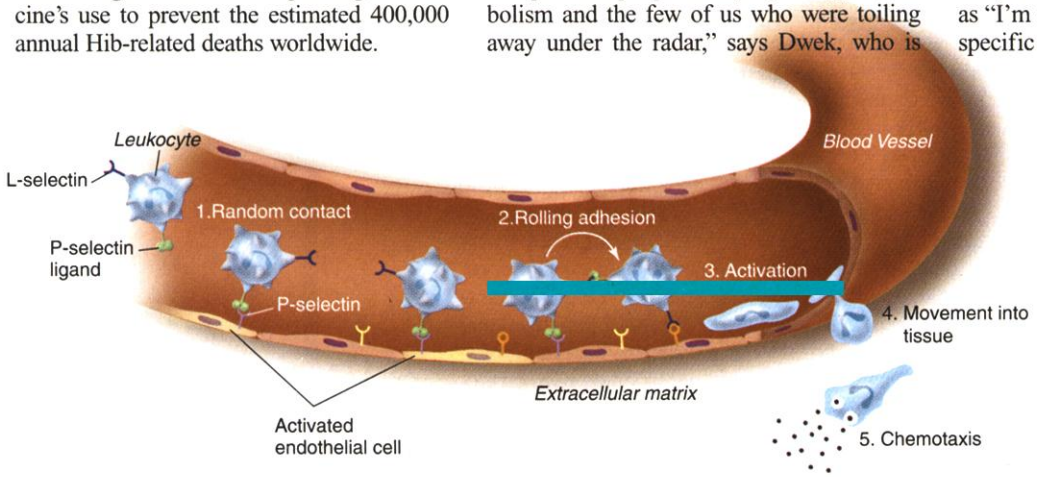
NEWS

# Searching for Medicine's Sweet Spot

**Long avoided by chemists and biologists, sugar-based drugs are suddenly on medicine's menu and garnering impressive early reviews**

Just over a decade ago, a bug called *Haemophilus influenzae* type b (Hib) wrecked the lives of some 25,000 children a year in the United States alone. Rather than causing the flu, as its name suggests, Hib produced bacterial meningitis in 60% of infected children, 10% of whom died. Those who survived often suffered permanent damage, ranging from mild hearing loss to mental retardation. But thanks to a sugar-based vaccine, Hib has been virtually eliminated from the United States, most European countries, and a growing number of developing nations. The World Health Organization is now pushing the vaccine's use to prevent the estimated 400,000 annual Hib-related deaths worldwide.




**Hold it.** Selectin proteins and their sugar-based targets slow the passage of white blood cells in circulation, allowing them to enter damaged tissue. Interrupting this binding may prevent inflammation.

The Hib vaccine isn't the only sweet success for sugar-based therapies. The anticoagulant heparin—a complex sugar, or carbohydrate—has long been the number-one–selling drug in the world. Top-selling protein drugs, such as the red blood cell booster erythropoietin (EPO), bristle with sugars that ensure that these molecules stay in circulation long enough to carry out their task. And two new flu drugs approved for use in 1999 work by attacking a sugar-busting enzyme that influenza viruses use to help them exit infected cells (see diagram, p. 2343).

But these triumphs have been hard won. Six decades elapsed between the discovery of the sugar groups on Hib that induce an immune response and the development of an effective vaccine. Amgen, the maker of EPO, regularly throws out as much as 80% of its protein because the attached sugars are incorrect. Meanwhile, the 1990s saw a series of biotech companies go belly up after their carbohydrate drugs failed in clinical trials (see p. 2340). "The field has struggled, there's no denying it," says David Zopf, vice president of Neose Technologies in Horsham, Pennsylvania.

This struggle has long confined research on carbohydrates to a small niche in biology. The field didn't even have a proper name until 1988, when Oxford University biochemist Raymond Dwek coined the word glycobiology during an appearance on a British morning television show. "In a world focused on nucleic acids and proteins, there really wasn't that much interest in carbohydrates except from people studying energy metabolism and the few of us who were toiling away under the radar," says Dwek, who is now director of Oxford University's Glycobiology Institute in the U.K. John Magnani, president of GlycoTech, a carbohydrate-focused biotech firm in Rockville, Maryland, says that even today, carbohydrate research is "the Rodney Dangerfield of pharmaceutical research. It gets no respect."

But recent advances in the study of carbohydrate chemistry and biology are beginning to turn the tide. Chemists have begun to crack a long-standing problem: producing carbohydrates in quantities large enough to study in biological systems. Like proteins and nucleic acids, carbohydrates are polymers of relatively simple molecules linked together. But unlike the amino acids in proteins and the nucleotides in nucleic acids that link up like boxcars in a train, sugar chains can branch and twist, giving them a multitude of three-dimensional shapes. That structural complexity makes carbohydrates difficult to analyze and extremely hard to make. But new automated methods of synthesizing large quantities of carbohydrates promise a dramatic change (*Science*, 2 February, p. 805); similar developments spurred the study of proteins and nucleic acids in the 1970s and 1980s.

Two decades of genetic and biochemical studies by researchers who toiled largely in obscurity have also spelled out many critical roles played by carbohydrates and identified potential drug targets. Sugars are ubiquitous in cells. They dangle from nearly all the protein and many of the fat molecules in the body. These combination molecules, called glycoproteins and glycolipids, dot the outer surfaces of all cells and serve as cellular identification tags to the surrounding world. The body uses them to say something as general as "I'm a human tissue, I belong here," or as specific as "I'm injured, send help from the immune system over here." Cancer cells use sugar groups on their surfaces to slip past the immune cells looking to do them in as they migrate through the body. Pathogens rely on the glycoproteins and glycolipids on cell surfaces to home in on their tissue of choice in their favorite host species and to spread themselves from cell to cell. Harmful inflammatory reactions are often triggered by carbohydrates, as is blood clotting.

"Carbohydrates are central to many processes that are at the core of important diseases, and now that we understand some of those roles, it's not surprising that this has become a hot topic at drug companies," says Christian Raetz, a glycobiologist who helped Merck get into the field before he returned to academia as chair of the biochemistry department at Duke University Medical Center in Durham, North Carolina.

Now, dozens of new carbo-related compounds are in clinical trials, aimed at treating conditions ranging from inflammation and tissue rejection to hepatitis and cancer. "The result is that glycobiology's future now looks pretty bright," says Zopf. "Glycobiology has finally become part of the mainstream," adds Hudson Freeze, a longtime glycobiology researcher and director of the glycobiology program at the Burnham Institute in La Jolla, California. "We're no longer a boutique science."

### Interrupting inflammation

One of the first critical roles played by carbohydrates began to come into focus in the late 1980s. Several groups independently cloned the genes for three human carbohydrate-

CREDIT: CARIN CAIN

binding proteins that play a role in attracting leukocytes, or white blood cells, to injured sites in the body. Called selectins, these proteins appear on the surface of the endothelial cells lining blood vessels after injured tissues nearby release powerful signaling compounds known as cytokines. The availability of the cloned genes led to a flood of publications in the early 1990s showing that selectins bind to a distinct carbohydrate structure, called sialyl Lewis x ($sLe^x$), on the surface of circulating leukocytes. This binding acts as a kind of brake that slows down leukocytes circulating in the bloodstream, causing them to roll along the injured blood vessel wall and allowing them to slip into the injured tissue (see diagram, p. 2338).

Although those leukocytes launch the healing process, they can also lead to inflammation, which itself can damage tissues. That prompted several groups to try to prevent inflammation by blocking the ability of selectins to bind to their $sLe^x$ targets. Early experiments by Ajit Varki and his colleagues at the University of California, San Diego (UCSD), offered initial hope. Their work in mice showed that blocking a selectin subgroup called L selectin from binding to $sLe^x$ reduced inflammatory responses in damaged blood vessels.

Since then, the track record for getting selectin inhibitors to work in humans has been spotty at best. In 1995, researchers at Boulder, Colorado–based NeXstar Pharmaceuticals, now a part of Gilead Sciences in Foster City, California, developed potent selectin inhibitors in collaboration with Varki's group. But when the company's inhibitors failed to work in animal disease models, the project died on the vine. Cytel, formerly of San Diego, made it farther. Its compound, intended to prevent damage to tissues after blood starts flowing again after a heart attack, stroke, or tissue transplantation—a condition known as ischemia reperfusion injury—made it through human safety studies. But final-stage clinical data showed no benefit from the drug, and the company closed shop. "Historically, this has been a tough area to be in," says Gray Shaw, who heads drug development for a selectin inhibitor at Wyeth, the pharmaceutical arm of American Home Products based in St. Davids, Pennsylvania.

Wyeth is betting it can do better with a compound called PSGL-1 that binds to another selectin subtype called P selectin. P selectin is expressed not only on endothelial cells but also on platelet cells, causing these red blood cells to stick to leukocytes and create blood clots. "By inhibiting P selectin, we hope to not only prevent ischemia reperfusion injury but also the subsequent clotting events that can reocclude a vessel that's just been opened," says Shaw. Wyeth is currently conducting phase II trials with a soluble recombinant form of PSGL-1, and Varki, for one, is optimistic. "I think that the Wyeth drug is the first really good selectin inhibitor that has been given a chance to prove itself," he says.

Inhibiting P selectin may also prove to be useful for stopping the spread of tumors. Metastasizing tumor cells grab onto P selectin on platelets and use them as a protective shield against immune system cells. In the 13 March issue of *The Proceedings of the National Academy of Sciences*, Varki and his UCSD colleagues reported that heparin treatment, which has been used with mixed suc-

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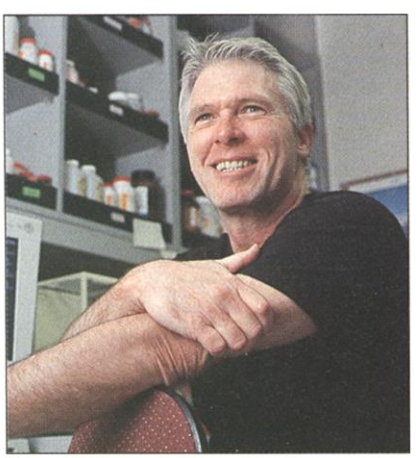
## Saving Lives With Sugar

The study of slime molds and the development of a lifesaving treatment for a rare genetic disorder may seem worlds apart. But a chance observation recently brought them together in dramatic fashion. Six years ago, Hudson Freeze noticed that cells from one of his molds (more properly known by the name of the organism, *Dictyostelium*) showed biochemical similarities to cells taken from a child with a disorder in the way cells manipulate sugars. The condition, known as congenital disorders of glycosylation type Ib (CDG1b), causes chronic gastrointestinal problems, including vomiting, diarrhea, bleeding, and blood clot formation. And though not always fatal, it's related to other inherited sugar-processing defects that can cause severe neurological problems and even death.

Children with CDG1b lack an enzyme called phosphomannose isomerase that converts the sugar fructose-6-phosphate to mannose-6-phosphate. The mannose compound is a critical intermediate needed to synthesize *N*-linked glycosylated proteins, which are involved in myriad biochemical functions. Freeze, who heads the glycobiology program at the Burnham Institute in La Jolla, California, was studying a strain of *Dictyostelium* engineered to produce no phosphomannose isomerase. He discovered that adding mannose to the mutants' culture medium corrected this deficit by allowing *Dictyostelium* to use an alternative route for making mannose-6-phosphate. On a hunch, Freeze added mannose to the CDG1b cells and got the same results. Hoping to use mannose to treat CDG1b, Freeze asked the U.S. Food and Drug Administration (FDA) for permission to test the safety of mannose therapy on healthy volunteers. In 1995, the FDA agreed.

Shortly after Freeze and his Burnham Institute colleagues began their initial safety tests with mannose, he got a call from a physician in Germany who had read a paper on the cell work. One of the doctor's patients, a young boy, was about to die from CDG1b—the child was bleeding to death and had already received 20 liters of blood. "I told him how much mannose to give the child and how often," says Freeze. "Six months later, the physician called back and told me that the boy was completely fine."

Freeze and chief collaborator Thorsten Marquardt of the Pediatric Clinic in Münster, Germany, published their findings in the April 1998 issue of the *Journal of Clinical Investigation*. Since then, Freeze has gotten two or three calls a week from physicians wanting to know more about mannose treatment for CDG. "Unfortunately, mannose only works for CDG1b," he explains with obvious regret, but then he adds that he and Marquardt have found that the sugar fucose works as a treatment for another glycosylation disorder that interferes with the body's ability to fight infections by altering the capacity of immune cells called leukocytes to stick to their targets. Both disorders are easily detected by a simple blood test.



CREDIT: SANDY HUFFAKER

**Sweet relief.** Hudson Freeze found that sugar therapy combats a rare genetic disease.

"The real importance of what Hud and Thorsten have done is not just that we can treat these syndromes, but that we have a new perspective on multisystem diseases that we had no way of understanding before," says William Balistreri, editor of the *Journal of Pediatrics* and head of pediatric gastroenterology, hepatology, and nutrition at the Children's Hospital Medical Center in Cincinnati. "The fact that a simple biochemical defect causes such a wide range of symptoms involving multiple organ systems has been a revelation."

Today, Freeze continues to study the biochemistry of CDG and other aspects of carbohydrate assembly. But his biggest passion is getting the word out about potential treatments for these disorders. "We're not talking about a lot of kids, but for the few hundred born every year, these therapies can change their lives," he says—and even save them. **–J.A.**

cess as part of chemotherapy, dramatically slows tumor metastasis by binding to P selectin on platelets before cancer cells can do the same. "This appears to markedly reduce the long-term organ colonization by tumor cells," says Varki. The response is inconsistent, however, perhaps because the chemical makeup of heparin is variable, and the clotting problems that can result from heparin therapy make it unlikely that heparin will be widely used as a chemotherapy agent. Other P selectin inhibitors may fare better, however.

**Cancer vaccines**

Stopping tumor cells from binding selectins isn't the only way researchers hope to use carbohydrates to block cancer. Transformed tumor cells hide from normal immune surveillance by displaying glycoproteins and glycolipids on their surfaces. Some researchers are now trying to turn the tables by using the carbohydrate chains from these glycomolecules as vaccines. "The idea is to manipulate the antigens in such a way that they become visible to the immune system," says Alan Houghton, chief of clinical immunology at Memorial Sloan-Kettering Cancer Center in New York City. "We and others have been able to do that, and the immune system will then make a concerted, and apparently successful, attack on tumors."

Several companies and universities are conducting clinical trials of carbohydrate-based anticancer vaccines. Houghton and his colleague Philip O. Livingston, for example, have been heading a team that is using synthetic carbohydrate antigens prepared by chemist Samuel Danishefsky and members of his laboratory at Sloan-Kettering and Columbia University.

While Danishefsky's team worked out how to synthesize carbohydrate cancer antigens with names such as Globo-H, which is associated with breast cancer, and Fucosyl GM1, which is isolated from small cell lung cancer, Livingston was figuring out how to boost their otherwise lousy ability to trigger an immune response. The solution was to link these carbohydrates to keyhole limpet hemocyanin, a strongly immunogenic protein isolated from a marine mollusk, and then deliver the twosome with another immune booster. The Sloan-Kettering group is now conducting phase II and phase III trials

## After the Fall

In the early 1990s, biotechnology companies experienced a brief sugar high. Two start-ups—Cytel and Glycomed—pushed new sugar-based drugs into late-stage clinical trials. Cytel's compound was intended to block inflammation by inhibiting the binding of proteins called selectins to white blood cells, thereby disrupting the ability of white blood cells to find damaged tissue. Glycomed tried to modify a natural complex sugar compound called heparin to improve its performance in slowing the proliferation of smooth muscle cells in patients with damaged blood vessels. If the drugs worked, they promised to usher in a new era of compounds targeting sugars and sugar-binding proteins, which together play key roles in infections, cancer metastasis, and immune system diseases. That era hasn't yet arrived. Both drugs faltered, as did the two companies, whose assets and technologies were eventually sold off to competitors.

"Those two companies looked pretty sexy, and both failed," says David Cox, CEO of Synsorb Biotech, a carbohydrate-based drug discovery company in Calgary, Alberta. That soured many investors and major pharmaceutical companies on the idea that sugars would make good drugs, and the

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# Sugar Separates Humans From Apes

Humans and chimps differ at the genomic level by 1% to 2%. Yet so far, the only identified gene that differs between humans and chimpanzees codes for an enzyme that makes a particular form of a sugar called sialic acid: Chimps, and all other mammals for that matter, have the gene, while humans do not.

To Ajit Varki and his wife and colleague Nissi Varki—both professors at the University of California, San Diego (UCSD)—this fact may provide a clue to how evolutionary pressure and molecular biology interact to produce changes that have multiple consequences. "Since many pathogens bind to sialic acids on cell surfaces, changing those sialic acids is one way for an organism to evade a particular kind of pathogen," says Ajit Varki. "Such a change could give a big selective advantage to an individual with such a mutation." But the Varkis, together with UCSD colleague Elaine Muchmore, are now exploring an even more intriguing possibility: Perhaps the change may also make the brain work better.

"Ajit and Nissi are asking a very important question, which is what are the consequences of a single gene change on the physiology, and therefore the evolution, of humans," says Bernard Wood, the Henry R. Luce Professor of Human Origins at George Washington University in Washington, D.C. "In terms of trying to understand how humans fit into the rest of the world, this work has a very important place. It's one of those bits of biology that will become a citation classic."

The gene in question codes for the enzyme CMP-sialic acid hydroxylase, which adds an oxygen atom to a sialic acid variant known as *N*-acetylneuraminic acid, creating *N*-glycolylneuraminic acid (Neu5Gc). All mammals except humans have this enzyme, so all mammals except humans have both forms of sialic acid in their cells. It turns out, however, that the distribution of this enzyme is always skewed: plentiful throughout the body, but present in only small amounts in the brain and central nervous system. "For unknown reasons, the expression of this sugar is selectively down-regulated in the brains of all mammals, while being widely expressed everywhere else in the body," says Ajit Varki. "We wonder if its total elimination in the human brain might then have prompted a further improvement in the brain."

Using knockout and transgenic models, the Varkis hope to look at the effect of eliminating or overexpressing this enzyme in the brains of other species. While this probably won't produce animal Einsteins, it may provide answers to the question of how one mutation presumably driven by environmental pressures, such as infection, might lead to other consequences that drive evolution. For example, these studies have already shown that humans' unique sialic acid profile has marked effects on the ability of immune system cells known as macrophages to home in on their targets. The effect can be positive or negative, depending on the target.

As it turns out, however, humans do have trace amounts of Neu5Gc in their tissues. This observation baffled the Varkis, because not only is the Neu5Gc-producing enzyme totally missing from humans, but there is no obvious alternative pathway for making the compound. Their hypothesis: "This is most likely coming from the diet, from humans eating meat, since plants, lower invertebrates, and bacteria don't make this sialic acid," says Ajit Varki. The Varkis are now studying whether these trace amounts have any biological significance. **–J.A.**

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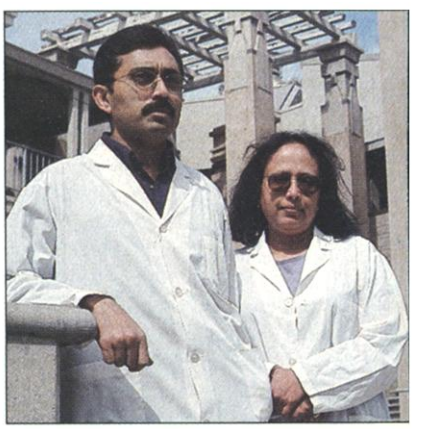

**Brain boost.** Ajit and Nissi Varki, probing impact of missing enzyme.

industry is still struggling to emerge from the shadow. "Sugars are now a show-me story, rather than a promise-me story," says Cox.

But carbohydrate specialists say biotech companies and a few venturesome pharmaceutical giants are regaining a bit of a sweet tooth. According to a review of the field last year by Ole Hindsgaul, a carbohydrate chemist at the University of Alberta in Edmonton, and Minoru Fukuda of the Burnham Institute in La Jolla, California, more than 30 carbohydrate-based drugs have recently been approved by the U.S. Food and Drug Administration or are in clinical trials (see table). That pales in comparison to the number of potential new drugs from traditional sources, small organic molecules and proteins. Still, "the field is really gaining momentum," says James Paulson, a carbohydrate expert at the Scripps Research Institute in La Jolla.

Until recently, that momentum has been held in check by a handful of challenges. For one, sugar-based drugs tend to take a weak hold on their targets. This means they must often be given in high doses to produce an effect. The molecules also tend to be broken down or cleared quickly from the body. Finally, their branching structures are difficult to make, raising the cost of manufacture.

To get around these problems, companies are adopting several strategies. The first is to overcome sugars' weak binding with sheer numbers. Synsorb Biotech, for example, is in the final stage of clinical trials with a sugar-based compound against a diarrhea-causing bacterial toxin. Because the compound binds to the toxin in the gut, it acts before the body has a chance to clear it. The drug, called Synsorb Cd, resembles a child's Koosh ball, with a solid core and thousands of sugar strands dangling off it. The toxin molecules it seeks each contain some 15 sites that normally bind to sugars on cells lining the gut. So when they encounter a sugar-coated Koosh ball, all 15 quickly get tied up. "The toxin can't get away" from the Koosh ball and is flushed from the body, says Cox.

Other companies are trying to develop compounds with the attributes but not the downsides of sugar molecules. Researchers at Rockville, Maryland–based GlycoTech, for example, are working to imitate the structure of carbohydrates in a small organic molecule of the type most often used for making drugs. The hope, says GlycoTech president John Magnani, is that through a combination of careful design and trial and error, researchers will hit upon a compound that binds to a specific carbohydrate docking site with a tenacious grip.

Taking a more fundamental approach, companies such as GLYCODesign of Toronto, Ontario, and Abaron Sciences of La Jolla are attacking the enzymes that build the carbohydrates in the first place. "Rather than try and physically interfere with carbohydrate-binding proteins with carbohydrate drugs, we try to block the synthesis of the structures they recognize," says Paulson, who is also an Abaron co-founder. Abaron, Paulson adds, is developing inhibitors against six glycosyl transferase enzymes that make carbohydrates recognized by white blood cells. The hope is that by temporarily shutting down the production of these carbohydrates, the drugs will disrupt the cycle of inflammation in chronic conditions such as rheumatoid arthritis and Crohn's disease.

New ideas are advancing by another route as well: Some traditional carbohydrate companies are offering their expertise to pharmaceutical firms. For example, Neose Technologies of Horsham, Pennsylvania—which makes sugars for bulking agents and food additives—uses naturally occurring sugar-linking enzymes to join the proper sugar groups to recombinant protein–based therapeutics. Those sugars typically play one of two key roles, either stabilizing the protein's shape so that it can bind to its target, or modifying the target binding site, says Neose's president Sherrill Neff. Drugs such as Amgen's top-selling protein drug erythropoietin would be ineffective without the proper sugars.

There is no guarantee that any of these strategies will pay dividends. Even the carbo supplier Neose, a company not dependent on the clinical success of its own drugs, is currently struggling to become profitable. But Cox argues that just one or two successes could inject new hope into the field. Says Cox: "There's nothing like success to silence your skeptics."

–ROBERT F. SERVICE

**SOME CARBOHYDRATE DRUGS IN CLINICAL TRIALS**

| Company | Indication | Class/Structure | Status |
|---|---|---|---|
| SafeScience | Cancer | Pectin-based oligosacch. | Phase II |
| GLYCODesign | Renal cell carcinoma | Swainsonine analog | Phase II |
| Progenics | Melanoma | $GD_2$/KLH conjugate | Phase III |
| Progenics | Cancer | $GM_2$/$GD_2$/KLH conjugate | Phase I/II |
| Austin Res. Inst. | Breast cancer | Mannan/mucin formulation | Phase III |
| Progen Indus. | Cancer | Sulfated oligosaccharide | Phase I |
| Synsorb Biotech | *C. difficile* inf. | Oligosacch. conjugate | Phase III |
| Synsorb Biotech | *E. coli* O157:H7 infection | Oligosacch. conjugate | Phase III |
| Biomira | Metastatic cancer | Sialyl Tn antigen conjugate | Phase III |
| Oxford Glycosci. | Gaucher's | Imino sugar analog | Phase III |

SOURCE: ADAPTED FROM M. FUKUDA AND O. HINDSGAUL, *MOLECULAR AND CELLULAR GLYCOBIOLOGY* (OXFORD UNIV. PRESS, OXFORD, U.K., 2000)

in late-stage patients for whom more conventional therapies have failed. Patients receive immunizations once weekly for a month and then every 3 months for the duration of the trial. Results are expected within the next year.

In the meantime, Danishefsky's group is pressing ahead on a next-generation vaccine: a chemically linked combination of several individual carbohydrates known as "polyvalent antigens." "Work in the mouse suggests that these polyvalent antigens will do a better job yet," says Danishefsky, who adds that making the molecules is the most complex synthesis project he has ever undertaken.

Biomira, a biotech firm in Edmonton, Alberta, is also nearing the end of its own cancer vaccine trial. The company's Theratope vaccine uses an antigen known as STn, part of a larger antigen known as mucin-1 found on breast cancer cells. This month, the company completed enrollment in a double-blinded phase III breast cancer trial that will test the vaccine on more than 950 women with metastatic breast cancer. "We'll get our first look at the data in about 6 months and take another look at these patients about 18 months from now," explains Mairead Kehoe, the company's director of clinical trials. While the waiting game continues, the mood among cancer researchers remains upbeat. "Our expectation is that carbohydrate-based vaccines will stop the metastatic spread of cancer and allow the body to control this disease," predicts Danishefsky.

**Viral deconstruction**

Researchers also have big hopes for carbohydrate drugs to stop another kind of invader: viruses. It turns out that even relatively minor interference with the sugars on proteins that make up the viral coats of two major scourges, the hepatitis B and C viruses, can produce big results.

Later this year, United Therapeutics of Silver Spring, Maryland, is expected to begin clinical trials on two antihepatitis drugs discovered as part of a collaboration between Oxford's Dwek and Timothy Block, a

viral hepatitis specialist and director of Thomas Jefferson Medical College's Jefferson Center for Biomedical Research in Doylestown, Pennsylvania. Both drugs are variants of natural sugars, and they work by gumming up two glycoprotein-processing enzymes in the endoplasmic reticulum (ER), the site where cells add carbohydrates to newly synthesized proteins.

When the hepatitis B virus invades liver cells, it depends on the ER's machinery to reproduce. But Dwek and Block found that when they added the compound *N*-nonyl deoxynojirimycin (NN-DNJ) to human liver cells, glycoprocessing was disrupted to a small but crucial extent. The result: The virus couldn't construct its M envelope protein, a critical coat component. These test tube studies show that "inhibition of as little as 6% of cellular glycoprocessing results in a greater than 99% reduction in the secretion of hepatitis B virus," says Dwek. "There seems to be no effect on the host [cells], but it's a lethal change for the virus."

Dwek and Block believe these drugs cause viral replication to go awry by preventing the envelope proteins from folding into the correct three-dimensional shape. "We know that there are proteins called chaperonins in the ER that grab onto a new protein's sugars and help the protein fold correctly," says Dwek. The two scientists suggest that only a small number of misfolded M proteins disrupt the symmetry characteristic of the hepatitis B virus coat and prevent it from escaping the endoplasmic reticulum.

Studies in woodchucks, the preferred animal model for testing potential hepatitis B drugs, confirmed that NN-DNJ stops viral replication cold, with no detrimental effects on the animal's health. "Better yet, we've been unable to find any mutant viruses that can escape this effect," says Block. "That's one big advantage of targeting a host enzyme and doing so at such a low level."

That could come as welcome news to hepatitis B patients, who commonly take a drug called lamivudine. The drug has serious side effects, and in 20% of patients the virus develops at least partial resistance within a year, a figure that rises to 53% after 3 years.

Using the same partial interference approach, Dwek and Block recently developed a second sugar compound, *N*-nonyl deoxygalactojirimycin, that has the same effect on hepatitis C virus in animal tests.

Although this approach has yet to prove itself in humans, a third sugar compound from Dwek's group is well on its way to demonstrating that a subtle adjustment in glycolipid synthesis can benefit at least some patients with Gaucher's disease, one of a class of inherited disorders known as glycolipid storage diseases. Gaucher's disease results from one of many genetic mutations that can either slow or prevent the breakdown of certain glycolipids, which accumulate in storage vesicles and eventually kill cells. Since 1994, Genzyme Therapeutics, headquartered in Cambridge, Massachusetts, has been selling a recombinant form of the enzyme at fault, glucocerebrosidase, under the trade name Cerezyme. The therapy is highly effective, but it requires a 2-hour intravenous infusion as often as three times a week and costs approximately $200,000 a year.

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## The Best of Both Worlds?

When drug companies search for novel pharmaceuticals, they pay close attention to how well candidate molecules bind to their targets. The tighter the binding, the smaller the amount needed to do the job and, consequently, the cheaper the drug is likely to be. Unfortunately, sugar-based compounds often don't follow this tidy rule of thumb: They typically bind to their targets with numerous weak handholds. They are also poor at crossing cell membranes and are expensive to make. As a result, many drug companies don't bother with them and look instead for more traditional small organic molecules to do the same job. But Ole Hindsgaul thinks sugars have something worth hanging on to.

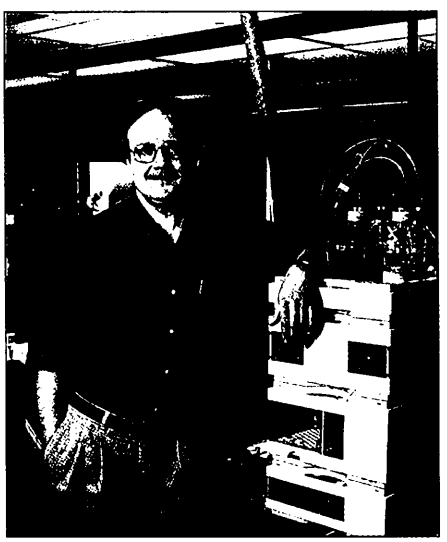
Hindsgaul, a carbohydrate chemist at the University of Alberta in Edmonton, has recently begun making what he calls "carbohybrids," molecules containing a sugar portion linked to other organic groups that are simple to make and typically bind more tightly to protein drug targets. A wide variety of proteins in the body bind oligosaccharides, compounds made up of multiple sugars. And although there are usually many bonds involved, one key sugar group typically embeds itself deeper in the protein than the rest, holding the oligosaccharide in place. "The proteins we're trying to inhibit evolved to bind sugars," says Hindsgaul. "If we give them one of the sugars that fits in the active site, it anchors the compound. As a result, you're more likely to hit only carbohydrate-binding sites."

The strategy is showing early promise. Two years ago, Hindsgaul and his Alberta colleagues devised a high-speed "combinatorial" technique for making hundreds of related carbohybrids. The hybrids were designed to bind to plant proteins that themselves bind oligosaccharides containing the sugar unit galactose. Each hybrid contained a galactose group linked to a different combination of other organic groups, giving it a slightly different shape. Hindsgaul's group tested their compounds against four separate galactose-binding proteins. "Out of the blue, one of these [hybrids] lit up," he says, showing 16-fold better binding ability than a conventional multisugar compound. What's more, the new compound contained groups that make it more lipid friendly, enabling it to cross cell membranes more easily.

The compound's binding ability "is still considered lousy by pharmaceutical standards," says Hindsgaul. "But it's good for sugars." Still, the approach "makes sense," says David Cox, president of Synsorb Biotech, a carbohydrate biotech company in Calgary, Alberta. "By manipulating the organic [groups] to the sugar, you can refine the binding that a sugar has to a protein," he says. If Hindsgaul's group can improve the hybrids' binding still further, says Cox, the molecules could find applications ranging from preventing cancer metastases and tissue rejection to combating viral infections.

Hindsgaul's group is testing further alterations of the hybrids. At a meeting last year, members reported finding a compound that inhibits an enzyme associated with cancer metastasis at a 0.3 micromolar concentration. That's still a lot, about 10-fold higher than the sweet spot for most pharmaceuticals. But the continued improvements mean that carbohybrids are starting to look enticing.

–R.F.S.



**Tight grip.** Ole Hindsgaul's hybrid molecules bind more strongly to targets than sugars do.

CREDIT: RICHARD SIEMENS

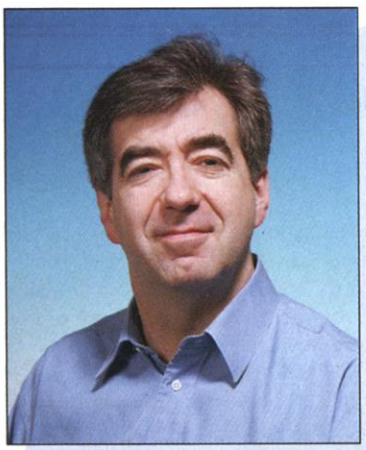
Dwek, along with Oxford colleagues Terry Butters and Frances Platt, reasoned that they might be able to restore the body's glycolipid balance—at least in patients whose mutations don't completely destroy the affected enzyme—by decreasing the synthesis of the glycolipids, which also occurs in the ER. This time, they chose a sugar variant called NB-DNJ as their weapon of choice. The results have been promising. "We don't shut down glycolipid synthesis completely, just enough to restore the proper balance between synthesis and degradation," says Dwek.

Oxford Glycosciences, an Oxford, U.K.–based biotech firm specializing in carbohydrates, has been conducting clinical trials with NB-DNJ, known more prosaically as Vevesca, both alone and in combination with Cerezyme. Last April, the company published initial clinical data in *The Lancet* demonstrating the drug's safety and hinting at its effectiveness. Last month, the company announced its preliminary analysis of a 6-month phase III study indicating that the compound—which is taken orally—was as effective as Cerezyme at maintaining healthy phospholipid levels. With these promising results in hand, the company is now pressing forward with clinical trials on related compounds for other lipid storage disorders, including Fabry's disease.

This new surge of interest in carbohydrate-based therapies is removing some of the sour taste of the earlier disappointments in the field. Now, glycobiologists are more confident that a spoonful of sugar will not only make the medicine go down, but replace it with something that works better.

**–JOSEPH ALPER**

Joseph Alper, a science writer in Louisville, Colorado, is managing editor of the online biotechnology magazine *dailytwist*.



**Protein pioneer.** John Collinge argues sugars may misguide protein folding.

PROFILE

## Bent Out of Shape

**LONDON**—When it was first proposed in the early 1980s, the notion that aberrant proteins called prions can replicate without DNA or RNA and cause infectious diseases was biological heresy of the first order. While still controversial, this hypothesis eventually won enough adherents that it earned its leading advocate, University of California, San Francisco, neurologist Stanley Prusiner, the 1997 Nobel Prize in physiology or medicine. Now, John Collinge, a neurologist at St. Mary's Hospital in London, is pushing the controversy a step further. Collinge contends that the differences between prion types, or "strains" as they are often called, is in large part determined by how many carbohydrate molecules bind to a protein. This claim has helped make Collinge one of the prion world's most high-profile scientists.

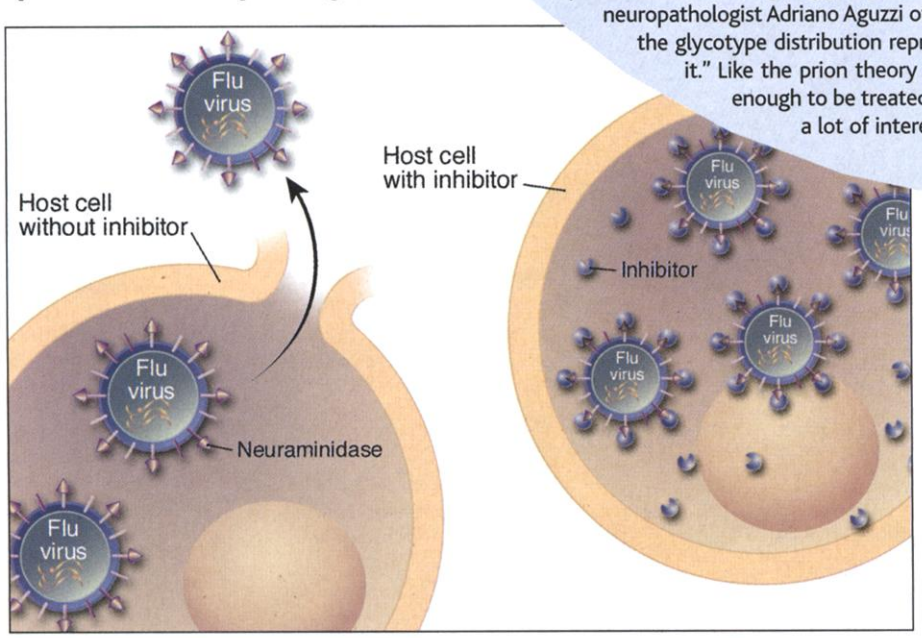
Collinge was introduced to prion diseases in the late 1980s when, soon after medical school, he began working in psychiatrist Tim Crow's lab at the Clinical Research Centre in Harrow, U.K. Shortly before, Crow's group, together with Anita Harding's team in London, had identified a gene mutation linked to an inherited form of Creutzfeldt-Jakob disease (CJD), an invariably fatal neurodegenerative disorder. Other workers had showed that this gene codes for a protein called PrP, of which prions are misfolded versions, and Collinge set to work characterizing this and other PrP mutations responsible for CJD. According to Collinge, the fact that the prions created by these mutations could then infect experimental animals was a "very powerful argument" that proteins alone could cause a transmissible disease.

In 1990, Collinge moved to St. Mary's, where he continued working on prion protein genetics and structure. Other researchers—including Richard Marsh of the University of Wisconsin, Madison, who worked on prion diseases in minks—had found a correlation between prion strains and protein structures. Collinge's group, working with the prions that cause human disease, found that strains also correlated with the pattern of sugar groups bound to the protein. For example, prions isolated from the brains of patients with "classical" forms of CJD were found to have a very different sugar pattern from those isolated from patients with a variant form of CJD that struck some patients much earlier in life. But the sugar pattern of variant CJD prions was identical to those that cause bovine spongiform encephalopathy—a finding key to establishing that variant CJD was in fact the human form of "mad cow disease."

Collinge believes that sugars known as glycosyl groups mark prion strains in part because they stabilize the proteins' aberrant shapes. "Certain [protein] conformations will associate with different glycosyl patterns," he says. And in early 1999, Collinge and his colleagues reported developing a diagnostic test that detects variant CJD in tonsil biopsies on the basis of their glycosylation patterns (*Science*, 22 January 1999, p. 469).

Given the continuing controversy over whether proteins alone can form infectious particles, many of Collinge's colleagues are intrigued but cautious about the role of sugars in prion diseases. Collinge's glycosylation work "is a very significant breakthrough," says neuropathologist Adriano Aguzzi of the University of Zurich. "But we do not know if the glycotype distribution represents the essence of a strain or a surrogate for it." Like the prion theory itself, Collinge's glycosylation theory is heretical enough to be treated with caution but intriguing enough to stimulate a lot of interest.

**–MICHAEL BALTER**




**No escape.** Newly approved carbohydrate drugs block the ability of flu viruses to exit infected cells by binding to neuraminidase, a viral protein required for the job.

CREDITS: (TOP) RAY YOUNG; (BOTTOM) CARIN CAIN