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

PROFILE

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.**

CREDIT: SANDY HUFFAKER

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