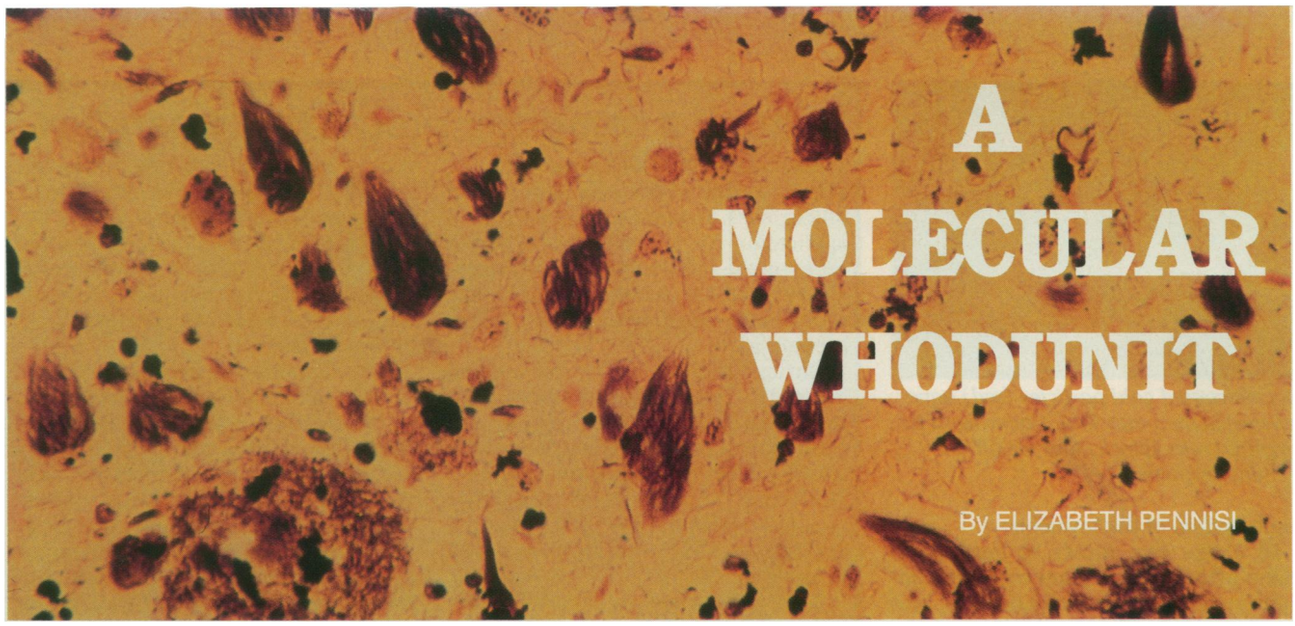


A MOLECULAR WHODUNIT

By ELIZABETH PENNISI

Coleman/Univ. of Rochester

New twists in the Alzheimer's mystery

Two hallmarks of Alzheimer's: Large, round plaques (dark blotch, lower left) and neurons filled with neurofibrillary tangles, also stained dark.

Alois Alzheimer's first observations of the telltale signs of the disease that bears his name were like those of detectives who come upon a murder scene long after the crime took place. In 1907, the German physician found microscopic bits of debris — now called plaques and neurofibrillary tangles — cluttering brain tissue taken from a 51-year-old woman who had suffered from dementia.

Since then, countless scientists have revisited the scene of the crime and attempted to reconstruct what causes some aging brains to slowly lose their ability to function.

Their efforts read like the early chapters of an Agatha Christie novel: Each time these science sleuths finger a suspect, baffling new findings poke holes in their case. Barely two years ago, for example, a particular piece of protein that lies at the core of the plaques that Alzheimer first described seemed the most likely culprit (SN: 3/7/92, p.152). But some results since then have raised the possibility that circumstantial evidence may have inappropriately implicated this protein piece, called beta amyloid peptide.

At this point in a Christie novel, Hercule Poirot enters the picture. To the chagrin of his fellow detectives, his clever insights let him discover what others have overlooked. Voila! The murderer is found.

In the Alzheimer's field, along came Allen D. Roses, a neurologist at Duke University Medical Center in Durham, N.C. In August 1993, his team raised eyebrows in the brain research community with data linking a protein that shuttles lipids through the body to increased risk of Alzheimer's late in life (SN: 8/14/93, p.108).

That protein, apolipoprotein-E4 (Apo-E4), is one of several forms of apolipoprotein-E. Each form is coded for by a different version, or allele, of the apolipoprotein-E gene. Because everyone inherits two copies of this gene, each of us has alleles coding for one or two forms of apolipoprotein E. Those with two genes for Apo-E4 are eight times as likely to develop Alzheimer's as people who inherit two genes for Apo-E3, the protein's more common form, says Roses.

This perplexing finding did not fit into standard theories about the disease. By November, however, a half dozen research teams had accumulated enough supporting data (see sidebar) to prompt the National Institute on Aging (NIA) to host a special briefing for neuroscientists gathering in Washington for the annual meeting of the Society for Neuroscience. During the four-hour session, several investigators presented evidence linking Apo-E to Alzheimer's, and Roses described how he and his colleagues think the crime that leads to dementia occurs.

"We believe this is a major discovery that moves Alzheimer's research to a new and higher level," Zaven Khachaturian, an Alzheimer's researcher at the NIA in Bethesda, Md., told reporters that evening. "It provides, moreover, an opportunity for very good molecular biologists and biochemists [to find] a way of blocking [Apo-E4's] action."

Unfortunately, whereas Agatha Christie wrote fiction, Alzheimer's disease is all too real to the 4 million people in the United States afflicted with it. And biomedical research efforts rarely read like mystery novels, where in the final pages all the elements of the story get tied up neatly.

Many long-time Alzheimer's scientists fault Roses for overzealous use of what Poirot called the "little gray cells." They argue that his theories lack supporting experimental evidence; a few scoff at Apo-E4 altogether. Others favor different suspects, such as the protein that lies at the base of the neurofibrillary tangle or calcium ions, which figure importantly in many aspects of cell function. Many doggedly pursue the beta amyloid plaque and its potential biochemical accomplices.

And while fans of country-house murders would have those naysayers proved wrong, realists among these researchers believe that each of the suspects may prove guilty — that several biological molecules or processes gone "bad" can cause Alzheimer's disease to develop.

"I think [we] all have a piece of the truth," says neuropathologist Michael L. Shelanski at Columbia University in New York City. "When we understand Alzheimer's, we may be able to tie all the pieces together."

Even before Roses joined the fray, the hunt for suspects in the Alzheimer's mystery was in apparent disarray. While scientists agreed that Alzheimer's dementia arises when certain groups of nerve cells stop working and start dying, they couldn't agree on why this happens. At first, researchers thought the hottest trail led to the neurofibrillary tangles, stringy masses that clog the inside of nerve cells in the parts of the brain important for memory and learning.

Then several breakthroughs shifted the focus to beta amyloid, which forms the core of the plaques that lie outside nerve cells in brains of Alzheimer's sufferers. Scientists determined the protein's composition and synthesized it for detailed study. Also, geneticists discovered defects in the gene for its precursor

protein in the small group of people who inherit a tendency to get Alzheimer's at very early ages. Exposure to beta amyloid can kill cells growing in test tubes, others found.

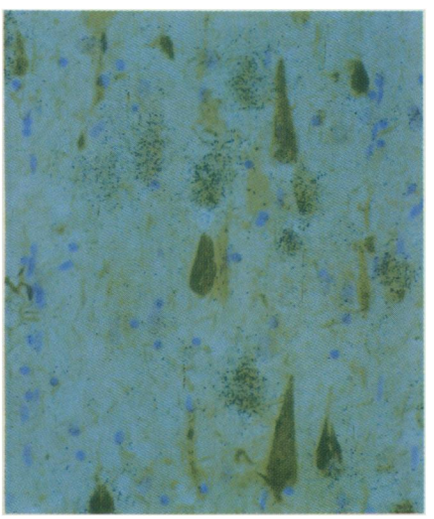
"There's converging evidence that the [beta] amyloid peptide is the bullet," asserts Joseph T. Coyle from Harvard Medical School in Boston. "The evidence is convincing [us] that amyloid is a very critical and precipitating event in the progression of Alzheimer's disease."

But careful examination of brain tissue has revealed that many older people develop plaques without being stricken with Alzheimer's. It turns out that the brain produces lots of amyloid precursor protein (SN: 10/10/92, p.237) and that beta amyloid usually exists in the brain but in a soluble form, not as plaques. "We have data that show there are perfectly normal nerve cells right in the middle of the plaques," says Paul D. Coleman at the University of Rochester (N.Y.) Medical Center.

Besides, the number and distribution of tangles — not plaques — correlate closely with the amount of brain function lost, says Bradley T. Hyman, a neurologist at Massachusetts General Hospital in Boston. "Yet we know amyloid is central to the disease process," he laments. "There is a basic paradox."

Data reported last November at the neuroscience meeting only add to this confusion. Previously, researchers had shown that the more severe the dementia suffered by Alzheimer's patients, the fewer the number of connections, or synapses, found in certain parts of their brains. Coleman studied the distribution of a protein critical to the functioning of synapses, using its presence as an indication that these connections existed. He and his colleagues have now shown that the density of synapses decreases most in nerve cells where tangles have developed.

"What's really a critical event in the progression of disease is the formation of neurofibrillary tangles," Coleman concludes. "[Beta amyloid's] relationship to Alzheimer's disease is tenuous."



Coleman/Univ. of Rochester

Cells darkened by stained neurofibrillary tangles have lost a protein (black dots) important for nerve-to-nerve connections.

A protein called tau lies at the root of these nerve-cell-clogging tangles. In cells, tau encourages copies of another protein, tubulin, to link up and form microtubules, "the metro system of the neurons," says John Q. Trojanowski at the University of Pennsylvania School of Medicine in Philadelphia. Like ties anchoring the rails of train tracks, copies of tau steady the microtubules, which guide molecules to and from the far reaches of a nerve cell.

As with many proteins, tau's shape and functioning depend in part on how many chemical side groups, called phosphates, attach to its amino acids and where these linkages occur. Some enzymes called kinases put phosphates on proteins, while other enzymes, namely calcineurin and other phosphatases, yank off phosphates.

"Things can be hopping on and off rather dynamically," Trojanowski says.

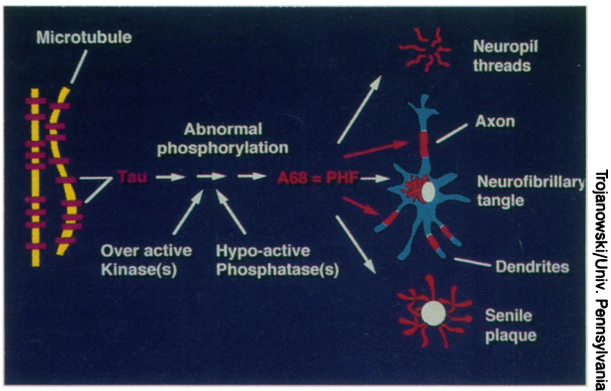
Using antibodies designed to link with tau that has phosphates attached only at particular sites, scientists can distinguish tau in different phosphorylation states. When too many phosphates attach, this "hyperphosphorylated" tau cannot stick to microtubules, says Eva-Maria Mandelkow, a cell biologist at the Max Planck Institute for Structural Molecular Biology in Hamburg, Germany. Thus, tau — when differentially phosphorylated — promotes the assembly, disassembly, and reassembly of microtubules, as needed, by the cell.

But if phosphatases slack off or if kinases become overzealous, then tau may get too many phosphates too quickly. These phosphates can cause tau to leave its post at the microtubule prematurely and to fail to help make more microtubules. Then, because tau dislikes the cell's watery environment, it may intertwine with another tau molecule to form a paired helical filament. These filaments congregate to create neurofibrillary tangles.

"It's like clogging up the cell from inside," says Mandelkow. "It's like constipation."

"We now have very good data to think about how hyperphosphorylation of tau, leading to tangle formation, can be damaging," adds Trojanowski. When tau falls off, the microtubule weakens. At first, the microtubule still functions, but just as the loss of too many railroad ties will cause a track to fall apart, the loss of too many taus will cause the cell's transport system to break down. "If you fail to transport vital and essential material, the axon can die or atrophy, and even the cell body would be at risk," says Trojanowski.

In their studies, Eva-Maria Mandelkow and Eckhard Mandelkow have homed in on several enzymes that can disrupt tau's role in stabilizing microtubules, they reported at November's neuroscience meeting. These enzymes can add phosphates at different spots on tau. "The important contribution is that we've discovered a class of phosphorylation sites that has to do with the way the cell talks to the outside," Eckhard Mandelkow told SCIENCE NEWS.



Trojanowski/Univ. Pennsylvania

Schematic suggests that when tau's phosphate balance gets out of whack, plaques and tangles develop that can disrupt nerve-cell function.

One of these enzymes — called mitogen-activated-protein kinase (MAPK), or externally regulated kinase — helps relay a gene-activating message from outside a cell to the nucleus. When the researchers mix this enzyme in a test tube with tau, "tau acquires features symptomatic of Alzheimer's tau," he adds.

"All these enzymes are present in normal tissue and are ubiquitous; most are involved in the signal transduction pathway," says Eva-Maria Mandelkow. "It appears in Alzheimer's that one or two of the pathways have gotten out of control." Those out-of-control pathways are also implicated in cancer development and in cellular suicide, called apoptosis (SN: 11/21/92, p. 344), she notes.

Other work bolsters the notion that tau alone is not the culprit. Tau from the brains of Alzheimer's victims cannot bind to microtubules. But, mysteriously, the phosphate-carrying tau in fetuses does link to microtubules, even though it is almost identical to the tau found in Alzheimer's brains, Trojanowski and his Pennsylvania colleague Virginia M.-Y. Lee report. These results further implicate enzymes, not tau by itself, as the source of the brain's trouble.

But what makes these enzymes misbehave?

This is the point in the plot where Roses offers a new twist. At the November NIA briefing, he reported on an analysis of Boston Alzheimer's patients that supported his earlier results. "If you do have Apo-E4, then it appears to act in a dose-responsive way," he says. The more Apo-E4, the earlier dementia appears in some people.

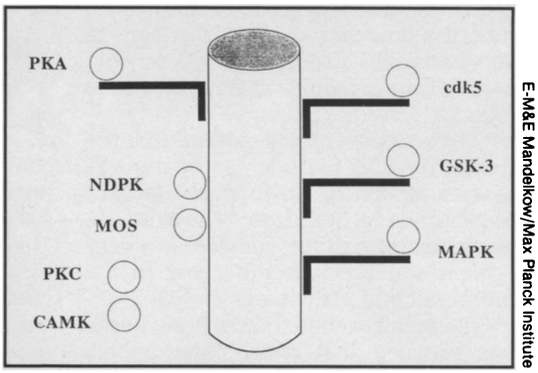
However, he has decided that it's not the presence of Apo-E4, but the absence of two other forms of this protein that increases the risk that Alzheimer's will develop in older people.

When Roses and his colleagues first began looking for Apo-E4 in the brain, they were surprised to find it associated with neurofibrillary tangles. Apo-E is not supposed to exist inside neurons. To understand this unusual finding, they started looking at how Apo-E interacts with tau by mixing these molecules in a test tube.

The more common Apo-E3 and Apo-E4 differ in only one of their amino-acid building blocks. But that difference means Apo-E3 binds easily and tightly to tau and Apo-E4 does not, Warren J. Strittmatter of Duke reported at the NIA session. Apo-E4 and Apo-E3 both fail to stick to tau that has taken on several phosphates, which it will do when an extract from brain tissue is added to the test tube, he adds.

In other experiments, Strittmatter and his colleagues mixed different fragments of Apo-E3 with tau and then fragments of tau with Apo-E3 to see which parts interact. They determined that the first half of Apo-E3 binds to the part of tau that also attaches to the microtubules. But if they boil Apo-E3, causing it to lose its shape, it will not link to tau, Strittmatter reports.

Thus Roses and Strittmatter suggest that Apo-E3, by sticking to tau, may help keep phosphates away, while Apo-E4 fails to do so. This failure leads to a gradual accumulation of tau proteins with phosphates, which coagulate into tangles.



E-M&E Mandelkow/Max Planck Institute

Diagram of microtubule with tau hanging off shows which enzymes link to tau (PKA, cdk5, GSK-3, and MAPK).

"Apo-E3, we think, functions as an intracellular neuroprotective cofactor, sequestering tau from phosphorylation but allowing tau to interact with tubulin to stabilize microtubules," Roses explains. That slight advantage can translate into years of clear thinking, he adds. His data suggest that people with two copies of Apo-E3 tend to develop dementia around 85 years of age, while those with two copies of Apo-E4 may be affected by age 69.

"If we don't have to correct something that's wrong, but just have to supply something that's missing, it leaves the great potential hope for a therapy," he adds. He thinks that researchers should try to find molecules that mimic Apo-E3. These mimics may prove useful for treating or preventing the disease in people who inherit the Apo-E4 allele.

Roses' critics point out that the test-tube experiments may not reflect what occurs in cells. They argue that Roses fails to place Apo-E at the crime scene at the right time. "Normally, Apo-E is outside the cell and tau is inside," says Kenneth S. Kosik from Brigham and Women's Hospital in Boston. Also, they point out that if Apo-E3 attaches to tau where microtubules bind, then tau should be unable to link to microtubules.

"You have what is totally unfounded speculation," Columbia's Shelanski complains. "The theory has some serious problems."

Other scientists think Apo-E4 more likely plays the role of an unwitting accomplice that helps set up lethal events, including plaque formation, outside the cell. People with Apo-E4 tend to have larger plaques than people with other types of Apo-E, Roses notes.

Still others, while accepting some association between Apo-E4 and an increased risk of Alzheimer's, argue that this association may have little to do with the disease itself. "I think the jury is out about [Apo-E's] physiological relevance," says

Apo-E4: A risk factor, not a diagnostic test

Several research groups now have confirmed a link between Alzheimer's disease and the protein apolipoprotein-E4 (Apo-E4). Not all the new epidemiological and genetic studies show as strong an association as those conducted by Allen D. Roses and his colleagues at Duke University Medical Center in Durham, N.C. But the data do confirm that a connection exists between this late-onset dementia and the gene that codes for the protein.

After hearing about Roses' results (SN: 8/14/93, p.108), Richard Mayeux of Columbia University's College of Physicians and Surgeons checked his Alzheimer's study population for Apo-E4. The odds of having two copies, or alleles, of the Apo-E4 gene are 17 times higher in people with Alzheimer's than in those without the dementia, he reported in November 1993 at a scientific briefing sponsored by the National Institute on Aging. Alzheimer's patients are five times as likely to have a single copy of the Apo-E4 gene as those not affected by the disease.

At that meeting, Gerard Schellenberg from the University in Washington in Seattle described results from two studies. He and his colleagues first evaluated the genetic makeup of 52 families with lots of older members affected by Alzheimer's. They also looked at the unaffected relatives.

"There's a highly significant genetic association between Apo-E and Alzheimer's disease in this group," says Schellenberg. But 10 percent of those families lacked the Apo-E4 allele altogether, he notes.

He and his colleagues then examined the occurrence of the Apo-E4 gene in a broader population — 23,000 people over age 60 who belong to a Seattle health maintenance organization (HMO). To find HMO members who might have Alzheimer's, the researchers scanned the medical records for people reporting memory problems and also considered potential Alzheimer's patients referred to them by the HMO's physicians.

Since 1987, they have identified more than 200 people with the disease, says Schellenberg. They compared the Apo-E data from these patients with those from other HMO members of the same age and background but without dementia. The results also indicated that the Apo-E4 gene shows up significantly more often in people with Alzheimer's, Schellenberg says.

He and others note, however, that having Apo-E4 does not automatically destine a brain to Alzheimer's. Apo-E4's presence simply increases the risk. "It's not dissimilar to the chances of a very heavy smoker getting lung cancer," says Robert Katzman, a neuroscientist at the University of California, San Diego, School of Medicine. "It's about a tenfold increase in risk."

Moreover, having Apo-E4 "does not appear to be of predictive or diagnostic value in the general population," Schellenberg cautions. Thus testing for the Apo-E4 gene would miss about 60 percent of those with Alzheimer's, and it would misdiagnose some of those without Alzheimer's as having the disease, he notes. — *E. Pennisi*

Rudolph Tanzi of Massachusetts General Hospital. "A gene nearby [Apo-E] could be the culprit," he cautions.

Not so, retorts NIA's Khachaturian, who is more convinced with each new study that Apo-E4 is a real risk factor.

"We're just so ignorant about how the normal brain works or whether Alzheimer's is two or 10 diseases that we can't squelch any ideas," adds Trojanowski, the University of Pennsylvania researcher.

Moreover, Khachaturian does not think Apo-E has to get into the cell to have the effect Roses describes. As a transporter of cholesterol, Apo-E can affect a cell's integrity by inserting these fatty molecules into the cell's membrane, perhaps altering the membrane's ability to keep calcium from leaking into the cell. Once calcium gets in, it can wreak havoc and set off a chemical cascade that leads to the phosphorylation of tau, the breakdown of microtubules, and the formation of tangles, Khachaturian proposes. It may also stimulate more production of the protein that is the precursor of beta amyloid.

Mark P. Mattson from the University of Kentucky Medical Center in Lexington also suspects that abnormalities in calcium concentrations ultimately kill these brain cells. Older cells are less able to control inflow of this ion precisely, and this may make them more vulnerable, he says.

Indeed, Apo-E4 may actually enter, or at least attach to, cells. Hyman's group at Massachusetts General Hospital finds that many cells in the brain, including nerve cells, contain docking sites, or receptors, for Apo-E. These receptors are densest where plaques exist, he and his colleagues reported in the October 1993 NEURON.

Because beta amyloid is hydrophobic—like fat-like molecules, it seeks to avoid water — Apo-E molecules may transport it, like cholesterol, back to cells for processing. If for some reason the transport or processing systems do not work efficiently, then beta amyloid might accumulate and eventually settle out of solution as a plaque.

Thus Apo-E might play a role in both tangles and plaques.

Alternatively, the connection between plaques and tangles could come down to a problem with the enzyme MAPK. "There's a whole cascade of reactions that ultimately results in the activation of MAPK; these start with the activation of a receptor on the cell," says the University of Rochester's Coleman.

That receptor may be sensitive to a secreted version of amyloid precursor protein, says Kosik. At the neuroscience meeting, he and his colleagues reported that this protein stimulates part of the MAPK pathway. However, plaques don't necessarily occur in the same parts of the brain as tangles, Coleman notes.

Hyman's group mapped the distribution of this enzyme — under the name of externally regulated kinase — in brain tissue taken from Alzheimer's patients who had just died. It shows up most at the sites of tangles, leading Hyman to suspect it as the overzealous enzyme that ruins tau and makes it form the tangles.

So even if Roses does not have Apo-E's connection to Alzheimer's quite right, some of his colleagues find his vision invigorating. "The broader idea of Apo-E and neural dysfunction is very appealing," says Hyman.

Eva-Maria and Eckhard Mandelkow, too, suspect that MAPK, or some element in the signal transduction pathway, links these two hallmarks of Alzheimer's disease. "From a scientific point of view, the beta amyloid and tau camps will have to merge eventually," says Eckhard Mandelkow.

That could take many more chapters and involve many more suspects, scientists warn. Rather than reading like a novel, their efforts play out more like a television soap opera, with cliff-hangers at the end of each episode.

"A great deal of progress has been made in understanding the pieces," says Shelanski. "But no one has the answer. Yet." □