the POWER of MILK

Breast-feeding boosts an infant's immune system and promotes a healthy gut. Scientists are finally isolating the compounds responsible. The result could be a health breakthrough for all ages.

PERLA LEWIS-TRUONG'S DUE DATE WAS MARCH 1. BUT THE DAY AFTER Thanksgiving, she was admitted to the hospital with severe preeclampsia, a disorder marked by a rapid rise in blood pressure that puts a mother's health and pregnancy at risk. A week later doctors had to deliver her daughter by cesarean section, 13 weeks early. Baby Celia weighed only a pound and a half. After two months, she is four pounds and still nearly translucent but healthy, lying in a small heated pod in the Children's Hospital of the University of California, Davis, in Sacramento. Celia was lucky to be born here, at a teaching hospital with an advanced neonatal intensive care unit. Premature babies face many potential problems, including necrotizing enterocolitis, in which intestinal walls deteriorate and bacteria invade. A quarter of infants with the disease die, and survivors may suffer neurological problems for years.

Mark Underwood, a neonatologist at U.C. Davis, is constantly seeking better treatments for his delicate patients. Contrary to traditional practice, his focus is not on drugs but on diet. Underwood believes that many cases of necrotizing enterocolitis could be prevented by giving preemies a special daily cocktail of probiotics (healthy bacteria) and prebiotics (the food those bacteria eat), all inspired by what might be considered the ultimate superfood: human milk.

"Milk is powerful as a preventer of disease and an enhancer of performance," says Bruce German, a food chemist at U.C. Davis. "By understanding how it does what it does, we can bring the principles, the mechanisms of action, and the benefits to everyone." Human milk's most important role could be preventing infant disease and

by FLORENCE WILLIAMS

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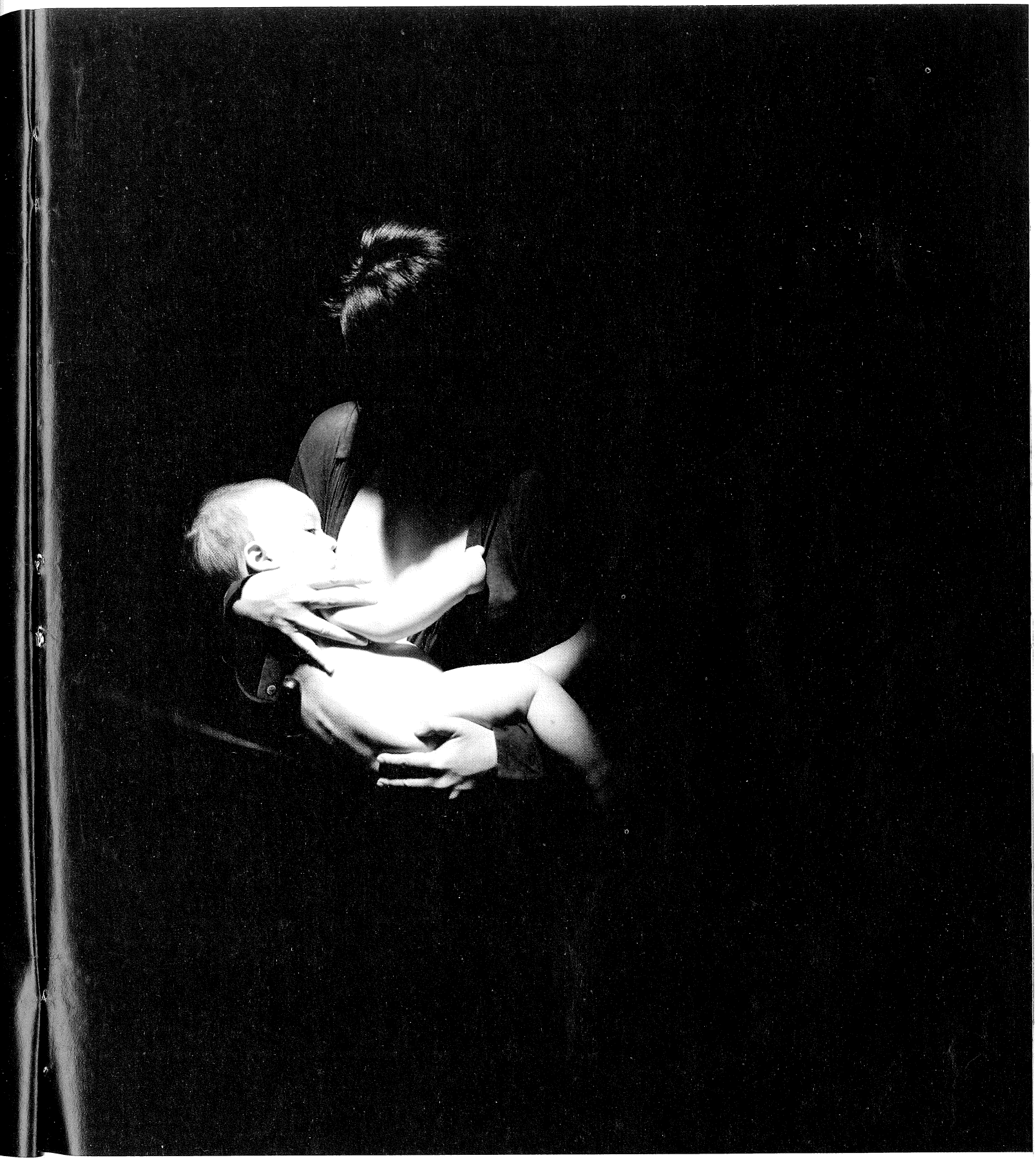

boosting immunity by cultivating a balance of microbes in the gut and the rest of the body, a kind of internal ecosystem called the microbiome. In fact, many researchers now believe that mammalian lactation originally evolved as a protective, not a nutritional, adaptation.

Consider baby Celia's situation at Davis. It is an impressive hospital, but even the best neonatal intensive care unit presents a challenging environment for a preemie. Fetuses are bacterially naive, with little exposure to pathogens or other microbes before birth. Then they get exposed in a very specific way: first through the birth canal, a wellspring of bacteria, and then through near-constant snuggling with their moms—ideally accompanied by immediate breast-feeding. Within days, microbes both good and bad start to colonize the baby and help educate the immune system. But Celia, born through a C-section and then placed in an isolette, acquired most of her bacteria not from her mother but from a hospital. Too young to breast-feed, she received nourishment, including liquid vitamins and a few drops of her mother's milk, through a catheter in her umbilical cord.

"We think that makes them sick," says Underwood, a soft-spoken man in glasses and a blue polo shirt. For preemies, infections come fast and furious, but those who receive breast milk are half as likely to suffer from necrotizing enterocolitis as their formula-fed peers. Such statistics are driving Underwood and his colleagues to peer deeper into human milk. And their findings are poised to improve health not just for babies, but for all of us.

Once we isolate the active ingredients of human milk, they might help fight everything from cancer to HIV.

FOR A SUBSTANCE SO IMPORTANT TO THE success of our species, human milk has, until recently, been largely neglected by researchers. For one thing, most infants in the developed world can now survive without it. Doctors and scientists long assumed most of its value was nutritional, in which case it could be replaced by commercial infant formula, which is now a $3.5 billion-a-year business in the United States alone.

Far more money has gone into improving efficiencies in the dairy industry or studying the cholesterol-reducing effects of red wine than into understanding human breast milk. "People should not underestimate how important the money is," says food researcher Bruce German from his office in the new, light-dappled Robert Mondavi Institute for Wine and Food Science on the Davis campus.

German had his lightbulb moment in 1994, when he decided that food scientists were "overblowing the red wine thing." He asked himself, what is the one food that's clearly meant to help humans? German, a ruddy man in a white sweater vest that looks knit by a relative, answers his own question effusively: "Milk!"

German took a sabbatical in Switzerland to work at food giant Nestlé, one of the world's leading sellers of infant formula. Where better to learn about human milk, he reasoned, than at a company so keen to mimic it? Nestlé researchers suspected that milk could "grab onto pathogens," flushing them out of the baby, and even act as an anti-inflammatory, calming hypervigilant, immature immune cells. But like other researchers, they didn't know the mechanism involved. If only the means of action could be decoded, and the healthy components isolated, identified, and produced in quantity, German thought, then they could be repurposed to treat everything from diarrheal diseases to cancer to HIV.

One class of substances in particular intrigued German: oligosaccharides. These sugar molecules, among the most common solid components of milk, are not digestible. Since we cannot metabolize them, he wondered, why are they there in such abundance? He had a hunch that the answer might be related to the human microbiome. If the molecules are not feeding us, he reasoned, maybe they are feeding the microbes that boost our health. On his return to Davis, German began collaborating with molecular biologists and chemists to isolate the oligosaccharides and test them against various bacteria.

To date, researchers have discovered more than 150 different human-milk oligosaccharides and believe there may be some 200 altogether. Built in combinations of 3 to 20 monosaccharides (simple sugars), these compounds are hard to fragment and analyze. German's colleague Carlito Lebrilla decided to attempt the job with the university's new nanoflow liquid chromatography time-of-flight machine, which identifies molecules by measuring the time it takes them to ping around a tube that looks like a stovepipe. In order to separate the 200-some compounds in breast milk so they could be analyzed individually, Lebrilla worked with a biotech company to develop a microchip that acts like a filter for the machine, allowing different compounds through at different speeds.

Another helpful technology was a superconducting magnet cyclotron—a million-dollar drum-shaped device that sends molecules racing around in circles. Researchers can blast the compounds apart with lasers and measure the mass of the molecular fragments that spew out, like smashing a geode to see what minerals are inside. Through painstaking work, German and his colleagues eventually identified dozens of new sugars that could be keys to human health and disease.

At another lab in Davis's food-science complex, the effort is on to see just what these sugars actually do. At the heart of the effort is microbiologist David Mills, who spent years growing finicky bacteria in test tubes laced with oligosaccharides in oxygen-deprived chambers that mimic conditions in the human gut. It is not a project for the squeamish: To recreate the biology as exactly as he can, Mills works with fecal bacteria collected from infant stool samples. In this way he discovered that *Bifidobacterium infantis*, one of the dominant bacteria present in the poop of healthy breast-fed babies, is particularly good at eating large oligosaccharides capped at the end with a particular kind of sugar unit. These molecules, called large fucosylated oligosaccharides, are plentiful in breast milk

despite the fact that humans cannot digest them at all. But *B. infantis* can. The microbe efficiently eats these sugars before other bacteria get to them, starving out bad bugs and aiding the infant that serves as its host.

At the Davis neonatal intensive care unit, concentrated breast milk rich with oligosaccharides is now being tested in babies unable to grow enough *B. infantis* on their own. The hope is that seeding their guts with regular doses of the bacterium and the sugar it eats will compensate for that lack. The *B. infantis* supplement is brewed in a food-grade facility and turned into a soluble powder. Underwood, German, and their colleagues believe it will have major potential, not just for preemies but for babies and small children in the developing world who suffer high rates of other gut infections leading to diarrheal disease.

Assuming this and several other related human trials currently under way are a success, the next step will be making enough of these crucial breast-milk sugars and doing it cheaply. One idea for ramping up production is through dairy cows, which naturally produce many of the same oligosaccharides as humans do, though in tiny quantities. Fortunately, California's dairy industry produces more than 3,000 tons of cheese a day, and a corresponding amount of liquid whey that is extracted from it. Supported by funding from the Gates Foundation, U.C. Davis is working to find an efficient way to partition out and concentrate the human-active compounds.

Other breast-milk oligosaccharides are also showing promise. At the University of California, San Diego, a sugar called disialyllacto-N-tetraose reduced the mortality rate from necrotizing enterocolitis in rats from 25 percent to only 5 percent. Nutritional scientist Lars Bode believes the compound may act by encouraging the growth of beneficial bacteria or by reducing inflammation of the gut.

Bode's lab has shown that the same family of compounds ably attach to a protozoan parasite and potentially lethal pathogen, *Entamoeba histolytica*. Bode believes these sugars, because they are indigestible, journey intact to the colon, where their structure mimics molecules on the surface of gut epithelial cells. When the parasite tries to hook onto the intestinal wall, it latches onto the milk decoy instead. The milk molecules then most likely flush the parasite out. Bode's finding could have big implications for both child and adult health, because *E. histolytica* is the world's third-leading cause of death by parasites. These particular oligosaccharides would be expensive to synthesize in a lab, but simpler sugars derived from cow's milk also appear to work well against *E. histolytica*.

Bode points to human-milk oligosaccharides performing other heroic medical feats. They inhibit *Streptococcus pneumoniae*, the bacterium frequently responsible for respiratory and ear infections, which may explain why breast-fed infants get fewer respiratory illnesses than formula-fed ones. And at Boston College, biochemist David Newburg and his colleagues have found that another oligosaccharide called 2'-fucosyllactose is effective at warding off *Campylobacter*, cholera, and enteropathogenic *E. coli*—a frequent cause of diarrhea—in animal models. He believes it has the same impact in humans.

COCKTAILS OF MILK-DERIVED COMPOUNDS could soon start moving from the lab to medical use. Newburg has cofounded Glycosyn, Inc. to test his oligosaccharide in humans within two years. The company makes 2'-fucosyllactose from yeast and bacteria and is working toward marketing a nutritional supplement for children in the developing world, where diarrheal diseases kill more than a million of them a year. Especially at risk are newborns and weaning toddlers, who lose the protection of mother's milk at the same time they are exposed to a wider variety of food pathogens. Newburg and his collaborators are also studying a human-milk fat that seems to inhibit HIV from infecting human cells, and yet another milk component that prevents HIV from disabling the host's immune cells.

The big infant formula producers are closely watching these promising breast milk studies. Abbott, Mead Johnson, the British company Aptamil, and the German company HiPP have begun offering formula with a prebiotic called GOS, for galacto-oligosaccharide, derived from cow's-milk lactose. At least two companies have started supplementing formula with probiotics. HiPP uses a strain of human-milk *Lactobacillus* to help ward off infections. And Nestlé's Gerber has introduced an infant formula with bifidobacteria to support infants' immune systems. "There's a whole lot happening," Newburg says. "The formula companies are all partnering up to get a supply, get prepared, and do big human testing."

New milk-inspired therapies may soon find their way into the intensive care unit, too. Back at U.C. Davis, Mark Underwood is working on his next project, developing lipids from human milk to bathe and protect preemie skin. Bruce German responds with characteristic enthusiasm. "It's better than Vaseline!"

Florence Williams is the author of *Breasts: A Natural and Unnatural History*, published this May by W. W. Norton & Company.

Breast Milk Therapies for Adults

The first round of milk-derived drugs are aimed at infants and children. But such compounds could soon also be aiding grown-ups–especially those whose internal population of microbes, or microbiomes, have been damaged due to illness, age, or antibiotics and other drugs.

CD14, a cell surface protein abundant in human milk, is present when the immune system learns to fight pathogens in the intestines. Nestlé researchers think the protein could turn out to help adult patients suffering from the immune responses that cause Crohn's disease.

LACTOFERRIN, a human-milk protein that binds iron, helps the body fight shigella, salmonella, *E. coli*, and other microbes that feed on iron. A supplement called Lactoferrin Gold 1.8, marketed by Nikken in Japan, is made from the milk of transgenic cows manipulated to have a human lactoferrin gene. The current process for producing lactoferrin is so inefficient that it requires nearly a gallon of cow's milk to create just one capsule of the stuff. Yet the treatment may be worth it for the most vulnerable. Already shown to work in pigs, whose immune systems are much like ours, lactoferrin could provide a boost for those with compromised immune systems such as infants, the elderly, and the chronically ill.

LYSOZYME, an enzyme found in low levels in human milk, has been shown to kill *E. coli* in mice and pigs; it could soon find medical applications. To boost production of the enzyme in animals, U.C. Davis scientists have transferred the human gene for the enzyme into dairy goats. Although regulations prevent the transgenic milk product from being sold, the researchers are working with the Brazilian government to test it in the northeastern part of the country, where childhood diarrhea is common and some areas have infant mortality rates up to 10 percent. F. W.