shuler claims that the animal on a chip is any sort of panacea for the complex and deeply challenging drug-development process. For one thing, the chips still have to prove in large-scale tests that they really do a better job than conventional cell cultures of predicting toxicity. But if they measure up, then the pills you take ten years from now may very well arrive thanks to the sacrifices of a silicon lab rat.

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