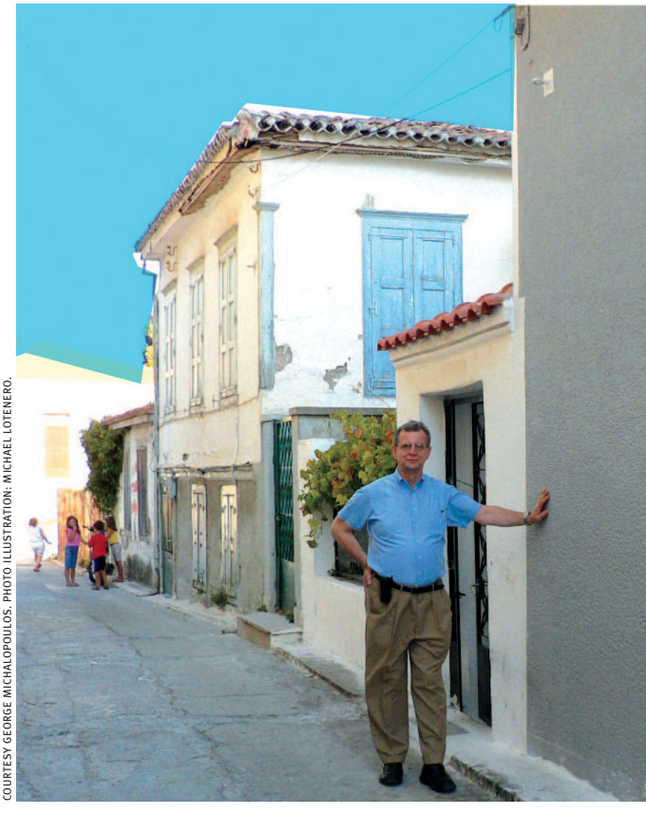
AN EPIC TALE OF INJURY AND RENEWAL

BY ELAINE VITONE

PROMETHEAN EFFORT

Among regenerative medicine types, liver research docs, and, really, any ardent fans of tissue biology, one often-cited story is the Greek myth of Prometheus, cocreator of humankind. Prometheus so loved us that he dared to defy Zeus, stealing fire from him to give to us. To put Prometheus in his place, Zeus chained him to a mountain, where, each day, a great eagle fed from his liver. Each night, the liver grew back and, the next day, this gruesome martyrdom began anew.

In Prometheus' modern-day cheering section, one of the most vocal fans is the University of Pittsburgh's George Michalopoulos, who leads Pitt's Department of Pathology and holds the Maud L. Menten Chair in Experimental Pathology. As you might guess, if not from his name then from his accent or from the way he loves to drop Greek etymology into conversation, Michalopoulos is Greek himself. ("My daughter tells me I'm worse than the father from, you know, *My Big, Greek, Fat*, what is it, *Wedding*?" says Michalopoulos, chuckling. "They make fun of me.")



COURTESY GEORGE MICHALOPOULOS. PHOTO ILLUSTRATION: MICHAEL LOTENERO.

Michalopoulos in his hometown on the island of Samos. OPPOSITE PAGE: The Greek word for liver, *hēpar*, comes from *hēpaomai*: "to repair." In this 6th century BCE depiction, Prometheus is bound and helpless while, each day, an eagle feeds from his liver. Each night, it grows back.

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One summer afternoon in 2013, sitting in his office on the fourth floor of the University's Biomedical Science Tower 3, the MD/PhD explains in his deep voice: Every tissue has its own regenerative capabilities—bone marrow, skin, intestine, and even brain and heart, we now know. But the liver leaves them all behind. You can cut away two-thirds of it, and within weeks, it will grow back to its original size. You can even cut out half of it and transplant it into someone else and, again within weeks, each half wills itself whole. The liver is the only organ that can do this.

Liver regeneration is seen in all vertebrates. Presumably, as Michalopoulos wrote in a 1997 *Science* review paper that put his favorite Greek god on the cover, this process evolved to protect animals, as liver loss has catastrophic results. The liver produces most of the blood's enzymes and all of its coagulants. It turns smithereens of food from the stomach into the soluble stuff that keeps us running. And, most famously, the liver detoxifies everything we put away, from beer to burgers to acetaminophen, as well as whatever else we might ingest. Without the liver, we truly are lost, within minutes.

In many cultures, if a person had fire in the belly, that burning center was thought to be the liver. The Zulu words for *liver* and *courage* are one and the same. In Persian, Urdu, and Hindi idioms, to "throw bits of your liver" is to give something your all. To set out and raise hell.

And the liver *is* stubborn. Block one molecular means of regeneration, and the organ will conjure up another means, then another, then another. It is a fighter. A *live*-er.

Much of what we now know about this incredible phoenix within the body, we know thanks to Michalopoulos and his team—notably, "from day one," he says, PhD professor of pathology Reza Zarnegar. Throughout their decades-long quest to understand this process, Michalopoulos has gathered around him the strongest group in the United States for studies of liver regeneration. Pitt's "hepatomaniacs" are working to harness this force of nature to treat cancer; to develop bioartificial liver devices; to ensure better outcomes after liver-transplant surgery; and to reverse liver failure, so that fewer transplants are needed in the first place.

They continue a great tradition of hepatomance-ing at Pitt. In the 1980s, Thomas E. Starzl, Distinguished Service Professor of Surgery, performed the first successful human liver transplants. Given Pitt's strength in this area, the hope was to cultivate a strong program in liver biology—hence Michalopoulos. And since he was recruited in 1991, Pitt's Department of Pathology has grown to one of the largest in the country, with more than 170 faculty members. They've been one of the top 10 National Institutes of Health–funded pathology departments for more than a decade.

In ancient Babylonia and neighboring countries, if you wanted to know whether the gods were on your side in battle, illness, or the whims of the weather, the place you looked for answers was the liver. Soothsayers sacrificed animals to read the scars on the organ's surface—evidence, we now know, of the liver's dogged will to survive.

Today, the liver's astounding capacity for self-healing informs regenerative medicine, transplant medicine, developmental biology, and cancer biology. The latter—regeneration's alter ego—is Michalopoulos' next challenge.

In 1971, Michalopoulos came to the United States, a newly minted MD who'd spent his teen years reading copies of *Science* and *Nature* at his local library in Athens, marveling at the impact of DNA structure on replicating cells. He began a combined pathology residency/PhD program at the University of Wisconsin, studying the effects of chemical carcinogens as part of an emerging school of thought that figured tumor growth must mimic the growth of normal tissue, but with one fatal flaw: It doesn't stop. The Wisconsin group's favorite model to study tissue growth, naturally, was the liver. Not only is it big (the largest organ in the body), soft, and easy to grind up and distill down to its proteins, but the liver is also easy to nudge into self-repair on a massive scale. Just surgically remove two-thirds of it from a rodent—partial hepatectomy, as it's called.

Well, that part was easy enough. But studying the process in cultured cells proved tricky. Out of the context of the body, these storied survivors simply give up and die. Michalopoulos spent two and a half years "trying everything under the sun" to make them grow. "That was fun, but it was frustrating," he says. (In the late '90s, he would succeed. Working with Bill Bowen and Joe Locker, he developed a medium called hepatocyte growth medium, or HGM, which is still used in liver-research studies all over the world.)

Michalopoulos joined the faculty at Duke University in 1977, followed by Zarnegar in the mid-1980s. In the 1970s, studies had shown that agents circulating in the blood of animals surgically divested of portions of their livers had the ability to trigger regeneration. So, they took blood from an hepatectomized animal and "fed" it to liver cells in culture. That worked. On Michalopoulos' office wall, right next to Prometheus' close-up, hangs the first photographic proof: Little black grains of radioactive thymidine tag the nuclei of these dividing crimson cells.

The two scientists moved to Pitt in 1991, where they spent six years separating out the circulating proteins and testing them one by one until they finally found what awakens the sleeping lion. Hepatocyte growth factor (HGF), which was discovered simultaneously by the Pitt duo and two other labs, subsequently opened several new fields, as it would also be found to play roles in wound healing and embryonic development, among other biological processes. As of this writing, the paper has been cited some 10,700 times.

In the early 2000s, Zarnegar's group was the first to show that HGF mutations occur in human cancer, a discovery that was made public in the *Journal of Clinical Investigation*. Zarnegar and colleagues later found that the HGF receptor system plays an important role in the regulation of hepatic glucose and fat metabolism. The findings, published in *Nature Medicine* in 2011, implicate a cross-talk between HGF receptors and their distant cousins in the insulin receptor system. This work carries implications for type 2 diabetes and fatty liver disease. "It's amazing. I'm still working on how HGF works, and what it does," Zarnegar says.

Discovering HGF put the team on the map, but it was just the beginning.

"We had grabbed the elephant by the tail, so to speak," Michalopoulos says.

In collaboration with Paolo Comoglio, a histology professor at University of Torino Medical School, Italy, Michalopoulos and Zarnegar helped identify the receptor for HGF in 1991.

And in a series of papers published throughout the late 1990s, the Pitt team figured out the answer to a question that had vexed liver researchers ever since their development of HGM (the medium that kept liver cells alive in culture): After a few days in the dish, the cells behaved strangely. They undifferentiated, losing their markers of liver cell-ness. The team found a solution: adding extracellular matrix to the mix. The extracellular matrix—the strong yet pliable protein "glue" that holds tissue together—has connections with receptors in the cells, Michalopoulos explains. It communicates with them.

They learned that, within an hour of liver

COURTESY REZA ZARNEGAR, PHOTO ILLUSTRATION MICHAEL LOTENERO

Michalopoulos and Reza Zarnegar (right) have spent decades unveiling secrets of liver regeneration.

injury, an enzyme called urokinase is released into a rodent's bloodstream (from where, no one yet knows). Urokinase signals a breakdown of the liver's extracellular matrix, which holds abundant stores of HGF. As the extracellular matrix breaks apart, HGF is released into the bloodstream. And then, *boom*, cell division.

At the time, the only thing we knew to behave this way (step one: matrix breakdown; step two: cell division; step three: matrix resynthesis) was cancer. And, in the ensuing years, scientists would learn that every other kind of tissue regeneration begins this way, too.

To ignite liver regeneration, the Pitt team found, HGF works synergistically with another growth factor, which circulates continuously throughout the liver, whether injured or not—it's called EGF (a.k.a., epidermal growth factor). Within 30 minutes of a partial hepatectomy, both growth factors activate, prompted by the breakdown of the extracellular matrix.

When the liver suffers a bad blow, levels of protein in the blood soar, and many of these proteins have been found to act as liver-regeneration helpers. The biggest helper of all is the hormone norepinephrine. In 1986 the team found that in the presence of even the tiniest levels of HGF and EGF, norepinephrine boosts levels of both of these growth factors and, in turn, liver regeneration.

The hepatomaniacs persisted, discovering layers of redundancies in the liver's bag of tricks. They learned that, even if growth factors are nowhere to be found, the injured liver has other molecular means to press on.

Among them is Wnt/ß-catenin, a group of proteins that serve as a pathway into the cell. As a postdoc at the Department of Veterans Affairs Medical Center, Washington, D.C., and subsequently as one at Temple University Hospital's Fels Institute for Cancer Research and Molecular Biology, Satdarshan "Paul" Monga studied Wnt/ß-catenin's role in fetal liver development. The pathway had also been shown to play a role in 20 to 30 percent of liver cancers, and Monga wondered if it might also have a role in liver-regeneration. So, in 1999, he showed up on the fourth floor of Pitt's BST South, an eager emerging scientist handing out copies of his CV. He went on to prove his hypothesis correct in those very halls.

Today, Monga, an MBBS who's now a Pitt professor of pathology and its Endowed Research Professor of Experimental Pathology, is simultaneously studying Wnt/ß-catenin's potential as a target for cancer treatment, for aiding recovery from acetaminophen overdose (the largest cause of acute liver failure), and for helping living donors for liver transplants recover more quickly.

"I've grown under George's tutelage," he says. "He's really like a father figure to me. … I would not be where I am now without his direction, mentorship, and support."

Monga is one of many mentees who speaks of Michalopoulos with the reverence and gratitude of an adoring son. More than one young researcher told this writer that Michalopoulos is the "best thing that ever happened" to him.

At the big, international hepatomeetings, Michalopoulos, a tall guy in a blue blazer, is easy to spot. Through conference sessions, he's the earnest pupil who always sits in the front row, furiously taking notes on his iPad. In the Q&A at the end, he's the sage who steps up to the microphone, invariably asking, in his booming voice, a question so brilliant it blows everyone away. And in the session breaks, he's the unabashed aficionado of science roaming the halls like a kid in a candy store, taking in the poster presentations and chatting up young researchers: *Hey, this is wonderful. I bet you what's going on here is this. … Have you tried it? If you need that reagent, we have it in our lab. We can give it to you.*

Michalopoulos likes to think that what goes around comes around. He often says, "Science is too big to be considered yours."

Udayan Apte (Fel '07, '08), a former Pitt postdoc twice over, says that in preparation for his leaving the nest, Michalopoulos gave him no fewer than 17 ideas to pursue in his independent research career—enough to last several researchers a lifetime. Apte is now pursuing them as an assistant professor of pharmacology, toxicology, and therapeutics at the University of Kansas. And lately, Michalopoulos has been guiding Apte's mentees, too. Students returning from conferences tell him, *Your mentor is so cool. He gave me this great idea …*

Alphonse Sirica, PhD professor of pathology and internal medicine and chair of the division of cellular and molecular pathogenesis in the Department of Pathology at Virginia Commonwealth University and friend of Michalopoulos' from way back, says that, years ago, discussions at these meetings sometimes got a little caustic (clashes over the confounding origins and behavior of liver cells). So, in 1990, the two organized a liver-regeneration summer research program for the American Society of Experimental Biology. Sirica recalls how Michalopoulos charmed everyone, fostering a respectful, collegial discussion—then corralled the herd for a fun dinner outing afterward.

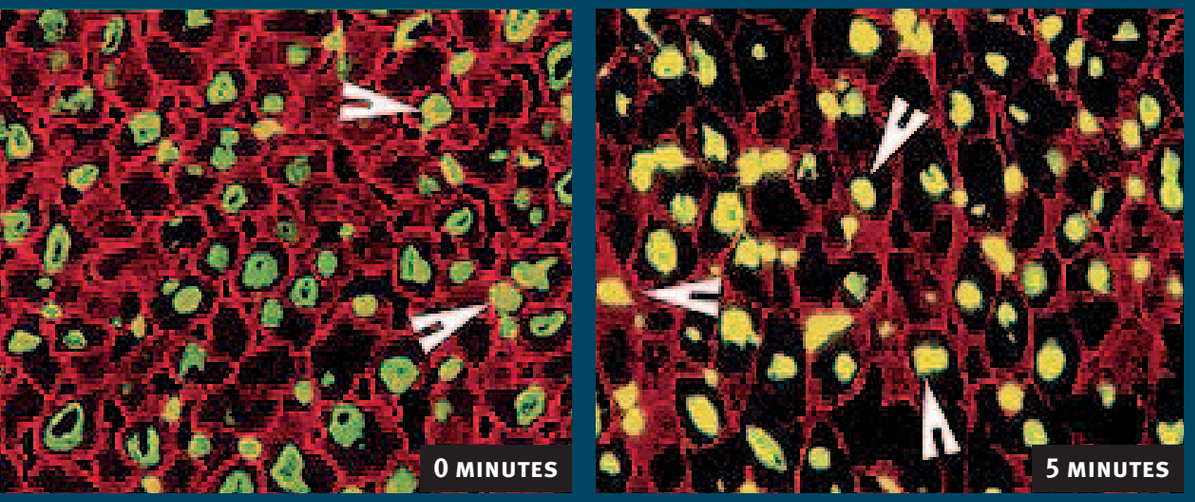
"He changed the demeanor," says Sirica. "I think he coined the term *hepatomaniacs*."

Joe Locker was on the Pitt pathology faculty from 1984 to 1999, then moved to Albert Einstein College of Medicine. Last December, he came back, bringing an expertise in next-generation–DNA sequencing with him. (The professor of pathology is now developing infrastructure for the department's newly acquired, million-dollar sequencer.) Locker returned largely because of Michalopoulos, for a couple of reasons: One, he's built a department that Locker sees as the best in the country at integrating basic and clinical science. (An MD/PhD, Locker gets his kicks from both flavors or science. Pitt just launched a personalized-medicine lab for cancer earlier this year, he's pleased to add.)

And two: Michalopoulos is such a "positive force" in the field, says Locker. "He's helped people all over the world."

Locker studies transcription control, the cellular process of reading the genome. Previously, he focused on a gene called alpha-fetoprotein, which becomes active in development, silences in adulthood, then reawakens in liver cancer. And now he studies transcription factors in liver regeneration, as well as another type of liver growth called hyperplasia (more on that later).

Pitt's pathology department is envied for



Pitt's Paul Monga discovered that Wnt/ß-catenin, a pathway involved in both embryonic development and in liver cancer, aids in liver regeneration, as well. Here, Wnt/ß-catenin, seen in red, hangs out in its usual digs, the cell membrane (left). Five minutes after partial hepatectomy, Wnt/ß-catenin moves inside the green nucleus, turning it yellow (right). Once inside the nucleus, Wnt/ß-catenin turns on genes that initiate cell division.

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its supportive environment. As Monga says, having a chair who is still very much an active researcher (to date, 35 years of NIH funding) means he can relate when things go south—not so in other institutions, unfortunately. "If you run out of funding, the warnings begin to pop in your e-mails. That never happens here. That's the time when you need support. That's when George says, 'What can I do for you?' I'm so used to hearing that. 'What can I do for you? How can I help?'"

For all his skill as a leader, Michalopoulos is a scientist first. That's clear enough when you sit down and look through the microscope with him, his colleagues and friends say.

"It's like you're looking with a young trainee who's experiencing it for the first time," says Locker. "He's just excited about it."

In 2002, Michalopoulos' team showed that the human liver not only has multiple molecular redundancies to incite tissue regeneration, but absent these, it can also reinvent itself at the cellular level. The two main types of liver cells—hepatocytes and biliary cells—can shape-shift like something out of mythology, turning themselves into each other as needed to fill in each other's gaps.

Previously, a team at the National Cancer Institute had shown this happens in animal studies, but many of the world's hepatomaniacs had remained skeptical. It must be due to some kind of liver stem cells, they said—even though no one had ever been able to prove such cells existed. Michalopoulos was in a unique position to put the question to rest, thanks to the massive tissue bank he and Rajiv Dhir set up at Pitt in 2000. The samples, which are taken from patients of Pitt's liver-transplant center, are an invaluable resource for the department, as well as to many other hepatomaniacs.

Under a microscope, using special markers, he found that these tissue samples showed "regenerative clusters," he says. "You have biliary cells in the periphery. You go down toward the center, and you have cells that are mixed hepatic/biliary markers. And you [reach] the center, and they're all hepatocytes."

Having elucidated much of the process of regeneration—how it starts, how it finagles workaround after workaround, seemingly no matter what challenges you throw at it (he would write another big review paper for *Cellular Physiology* in 2007)—four years ago, Michalopoulos asked: What makes liver regeneration stop? Because when the liver stubbornly insists on growing, it doesn't do so willy-nilly. It's smart about it.

In pregnancy—a time when circulating blood increases by 40 percent to accommodate another kind of mythic growth—the liver doubles in size to metabolize, detoxify, coagulate, and produce critical proteins for two. And then, about a month after the baby is born, it shrinks back down to its original size.

And when faced with certain toxins, the liver adapts, growing bigger and producing extra enzymes so it can work more efficiently. Then, when the threat is over, the liver shrinks back down. (This particular kind of liver growth, known as hyperplasia, has been detailed in the literature by Locker and others at Pitt.)

And after any vertebrate suffers a liver injury, the organ grows back to its original size—no more, and no less. The organ seems to have its hand on the dial, controlling the greater bodily machine running the process—the "hepatostat," as Michalopoulos calls it. And he wanted to know how it works.

The team turned back to the extracellular matrix. Because that's what tells the liver cells when to start the process, they wondered: Could it be what tells them to stop, too?

And, in 2001, they found this was exactly the case. Working with Chuanyue Wu, a PhD who holds the Lombardi and Shinozuka Experimental Pathology Research Chair, they produced a mutant mouse that lacked an enzyme called ILK (integrin-linked kinase)—a key, shot-calling protein they'd identified within the extracellular matrix. And, without ILK, the liver cells not only undifferentiated, losing their markers of liver cell-ness, they also didn't know when to stop growing. The livers of these mice ballooned to two-and-a-half times their normal size. Michalopoulos' team showed that when the extracellular matrix resynthesizes, it signals to the cells that it's time to stop growing. And not much else is known about what terminates regeneration, Michalopoulos says.

"And that got us into liver cancer."

Three years ago, the team partnered with Jianhua Luo, a Pitt MD/PhD professor of pathology, to study the genes expressed in—and proteins produced by—liver cancer. Among these, they found one protein in particular that

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liver tumors pumped out like mad: glypican 3.

And Michalopoulos, a world authority in stubborn tissue survival, had never heard of it. Which was weird. And when he looked it up in the literature, he found glypican 3 wasn't a growth stimulant, as he was expecting, but a growth suppressor. Which was even weirder.

Why would cancer cells, by nature hell-bent on growing, produce massive amounts of stuff that would prevent them from doing that very thing? To begin figuring that one out, they went back to their old, familiar model of normal tissue growth—liver regeneration after partial hepatectomy—and saw that glypican 3 was indeed produced in that setting, too. Its levels climb throughout the process, peaking at the end. Glypican 3, it seemed, is one of the brakes.

Or part of them, anyway. Luo did further tests on yeast to determine glypican 3's binding partner. And wouldn't you know, another protein Michalopoulos had never heard of popped up: CD81.

"I said, 'What the heck is CD81?!'"

Back he went to the literature, where he discovered this regenerative-medicine no-name was actually one of the two proteins that are necessary for infection of … wait for it … hepatitis C.

Yes, the same hepatitis C that is associated with some 95 percent of liver cancers, for reasons yet unknown. Now, the team has a hypothesis: Hep C tricks liver cells into becoming cancerous by producing a protein (dubbed E2) that suppresses the effects of glypican 3.

"This is where having a regeneration background helps you," Michalopoulos says. You can draw connections in studying cancer growth, because cancer regeneration is just like normal tissue regeneration. Both use certain pathways to make growth factors and set up shop, building blood vessels, connective tissues—everything they need to thrive as a tissue.

"Cancer is irregular, [nearly] autonomous growth of tissue. That's the definition," he notes.

A few years ago, the team began comparing liver-cancer DNA with the healthy DNA of patients and looking for the differences between them. They found 25 genes that were present in more than 10 percent of the tumors, which they published in the *American Journal of Pathology*. When Michalopoulos first looked at the genes, none of them seemed to be big players in liver regeneration. But upon closer study, he realized they were all "close cousins" of such players—proteins they interact with during this process.

Which made a lot of sense. Cancer typically isn't the result of just one or two big, fat, molecular screw-ups, he explains. "Then, it wouldn't be a cancer cell. It would be a dead cell." But cells can afford mistakes in their critical proteins' first cousins. "Then, the configuration of the whole complex of that signaling can change. And the cell can grow faster."

In his office, Michalopoulos prints out a translation of the Prometheus myth in its earliest written form, penned by Hesiod, the Chaucer of Greece. Michalopoulos just looked up the original text last week, he explains, in preparation for a speech back home (the University of Athens awarded him an honorary doctorate in August). In his Word document, he has underlined his favorite part:

And ready-witted Prometheus he [Zeus] bound with inextricable bonds, cruel chains, and drove a shaft through his middle, and set on him a long-winged eagle, which he used to eat his immortal liver; but by night the liver grew as much again everyway as the long-winged bird devoured in the whole day.

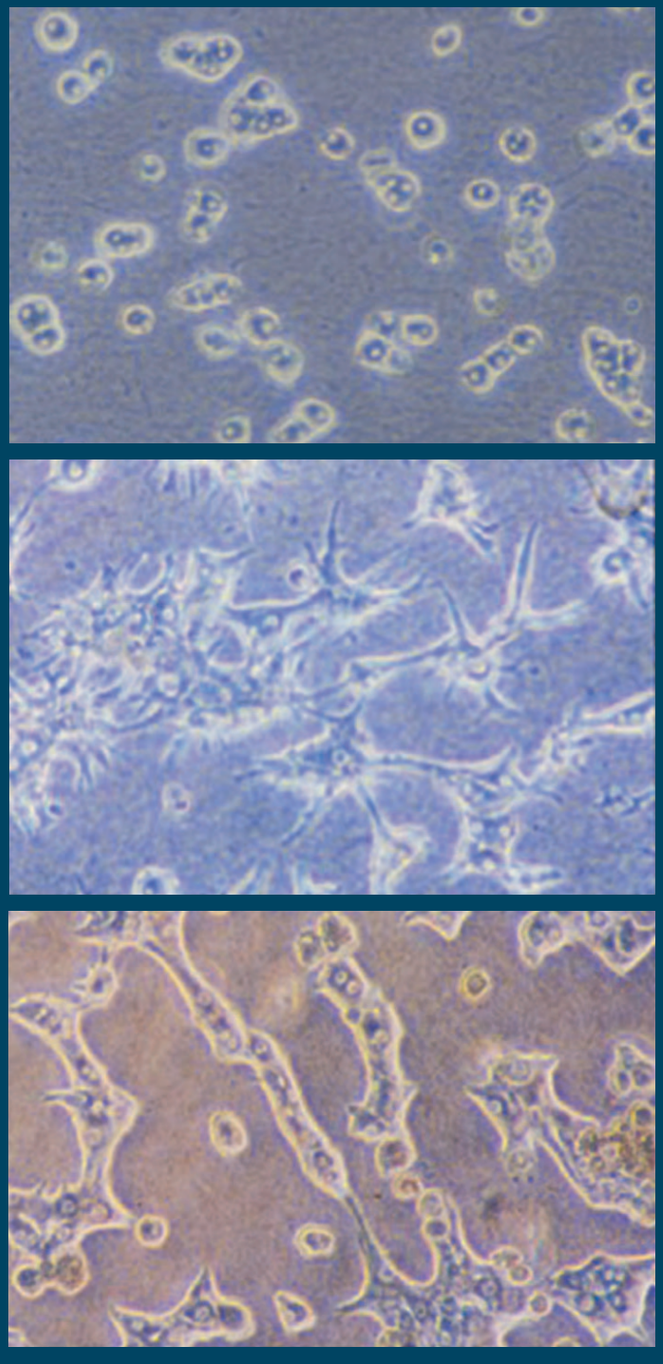
When Michalopoulos saw these poetic lines from the ancients, all but spelling out a very modern idea—the hepatostat—this scientist "freaked out."

"I said, 'My God. How did they know that?' I take no responsibility," he says, laughing.

Throughout the ages, Prometheus has been an archetype for human striving. It's a fitting backdrop for Michalopoulos' brand of selflessness: giving away ideas in the hallway, and even donating a whole lobe of his lab to give a young mentee a new start.

"I can't pursue everything I think I could possibly do. If all of us throw ideas at each other, it's conceivable some of these things might actually happen in one's lifetime. I'm 67. Will I be able to see everything? There's so much to be discovered yet.

"This is like the end of antiquity, in regards to the biological sciences. We just became capable of doing massive screening in the last 10 years. So now we've got to get everything defined and know what the jigsaw puzzle looks like. Before, we could only see little pieces here and there and make a mechanistic hypothesis. Now it's all there. We just have to find out what it means." ■



COURTESY GEORGE MICHALOPOULOS

Cells of the liver, famous for their regenerative capacity, refuse to divide in culture (top). In 1987, Michalopoulos, Zarnegar, and team discovered the missing ingredient, which circulates in the blood: HGF. Fueled by this growth factor, hepatocytes begin actively dividing within 24 hours (middle). By 72 hours, they arrange themselves into long, platelike structures, just as in normal liver tissue (bottom).