SCIENCE NEWS Online
THE WEEKLY NEWSMAGAZINE OF SCIENCE

Week of April 13, 2002; Vol. 161, No. 15

The True Sweet Science

Researchers develop a taste for the study of sugars

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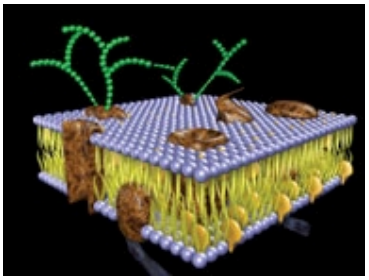
"A spoonful of sugar helps the medicine go down," sings Julie Andrews in the popular Disney movie *Mary Poppins* . The musical nanny doesn't seem to realize that sometimes the sugar is the medicine. Consider the blood-thinner heparin, the best-selling drug in the world. This complex sugar, or carbohydrate, is a reminder that there's more to life than DNA and the proteins it encodes. Complex sugars coat almost every cell in the body, as well as microbes that cause disease. And many proteins acquire a covering of sugars.

Although scientists once thought such sugars were mere decoration, they now appear to play a vital role in the function of glycoproteins (from *glukus* , the Greek word for sweet). That influence can make a difference in the body or in a drug-manufacturing plant. The California-based pharmaceutical company Amgen, for example, discards significant amounts of its yield of red blood cell-boosting erythropoietin because improper gylcosylation during its manufacture creates a less effective drug.



BRANCHING OUT. Glycoproteins perform myriad roles for cells. In this representation, the protein portions (brown) sit within a cell membrane and branching chains of simple sugars (green hexagons) extend into the space between cells.
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As erythropoietin and heparin attest, the study of sugars, a field known as glycobiology, can be big business. Nonetheless, because of the difficulties associated with studying sugars, it's only been in the past decade or so that the discipline has grabbed the attention of more than a small cadre of biologists and chemists.

"Historically, people focused on DNA and proteins. Sugars were a nuisance," notes Ram Sasisekharan, a chemist at the Massachusetts Institute of Technology (MIT).

Physicians were also slow to realize the importance of sugars to human health. They now know that a variety of birth defects, potentially fatal syndromes, and other illnesses stem from problems with sugars.

"Glycobiology did not earn the respect it deserves until the recent descriptions of several human congenital diseases with defects in the glycosylation of proteins," Harry Schachter of the University of Toronto notes in the December 2001 *Journal of Clinical Investigation* . Schachter says he expects that many more such illnesses will soon be recognized. "It is likely that we have seen only the tip of the iceberg," he contends.

Indeed, scientists have begun to reveal myriad roles for complex sugars. One recent study, for example, suggested that molecules in heparin's family are crucial to the embryonic development of animals. Another found that the coating of sugars on tumor cells helps determine whether those cells will spread in the body.

Reflecting the desire of the scientific community to push glycobiology forward, the National Institute of General Medical Sciences last fall committed $34 million over 5 years to certain areas of carbohydrate research. This glycobiology renaissance springs from newly developed tools and techniques that have enabled investigators to more quickly decipher the complex structures of sugars and to synthesize the molecules in more-efficient ways.

"The technology has begun to fall in place," says Sasisekharan.

Sugarcoated chips

Whereas the building blocks of DNA and proteins bond in a few, predictable ways, there are many ways that two simple sugar molecules may join. This flexibility greatly increases the complexity of the resulting, more complex sugars. For example, two glucose molecules can join in almost a dozen ways, and three hexose molecules can combine into several thousand distinct forms.

When different simple sugars are combined, the possibilities seem endless. One family of carbohydrates similar to heparin's uses 32 simple sugars in its construction. Adding to the complexity, carbohydrates can consist of a linear chain of sugars or contain multiple chains branching off individual sugars.

Given all that, it would be surprising if scientists could simply isolate a complex sugar from a tissue sample, pop it into a machine, and quickly read the sequence of simple sugars that constitute it. Although such tools have long been available for sequencing the building blocks

of DNA and proteins, glycobiologists haven't had the same luxury. They're making progress, however.

Most sugar investigators are turning to various forms of mass spectrometry, a technique in which complex molecules are vaporized into ions. These are then sorted and analyzed by mass, charge, and other characteristics. In 1999, for example, Sasisekharan and his colleagues described a new mass spectrometry strategy to sequence the sugars in heparin's family, called the heparan sulfates. These sugars coat cells and are also an integral part of the matrix of proteins and other molecules that occupy the space between cells. Among their other roles, heparan sulfates help regulate the release of growth factors sequestered in this matrix.

Sasisekharan's team has employed the new strategy to study updated versions of the drug heparin. Traditional heparin has been used since the 1930s to prevent blood clots that lead to strokes or heart disease. The drug has side effects, however. So, to retain the blood-thinning properties but avoid the problems, scientists have recently made experimental drugs out of fragments of heparin.

The sequence of sugars of one of the less effective of these so-called low-molecular-weight heparins was different from what scientists had thought. This work revealed that the fragment was missing some of the heparin-molecule region that's key to the drug's anticoagulant properties.

Sasisekharan notes that his group's technique may help manufacturers ensure the purity of heparin and its derivatives. These companies haven't had an easy way until now to check whether the sugar molecules they were making were uniform in structure from one production batch to the next, he says.

Considering how difficult it is to take complex sugars apart for analysis, it's not surprising that glycobiologists have also had a hard time synthesizing complex sugars in a precise way. There are several laboratory strategies for building a carbohydrate out of sugars. The most natural and efficient way is to exploit the same enzymes that cells use to construct their complex sugar molecules.

"The problem is we don't have all the enzymes," says Peter H. Seeberger of MIT.

With their incomplete set of molecular tools, scientists and manufacturers have had to synthesize carbohydrates through a series of complicated chemical reactions. Seeberger and his colleagues recently developed a more efficient, enzyme-free strategy for making complex sugars to order. In the Feb. 23, 2001 *Science* , they describe using a technique called solid-phase synthesis to create carbohydrates with as many as 12 simple-sugar building blocks. "Some of those structures used to take 6 months to a year to make. We can make them in about 2 days now," notes Seeberger.

Chi-Huey Wong of the Scripps Research Institute in La Jolla, Calif., and his colleagues have developed their own automated technique for enzyme-free carbohydrate synthesis. They recently tested it by building a six-sugar compound that sits on the surface of some tumor cells. Other scientists are testing whether this carbohydrate can stir the immune system to fight off cancer.

Although chemists have made great strides in carbohydrate synthesis recently, Wong cautions that it will be several more years before biologists will easily make any complex sugar they might want to study.

Learning to decipher the structures of sugars and to synthesize them efficiently is important, but scientists are also eager to learn with what substances these molecules interact. That's where another technological advance comes into play—the carbohydrate chip. It's not a starchy snack but a glass slide or other material onto which researchers attach an array of hundreds to thousands of sugar-containing molecules. With such chips, scientists can learn what proteins or other molecules in tissue or fluid samples naturally interact with the sugars. Biologists have previously created chips with arrays of proteins or DNA strands to identify protein-protein interactions or to measure the activity of genes in a cell or tissue (SN: 3/8/97, p. 144).

Several research groups are developing carbohydrate chips. In the March *Nature Biotechnology* , Denong Wang of Columbia University and his colleagues describe a simple method for building such instruments. Without ruining the carbohydrate array's ability to interact with other substances, Wang's group found they could affix thousands of complex sugars to glass slides coated with the carbohydrate nitrocellulose. Such carbohydrate chips should be much more stable than those bearing proteins or DNA.

"You can produce a [carbohydrate] chip and use it for many years. There's no special handling required," says Wang.

To test their invention, the investigators created a chip out of the sugar molecules and glycoproteins that normally coat certain microbes. The researchers showed that when they exposed this chip to a blood sample, antibodies would stick to the sugars of specific microbes. Such a chip, says Wang, could serve as a quick diagnostic test for active infections.

Medical sweet spots

As scientists become more adept at making and analyzing carbohydrates, they should learn what these complex molecules do inside and outside cells. It's already clear that sugars mediate many forms of cell-to-cell interactions. For example, immune cells bristle with many different sugars. When a tissue suffers an infection or injury, nearby blood vessels make sugar-binding proteins that grab onto some of the immune-cell sugars and guide the cells to the damaged area.

Sugars also help explain how a string of amino acids consistently folds into a protein's three-dimensional shape—one of the more enduring mysteries in biology. Studies have

revealed that sugars can regulate when a newly minted protein interacts with so-called chaperone molecules, which help it fold.

The sugars "actually help a protein to fold," says Raymond Dwek, director of the Oxford University Glycobiology Institute. "That is one of the most significant discoveries in glycobiology."

Sugars are also now seen as crucial players in the growth of an embryo. In the Jan. 18 *Journal of Biological Chemistry* , Scott Saunders of Washington University School of Medicine in St. Louis and his colleagues report that a protein called Noggin binds to heparan sulfate proteoglycans, molecules combining heparan sulfate and a protein. Noggin normally governs development by inactivating proteins that diffuse through an embryo and establish its body plan. From his group's work, Saunders concludes that heparan sulfates indirectly influence how the embryo develops.

That finding may be of more than academic interest. Saunders studies kids with Simpson-Golabi-Behmel syndrome, a rare disease that has been traced to a defect in the production of heparan sulfate proteoglycans. Children with the syndrome typically develop an enlarged head or body and fused fingers or toes. They also experience certain childhood cancers more often than normal.

Cancer researchers, too, are increasingly paying attention to sugars. It's been known for a long time that the sugar coating of a cell usually changes when the cell becomes cancerous. It isn't clear, however, whether that's a byproduct of the cancerous transformation or a factor that helps the tumor.

In the Jan. 22 *Proceedings of the National Academy of Sciences* , Sasisekharan and his colleagues offer evidence that some heparan sulfate fragments promote the growth and spread of tumors, while others inhibit those processes. In mice carrying melanoma or lung cancer cells, the investigators tested the effects of two enzymes, each of which cleaves heparan sulfates in a different spot. When the researchers injected one enzyme into the rodents, the cancers grew and spread more readily than normal. The other enzyme, however, produced the opposite result. Cancer growth and spread was inhibited.

The researchers obtained similar results when they injected mice with the heparan sulfate fragments themselves, rather than the enzymes. The results are intriguing, says Sasisekharan, especially since there have been some hints in the tests of heparin that the blood thinner may reduce mortality in cancer patients.

Infectious disease is another area of human health that has come to the attention of glycobiologists. Two recently approved drugs for the flu, for example, inhibit a viral enzyme that strips a sugar off a glycoprotein on the surface of cells. In doing so, the drugs prevent influenza viruses from infecting cells.

Dwek and his colleagues are also developing antiviral agents that exploit the role of sugars in protein folding. They've identified compounds that inhibit one of the cellular enzymes that removes sugars from a new, unfolded protein. In infected cells, the compounds appear to prevent the proper folding of viral proteins without harming the cells. The researchers hope this year to begin testing the drugs on people infected with hepatitis virus.

To Dwek, who coined the term glycobiology more than a decade ago, the increasing scientific and medical interest in the field is welcome. He says, "It's a boom time, as far as I'm concerned."

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From *Science News* , Vol. 161, No. 15, April 13, 2002, p. 232.