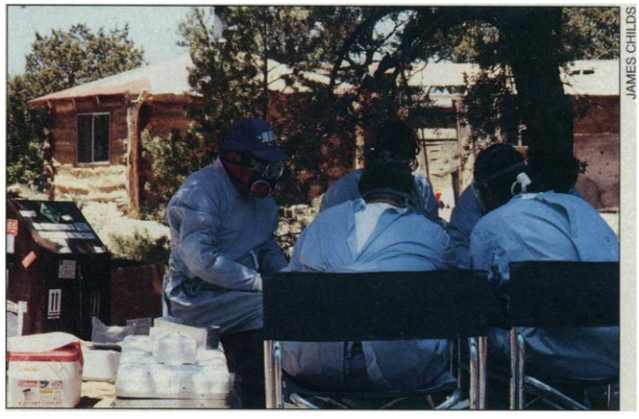
Hantavirus Outbreak Yields to PCR

Researchers have yet to isolate the virus that has killed 26 people in the United States this year, but they have its genes, they know where it hides, and they are working out its modus operandi

Fall weather is bringing chilly nights and sparkling, crisp days to the southwestern United States. It may be a relief from the blistering summer, but for Gary Simpson, New Mexico's chief infectious disease officer, the change of seasons is accompanied by a nagging concern. The cold is driving small rodents indoors, and Simpson fears that, as the animals hunker down next to people, they could trigger a second outbreak of a rodent-borne hantavirus disease that spread panic throughout the state earlier this year. New cases, if they appear this fall, would come smack in the middle of flu season, when it would be hard to distinguish patients who need critical care from those who should be sent home with aspirin. "We really have no idea" what to expect this autumn, says Simpson, since the disease was unknown until a few months ago.

As Simpson makes contingency plans, researchers around the country are busy taking stock of the virus itself. The fact that they have a culprit to study at all is the result of an extraordinary bit of detective work—as James Hughes, Stuart Nichol, and their colleagues at the Centers for Disease Control and Prevention (CDC) in Atlanta outline in a perspective and report in this issue (see pages 850 and 914). Just 30 days after the first reported death from a mysterious pulmonary disease in New Mexico, CDC had identified the perpetrator. Using polymerase chain reaction (PCR) to amplify viral genes from victims' tissue, Nichol's team pinned the blame on a previously unknown strain of hantavirus—a member of a family of viruses long known in Asia and Europe where they have been associated with hemorrhagic fevers and renal disease (see page 835).

Researchers at CDC and the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) in Frederick, Maryland, are now furiously trying to culture the virus in cells but, like other members of its family, it is proving hard to isolate. Once the organism is grown in vitro, researchers will be better able to study its modus operandi, develop cheap diagnostic tests, and work on therapies and vaccines. But even without a "tame" virus, CDC researchers, in an impressive demonstration of the power of modern genetic techniques, have extracted much of the organism's genome from infected tissue (see box, p. 834). Using immunological tests and these genetic data, they have developed probes and shown that the organism is carried primarily by deer mice, whose habitat includes most of North America. These probes have also enabled CDC to link 42 cases and 26 deaths to the virus. Most were in the Southwest, where an explosion in the deer mouse population early this year appears to have been a key factor (see facing page), but some have been found as far afield as North Dakota and California.



JAMES CHILDS

Animal roundup. CDC researchers dissecting animals trapped around a house where the disease struck.

Like other hantaviruses, which are named after a prototype identified near Korea's Hantaan River in the 1950s, this virus does not appear to be passed directly from one person to another. But the new strain—initially dubbed the Four Corners strain* after the region of New Mexico, Utah, Arizona, and Colorado, where the first cases occurred—differs from other known hantaviruses in three key respects: Its effects are more rapid and more lethal—apparently killing about two-thirds of those it infects compared to 5% to 20% for the Hantaan strain—and it destroys the lungs rather than the kidneys.

The big question researchers and public officials like Simpson are now grappling with is: Just how serious a threat does this virus pose? Researchers are now using PCR to scour old tissue samples for clues to how long the virus has been in the deer mouse population and whether it is genetically stable. Few virologists are yet willing to stick their necks out, but many agree with CDC epidemiologist James Childs, who says, "I think we're looking at a very well-established virus-rodent relationship in which the virus has been a parasite of these mice for a very long time." If he's right, the organism has had plenty of time to cause human disease, yet major outbreaks have not been observed.

The virus may, however, cause occasional deaths that are lost in the background noise. They could easily get lost: Depending on whether you prefer CDC or the National Institutes of Health (NIH) estimates, the number of people in the United States who die each year of unexplained respiratory disease is somewhere between 50,000 and 150,000. "I think it's a fairly rare illness, but when it occurs the results can be devastating," says Jay Butler, a CDC medical epidemiologist.

Yet the behavior of any organism such as this, notes former CDC virologist Karl Johnson, is hard to predict. Johnson is a senior figure in hantavirus research, part of the team that isolated Hantaan. He considers the Four Corners strain a classic "emerging virus" (*Science*, 6 August, p. 680.) If deer mice are chronically infected with it, he says, "I would be very surprised if we don't find it everywhere" we find deer mice. Does that mean the disease will expand? Not necessarily, Johnson says. Perhaps the virus has made only a fleeting appearance as it "boiled up from the bottom of the cauldron" of pathogens existing in the wild. It is too soon to tell whether it will sink back down or resurface in regular cycles.

Early warning

One researcher who has long been convinced that hantaviruses are endemic in the United States and that they might be causing disease is Richard Yanagihara, a virologist and longtime hantavirus hunter in the lab of D. Carleton Gajdusek at NIH. His conviction stems from Gajdusek's four decades of studying Asian strains of hantavirus—work that provided some of the basis for the rapid identification of the Four Corners strain.

* Tradition calls for naming a new virus after the place of discovery, but to avoid offending local people, CDC is now calling this one Pulmonary Syndrome Hantavirus.

The Asian form of the virus, as Gajdusek pointed out in the early 1960s, is related to other viruses in Russia and Scandinavia (the Puumala group), which are carried by voles and cause a mild kidney disease. Although there had been no reports of such disease in the United States, Gajdusek and Yanagihara thought it likely that North American rodents carried a Hantaan-like virus, and they set out to find it.

Yanagihara recalls how he, Gajdusek, and 10 of Gajdusek's adopted children from New Guinea set out small rodent traps on Gadjusek's property, called Prospect Hill, in Frederick, Maryland, in the early 1980s. They tested blood from voles, noting a positive reaction with Hantaan and Puumala virus. In 1982, they announced the first domestic hantavirus—the Prospect Hill strain. But they couldn't link it to any human disease.

Yanagihara continued looking. In 1983, he bled 203 mammalogists at their convention in Florida, and found that four had an antibody response to Prospect Hill strain. That led Yanagihara and his colleagues to warn in 1984 that some types of hemorrhagic virus "may go unnoticed in the United States" and that "persons having frequent contact with wild rodents...might constitute a high-risk group." They called for further studies on the subject.

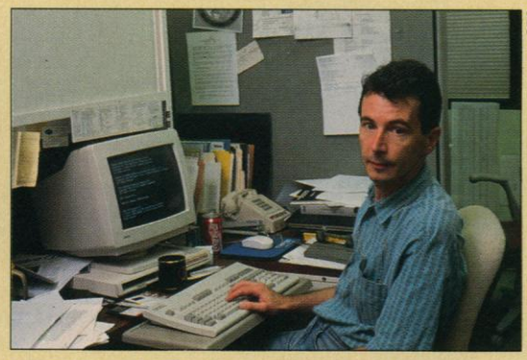
Scientists did do more studies, financed by an outfit with pockets deep enough and a mission broad enough to cover such an arcane topic—USAMRIID. Childs, then at Johns Hopkins University, was among the most active grantees. He surveyed the rats of Baltimore and found that many were infected with a variant of the Seoul virus. Rats in other cities tested positive, too, and after scouring public health clinics, Childs has found three mild cases of hemorrhagic disease.

At the cellular level, USAMRIID virologist Connie Schmaljohn made an important contribution at this time: In 1986 she was the first to sequence Hantaan's genetic code. She also sequenced part of Prospect Hill, and Mark Parrington and Yong Kang at the University of Ottawa obtained the full sequence in 1989. CDC researchers credit this work as being a key to the quick identification of the Four Corners strain as a new hantavirus.

Ironically, USAMRIID has recently been the focus of public suspicion rather than approbation for its hantavirus research, thanks to its former involvement in classified biological defense work. When the new hantavirus appeared this year in the Southwest, some people suspected it might have escaped from the Army's labs. The speculation inten-

Virology Without a Virus

"We didn't know what we had on our hands when we started getting samples in," says Stuart Nichol, a virologist at the Centers for Disease Control and Prevention (CDC) in Atlanta. "All we knew was that people were dying of something." That was on 21 May. Just a week earlier, an observant physician in New Mexico, Bruce Tempest, had alerted state officials about some unusual deaths. A young Navajo long-distance runner had died in the clinic of sudden pulmonary failure after attending the funeral of his fiancée. The fiancée had died similarly a few days earlier. No one could determine why they had died.



CDC

Disease detective. CDC virologist Stuart Nichol.

Tempest began calling around and quickly identified three more cases that looked similarly suspicious. He thought it might be plague, but tests showed that the plague bacterium wasn't the cause. Nor could New Mexico officials identify a cause. They sent blood and tissue specimens to CDC, where researchers discussed strategies for a week, then began processing samples on 31 May in the high-security containment labs. CDC virologists Thomas Ksiazek and Pierre Rollin ran a battery of tests on blood samples, looking for antibodies to known pathogens. The results were negative, except for signals for the Puumala hantavirus—which causes a relatively mild disease in Europe that affects the kidneys. CDC scientists then were at a critical juncture, says CDC medical epidemiologist Jay Butler: "We asked ourselves, Do we try to focus in on hantavirus, or is that a red herring?"

That weak signal, however, turned out to be anything but a red herring. Relying on research done by the Army, the National Institutes of Health, and by CDC itself, CDC pathologist Sherif Zaki probed the tissue samples with monoclonal antibodies that react with other hantaviruses. At the same time, Nichol synthesized pieces of shared gene sequences of two known hantaviruses—Prospect Hill and Puumala—to use as "primers" for polymerase chain reaction (PCR). His aim was to amplify traces of hantaviral genes that might be present in tissues of disease victims. Using molecular and immunological techniques, by 9 June CDC proved that a new hantavirus was killing people in the Southwest.

Nichol used the PCR process to pull out information from tissue samples about the unique genetic structure of the virus. As more of the sequence came to light, Nichol incorporated it into targeted PCR primers, enabling him eventually to get pieces of the new virus's genome.

Nichol's group inserted the new genetic material into bacteria, causing them to produce viral proteins that could be used as probes for antibodies specific to this new virus. After exposing disease tissues to staining antibodies, Zaki began looking for signs of infection. He found that lung tissues were full of antigens, especially along the capillary walls. He and other CDC researchers conclude that the virus targets endothelial cells. While Asian strains of hantavirus seem to focus on endothelial cells in the kidney, the U.S. strain goes to the lungs.

Scientifically, the identity of a virus usually remains in limbo until it has been grown in the lab and its effects replicated. But this hantavirus, like other members of the hantavirus family, has proven uncooperative, and it has not yet been isolated in cell cultures. Even in the absence of a cultured virus, however, the PCR method has firmly established the identity of this virus. One remarkable example of its specificity came to light as CDC was reviewing the case of a man who died in Snowflake, Arizona. When Nichol studied the genetic sequence of the virus that killed this man, he noticed that it was slightly different from others in Arizona and more like sequences from Colorado. Did CDC have the wrong address for the patient? A second check revealed that the victim had been in Arizona only 2 weeks, and that he lived in Colorado. The lab then tested a deer mouse caught near his home in Colorado. It was infected with hantavirus. Even more striking, the viral sequence from the mouse was identical to the sequence obtained from the man. In the age of PCR, a lot of virology can clearly be conducted without a virus.

–E.M.

sified when it became clear that the virus whose genetic structure most closely resembled that of the lethal southwestern version was the Prospect Hill strain, discovered practically in USAMRIID's back yard in Frederick, Maryland. *Scientific American* reported on the speculation this month in an article headlined: "Were Four Corners Victims Biowar Casualties?" Those who know the virus well say such speculation is wrongheaded. "Totally ridiculous," scoffs Yanagihara. "It disturbs me a great deal that people have this Andromeda strain mentality.... Even some of my scientific colleagues seem to think a monstrous mutational event" was needed to make the virus pathogenic.

Yanagihara doesn't think any monstrous mutation was needed to make the Four Corners strain of hantavirus deadly. In fact, he thinks the virus has been around for a long time in pathogenic form. His theory will be put to the test over the next few months, as researchers begin to recheck blood and tissue samples they collected and filed away long ago. For example, they will tap into decade-old deer mouse collections in New Mexico and Pennsylvania. Yanagihara himself has already reanalyzed samples of lung tissue from deer mice trapped in the early 1980s from Mammoth Lakes, California, near the spot where a young field biologist contracted fatal respiratory disease this year. In a recent letter to *Lancet*, Yanagihara reports finding hantaviral genes in these samples, and he believes that this 10-year-old virus will turn out to be essentially the same as the one that caused deaths in the Four Corners area.

Attacking the virus

Now that the characteristics of U.S. hantaviral disease are coming into focus, a key task is to find the means of combating it. The virus's attack is insidious. At first, it causes ordinary flu-like aches and pains. But within a few days it begins to wreak havoc in the lungs, causing capillaries to leak. After about a week, says Frederick Koster, a physician who saw 12 victims last summer at the University of New Mexico Hospital in Albuquerque, people enter a "crisis phase." Over a span of hours, they find it harder and harder to breathe. He recalls one 12-year-old boy who came to the clinic with what looked like ordinary flu, then went home. His father brought him back again, and within hours, the boy stopped breathing. Even if assisted by a ventilator, many die from blood loss.

It's a frightening syndrome, made worse because researchers don't know how to stop the infection once it begins. The CDC has obtained permission from the Food and Drug Administration for "open label" use of the broad anti-viral agent ribavirin (a nucleoside). It amounts to compassionate use, with no statistical results anticipated. One study of ribavirin published in 1991 found it

A Rogues' Gallery of Hantaviruses

The hantavirus nailed as the cause of 26 deaths in the United States this year belongs to a broad family of viruses responsible for widespread illness in Asia and Europe. National Institutes of Health virologist Richard Yanagihara estimates that each year diseases associated with hantaviruses strike some 200,000 people (half of them in China) and kill between 4000 and 20,000.

Western interest in hantaviruses dates to 1951, when United Nations troops in the Korean war began to come down with an illness characterized by high fever, severe headache, muscle pain, vomiting, and hemorrhaging that ends in renal failure and death in about 7% of cases; in 3 years some 3000 troops were diagnosed with Korean hemorrhagic fever with renal syndrome (HFRS). As soldiers were falling ill in Korea,

Recognized Hantaviruses

Virus	Disease	Principal Reservoir	Distribution
Hantaan	HFRS*	striped field mice	Asia
Seoul	HFRS	rats	Cities worldwide
Belgrade	HFRS	yellow-necked mice	Yugoslavia
Puumala	nephropathia epidemica	bank voles	Europe
Prospect Hill	none described	meadow voles	United States
Thottapalayam	none described	shrews	India
Thailand	none described	bandicoots	Thailand

* Hemorrhagic fever with renal syndrome. SOURCE. Ted Tsai

Western virologists began to realize that similar hemorrhagic fevers were prevalent outside Southeast Asia. They saw parallels between HFRS and illnesses such as Tula fever in Russia and a mild form of HFRS in China. In addition, in 1953 NIH virologist D. Carleton Gajdusek, then with the U.S. Army, proposed a kinship between HFRS and nephropathia epidemica, a mild kidney illness first seen in Scandinavia.

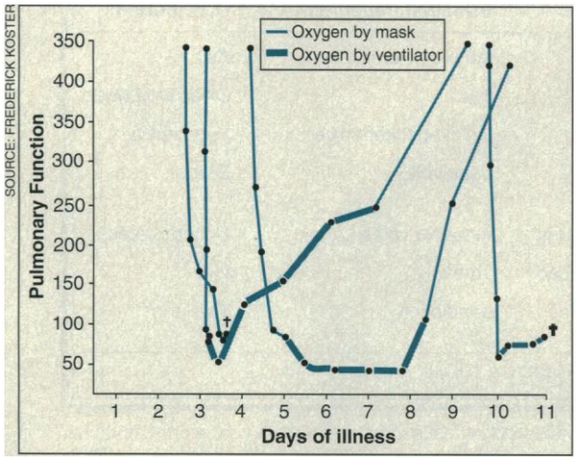
Efforts to identify the agents responsible for these diseases were stymied until 1976, when virologist Ho Wang Lee of Korea University College of Medicine in Seoul isolated a virus from striped field mice (*Apodemus agrarius*) captured near the Hantaan River in South Korea. All viruses of this genus have resisted culturing in the lab; it wasn't until 1981 that Army virologist George French, Lee, and colleagues got Hantaan into a continuous cell line. Researchers quickly flushed out other hantaviruses. In 1982, Gajdusek's team isolated a hantavirus, which they called Prospect Hill, from meadow voles (*Microtus pennsylvanicus*) in Frederick, Maryland; they couldn't link it to any human disease, however. Later that year, Lee isolated a hantavirus from rats (*Rattus norvegicus*) in Seoul that causes a mild form of HFRS. This finding led researchers to believe that rats on trading ships had dispersed hantaviruses worldwide. Indeed, the Seoul serotype appears to cause much of the HFRS in China, and in 1985 a group led by microbiologist Ted Tsai of the Centers for Disease Control and Prevention found a virus similar to Seoul—which they labeled Tchoupitoulas—in a New Orleans rat. After that, Tsai says, "virtually everywhere we looked—West Coast, East Coast, Cincinnati, New York City—we found infected rats."

Later in 1985, a research team led by virologist Joel Dalrymple at the U.S. Army Medical Research Institute of Infectious Disease (USAMRIID) isolated from bank voles (*Clethrionomys glareolus*) in Sweden the Puumala serotype (tentatively identified 5 years earlier by Finnish researchers) that causes nephropathia epidemica. Other hantaviruses, still poorly characterized, appear to have been isolated from a patient in Greece, a house mouse in Texas, house shrews (*Suncus murinus*) in India, bandicoots (*Bandicota indica*) in Thailand, and yellow-necked mice (*Apodemus flavicollis*) in Yugoslavia. "This is a moving train in terms of our knowledge base—I think we're going to find new hantaviruses as time goes on," says Ernest Takafuji, USAMRIID's chief.

–R.S.

effective in reducing deaths from Hantaan virus. But the drug must be given to patients early to prevent hantaviruses from doing catastrophic tissue damage, and the Four Corners strain may move too rapidly for ribavirin to be effective. Few experts are optimistic about ribavirin. Johnson says that prescribing it is just an "emotional" gesture. CDC's Butler notes, "So far, we don't have any evidence it changes the course of disease."

It might be possible to develop a vaccine against the Four Corners strain, once the virus has been cultured in the lab or its entire genome extracted. For the past decade, Army microbiologist Schmaljohn has been developing a vaccine to combat the Hantaan virus in Asia, and she plans to begin a large field trial in China next year. But developing a similar weapon against the Four Corners strain could take years.



Rapid killer. Plots of pulmonary function of four patients show sharp drop followed by death in cases 1 and 4 and slow recovery in the other two.

Meanwhile, two researchers at the University of New Mexico—Brian Hjelle and Steven Jenison—have been working on a quick diagnostic test based on "Western blot" technology to distinguish flu sufferers from hantavirus victims in the clinic. They began work on it in a rush in June, "as soon as we heard about hantavirus." After downloading viral sequence data by computer from federal databanks, they got help from CDC and learned to pull pieces of viral genome from patient tissue. They spliced these genes into bacteria, which expressed viral proteins that could be used to test human serum for antibodies to the virus. They claim their test can provide a reliable identification of hantavirus infection within 26 hours. But CDC staffers feel the test is still too complex to be used on a mass scale.

Attacking the vector

If researchers can't yet cure those who are infected, they aren't entirely helpless against this new viral threat. The best route of attack against the virus is preventing it from being transmitted from rodents to people. CDC researchers have concluded that it is most likely transmitted by aerosol from the rodents' urine and feces. The evidence, says CDC immunologist Pierre Rollin, is anecdotal and is derived from experience with other hantaviruses. He recalls the case of the doctor who made a short visit to a barn in France where infected rodents lived, saw no animals, but came down with a fatal infection of Puumala. C.J. Peters, chief of CDC's special pathogens branch, recalls the case of a Korean restaurateur who used a broom to beat a rat to death in his bedroom, then got sick. No one knows exactly how the U.S. victims acquired their infections, but many lived around rodents, and transmission by aerosol seems likely, says Peters. For that reason CDC is telling people to "avoid contact" with deer mice by keeping them out of homes.

Public health officials in New Mexico have already run a massive mouse roundup, and some officials have advised people to trap rodents and disinfect them with Lysol. CDC staffers aren't keen on the idea of having people handle rodents, however, since they often excrete fluids around the trap. But for now,"We don't know if it's safer" to trap or not to trap, Childs says.

Most people are hoping that 1993 will prove to have been an extraordinary year, and that the virus will not return. "But we would be remiss not to be prepared" for a resurgence, Simpson says, as he braces for the unknown. He's not alone in his concern. "We're worried that there's a good possibility we'll see more hantavirus infections in the area this fall," says Childs. One reason to worry is that the Hantaan and Puumala viruses exhibit two epidemic peaks: one in summer and a second in late fall between November and January.

One hopeful sign is that the deer mouse population in New Mexico declined in late summer, although it is still above 1992 levels. But just in case the Four Corners strain mimics Hantaan, New Mexico has set up an emergency team involving the University of New Mexico and the CDC to sort emergency cases from ordinary flu sufferers. The aim is to perform rapid triage if necessary. It's possible that none of this planning will be needed. In spite of the rapid progress made so far in identifying the culprit, developing initial diagnostic tests, and providing advice to the local populace, even CDC's experts cannot predict what course the virus will take next.

–Eliot Marshall

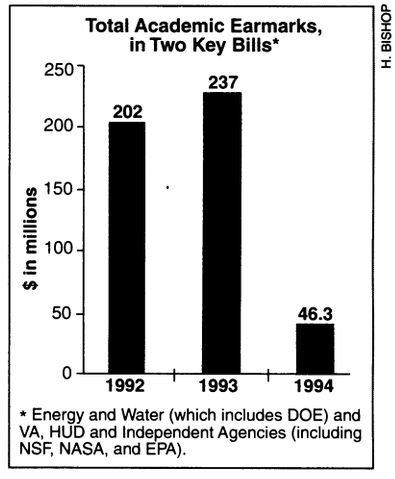
With reporting by Richard Stone.

THE 1994 BUDGET

Better for Science Than Expected

When Congress decided last month to kill the Superconducting Super Collider (SSC), some researchers took the vote as a sign that the United States no longer cared about basic research (*Science*, 29 October, p. 644). But the flurry of appropriations bills passed by Congress in the past couple of weeks tell a different story: Most research agencies have received increases, of varying sizes, for the fiscal year that began last month.

Although in some cases these increases are less than the Administration requested (see table), they are welcome news in a year in which Congress made cuts in almost every other sector. Increases for basic science can be found throughout the discretionary portion of the 1994 federal budget. The National Science Foundation (NSF) received $293 million more, to $3.027 billion. The $11 billion National Institutes of Health (NIH) got a boost of $617 million, double the increase President Clinton requested.



Pork light. A relentless campaign by Representative George Brown (D–CA) against congressional earmarking of academic facilities appears to be making some headway. The amount of money set aside for pork-barrel projects in two 1994 appropriations bills important to science—Energy and Water, and Veterans Administration, Housing and Urban Development, and Independent Agencies—dropped sharply after skyrocketing in 1992 and 1993, according to figures released last week by Brown's science committee.