Blood from Stones

Tests for prehistoric blood cast doubt on earlier results

By JOCELYN KAISER

Conservation Analytical Laboratory, Smithsonian Inst.

Tuross and Dillehay detected hemoglobin on an edge of this black basalt stone tool unearthed in southern Chile. They think an overlying layer of peat and silica gel in the surrounding soil helped preserve the protein for at least 13,000 years.

Forensic experts aren't the only scientists who mine bloodstains for clues. For more than a decade, archaeologists have been borrowing crime-lab techniques to hunt for ancient blood on scraps of stone.

Using antibodies to detect blood and the species it came from, some researchers have seemingly obtained astonishing results. Margaret E. Newman of the University of Calgary in Alberta and her colleagues reported finding buffalo blood on stone knives at a 5,600-year-old butchering spot in Canada. Thomas H. Loy's team picked up human blood in paint dating to 20,000 years ago on a cave wall in Australia. And at an Iraqi site, Loy says, he detected 180,000-year-old blood spilled by a man whittling wood.

It seems that dirt stuck in the grooves of a stone scraper or a dark spot on a rock slab can reveal such secrets as what creatures early peoples sacrificed and when they turned from hunting to farming.

But just as discoveries of ancient DNA have met with skepticism, researchers' zeal for archaeological blood tests, known as residue analysis, has begun to fizzle. In a recent spate of papers, scientists question not only one another's findings, but whether it's even possible for traces of buried blood to survive thousands of years.

"People are getting very capricious and puzzling and different results," says Christopher Chippindale, editor of ANTIQUITY, a journal on whose pages the debate is unfolding. "There's something in the biochemistry that is giving false positives. That really puts quite a question mark on the various studies."

Loy, now at the University of Queensland in Australia, leads the field in archaeological blood claims, having reported ancient blood on more than 1,000 tools since 1983. Initially, he identified prehistoric hemoglobin, a protein in blood, by crystallizing it. That test has come under heavy criticism, but Loy stands by his results.

When he and others began using immunological tests, they seemed to move to firmer ground. These tests, which detect blood proteins, date back more than 40 years. (Archaeological DNA tests, used since the 1980s, decode genetic material.) To devise a test for, say, deer blood, scientists inject fresh deer blood into a rabbit, which makes millions of antibodies to the blood. The antibodies in rabbit serum, called antiserum, can then be used to search for deer blood.

To test a stone tool for traces of such blood, a researcher would generally wash the tool, then pour the washing extract onto a solid to which the blood proteins stick—a plastic membrane, for example. At that point, he or she rinses the solid with deer antiserum, then with a second antibody that sticks to the antiserum. Because this second antibody is tagged with a fluorescent molecule or some other marker, it flags any deer blood in the sample.

In practice, the assays are more complicated. Because closely related species have similar blood proteins, the antiserum for, say, elk can react with blood from a deer or cow. So it's necessary to test each antiserum against many other species' blood for cross-reactions and to be aware of these reactions when testing a piece of stone.

The test itself varies from one laboratory to the next. Some people buy commercial antisera, while others make their own. Some testing methods are a thousand times more sensitive than others. An antiserum can be made to react with a single protein, such as albumin or hemoglobin, or even with one region of a protein instead of the many proteins in whole blood.

A chemist for 27 years, Judith A. Eisele had these things in mind 4 years ago when she began looking at blood residues on tools for an anthropology master's project at the University of Nevada at Reno. Working with biochemist Roger A. Lewis, she used a dozen antisera, from turkey to bear, to test for blood on more than 150 flaked stone tools from the Southwest.

When only seven tools tested positive for blood and these results proved ambiguous, she tried another experiment. She coated clean stone tools with deer blood and buried them for several months. The results, published in the March ANTIQUITY: Tools buried in dry dirt tested positive for blood for only 10 months. As for tools stored in damp dirt, the blood couldn't be detected after just a single month.

Eisele's adviser, archaeologist Donald D. Fowler, sent her master's thesis to researchers across the country a year ago. "The dovecotes were definitely fluttering," says Jerold M. Lowenstein, an immunologist at the University of California, San Francisco. Other reports added to these doubts. Researchers from the United Kingdom buried stone tools daubed with blood; only one tested positive for blood a year later. When scientists in Texas and New Mexico recently sent 54 tools dipped in *fresh* animal bloods to a commercial laboratory, it incorrectly identified half the samples.

Lowenstein and retired Boston physician Elinor F. Downs also reported confusing results in the January/February JOURNAL OF ARCHAEOLOGICAL SCIENCE. They split up washings from a set of stone tools, sending one-third to another university laboratory and keeping one-third each for themselves. Downs used crossover immunoelectrophoresis, Lowenstein radioimmunoassay, and the third group a dipstick clinicians use to detect hemoglobin in urine.

While the three groups agreed about tools that held fresh blood or nothing, their results for ancient blood didn't match. On a particular tool, for instance, one team found human blood, another bear blood, and the third nothing at all.

So is it possible to get ancient blood from a stone? The answer depends on whom you ask. Howard Ceri of Newman's group at the University of Cal-

gary argues that the immunological techniques are valid, even on aged blood. "Look at the wealth of forensic evidence that's out there," he says.

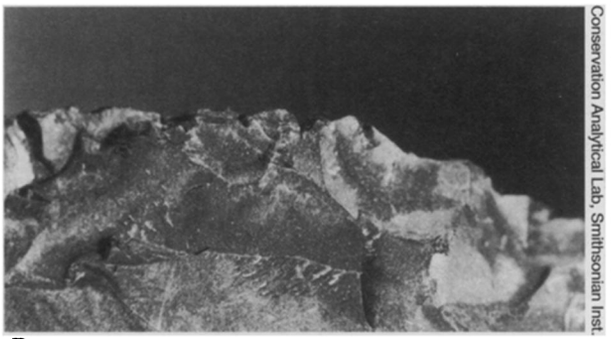
Loy says others have gotten negative results because their tests aren't sensitive enough to detect minuscule amounts of blood and because they don't begin by screening for blood visually.

"They're either archaeologists using techniques that they really don't understand in terms of chemistry or immunology," he says, or they are immunologists who have never "actually looked at a tool." Loy tests for a single region of immunoglobin, and he is among the few who claim to have seen red blood cells on artifacts through a microscope.

At the other extreme is Eisele, who wonders whether blood can endure in prehistoric bone, much less on stone tools, except under freezing conditions. Those who think they've found blood on artifacts, she says, more likely have picked up proteins from microbes or plants.

Lowenstein remains confident that he has detected blood on some tools. So does biochemist Noreen Tuross of the Smithsonian Institution in Washington, D.C. In the spring JOURNAL OF FIELD ARCHAEOLOGY, she and Tom D. Dillehay of the University of Kentucky, Lexington, reported a strong indication of hemoglobin, possibly from a mastodon, on a tool from a site in Chile dated to 11,000 B.C.

But Tuross speaks for many when she says, "Immunoreactivity to ancient, degraded molecules is an area we don't fully understand. To take modern techniques and to apply them to ancient results is inappropriate." Antibodies designed to find fresh, folded proteins could yield misleading results when used on old proteins that have lost their shape and broken into fragments or formed denser shapes, Tuross warns.



Conservation Analytical Lab, Smithsonian Inst.

Detail of black basalt stone tool on p.376.

Adding to the confusion, there's no consistency across groups on how they test, how they deal with cross-reactions, or even how they wash possible blood from tools. And unlike chemists, researchers who publish in archaeological journals aren't accustomed to describing their procedures, Eisele says.

Blind tests may help iron out these problems. One has just been set up by University of Colorado Health Sciences Center in Denver researchers and a Golden, Colo., company, Paleo Research Laboratories. The group sent stone tools covered with modern blood to a half dozen research teams, which will analyze them using their usual methods and send back the results.

Lowenstein, a participant, says the study is a good first step. "I think we're just getting into the scientific phase of this work, and I think it's badly needed." But the real check will be blind tests for ancient blood, he says.

As complicated as ancient blood analysis is proving, it's a goal worth pursuing, Tuross adds. "The excavation record of early man is so overwhelmingly dominated by stone tools," she says. "Archaeologists really want this work done. What captivates them is that this is material they would normally [wash] away."

Such debris may yet yield intriguing surprises—the world just may have to wait a few years to be sure. □