Blood Trails, DNA, and O. J.

Since 1985, with Alec Jeffrey's discovery of the uniqueness of portions of the DNA structure of certain genes, investigations involving blood have taken an entirely new turn. While the ultimate goal of the analysis of proteins and enzymes was to individualize blood, that's pretty much established with DNA technology. Within a year of the discovery, DNA typing was being put to the test in criminal cases. It not only cleared one man who had confessed to a crime, but also led to the conviction of the actual killer in the same crime.

DNA can narrow down suspects in a hurry, but it's not foolproof. It can be challenged in court on the basis of sloppy evidence collection and the corruption of samples during testing. That was the tactic that O. J. Simpson's defense team used to win for him an acquittal in his double murder trial. Just how did they manage to accomplish this? To trace their strategy, let's look at the case.

On the night of July 12, 1994, Nicole Brown Simpson and Ronald Goldman were slaughtered outside her Brentwood, California home. Nicole was the former wife of football celebrity O. J. Simpson, and he was called in from out of town for questioning. Going to his home on the night of the murder, detectives had noted a bloodstain on the door of his white Ford Bronco and a trail of blood leading up to the house. That was suspicious enough to start asking questions.

When Simpson returned to Los Angeles, investigators noticed a cut on a finger of his left hand. He told several conflicting stories about how he had gotten it, which boxed him in later when blood at the crime scene indicated that the killer had been cut on his left hand and had trailed blood outside the gates. That hardly seemed coincidental.

Then when several droplets of blood at the scene failed to show a match with either of the victim's blood types, Simpson's blood was drawn for testing (after the droplets had already been collected). Comparison between his DNA and that of the blood at the scene showed strong similarities. The tests indicated that the drops had three factors in common with Simpson's blood and only one person in 57 billion could produce an equivalent match. In addition, the blood was found near footprints made by a rare and expensive type of shoe-shoes that O. J. wore and that proved to be his size.

Next to the bodies was a bloodstained black leather glove that bore traces of fiber from Goldman's jeans. The glove's mate, stained with blood that matched Simpson's, was found on his property. There were also traces of the blood of both victims lifted from inside Simpson's car and house, along with blood that contained his DNA. In fact, his blood and Goldman's were found together on the car's console.

Forensic serologists at the California Department of Justice, along with a private contractor, did the DNA testing. Then other evidence emerged, such as the testimony of the limousine driver who came to pick Simpson up for the ride to the airport: On the night of the murder, while he waited for Simpson, he had seen a black man cross the driveway and go into the house. Then Simpson claimed that the driver had been unable to get him on the intercom because he had "overslept." So then who was the black man who had entered the house?

When arraigned, Simpson pleaded Not Guilty and hired a defense team of celebrity lawyers. Barry Scheck and Peter Neufeld from New York were the DNA experts, renowned for their work on the Innocence Project, which used DNA analysis to defend the falsely accused. Scheck felt confident that they could produce challenges before the jury that would both educate and persuade them.

The reliability of this evidence came to be called the "DNA Wars," and three different crime labs performed the analysis. All three determined that the DNA in the drops of blood at the scene matched Simpson's. It was a 1 in 170 million match, using one type of analysis known as RFLP, and 1 in 240 million match using the PCR test.

Nevertheless, criminologist Dr. Henry Lee testified that there appeared to be something wrong with the way the blood was packaged, leading the defense to propose that the multiple samples had been switched. They also claimed that the blood had been severely degraded by being stored in a lab truck, but the prosecution's DNA expert, Harlan Levy, said that the degradation would not have been sufficient to prevent accurate DNA analysis. He also pointed out that control samples were used that would have shown any such contamination, but Scheck suggested that the control samples had been mishandled by the lab-all five of them---and the jury bought it.

The evidence was damning, but the defense team managed to refocus the jury's attention on the corruption in the Los Angeles Police Department. They then disputed the good reputation of the forensics labs, insisting that the evidence had been carelessly handled. Deliberating less than four hours, the jury freed Simpson with a Not Guilty verdict. They simply failed to understand how damning the DNA evidence really was and how ill fitting was the defense's logic about certain aspects of the blood at the crime scene.

Nevertheless, it can certainly be the case that what appears to be overwhelming blood analysis evidence still fails to tell the whole story. We can see that in the next case, a family tragedy that happened in Australia.

Analysis Gone Wrong

It should be kept in mind that analysis always involves interpretation. In the case below, an interpretation of the bloodstain evidence helped to convict a woman and stood up to two appeals, but turned out to have been in error.

In Australia in 1980, Lindy and Michael Chamberlain took their three children camping near Ayers Rock. The youngest was nine-week-old Azaria.

One evening, according to Lindy and Michael, they were preparing dinner at the camp barbecue site when they heard a sudden sharp cry from the tent in which Azaria was sleeping. Lindy went to check and saw a dingo, or wild dog, backing out, shaking something large in its jaws. It ran away and that's when Lindy discovered that Azaria was gone. The dog had taken her!

Trackers searched the area to no avail. There was no sign of the missing baby or the dingo, except for footprints leading to the road and beyond. The parents grieved deeply, but eventually accepted their fate as the will of God. They assumed she was dead.

Eight days later a hiker discovered baby Azaria's clothing in a crumpled heap west of Ayer's Rock. Only the baby's jacket was missing, but oddly, her undershirt was inside out and the booties were neatly laced up inside the jumpsuit. On the neck of the jumpsuit and undershirt were bloodstains that were later thought to be consistent with the type of stain that would result from a knife cut, not a bite. There were also no tooth marks on the clothing.

Around the scene, investigators found no sign of human remains, no dog hair, and no indication that violence had occurred between an animal and a baby. No dingo saliva was found on the clothing. Investigators did some experiments with caged dingoes and concluded that whatever had happened to the child had not involved a wild dog. That left human involvement---someone who left the child's clothing several miles from where she was taken. Suspicion turned to the parents, and then more specifically to Lindy.

Lest there was doubt about whether the clothing belonged to the child, blood tests were done to determine type (no DNA testing was available then), and then compared to the Chamberlain's blood types. The conclusion was that the clothing had belonged to Azaria. Another test showed that the undershirt had been worn the right way when the wound was made, but then someone had removed it, leaving it inside out. There also appeared to be two bloodstained prints on the jumpsuit made by the hands of a small adult, like a woman.

A search of the Chamberlain's car produced what appeared to be the blood of an infant on the seats and on a pair of scissors in the vehicle. After that, the Chamberlains were arrested and tried for the murder of their baby daughter. They insisted they were innocent, but the evidence appeared to say otherwise. Lindy was convicted of murder and Michael was declared an accessory to the crime. Lindy went to prison.

On her behalf, many people began movements to bring out the errors made in the interpretation of evidence---particularly the experiments done with dingoes and the blood analysis. The substance found in the car, for example, was not conclusively proven by any tests to be blood. Nor was the stain on the T-shirt proven to be from a cut rather than from an arterial bleed. If the dingo had grabbed the baby by the head or neck, there would be no teeth marks on the clothing.

Then in 1986, four years after the trial, Azaria's missing jacket was finally located-partly buried in sand

near a dingo cave not far from the campsite. It was torn and bloodstained, but in good enough condition to be identified as the one Azaria wore the last time she was seen. It was sufficient for reasonable doubt and Lindy was released. The following year, the couple was officially pardoned. Not long afterward, their convictions were quashed.

No matter how sophisticated the tests, interpretation is often subject to the narrative that the investigators build, especially if the evidence is ambiguous. It can only be hoped that future technology will eliminate the gray areas and provide more conclusive proof.
http://www.crimelibrary.com/criminal_mind/forensics/serology/

The "Fatal Vision" Murders

February 17, 1970. It was one of the worst crime scenes that the Fort Bragg military officers had ever seen. Army doctor Captain Jeffrey MacDonald lay still but conscious on the floor of his master bedroom, while his murdered family was sprawled all over the house. He weakly asked how his kids were and said that he'd heard them crying.

Colette, MacDonald's twenty-six-year-old pregnant wife, had been stabbed numerous times in the chest, and she lay bleeding underneath a torn blue pajama top that apparently had been worn by him. Above them both, written in blood on the headboard of the bed, was the word Pig, which was reminiscent of the vicious Manson murders in California only the year before. Colette's head showed evidence of severe blows with a blunt instrument, and both of her arms were broken.

Down the hall in one bedroom was two-year-old Kristen. She had been stabbed thirty-three times in the chest and back, and her sister, five-year-old Kimberly, had been repeatedly stabbed and hit. Both were dead, and next to Kristen was a pool of blood near which a bloody footprint was visible.

MacDonald's wounds were relatively minor (though he later claimed he'd been stabbed twenty-three times) and as police searched the house he told them what he could remember of the blitz attack. According to him, he'd been sleeping on the living room couch when Colette's cries woke him. Three men and a woman were standing in the living room, dressed like hippies and chanting, "Acid is groovy.kill the pigs." MacDonald said that he then tried to fight them, but they slashed him with an ice pick. In the process, he'd torn his pajama top and had then wrapped it around his hand to buffer the blows. Eventually the intruders knocked him unconscious with a baseball bat. When he came to, he found his wife and daughters bleeding from wounds and unresponsive to his attempts to revive them. He then made the emergency call that brought the MPs to his door.

Detectives William Ivory and Franz Grebner made a thorough investigation of the crime scene and determined that MacDonald's story just didn't add up. The relatively minor disorder in the living room failed to support the description of a struggle between MacDonald and four other people. Even more suspicious to them was the fact that one of the magazines on the table had extensive coverage of the Manson murders, which bore obvious parallels to this incident. The whole thing was beginning to look staged. To the detectives' minds, it raised questions about why this gang of hippies on acid who'd stabbed MacDonald's family in such frenzy had allowed him to survive relatively unscathed, with some slight stab wounds and a few bruises.

It was also troubling that MacDonald, who had terrible eyesight without his glasses, had been able to give such detailed descriptions of the perpetrators in his home. And why had he dialed the phone in the dark? When the MPs arrived, the house was dark.

Then more evidence began to put MacDonald into a more suspicious light. On the bed where Colette had been attacked was the torn finger from a latex glove such as surgeons wears, and a knife that MacDonald claimed to have pulled out of his wife was clean of fingerprints. It also proved not to be the knife that had stabbed her. Also free of prints were both of the phones that MacDonald said he'd used to call for help. Even more troubling was the fact that several blue threads from the pajama top were found beneath Colette, although MacDonald had claimed to have simply laid the garment on top of her. Quite a few fibers were discovered below the headboard of the bed where "PIG" had been written by a right-handed person wearing something that resembled a glove. More fibers were found on a bloodstained piece of wood found in the back yard. Out there, they also found an ice pick and another

knife, both wiped clean. Then more blue threads turned up in the children's bedrooms, including one under Kristen's fingernail, but none were found in the room where MacDonald claimed he was sleeping, and where the jacket had supposedly been torn.

The most damning evidence was the blood pattern analysis. Each family member had a different blood group, which made it possible to track where they had been. MacDonald's blood showed up in the kitchen next to a cabinet that contained surgical gloves. It was also in the bathroom, but there was none in the living room where he'd claimed to be stabbed, except for a tiny smear on a magazine, nor in the hall where he's said that he'd lain unconscious. A footprint in Kristen's bedroom was made with Colette's blood. Blood spatters on the ceiling indicated that a club had been raised back with force after hitting Colette.

Added to this was the lack of evidence that four intruders had not only entered the house but had struggled with all of the victims. This scenario is close to impossible.

Nevertheless, when it was found that the evidence had been mishandled and some of it lost, the charges were dropped. Then the investigation came alive again.

One of the initial FBI analysts, Paul Stombaugh, had determined that Colette had been covered with a sheet and moved by someone wearing the blue pajama top. He also examined MacDonald's pajama top and observed that all forty-eight holes made by the ice pick had smooth edges and a round shape. He believed that this could only have occurred if the garment had been stationary. It would not have happened if the scenario were as MacDonald described it-that he'd wrapped his jacket around his hand to ward off blows. (A demonstration showed that an ice pick stabbed at a moving target would make jagged edges.)

Then Stemberg noted two bloodstains from Colette on two parts of the torn jacket, and when they were held together, they matched, which meant the stain had been made before the jacket was torn. So MacDonald had not placed it on her after he found her lying dead on the floor, but had gotten her blood on himself before the shirt was torn.

Stemberg then folded the jacket to see how the stab marks matched and was able to show that the forty-eight punctures could have been made by twenty-one thrusts of the pick, which matched the number of stab wounds found on Colette's body.

This startling evidence helped the prosecution to win one first-degree and two second-degree murder convictions against Jeffrey MacDonald, and he is currently serving three consecutive life terms.

However, that was not the end of it. The evidence has been questioned backwards and forwards by those who believe that the MacDonald investigation was shoddy, and problems have emerged.

The defense had pointed out that Paul Stombaugh had little experience with blood analysis, but the jury had not heard this challenge to his credibility. Also, the defense had not been allowed to examine the bloody pajama top to make their own analysis of the bloodstains. On visual inspection, they did not buy Stombaugh's ideas, and years later they discovered that another lab technician had come to a different conclusion about the torn shirt: She thought the shirt had been stained after it was torn. (Nevertheless, all of this was vigorously debated at trial.)

It was also proven that although Stombaugh claimed that he'd folded the pajama top in a manner that matched how it was found at the crime scene, in fact the photos of the scene did not match the photos of his own experiments. He'd had to change the position of the shirt to get the holes to line up as he thought they should. It was also pointed out that the garment would not stay so neatly lined up as the

pick was being used to make one stab wound after another. The experiment also failed to account for the punctures in Colette's own pajama top, which was between her wounded chest and the jacket that her husband had laid on her.

Even so, another prominent blood pattern analyst, Judith Bunker, had declined to serve on the defense, because she felt that her conclusions would not support their position.

To understand how bloodstains at a crime scene can be open to debate, it's instructive to see where forensic blood analysis got its start. http://www.crimelibrary.com/criminal_mind/forensics/serology/

Putting it All Together

Several different blood analysis techniques came together in the Caren Campano case to provide enough evidence for an arrest. She was missing and there seemed to be nothing amiss in the home at first.

Her husband, Chris, admitted that they'd had a fight just before she had disappeared on July 1, 1992, from their Oklahoma City home. He offered to let investigators look around, which was his first mistake. A huge brownish patch on the bedroom carpet alerted them to the possibility that it was blood. They used several techniques to find out more:

1. A hema stick (microcrystal test) This stick is coated with a blood-sensitive chemical which, when touched to a substance and then sprayed with distilled water, indicates the presence of blood. Later in the lab, they determined that it was human blood.

2. Luminol Although the house appeared to be spotless, when this highly sensitive chemical was sprayed around the room in the dark, it illuminated so many areas that it was clear that a virtual bloodbath had taken place.

When blood flies through the air, the pattern in which it lands can determine its track, as well as the location and position of the weapon that inflicted the blow. It can also provide an estimate of how many blows were struck. Investigators found spatters on the walls, doors, and even across the ceiling. There was also a blood trail through the house and down the back outside steps. Piecing together from the splatter patterns what might have occurred, they felt certain that the victim had received numerous blows to the head with a blunt object, which collectively would have been fatal.

3. Blood Volume Test through stain recreation On the same rug, they poured the amount of blood that would have been needed to make a stain the same size as the one they found, and then estimated that a person the size of Caren Campano would have lost at least 40% of her blood. She could not have survived that.

4. DNA analysis - Reverse paternity test Although Caren's father was deceased and they had no samples of her DNA, they took blood samples from as many members of her family as they could find, hoping for a partial match with all of them. Finding it, the police had enough for an arrest on the suspicion of murder.

Finally, a year after her disappearance, they located Caren's remains, which by this time were mostly skeletal. The dental records matched and the story told by the many fractures to the skull confirmed the theoretical scenario pieced together from the blood spatters in the Campano bedroom. Chris Campano was then convicted of the murder of his wife.

The various tests used on blood and blood patterns can offer crucial evidence in the reconstruction of a crime scene and even the proof of murder without having the body.
http://www.crimelibrary.com/criminal_mind/forensics/serology

The Mad Carpenter

It was the brutal murder and dismembering of two young boys on the island of Rugen, off the coast of Germany, that turned the authorities' attention toward Ludwig Tessnow, a carpenter from Baabe. The year was 1901, and the two boys had run out to play. When they failed to return, a search was organized. It wasn't long before their body parts were found scattered over a wide area, and eventually the searchers located their disemboweled remains.

Earlier that day, Tessnow had been seen talking to them, and although he denied any involvement, a search of his home turned up recently laundered clothing that had suspicious stains. He claimed that they were from wood dye, which he used almost daily in his profession. Unable to prove otherwise or to find other incriminating evidence, the police left him alone until one investigator recalled a similar crime.

Three years earlier in Osnabruck, Germany, two young girls had been found in the woods, butchered in a style similar to the boys. The man seen loitering near the woods, his clothing stained, was Tessnow. At that time, too, he had claimed that the stains were from wood dye.

The local prosecutor then heard a farmer's report that a man who looked like Tessnow was seen fleeing from his field, and he then found seven of his sheep slaughtered. Their legs had been severed and tossed about the field. Tessnow was brought in for a line-up and the farmer had no trouble picking him out as the man who had run from his field.

Still, the police needed better evidence to tie Tessnow to the murders. Then they heard about a test recently developed by a biologist, Paul Uhlenhuth, that could distinguish blood from other substances, as well as mark the difference between human and animal blood. Tessnow's clothing was given to Uhlenhuth for thorough examination and his conclusions marked a turning point in the history of forensic science. He found dye, but he also detected traces of both sheep and human blood.

With this evidence, Tessnow was charged, tried, convicted, and executed.

Shortly thereafter, forensic blood analysis began to progress rather rapidly in several directions. http://www.crimelibrary.com/criminal_mind/forensics/serology/

Blood Analysis: A sample of suspected human blood is placed on a glass slide with a similar biological reagent. An electrical current passes through the glass and the protein molecules migrate toward each other. If a precipitate line appears indicating a reaction between antigen and antibodies, the sample is human blood.

The Poisoned Needle

How techniques for separating mixtures helped solve a deadly mystery

One morning in the summer of 1961, hundreds of crazed birds attacked the seaside town of Capitola, California. The birds "cried like babies" as they dove into streetlamps, crashed through glass windows, and attacked people on the ground. Most of the birds were sooty shearwaters, a normally non-aggressive species that feeds on small fish and comes ashore only to breed.

The incident fascinated Alfred Hitchcock, who frequently vacationed in nearby Santa Cruz. He included newspaper clippings about the Capitola attack in his studio proposal for The Birds, which appeared in cinemas two years later.

In the winter of 1987, the agent that is now believed to be responsible for the Capitola incident struck on the opposite shore of the continent. This time, it struck higher on the food chain. Over a hundred people became extremely ill within hours after dining on cultured blue mussels in restaurants around Prince Edward Island in Canada. It quickly became apparent that this was no ordinary outbreak of food poisoning. Vomiting, cramps, diarrhea, and incapacitating headaches were followed by confusion, loss of memory, disorientation, and (in severe cases) seizures and coma. A few exhibited emotional volatility, with uncontrolled crying or aggressiveness. Three elderly victims died. [Perl].

A tragic symptom of poisoning was the destruction of short term memory in about one quarter of the survivors. They could remember nothing that happened after the poisoning. Some were unable to recognize their surroundings or relatives. They could learn no new facts or skills. The most severely affected lost memories several years old. For twelve of the victims, the loss of short term memory was permanent.

The mysterious syndrome was called "amnesic shellfish poisoning". This sort of neurological damage due to food poisoning had never been encountered before. To prevent further injury and loss of life it was imperative that the toxic agent be isolated and identified as quickly as possible. A team of marine biologists and chemists was assembled by Canada's Department of Fisheries and Oceans (DFO) to work on the problem.

But quick resolution of the mystery was unlikely. An initial screening of the sample for known bacterial and viral pathogens revealed nothing. Tests for heavy metals, pesticides, and PCBs also were negative. The mussel samples were extremely complex, containing thousands of different chemical compounds. How can one component be isolated from a such a complex mixture, without knowing anything about its physical or chemical properties? How do you find a needle in a haystack, when you've never seen a needle before?

Suppose a test could be devised for the presence of the needle in a haystack. The haystack could be divided in half, and the half that tested negative for the needle could be discarded. Repeating this divide-and-discard process over and over again should eventually result in a pile with only one thing left: the needle.

That was the strategy the researchers used to isolate the toxin. A reliable but gruesome biological test was developed. Injection of a small amount of the sample into mice produced a very distinctive neurological reaction if the toxin was present: the mice involuntarily scratched their shoulders with their hind legs. [Teitelbaum]

Standard physical methods for separating complex mixtures were applied to the poisoned mussel

samples. At the same time, uncontaminated mussels were subjected to the same separations, to allow the analysts to compare fractions. Any differences in spectra or chromatograms between the control and toxic samples might be valuable clues in the search for the toxic agent. Mice were exposed to each fraction of the separation. Fractions found to be toxic were retained for further analysis. The others were discarded. If chromatograms and spectra indicated that the toxic fraction was still a complex mixture, another separation technique was applied.

Separation by solubility and volatility Most drugs and poisons are either fat soluble or water soluble, so a logical first step in the isolation was solvent extraction. To prevent potential decomposition of the compound by heat or harsh solvents, ground mussel samples were extracted at room temperature with aqueous methanol, a mild solvent. The extraction was inefficient but successful: mice had the same neurological reaction to the methanol extract that they had to the original mussel samples.

The extract was concentrated by evaporation. The vapor was not toxic, but the residue after evaporation was. The poison apparently was nonvolatile, which could indicate a high molecular weight compound, or a compound that ionized in solution.

A second extraction was performed by shaking the concentrated extract with a mixture of a nonpolar solvent (dichloromethane and water, which is polar. The two solvents don't mix; they settle into two easy separable layers.

The dichloromethane fractions for the toxic mussels contained several colored substances absent in the control mussels. The visible light absorption spectrum revealed a pattern of absorptions that are characteristic of phytoplankton pigments. An initial examination of the toxic mussels revealed that they were engorged with green plankton, while the nontoxic mussels weren't. This was an important clue in the search for the origins of the toxin.

But the pigments themselves were not poisonous. The dichloromethane fraction gave a negative result in the mouse bioassay. The aqueous layer contained the toxin, indicating that it was probably a polar, ionizable substance. This was a lucky break, because the researchers could discard the complex dichloromethane fraction and concentrate on the much simpler aqueous fraction.

Separation by polarity Column chromatography was used to separate the aqueous layer into simpler components. The sample was passed through a narrow tube packed with beads of a resin called XAD-2, which grabs the nonpolar parts of passing molecules, but lets ions pass freely.

XAD-2 chromatography is particularly effective for separating organic acids and bases. Flushing the resin with a strong base ionizes acids in the sample. The ionized acids will pass through the column before other organic compounds because the resin won't retain them in their polar ionized form. Flushing with a strong acid gives organic bases in the sample extra hydrogen ions (and a positive charge); any organic bases adsorbed onto the resin will be washed out of the column.

Of the many fractions that passed out of the XAD-2 column, only one was toxic. For the final stage of the purification, the toxic fraction was separated with high performance liquid chromatography (HPLC). Again, a polar solution containing the sample was passed through a column packed with a nonpolar stationary phase. A single, highly purified fraction collected from the HPLC column accounted for all of the toxicity present in the original mussel sample. The toxin was isolated.

Separation by charge, size, and molecular shape The researchers had to ensure that the final HPLC fraction was indeed the isolated toxic component. They separated the aqueous XAD-2 fraction again, using a completely different technique: high voltage paper electrophoresis.

Electrophoresis is a technique for separating ions based on their charge-to-mass ratios. Ions placed between a positive and a negative electrode will move towards the electrode with the opposite charge. Generally, the higher the ion's charge-to-mass ratio, the faster it moves towards the electrode. The smallest and most highly charged ions move ahead of larger ions with lower charges. Molecular shape also affects the rate of migration; shapes with more concentrated charges tend to migrate faster, all other things being equal.

The sample is applied to a piece of blotting paper. The ends of the paper were dipped in pH buffer solutions; an electrode was placed in each buffer solution. Ions for separate substances migrate at different rates and were resolved as separate bands across the paper. A developing agent (ninhydrin) was sprayed on the paper to stain the bands to make them easier to see.

A band very close to the band for glutamic acid was observed in the electrophoresis of the toxic XAD-2 fraction, but not in the control fraction. It stained a distinctly different color from the glutamic acid. When the material in the band was collected and injected onto the HPLC column, it took exactly the same amount of time to move through the column as the toxic component found by the HPLC analysis. It also produced exactly the same amount of toxicity as the HPLC fraction had.

Identification of the toxin Mass spectrometry was used to determine the compound's molecular weight (312 g/mol) and molecular formula ($C_{15}H_{22}NO_6$). Spectroscopic analysis revealed the presence of conjugated double bonds and features characteristic of an amino acid. By matching the spectra with those from STN International's Registry system, the compound was unambiguously identified as domoic acid, an triprotic amino acid.

Some scientists insisted that domoic acid could not be responsible for the poisonings, because it had been used as a folk remedy for intestinal worms in Japan for many years. There were no previous reports of toxicity from seaweed or seaweed extracts known to contain domoic acid in the medical literature. However, the seaweed extracts used in the remedies contained a total dose of no more than 20 mg of domoic acid, while some of the victims of amnesic shellfish poisoning consumed some 290 mg [Perl]. Many substances that are harmless or even beneficial at low dosages can have toxic effects at higher levels.

Domoic acid is a molecular Trojan Horse. Nerve cells mistakenly recognize domoic acid as glutamic acid- a fatal error. Glutamate (the ionized form of glutamic acid) is a neurotransmitter, a molecule used to send a message from one nerve cell to another. When the glutamate molecule binds to a glutamate receptor embedded on the membrane of the receiving nerve cell, the receptor opens channels in the membrane that allow calcium ions to flow into the cell. The influx of charge causes a voltage to build up across the cell membrane, and the nerve cell fires, passing the signal on to the next nerve cell. Frequent stimulation can cause new connections to grow between the neurons, so glutamate plays a fundamental role in thought, learning and memory.

It is possible to have too much of a good thing, however. Glutamate at high concentrations acts as an excitotoxin -a compound that kills cells by literally exciting them to death. Excess glutamate keeps the gates that allow calcium ions across the cell membrane open too long. Calcium ions flood into the cell, causing it to fire uncontrollably. The neuron swells and eventually bursts. The damage cascades to nearby neurons because the damaged and ruptured neurons release their glutamate and other excitatory amino acids, overstimulating nearby cells. The excess calcium inside the cell stimulates certain protein-cutting enzymes, which produce large quantities of free radicals as a by-product. The free radicals are extremely reactive, and damage any biochemical structure they come into contact with [Berman]. This excitotoxic cascade is thought to play an important role in brain injury and neurodegenerative diseases.

Domoic acid's structure is obviously similar to glutamic acid. But its five-sided ring makes it less flexible than glutamate, which causes it to bind very tightly to glutamate receptors. As a result, the excitatory effect of domoate is 30 to 100 times more powerful than that of glutamate [Perl].

How did the domoic acid get into the shellfish (and the anchovies eaten by the birds at Capitola)? Remember that phytoplankton pigments were found in the aqueous layer after solvent extraction. This wasn't quite a smoking gun, but it was definitely a fingerprint of the killer. An extensive investigation traced the domic acid to an obscure species of needle-like diatom*, called Pseudo-nitzschia pungens. Pseudo-nitzschia has been found in oceans around the world, so further outbreaks are possible in many locations. Commercial shellfish and seafood is now monitored regularly for domoic acid, using HPLC to identify the toxin. The screening and testing procedures have so far been successful- not a single instance of domoic acid poisoning in humans has been reported since the 1987 outbreak.

The original article and its references can be viewed at this address.