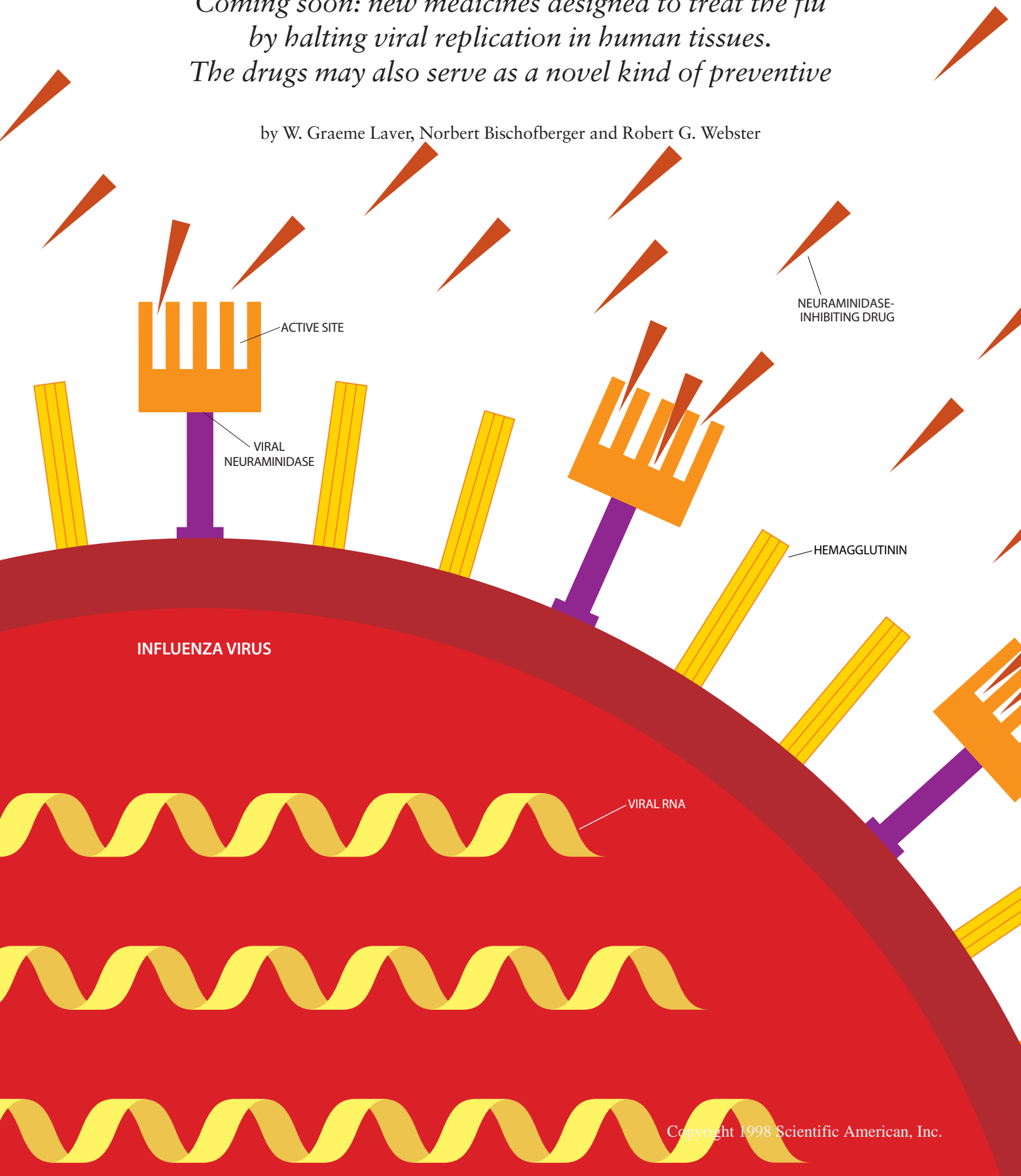


# Disarming Flu Viruses

*Coming soon: new medicines designed to treat the flu by halting viral replication in human tissues. The drugs may also serve as a novel kind of preventive*

by W. Graeme Laver, Norbert Bischofberger and Robert G. Webster



INFLUENZA VIRUS

ACTIVE SITE

VIRAL  
NEURAMINIDASE

NEURAMINIDASE-  
INHIBITING DRUG

HEMAGGLUTININ

VIRAL RNA

Every so often, a strain of influenza unfamiliar in humans suddenly begins passing from person to person. Because the virus is so unusual, few if any people have built-in immunity from past exposures. Even the vaccinated have no defense; flu shots shield against influenza variants that health experts have anticipated will be active in a given flu season, not against other, unforeseen kinds. Finding no deterrent, the new strain spreads unabated, causing illness—and death—on a global scale.

The worst worldwide epidemic, or pandemic, on record struck in 1918 and killed more than 20 million people, sometimes within hours after the first symptoms appeared. This disaster, traced to the so-called Spanish influenza virus, was followed by epidemics of Asian flu in 1957, Hong Kong flu in 1968 and

Russian flu in 1977. (The names reflect popular impressions of where the pandemics began, although all four episodes, and perhaps most others, are now thought to have originated in China.)

Public health experts warn that another pandemic can strike any time now and that it could well be as vicious as the 1918 episode. In 1997, when a lethal influenza variant afflicted 18 people in Hong Kong, contributing to the death of six, officials feared the next wave had begun. Authorities in the region managed to contain the problem quickly, however, by finding the source—infected chickens, ducks and geese—and then destroying all the poultry in Hong Kong.

Next time, humankind may not be so fortunate. If a virus as deadly as that Hong Kong strain tore through the world's crowded communities today, 30 percent of the earth's population could conceivably be dead (from the virus itself or from secondary bacterial infections) before a vaccine became available to protect those who initially managed to escape infection. Vaccines against any given influenza variant take about six months to produce, test for safety and distribute—too long to do much good in the face of a fast-moving pandemic.

If the feared pandemic does not materialize until next year or beyond, though, new methods for limiting sickness and death could be available. Later this year two drugs being tested in large clinical trials could be approved for sale as new missiles in the fight against the flu. The agents—called zanamivir (Relenza) and GS 4104—show great promise for preventing influenza infections and for reducing the duration and severity of symptoms in people who begin treatment after they start to feel sick.

Unlike vaccines (which prime the immune system to prevent viruses from gaining a foothold in the body) and unlike standard home remedies (which ease symptoms but have no effect on the infection itself), these drugs have been

**“PLUG DRUGS”** (*red wedges*) are showing great promise for preventing and treating the flu. They work by plugging the active, catalytic site of an enzyme called neuraminidase that protrudes from the surface of influenza viruses. This enzyme enables newly formed viral particles to travel from one cell to another in the body. With the enzyme inactivated, the virus is stopped in its tracks.

BRYAN CHRISTIE

designed to attack the influenza virus directly. They hobble a critical viral enzyme, called neuraminidase, and in so doing markedly reduce proliferation of the virus in the body. Additional neuraminidase inhibitors, not yet evaluated in humans, are under study as well.

As many people know, two anti-flu drugs, amantadine and rimantadine, are already on the market. But those agents, which work by a different mechanism, have serious flaws. They can cause confusion and other neurological side effects, and they are ineffective against one of the two major influenza classes (type B) that afflict people. Moreover, influenza viruses seem to become resistant to the drugs fairly easily. Therefore, individuals treated in the first phases of an epidemic can spread a drug-resistant version of the virus to other people, who will then prove unresponsive to the medicines. This last problem is particularly acute in “closed” communities, such as nursing homes.

The story of how the newer drugs were developed involves a wonderful combination of luck and logic. The breakthrough that led most immediately to their design was the deciphering, in 1983, of neuraminidase's three-dimensional structure. Yet it was a series of earlier discoveries that enabled scientists to realize that a specific part of the neuraminidase molecule was probably an Achilles' heel for all influenza variants—a weakness that thoughtfully constructed drugs might exploit.

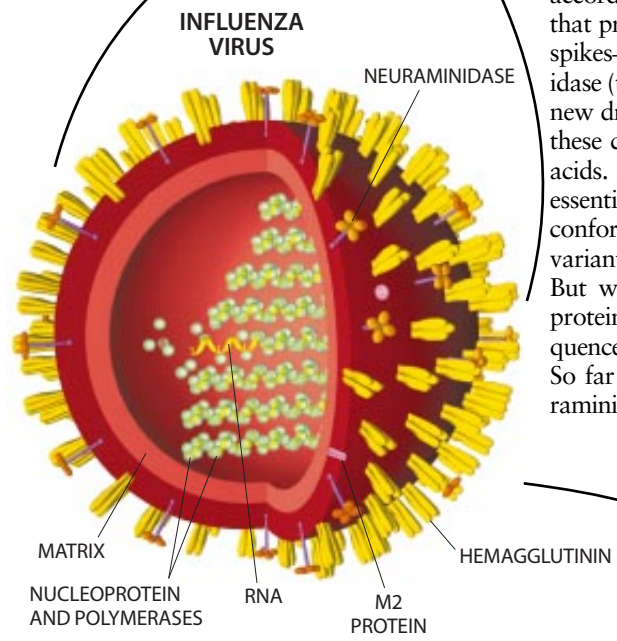
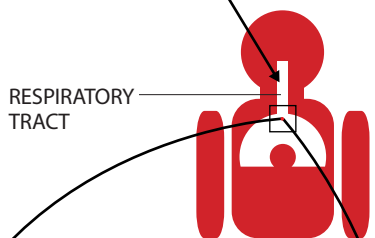
### Understanding a Scourge

One line of that early research uncovered some essential properties of influenza viruses and of their strategy for survival. Biologists have long known that viruses are basically genes wrapped in proteins that either protect the genes or help the viruses to reproduce in the body. Sometimes, as is true for influenza, these various constituents are further enveloped in a fatty (lipid) membrane. When any virus causes disease, it does so by invading selected cell types, replicating within those cells and then pouring out of the cells to infect others. Symptoms arise both because viral proliferation disrupts the colonized cells and because the immune system attempts to contain the infection, in the process causing local inflammation and systemic aches and fever.

Influenza strains that colonize humans have a particular affinity for the



THOMAS BROCK. Courtesy of Michael Madigan



epithelial cells that form the lining of the respiratory tract. Successful infection typically leads after a day or two to such classic symptoms as runny or stuffy nose, dry cough, chills, fever, aches, deep tiredness and loss of appetite. Historical descriptions based on symptoms indicate that flu epidemics have probably plagued human populations since well before the 5th century B.C.

Scientists isolated an influenza strain from a human for the first time in 1933. Since then, they have learned that influenza viruses come in two main “flavors”—types A and B—that differ in certain of their internal proteins. A third type (C) does not seem to cause serious disease.

Virologists further group type A forms according to variations in two proteins that protrude from the viral surface like spikes—hemagglutinin and neuraminidase (the enzyme that is the target of the new drugs). As is true of other proteins, these consist of folded strings of amino acids. All hemagglutinin variants adopt essentially the same three-dimensional conformation, and all neuraminidase variants take on a characteristic shape. But within each group, the individual proteins can differ markedly in the sequence of their constituent amino acids. So far 15 hemagglutinin and nine neuraminidase subtypes have been identified

on type A influenzas, which are named according to the hemagglutinin and neuraminidase molecules they display: H1N1, H1N2, H2N2 and so on.

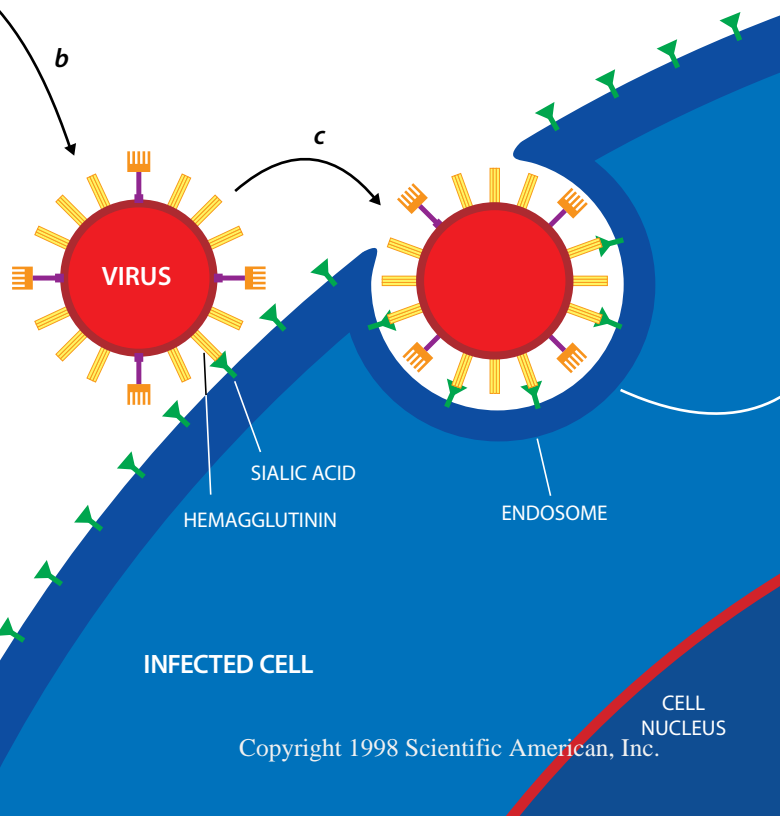
Type B viruses are a more uniform lot. They carry one form of hemagglutinin and one of neuraminidase, although the amino acid sequences can differ slightly from one B strain to another. Similarly, each influenza A subtype can also come in slightly varying strains.

Aside from their chemistry, type A and type B influenzas differ in their range of activity. Type B viruses infect only humans, and they cause regional epidemics rather than pandemics. Type A influenzas, in contrast, affect pigs, horses, seals, whales and birds as well as humans, although not all strains infect all species. (Indeed, only four subtypes have been found in humans.) They are also responsible for all of this century’s pandemics.

In spite of their differences, both influenza types have the same basic life cycle. For a single copy of an influenza virus, or particle, to enter a human cell, hemagglutinin on the virus must link to a sugary molecule, sialic acid, on the surface of the cell. This binding induces the cell to engulf the virus, which is initially sequestered within a kind of bubble. Soon, though, the viral genes (consisting of strands of RNA) and internal pro-

**LIFE CYCLE** of the influenza virus often involves transmission from one person’s airways to another’s via water droplets emitted during a sneeze (a). An individual virus (detail, above) enters a cell lining the respiratory tract after a molecule called hemagglutinin on the virus binds to sialic acid on the cell (b). This binding induces the cell to take up the virus (c), which soon dispatches its genetic material, made of RNA, and its internal proteins to the nucleus (d and e). Some of those proteins then help to duplicate the RNA (f) and to produce messenger RNA, which the cell’s protein-making machinery uses as a template for making viral proteins (g and h). Next, the viral genes and proteins assemble into new viral copies, or particles (i), and bud from the cell.

The particles emerge coated with sialic acid. If that substance remained on the virus and on the cell, the hemagglutinin molecules on one particle would soon attach to the sialic acid on other particles and on the cell, causing the new viruses to clump together and stick to the cell. But neuraminidase on the virus clips sialic acid from the offending surfaces (j), leaving the new particles free to travel on (k) and invade other cells.



teins are freed, and they work their way into the cell nucleus.

There some of the viral proteins set about replicating the viral RNA strands and also constructing a form (called messenger RNA) that can be read out and translated into proteins by the cell's protein-making machinery. Eventually the newly made genes and proteins come together and bud from the cell as new viral particles.

Inconveniently for the virus, the emerging particles are coated with sialic acid, the very substance that binds influenza viruses to the cells they attempt to invade. If the sialic acid were allowed to remain on the virus and on the surface of a virus-making cell, hemagglutinin on the newly minted particles would bind to the sialic acid, causing the particles to clump together on the cell, like insects trapped on flypaper. So trapped, they would be unable to spread to other cells.

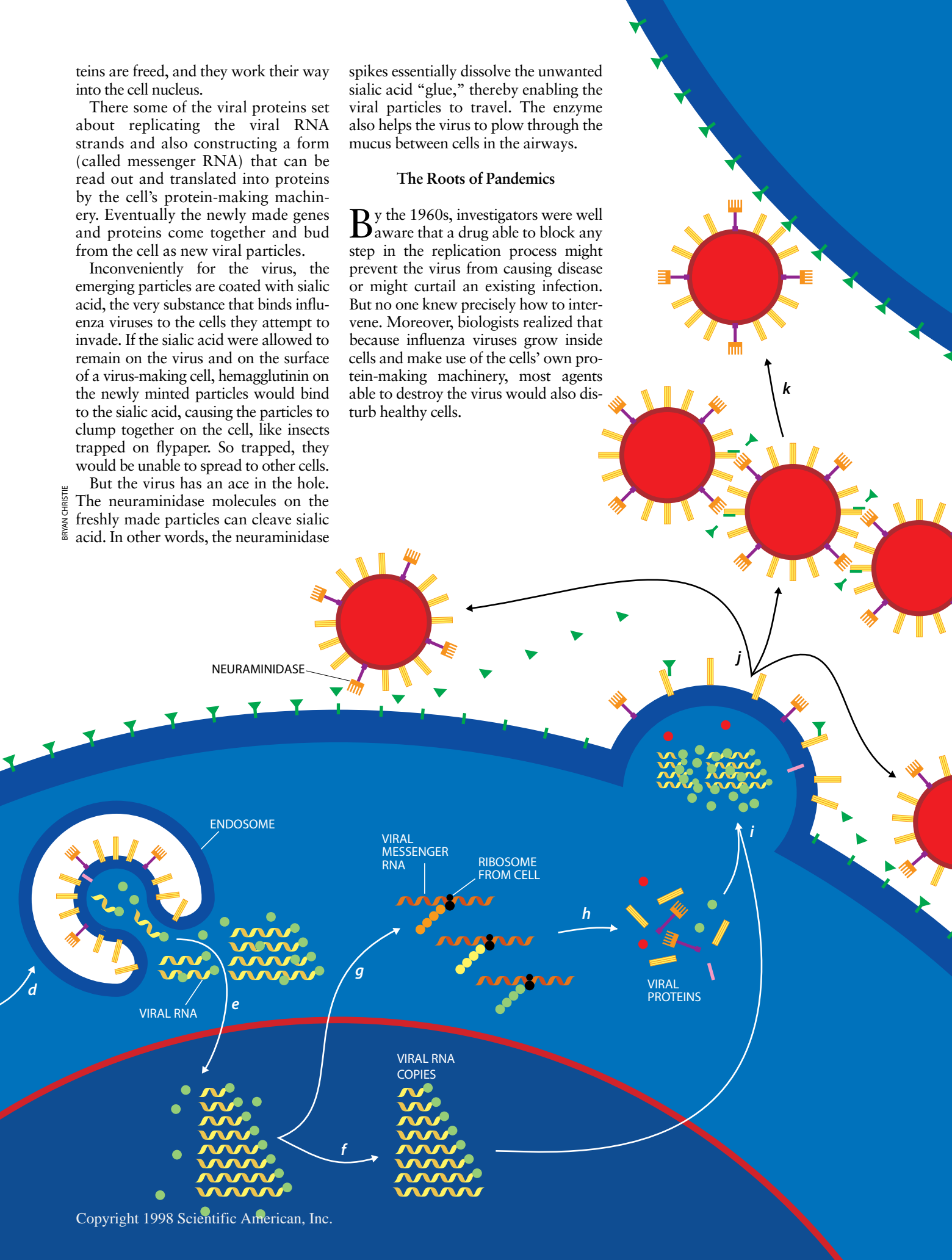
But the virus has an ace in the hole. The neuraminidase molecules on the freshly made particles can cleave sialic acid. In other words, the neuraminidase

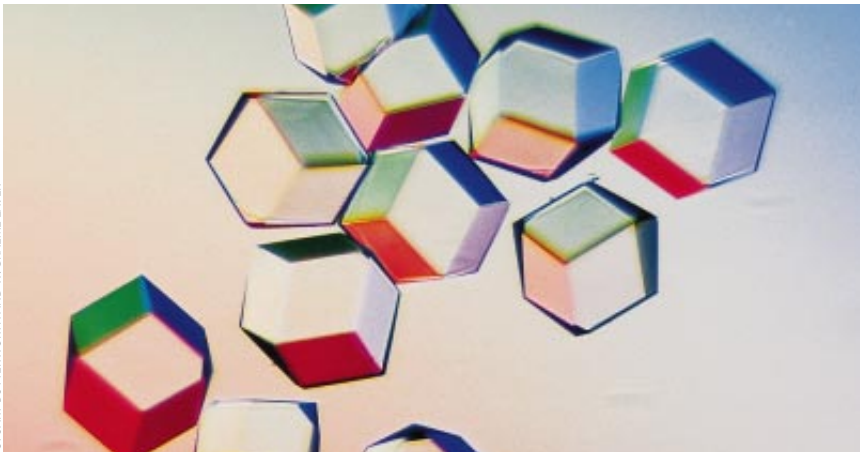
spikes essentially dissolve the unwanted sialic acid "glue," thereby enabling the viral particles to travel. The enzyme also helps the virus to plow through the mucus between cells in the airways.

### The Roots of Pandemics

By the 1960s, investigators were well aware that a drug able to block any step in the replication process might prevent the virus from causing disease or might curtail an existing infection. But no one knew precisely how to intervene. Moreover, biologists realized that because influenza viruses grow inside cells and make use of the cells' own protein-making machinery, most agents able to destroy the virus would also disturb healthy cells.

BRYAN CHRISTIE





**CRYSTALS OF NEURAMINIDASE** were obtained from an influenza virus found to infect wild birds on the Great Barrier Reef of Australia. Neuraminidase crystals have enabled scientists to determine the three-dimensional structure of the enzyme and to build drugs designed to plug its active site. The hues are reflections of colored lights.

As workers confronted this difficulty, they also continued trying to understand why some strains of influenza produce localized epidemics but others cause full-fledged pandemics. This research would eventually reveal that to be most useful, an influenza drug would have to be able to attack all influenza variants, including ones not known to cause disease in humans.

An influenza strain can produce a local or global epidemic only if the people exposed to the virus lack adequate immunity to it. For instance, when someone has the flu, the immune system produces molecules known as antibodies that recognize specific segments of hemagglutinin and neuraminidase on the viral surface. If the person is later reexposed to the same strain, these antibodies (primarily those directed at hemagglutinin) bind promptly to the virus and prevent it from causing a repeat infection.

If the virus never changed, as is the case with measles or mumps, the antibodies raised during infection or by a vaccine could provide durable immunity. But influenza viruses revise themselves all the time. In consequence, antibodies elicited in one year may well be less effective or useless if they encounter a differing form of the virus in the next flu season. The extent of change largely determines whether an epidemic becomes relatively contained or extends across the globe unchallenged.

One way influenza viruses change is by antigenic “drift,” gradual revision of the amino acid sequence in a protein (antigen) able to elicit an immune response. These alterations arise through small mu-

tations in the gene that constitutes the blueprint for that protein. Sometimes a mutation makes little difference in the protein’s stability or activity. Sometimes it damages the protein and reduces the viability of the virus. Other times, though, it enhances survival, such as by reconfiguring a site on hemagglutinin that was formerly recognized by an antibody.

When the hemagglutinin or neuraminidase genes and proteins accumulate several alterations, they can become virtually unrecognizable to most of the antibodies in a population and may initiate a new epidemic. The epidemic finds boundaries, however, when it reaches groups whose immune systems have already “seen” many of the alterations before.

Influenza B viruses seem to change exclusively through such antigenic drift, evolving gradually in their human hosts as they attempt to become less recognizable to the immune repertoire of a population. Influenza A strains, in contrast, can additionally undergo a more dramatic change, known as antigenic “shift,” that enables them, alone, to cause pandemics.

When antigenic shift occurs, strains crop up bearing a totally new hemagglutinin spike, and sometimes also a new neuraminidase molecule, that most people have never encountered. As a result the virus may evade the antibody repertoire carried by all populations around the globe and trigger a pandemic. In today’s jet-linked world, people can spread a dangerous new virus from one part of the earth to another in a day.

Such a drastic metamorphosis cannot

occur through simple genetic mutation. The best-studied process leading to antigenic shift involves the mixing of two viral strains in one host cell, so that the genes packaged in new viral particles (and their corresponding proteins) come partly from one strain and partly from the other. This reassortment can take place because the genome, or genetic complement, of the influenza virus consists of eight discrete strands of RNA (each of which codes for one or two proteins). These strands are easily mixed and matched when new influenza A particles form in a dually infected cell. For instance, some influenza viruses infect both people and pigs. If a pig were somehow invaded by a human virus and by a strain that typically infected only birds, the pig might end up producing a hybrid strain that was like the human virus in every way except for displaying, say, a hemagglutinin molecule from the bird virus.

Scientists have recently learned that antigenic shift can also occur in a second way. In this case, an animal influenza virus that has not previously been able to produce infections in people makes a direct leap into human beings.

No one knows which form of antigenic shift led to the Spanish flu pandemic of 1918, which was caused by the H1N1 subtype of influenza A. Reassortment has, however, been proved to account for the 1957 Asian flu and 1968 Hong Kong flu pandemics, which were triggered, respectively, by H2N2 and H3N2. Some work suggests that aquatic birds might have contributed the unfamiliar genes and that pigs probably served as the mixing vessels. If pigs do sometimes serve this function, their involvement might help to explain why pandemics commonly originate in China: millions of birds, pigs and people live closely there.

The virus that killed six people in Hong Kong in 1997 (H5N1), in contrast, was not a reassortment virus. It caused human disease after jumping directly from birds to people—a phenomenon that had never been seen before. H5N1 was unable to pass from human to human. Had it been given time to acquire transmissibility through mutation or reassortment, though, it might have become uncontrollable quickly.

The close call of 1997 has now convinced many public health experts that influenza cases need to be monitored not only in people (as is done now) but also in animals. Those animals should

certainly include migratory birds, because they probably serve as a year-round reservoir of influenza A viruses that then spread to domestic birds and other species. Prompt identification of animal strains with potential for harming people could help avert a public health disaster.

The incident in Hong Kong has also lent new urgency to research into the nature of the so-called species barrier that prevents many influenza strains from crossing from one kind of animal to another. If the barrier were better understood, scientists might be able to seal the leaks that now allow certain animal strains to breach the barrier and cause human disease.

### Eureka

Taken together, studies of influenza biology conducted before the early 1980s indicated that, in addition to blocking the activity of some molecule involved in the virus's reproductive cycle, an ideal anti-flu drug would do so by acting at a "conserved" site on the targeted molecule. That is, it would home in on an area formed from amino acids that are held constant across all strains of the virus. A drug that targeted a conserved region would presumably work against any influenza virus that turned up in people, including ones that spread abruptly from animals.

Interestingly, the structural work that enabled researchers to design neuramin-

idase inhibitors grew out of an accidental discovery. Back in the late 1970s, one of us (Laver) was attempting to determine whether the N2 spike on the virus that caused the 1968 Hong Kong flu pandemic (H3N2) had come from the strain responsible for the 1957 Asian flu pandemic (H2N2). As part of that effort, he wanted to compare the amino acid sequences of the molecules. To start, he had to isolate and concentrate their heads—the domains that protrude from the viruses.

When Laver freed the neuraminidase heads from purified viruses and concentrated them in a centrifuge, he found to his surprise that the resulting pellet of material was not amorphous, as proteins usually are. Instead it consisted of crystals. Crystals, which are highly ordered arrays of molecules, are essential for deciphering the three-dimensional structure of large proteins. Hence, the unexpected production of neuraminidase crystals implied that the structure of neuraminidase could perhaps be deciphered.

In 1983 Peter Colman and his colleagues at the Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia did just that. The work revealed that the neuraminidase spikes on influenza viruses consist of four identical molecules, or monomers. The resulting tetramer resembles four squarish balloons atop a single stick. The stick is embedded in the viral membrane, and the balloons protrude. Colman's group soon discovered that each neuraminidase

monomer in the foursome has a deep, central dent, or cleft, on its surface.

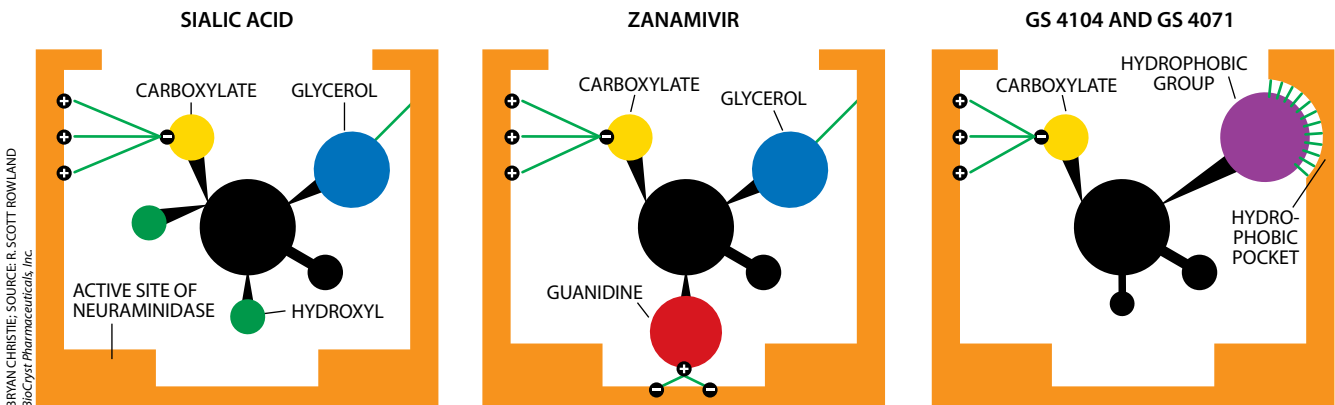
The team also found that even though influenza neuraminidase molecules could differ in the precise amino acids they contained, all known versions—including those from type A and from type B viruses—had a striking commonality. The amino acids that lined the wall of the cleft were invariant.

When parts of molecules resist change, the constancy usually implies that the unchanged components are essential to the molecules' functioning. In this case, the uniformity suggested that the cleft formed the active, sialic acid cleaving site of neuraminidase and that the unchanging, or conserved, amino acids in the cleft were critical to maintaining catalytic function. Subsequent work confirmed this suggestion.

Given that influenza viruses cannot spread readily from cell to cell without help from neuraminidase, the new discoveries implied that a drug able to occupy, and jam, the active site would inhibit neuraminidase in all versions of the influenza virus. That is, such a "plug" drug might serve as a universal cure for the flu.

### The Making of a Plug Drug

Pursuing this tantalizing idea, Colman's group identified the amino acids in the active site that normally contact sialic acid. They also looked for amino acids in the cleft that did not



NEW DRUGS clog the active site of neuraminidase by binding to it more readily than sialic acid—the substance it normally cleaves. Sialic acid (*left*) is held in the site, a cleft, mainly through its glycerol and carboxylate groups, which form bonds (*green lines*) with amino acids in the active site. Zanamivir (*center*) adds other bonds by replacing the hydroxyl of a sialic acid derivative with a large, positively charged guanidine, which

forms strong attachments to two negatively charged amino acids at the bottom of the cleft. GS 4104 is converted to GS 4071 in the body. The resulting molecule (*right*) retains the carboxylate bonds made by sialic acid but also makes use of a hydrophobic group. This group induces the binding cleft to form a similarly hydrophobic pocket, which holds the drug in place through hydrophobic attractions (*short green lines*).

## Building Better Vaccines

When contemplating the prospects for flu drugs, observers might reasonably wonder whether the disease would be better controlled by a universal vaccine—one able to prevent infection by inducing the body to mount a protective immune response against any influenza strain that might appear. Regrettably, no such all-purpose vaccine has yet materialized.

Nevertheless, immunologists are honing ways to speed vaccine production, so that immunization can be carried out swiftly if a virulent epidemic starts abruptly. They are also working on injection-free vaccines, to improve acceptance and to encourage immunization of children. Although the elderly tend to become sickest when they have the flu, children account for much of its spread.

Flu vaccines have been common since the 1940s. Today the manufacturing process begins after influenza samples collected by 110 surveillance sites around the world are analyzed. In February the World Health Organization pinpoints three strains—two type A and one type B—that seem likely to account for most of the flu that will occur in the upcoming season (November to March in the Northern Hemisphere). These become the basis for the vaccine.

A simple way to make a vaccine would be to grow vast numbers of the selected strains, inactivate them so that they cannot cause infection and combine them in a single preparation. Unfortunately, the strains that are selected tend to grow slowly in the laboratory

and are thus difficult to mass-produce. To overcome this obstacle, scientists begin by basically inserting immune-stimulating proteins—hemagglutinin and neuraminidase—from the surface of selected strains into a form of influenza virus that will grow quickly in the lab. For each strain, they infect chick embryos with both the fast-growing and the chosen virus. Many of the virus particles made by the embryos grow rapidly but now display the hemagglutinin and neuraminidase spikes of the strains expected to cause this year's epidemics. These high-growth reassortments are then isolated and delivered to vaccine manufacturers, who mass-produce them in more chick embryos.

At one time, inactivated forms of these viruses, including reassortments for all three of the selected strains, served as the vaccine. Now most manufacturers take the process a step further. They break the viruses apart and compose the vaccine of the viral proteins. The proteins elicit immunity but are totally unable to cause any kind of infection. In both cases, the vaccines prod the immune system to make antibodies able to bind to, and help eliminate, infectious viruses bearing those same proteins.

In an alternative approach, investigators are testing vaccines made of weakened live viruses, because live viruses evoke production not only of antibodies but also of white blood cells known as T

bind sialic acid but might be exploited to help anchor a plug drug. They noted, for instance, that the cleft included three positively charged amino acids that held tightly to a negatively charged group (carboxylate) on sialic acid.

In addition, at the bottom of the cleft they spotted a small pocket containing two negatively charged amino acids. These amino acids—glutamates—made no contact with sialic acid but were nonetheless present in all influenza neuraminidases examined. A hydroxyl (OH) group on the bound sialic acid pointed down toward that extra pocket but did not reach it.

These features suggested that replacing this OH with a large, positively charged atomic grouping might yield a tight-binding derivative. The positive group would presumably nestle into the

extra pocket at the bottom of the active site and would lock itself there by binding to the previously unused, negatively charged glutamates in the pocket.

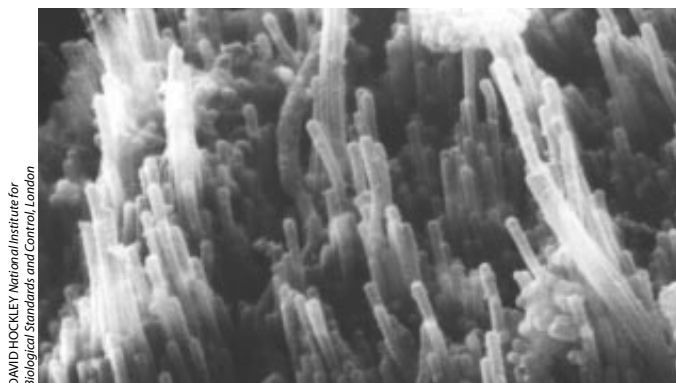
After some trial and error, in 1993 Mark von Itzstein and his colleagues at Monash University in Melbourne found that substituting a guanidino group (which is both large and positively charged) for the OH group on sialic acid produced an extraordinarily potent inhibitor of influenza neuraminidases. Further, the inhibitor had little effect on related enzymes made by bacteria and mammals, a sign that the compound probably would not disrupt human cells.

Studies in animals and preliminary trials in humans then revealed that the substance—zanamivir—prevented flu symptoms in individuals subsequently infected with influenza viruses and also

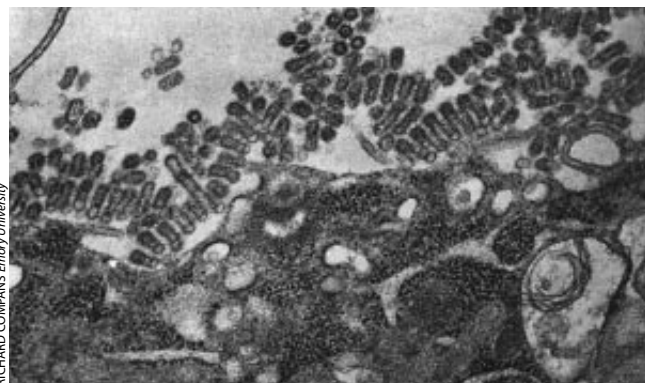
reduced the severity of symptoms in those who took the drug after being infected. The compound, however, did not work if swallowed as a pill; it had to be inhaled into the respiratory system through the nose or mouth.

Ironically, the guanidino group that makes zanamivir such a good inhibitor is the reason the substance cannot be taken as a pill. An ingested drug has to cross the cells lining the intestine and migrate into the bloodstream before traveling to other parts of the body. But charged molecules have difficulty crossing cell membranes, which are fatty and permeable mostly to noncharged substances.

Because inhalation is a common way to take medicine meant to work in the respiratory tract, Glaxo Wellcome in Stevenage, England, initiated further human testing of zanamivir. Yet be-



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**ANNUAL VACCINATION** is currently the best way to evade the flu. For some, shots may one day give way to a nasal spray vaccine.

lymphocytes. These cells recognize and eliminate virus-infected cells. T cells turn out to respond to closely related strains of influenza, not just to the single strains recognized by individual antibodies. Hence, they could potentially provide immunity for a while even after an influenza strain underwent small changes in the structure of its surface molecules.

A live-virus vaccine that is delivered as a nasal mist has been developed by Aviron in Mountain View, Calif. It has tested well in people, including children, and will probably be on the market in a year or two. Unfortunately, live-virus vaccines cannot be produced much

more quickly than killed-virus types, and so they probably would not provide a rapid defense against a sudden pandemic.

To shorten production time, scientists are examining manufacturing methods that sidestep the need for acquiring large numbers of fertilized eggs. One approach inserts hemagglutinin and neuraminidase genes from selected influenza strains into another kind of virus, such as a baculovirus, that grows readily in cultured cells—something influenza viruses do only poorly. As the genetically altered viruses reproduce in the cells, they also make large quantities of the encoded influenza proteins, which can then be purified for use in vaccines. Recombinant vaccines can be prepared and distributed in just two or three months, but their effectiveness is still being evaluated.

Yet another vaccine strategy, able to yield a product even faster, relies on “naked” DNA. In this scheme, investigators fit desired hemagglutinin and neuraminidase genes into rings of DNA known as plasmids. In theory, if such plasmids were injected into skin or muscle, cells in the vicinity would take them up and use them to make influenza proteins. These proteins would then be displayed on the cells’ surface, where cells of the immune system could spot them. In response, the immune system would deploy antibodies and T cells able to neutralize free virus and eradicate any infected cells. Naked-DNA influenza vaccines have worked well in lab animals but have yet to be tested in people. —W.G.L., N.B. and R.G.W.

cause some patients prefer to take a pill, Gilead Sciences in Foster City, Calif., joined with F. Hoffman–La Roche in Basel, Switzerland, to search for a neuraminidase inhibitor that could be taken as a tablet. A systemic drug that traveled in the blood could also conceivably help fight any highly virulent influenzas that infected cells outside the respiratory tract.

### A Second Candidate Emerges

After synthesizing and testing many compounds, Gilead and F. Hoffman–La Roche finally found one, given the working name of GS 4071, that was as potent as zanamivir. Structural analyses revealed that it retained one major neuraminidase-binding group also found in zanamivir: a negatively charged carboxylate group that bound to positively charged amino acids in the enzyme’s active site. In addition, the interaction of GS 4071 with the active site somehow caused one of the amino acids in the cleft to rotate and create a new hydrophobic

(water-hating) pocket. This pocket became an anchor for a similarly hydrophobic component (a chain of carbons and hydrogens) of the drug.

Initially the substance functioned well in the test tube and did no harm to healthy cells. But it failed a crucial test: the negative carboxylate group kept it from crossing out of the gut into the bloodstream in animals. Happily, though, a small adjustment—in essence, placing a mask over the negative group—fixed that problem. The masked form, dubbed GS 4104, passed easily into the bloodstream, after which it was unmasked, primarily in the blood and liver. Having been converted back to its original (GS 4071) form, it proceeded to inhibit the activity of neuraminidase and the spread of the virus in the respiratory tract of test animals. Like zanamivir, it also showed signs of functioning well in people.

Last fall reports from large, controlled human trials of zanamivir and GS 4104 confirmed and extended the earlier findings. If begun within about a day and a half after symptoms develop, both the inhaled zanamivir and the swallowed GS 4104 can reduce the time that people feel ill by about 30 percent (1.5 to three days). The compounds also reduce the severity of symptoms. In a trial of GS 4104, for instance, rating diaries kept by patients indicated that symptoms were 25 to 60 percent milder than in those pa-

tients who took a dummy pill. Further, the drugs lower the risk for potentially lethal secondary bacterial infections, such as bronchitis, by half or more. Such complications are a major cause of flu deaths, especially among the elderly and people with concurrent disorders.

A small study of zanamivir has recently indicated that an injected form of the drug is helpful as well. Separate investigations into prevention are encouraging, too. When zanamivir was tested, 6 percent of nonusers but only 2 percent of users came down with the flu. GS 4104 produced comparable results. So far neither drug has caused serious side effects.

On the basis of such studies, Glaxo Wellcome has requested approval to market the inhaled form of zanamivir in Australia, Europe, Canada and the U.S. Gilead and F. Hoffman–La Roche are expected to submit a similar application for GS 4104 in the U.S. and Europe this year. Other neuraminidase inhibitors developed by BioCryst Pharmaceuticals in Birmingham, Ala., have done well in studies of animals and have been licensed to Johnson & Johnson for further development.

To the casual observer, shaving a few days off a bout of the flu may seem a minor accomplishment, yet the achievement is more profound than it first appears. The fatigue and other discomforts felt during the last days of the flu

**NEWLY MADE VIRAL PARTICLES** can emerge from the cell as filaments (*left*), although they can also be spherical or any shape in between. The particles can be seen to cluster ineffectually on the cell surface (*small rods and spheres at top of right micrograph*) when their neuraminidase molecules are inactivated.

## What Accounts for Virulence?

When the flu kills, it usually takes the lives of people whose immunity is already compromised, such as by advanced age or some preexisting disorder. At times, though, it cuts down vigorous young people as swiftly and surely as it does the infirm.

Such was the case in 1918, when the “Spanish flu” pandemic killed more people than died fighting World War I. During that pandemic, even robust soldiers perished. Some felt a bit sick in the morning, went to bed in the afternoon and were dead by nighttime. Vigorous young individuals also became victims in 1997, when six of 18 people stricken by a novel strain of influenza died in Hong Kong.

What makes one strain inherently more lethal than others? Part of the answer seems to be an ability to infect a number of different tissues instead of the restricted set usually preferred by influenza viruses—namely, the respiratory tract in mammals and the gastrointestinal tract in birds. Many investigators interested in understanding the transformation to virulence are therefore hunting for features that enable some strains to become promiscuous, or pantropic, in the cells they attack.

About 15 years ago one of us (Webster) and his colleagues at St. Jude Children’s Research Hospital in Memphis uncovered a possible clue. In 1983 a virus that had been causing mild gastrointestinal disease in chickens in Pennsylvania suddenly began killing entire commercial flocks. The team found that substitution of just one amino acid for another in a viral surface protein—hemagglutinin—was the culprit. That small change had somehow enabled the virus to replicate in, and damage, organs throughout the birds’ body.

Subsequent work revealed why this tiny structural change had such

a profound effect on viral activity. When an influenza virus first enters a cell, the virus is initially sequestered in a kind of intracellular jail (an endosome). The virus manages to reproduce nonetheless because hemagglutinin molecules on the viral surface help the viral membrane to fuse with the endosome cage. As fusion occurs, viral genes and proteins escape from the endosome and set about mass-producing new copies of the virus. Hemagglutinin can facilitate fusion only if it has been cleaved into two parts before the virus enters the cell. This cleav-

**“SPANISH FLU” PANDEMIC that began in 1918 not only killed at least 20 million people, it also sent family members of flu victims to food lines. The scene below occurred in Cincinnati.**



CORBIS-BETTMANN

stem mainly from the lingering activity of the immune system after it has eliminated most of the virus in the body. The anti-flu drugs cannot affect that activity. But by halting viral replication, the compounds can certainly minimize the duration and severity of the early, most miserable part of a flu episode.

As a rule, the earlier a person begins therapy, the better the outcome is—partly because the body will harbor a smaller, more manageable amount of virus and partly because the drugs cannot undo tissue damage caused by the virus before treatment starts. This finding, combined with the research into prevention, implies that the best way to use these drugs may be in conjunction with instant tests that will signal the presence of an incipient infection before a person becomes symptomatic. It is quite conceivable that in the near future people will, say, dab their tongue each morning with a small strip of influenza-detecting test paper. If the strip turns a color, the individuals will know they harbor the influenza virus and need to begin taking a flu-fighting drug to ward off symptoms. Quick detection kits are now becoming available for use in doctors’ offices. Manu-

facturers are trying to perfect tests for home use as well.

Determining that a person has contracted the flu and not merely a cold or some other disorder is important because the drugs have power only against influenza viruses. They would be wasted if taken to combat colds (which are caused by other kinds of viruses), allergies or bacterial infections that produce flulike symptoms.

### Questions

Although the neuraminidase-blocking agents are generating great excitement, certain questions remain. Logic dictates that inhibition of viral replication and reductions in secondary complications should save lives, but whether the drugs will actually prevent deaths is not yet known.

Further, almost all drugs that humans lob at viruses and bacteria eventually become undermined by drug resistance. As the microorganisms struggle for survival, those that alter the drug target and make it unrecognizable can escape the drug’s effects [see “The Challenge of Antibiotic Resistance,” by Stuart B. Levy; *SCIENTIFIC AMERICAN*, March 1998]. Amanta-

dine and rimantadine are among those plagued by the resistance problem. Are there any reasons to think the new flu drugs will escape this fate?

In fact, there are. Scientists have tried diligently to produce strains of influenza in the laboratory that are resistant to zanamivir and GS 4104. So far they have had only limited success. Some strains gained resistance, as might be anticipated, by altering amino acids in the active site of neuraminidase. But these changes made the enzyme less stable or less active than usual, suggesting that viral survival in the body would be impaired.

Other strains became resistant not by altering neuraminidase but by changing the chemical makeup of hemagglutinin. Recall that neuraminidase is needed to strip sialic acid off nascent viral particles, so that hemagglutinin will not stick to sialic acid on neighboring particles and prevent the virus from spreading to other cells. The change in hemagglutinin reduced its affinity for sialic acid, thereby obviating the need for neuraminidase to intervene. Although it thrived in cell culture, this second group of mutants showed no resistance to the drugs in animals. Conceivably, the impaired binding

age is accomplished by particular enzymes, in the serine protease family, that are made in the avian digestive tract and the mammalian respiratory tract but are less evident in most other tissues.

The amino acid substitution found in the lethal avian virus altered the cleavage site on hemagglutinin in a way that made it accessible to cutting by enzymes (furinlike proteases) that are common in tissues throughout the body. Such increased susceptibility to cleavage enabled the virus to infect tissues systemwide.

This discovery suggested that the 1918 human pandemic might have become deadly because the responsible influenza strain car-

**CHICKENS** were trucked to Hong Kong in February 1998, replacing some of the millions killed to halt the spread to humans of a lethal influenza virus. Officials were about to test blood from the birds.



ROBYN BECK/AP Photo

ried a mutant form of hemagglutinin that was susceptible to cleavage by common proteases found outside human airways. To address this possibility and to search for other sources of virulence, Jeffery Taubenberger and his colleagues at the U.S. Armed Forces Institute of Pathology have been studying genetic material recovered from three victims of the long-ago pandemic: two soldiers (from whom tissue samples had been saved) and an Inuit woman whose body was exhumed from the permafrost of Alaska in August 1997.

The genetic work has revealed the amino acid sequence of the virus's hemagglutinin molecule, and Taubenberger's group has almost completed work on the sequence of a second surface molecule: neuraminidase. The hemagglutinin molecule turns out to be unremarkable at the cleavage site. In addition, Taubenberger says his as yet unpublished analysis of the neuraminidase gene indicates that neuraminidase lacks another kind of mutation that had been proposed as a possible route to viral promiscuity. That intriguing proposal suggested that a particular mutation in neuraminidase would basically allow it to hoard serine proteases and use them to cleave hemagglutinin molecules in tissues that did not provide those proteases.

For now—and perhaps always—the reasons for the extreme virulence of the 1918 pandemic will remain mysterious. But investigators do have a sense of why the influenza strain that appeared in Hong Kong in 1997 was so deadly.

That virus, which originated in fowl, did possess a form of hemagglutinin that is highly susceptible to cleavage. Still, researchers do not have absolute proof that this form of hemagglutinin accounts for the virulence. For that reason, they are continuing to comb the viral genes for hints to other explanations. —W.G.L., N.B. and R.G.W.

between hemagglutinin and sialic acid reduced the infectivity of the strains by weakening their ability to dock with cells.

Neuraminidase-blocking drugs are not the only ones under study. Scientists have long been trying to build plug drugs that target the sialic acid binding site on hemagglutinin, for instance, but up to now the effort has failed. Amantadine and rimantadine, the drugs already on the market, were found to work before their mechanism of action was known. It is now evident that they interfere with the activity of a viral pro-

tein called M2, which functions as an ion channel. This inhibition of M2 explains why the drugs have no effect against type B influenzas: those viruses do not carry an M2 molecule. As work on new drugs continues, so, too, do efforts to improve flu vaccines [see box on pages 84 and 85].

Between pandemics, smaller but still substantial influenza epidemics are common. In 1994, according to the U.S. Centers for Disease Control and Prevention, an estimated 90 million Americans (about 35 percent of the population) contracted the flu. Collectively, those

people spent 170 million days in bed and lost 69.3 million days of work. More typically in the U.S., the flu afflicts 10 to 20 percent of the population every year and causes some 20,000 deaths from influenza-related complications.

These numbers, like those associated with pandemics, are likely to fall in the next several years as new anti-flu drugs become available and as vaccines become more widely used and faster to produce. Indeed, society may finally be heading for an era in which the human species gains the upper hand against the much dreaded influenza virus. SA

### The Authors

W. GRAEME LAVER, NORBERT BISCHOFBERGER and ROBERT G. WEBSTER have all contributed to progress in the control of influenza epidemics and pandemics. Laver, professor of biochemistry and molecular biology at the Australian National University in Canberra, produced the first crystals of neuraminidase and continues to provide them to researchers who are designing new neuraminidase inhibitors. Bischofberger is senior vice president of research at Gilead Sciences in Foster City, Calif. Webster, who with Laver first traced pandemic strains of influenza viruses to lower animals, is Rose Marie Thomas Professor and chairman of the department of virology and molecular biology at St. Jude Children's Research Hospital in Memphis. He is also director of the World Health Organization's Collaborating Laboratory of the Ecology of Influenza Viruses in Lower Animals and Birds.

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