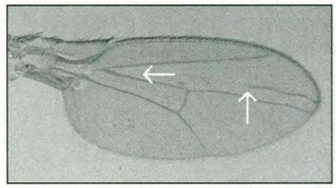
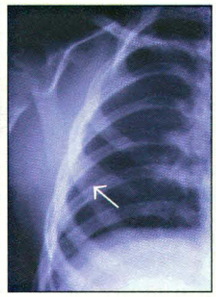
researchers know, for example, that the protein made by *hedgehog* sets in motion a train of events that ultimately turns on or revs up several genes, *patched* among them. Patched protein builds up until it interrupts transmission of the Hedgehog signal traveling from the cell membrane to the nucleus. By turning off the genes that Hedgehog turned on, the Patched protein keeps the system in check. It's "the balance between these two things that determines what goes on," Scott explains.

JOHNSON *ET AL.*, *DEVELOPMENT* **122**, 4161.





ALLEN BALE

Subtle but significant. In the fruit fly, *patched* mutations cause faulty wing veins *(arrows)*, and in humans they cause defective ribs *(arrow)*, as well as skin cancer.

When *patched* isn't functioning correctly, the genes stimulated by Hedgehog may be abnormally active, possibly prompting cells to continue to proliferate rather than differentiate. "You can wind up with uncontrolled growth," says Konrad Basler, a fruit fly developmental biologist at the University of Zurich in Switzerland.

Indeed, previous work had already suggested that malfunctioning of genes associated with the hedgehog path might lead to cancer. The *Wnt1* gene of mice—a counterpart of the fruit fly *wingless* gene, which codes for a protein that stimulates Hedgehog production in the target cell—can cause mammary tumors when it's overactive. In addition, a human *GLI* gene, which Bert Vogelstein's group at Johns Hopkins University School of Medicine discovered as an oncogene in a rare human brain tumor, is the counterpart of the *cubitus interruptus* gene of the fly. Studies of this gene, which encodes a transcription factor (a protein that turns genes on or off), indicated that it also interacts with *hedgehog* and *patched*, although its role in the path was unclear.

But now in a Research Article in this week's issue, Basler and his colleagues have helped clear up the mystery, also providing more clues about how problems in this pathway can lead to cancer (see p. 1621). When they created fruit flies in which some cells contained a mutant form of the *cubitus interruptus* gene, the Swiss group found evidence that the Ci protein serves as a key intermediary between Hedgehog and Patched. They found, for example, that Hedgehog leads to an increase in the amount of Ci protein, which in turn revs up the expression of genes normally activated by Hedgehog. Conversely, Patched causes a decrease in Ci, which may cause those genes to be turned off.

It all implies, says Philip Ingham of the Imperial Cancer Research Fund in London, that the uncontrolled cell growth of cancer results either when excessive Gli/Ci causes the genes controlled by Hedgehog to become overactive or when a disabled *patched* cannot rein in Ci activity. "Excess Ci is the same as not having Patched," says Ingham. "In fact, with enough Ci, the Hedgehog signal is transmitted even if there's no Hedgehog."

How that translates into different cancers is unclear, but as this picture gets sharper, researchers hope to pick out potential targets for different cancer therapies. Although basal cell carcinoma can be cured surgically, Katz points out that the new understanding suggests a less drastic alternative. Because the skin is so accessible for treatments, it might be possible "to interfere with the adverse effects [of mutated *patched*] chemically."

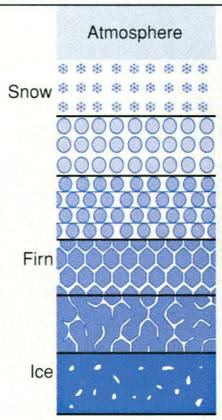
Researchers caution that even with the new information, however, they still don't know everything about how the Hedgehog pathway works. But with these new links to cancer, "all the biochemistry is going to come in floods now," Ingham predicts.

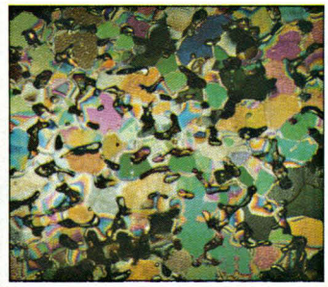
–Elizabeth Pennisi

CLIMATE CHANGE

Ice Bubbles Confirm Big Chill

SOURCE: J. SEVERINGHAUS





R. ALLEY

A deep squeeze. Bubbles trapped in glacial ice *(above*, in polarized light) as snow gradually compacted *(left)* hold climate clues.

Twelve thousand years ago and more, Greenland suffered a series of climate catastrophes. Clues buried in its ice sheet show that during the last ice age the climate repeatedly warmed sharply, only to slide into a renewed chill lasting thousands of years. Just how drastic the warmings and coolings were, and how much of the world they affected, has been a matter of debate. Now, new indicators derived not from the ice itself but from trapped bubbles of ancient gases—nitrogen and methane—show that at least the last of these catastrophes fully deserves the name. Known as the Younger Dryas, this cold snap 12,000 years ago was twice as severe as was thought, and it seems to have gripped much of the world.

"I'm completely in awe of the scale of the change and its speed," says isotope specialist James White of the University of Colorado. Glaciologist Richard Alley of Pennsylvania State University (PSU) adds that the result, presented at last month's meeting of the American Geophysical Union by Jeffrey Severinghaus of the University of Rhode Island (URI) and his colleagues, leaves "no way anyone can argue" that it was only Greenland that suffered the chill, 14 degrees Celsius colder than today: "A big piece of the world's climate was switching to a new mode." That deepens the mystery of these climate swings, but the new indicators in the ice may eventually help researchers home in on a cause.

Existing paleothermometers had been enough to show that Greenland's climate oscillated sharply during the last ice age. The evidence came from the ratio of oxygen isotopes in the deep ice, which preserves the isotopic makeup of ancient snowfalls. The relative abundance of lighter oxygen can serve as a thermometer because the colder it gets, the more water vapor containing the lighter isotope condenses to form snow. But although the oxygen isotopes clearly showed climate swings in Greenland during the last ice age, their magnitude was in doubt. The reason: The oxygen thermometer also reflects temperature far from Greenland, at the source of the water vapor.

Evidence that its readings were off came last fall, when Kurt Cuffey of the University of Washington and his colleagues used the temperatures in deep-ice bore holes to estimate the average chill of the ice age in Greenland. Like a cellar that holds last winter's chill, the deep ice preserves the ice-age cold. From the ancient chill, Cuffey and his colleagues estimated that ice-age Greenland was 20°C colder than today instead of the 10°C suggested by the oxygen isotopes.

The climate shifts that punctuated the last ice age were too brief to show up in the bore hole records, however. To measure them, Severinghaus, Edward Brook of URI, Todd Sowers of PSU, and Alley developed another isotope method that relies on the ratio of nitrogen-15 and nitrogen-14 trapped far below the surface, where snow compacts to ice. That ratio, they knew, would be altered any

time the temperature of the surface changed abruptly, creating a region of changing temperature in the compacting snow. The lighter nitrogen isotope would tend to diffuse through the snow's nooks and crannies, up the thermal gradient toward the surface, while the heavier nitrogen would tend to move downward. When the nooks and crannies finally closed off, they would trap nitrogen with a distinctive isotope ratio. And Severinghaus found a sharp peak in the ratio of the two isotopes at the end of the Younger Dryas, 11,600 years ago.

This isotopic spike alone doesn't provide a temperature, but the thickness of ice that separates it from the oxygen isotope shift that also marks the warming does. The oxygen isotope shift was recorded at the surface, in the snow that fell at the time of the warming, but the nitrogen isotope spike formed at the depth where snow was changing to ice. The gap indicates how long the conversion of snow to ice took—826 years, at the end of the Younger Dryas. And that interval depended on the temperature, among other things. After accounting for those factors, Severinghaus found that the air over Greenland was 14° ± 3°C colder than today at the end of the Younger Dryas, twice the 7°C indicated by oxygen isotopes.

Not only did the nitrogen thermometer show that this last cold snap in Greenland was twice as deep as thought, but another gas in the bubbles, methane, suggested that it must have extended far beyond Greenland. Although signs of the Younger Dryas have been turning up around the world (*Science*, 24 December 1993, p. 1972), some researchers have argued that it might have been strong only around Greenland. Methane seemed to offer an answer, because the gas comes from plant decomposition—which is sensitive to temperature and moisture variations—far from glacial ice. Researchers had already shown a sharp rise in trapped methane at about the end of the Younger Dryas, implying a widespread climate shift. Now the nitrogen isotope spike in the bubbles has allowed Severinghaus to show that the methane rise, as measured by Brook and Sowers, began within 20 years of the warming that ended the Younger Dryas in Greenland. So the warming must have affected a broad area of the middle latitudes, says Severinghaus, if not the entire hemisphere.

"Most of the warming in Greenland occurred in about 50 years. The gas rose in a century or so," says White. "That's pretty remarkable; it's a fundamental shift. We've seen nothing like that in the last several thousand years." Now he and other researchers will be applying the new thermometer—and any others they can get their hands on—to work out what threw Earth's climate into crisis.

–Richard A. Kerr

IMMUNOLOGY

Simple Mice Test Antibody Complexity

The world, from an immune system's view, is filled with enemies: a mind-boggling multitude of bacteria, viruses, and parasites just waiting to pounce. And the system has to maintain levels of a staggering range of antibodies tailored to take on these threats. Antibody-producing cells called B lymphocytes somehow mutate their antibodies to produce this diversity, but there are puzzling questions about how this process is controlled. The intricacy of the immune system makes them hard to answer, however. So researchers have been looking to make a simple system suitable for a rigorous test.

Now, on page 1649, Matthias Wabl's group at the University of California, San Francisco (UCSF), reports doing just that. They have "knocked out" various antibody-producing genes from mice and "knocked in" a preformed genetic package so that the animals are born with the ability to make only a few antibody types that recognize just one invader, or antigen. Yet despite this genetic near-uniformity, these so-called "quasi-monoclonal" mice developed, at first look, a near-normal repertoire of antibodies as they grew.

ELEMENTS OF ANTIBODIES

	Normal mouse		Quasi-monoclonal mouse
Heavy chain locus	250 $V_H \times 12\ D \times 4\ J_H$	= 10,000	1
κ locus	200 $V_\kappa \times 5\ J_\kappa$	= 1,000	0
γ locus	$V_\gamma J_\gamma$	= 3	3
Antibody repertoire	Heavy × light chains	= 30,000,000	3

Born to lose. The genetically altered "quasi-monoclonal" mouse has fewer components with which to construct antibody diversity.

Although the mice may be simple, scientists think they have a lot of potential. "This is a poorly understood facet of immunology, and this is a very clear result," says immunologist David Nemazee of the National Jewish Hospital in Denver. Although researchers are still in the dark about the apparatus controlling antibody diversity, the immunologically simple mice should help them shed some light on the matter. These mice, says Nemazee, "provide a potential model for studying what the control mechanisms are."

In previous work on other experimental animals, researchers "knocked out" or "knocked in" antibody-producing genes to restrict the diversity of the animals' immune systems, yet many developed a near-normal repertoire of antibodies. But it was hard to establish valid controls for many of these genome disruptions.

Wabl's group elected to manipulate the genome more precisely, to eliminate as many antibody-producing genes as possible while leaving the ability to produce antibodies to just one antigen. Antibody molecules are made up of elements called light and heavy chains, and the mouse genome normally contains genes for thousands of varieties of each, which combine to form millions of different types of antibodies. The UCSF researchers deleted or inactivated all the genes for the heavy chain and all but three of the light chain genes, and inserted a gene construct for just one heavy chain. The resulting combinations produced antibodies capable of binding only to a chemical called NP.

Yet by the time the mice reached adulthood, about 20% of the B cells were making different antibodies—they no longer bound NP. Overall, the UCSF team found that the amounts of different antibody types in the quasi-monoclonal animals were similar to those of genetically normal litter mates. When the scientists examined the serum for antibodies secreted by B cells, they found few antibodies that were specific for the original NP antigen, suggesting a greater diversity of antibodies had emerged in the circulation. The results implied that the animals were able to switch from just one major antibody type, which occurs early in development, to several types in adulthood—just like normal mice. "These results show that once a B cell expresses an antibody, the specificity is not etched in stone—it still has other options to produce other antibodies, but we don't know how," says immunologist Martin Weigert at Princeton University.

One test that's now possible with this new model is to examine how the interaction between antibodies and other cells in the immune system, such as the T cells that actively direct various aspects of immunological function, might do the job. Wabl hopes to tease these out by boiling the mouse system down still further, perhaps by coming up with an animal with a restricted range of T cells as well. Such simplicity, he hopes, will hold the key to some complex problems.

–Nigel Williams