# Major Setback for Alzheimer's Models

*Two of the three published mouse models are now being retracted—and NIH is beginning an inquiry to see whether a misconduct investigation is needed for one of them*

ONLY A FEW WEEKS AGO EXCITEMENT WAS running high in the Alzheimer's community. After repeated efforts in many labs, three groups had independently come up with what looked like good mouse models for the neurodegenerative disease. As the news media pointed out in the first flush of apparent success, mice with Alzheimer's would permit researchers to test highly experimental therapies and perform other studies not appropriate or possible on humans. But in the past few weeks, two of those mouse models have gone down in flames, one because it turned out to be an artifact (see box on facing page), the other amid concerns that one of the researchers may have presented human Alzheimer's tissue as mouse tissue.

Those concerns have already led the National Institutes of Health (NIH) to initiate an inquiry into the mouse model paper written by Shigeki Kawabata of the Yamanouchi Pharmaceutical Co., Ltd., in Tokyo, Gerald Higgins of the National Institute on Aging's Gerontology Research Center in Baltimore, and Jon Gordon of Mt. Sinai School of Medicine in New York City. The authors have sent a letter to *Nature*, the journal that published the paper on 12 December 1991, in which they retract it on the grounds that they could not reproduce the pathology results in additional mice. The NIH inquiry, however, will go beyond the question of reproducibility to the more serious question of whether Higgins may have substituted human Alzheimer's tissue for mouse tissue—a charge he adamantly denies.

But whether or not the committee that will conduct the NIH inquiry eventually agrees with Higgins, the loss of the mouse model has dealt Alzheimer's research a significant setback. The kinds of studies needed to work out just what causes the brain degeneration of Alzheimer's patients can't be done in the patients themselves. And while aged nonhuman primates also develop pathological brain changes, including memory deficits, similar to those of Alzheimer's disease, they are too scarce and too expensive to be used for routine research. So researchers were pleased with the prospect of having three mouse models to choose from.

Now they're left with only one: from Barbara Cordell and her colleagues at California Biotechnology Inc. in Mountain View, California. And the loss of the Kawabata-Gordon-Higgins model is particularly frustrating because it appeared to be by far the best.

In all three cases the researchers created their mouse models by transferring into the animals human genes for making β-amyloid, a protein found in the abnormal structures known as plaques that stud the brains of Alzheimer's patients and constitute one of the characteristic pathological features of the disease. Many, although by no means all, researchers think that abnormal β-amyloid deposition may cause Alzheimer's. So the researchers hoped that by inducing mice to overproduce the protein, they could reproduce the pathology in the animals. Indeed, the Gordon group seemed to have succeeded in doing so.

Whereas the other two groups saw what appeared to be signs of abnormal amyloid deposition in the brains of their transgenic mice, they did not see other characteristic features of Alzheimer's brains, such as neurofibrillary tangles, which are abnormal bundles of protein filaments in the nerve cell cytoplasm. But Higgins' photomicrographs of tissue purportedly from his group's transgenic mouse brains showed a full range of Alzheimer's pathology, including well-developed plaques, tangles, and degenerating neurons. "We all said the same thing. It looks amazingly like Alzheimer's disease," recalls neurologist and Alzheimer's expert Dennis Selkoe of Harvard Medical School. The *Nature* paper was based on a small number of animals, however.

When the Kawabata-Gordon-Higgins group wrote it, they included histopathological data from four transgenic mice from the same line. Two of the animals were 4 months old and the other two were 8 months, and only the older pair showed the strong Alzheimer's resemblance. But Selkoe says he was assured by Gordon and Higgins that they had additional transgenic mice with the pathology—and, he says, partly as a result of that assurance he wrote a "News and Views" article to go with the paper, in which he described the model as the most useful yet.

Some Alzheimer's experts, however, thought the resemblance to human Alzheimer's disease might be a bit too amazing. "I would like to see evidence that figures 3a to 3f [the photomicrographs showing the pathology] are coming from a mouse. I have difficulty accepting that," says neuropathologist Henry Wisniewski of the New York State Institute of Basic Research on Staten Island, who has been involved in Alzheimer's research for many years. Indeed, Wisniewski was sufficiently concerned by what he saw to contact Andrew Monjan, who is deputy associate director of the Neuroscience and Neuropsychology of Aging Program at the National Institutes on Aging (NIA), in early January. Monjan in turn mentioned Wisniewski's concerns to NIA colleague Richard Sprott, who asked a neuropathologist friend, Roderick Bronson of Tufts University, to take a look at the photomicrographs in *Nature*. Bronson's conclusion: While the pictures showed the classic plaques and tangles of Alzheimer's, it was impossible to determine from the pictures alone whether the brain tissue was mouse or human. "I couldn't tell and I don't think anyone else could either," he says.

Selkoe concurs. The problem is, he ex-

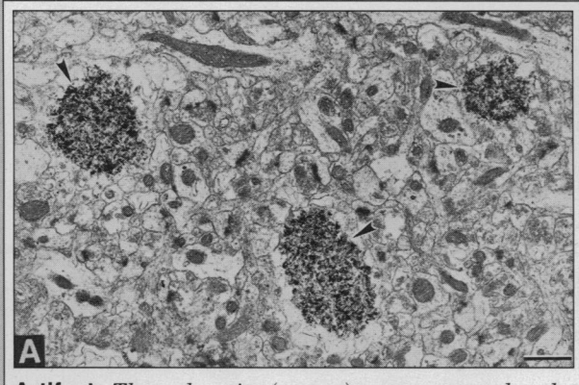
**The Published Transgenic Mouse Models**

| Laboratory | Gene Construct | Abnormalities | Location |
|---|---|---|---|
| Cordell | Full-length amyloid precursor protein (APP) | Extracellular amyloid deposition | Cortex and hippocampus |
| Kawabata/ Gordon/ Higgins | C-terminal 100 amino acids of APP (includes β-amyloid) | Plaques, tangles, degenerating neurons | Cortex and hippocampus |
| Wirak | β-amyloid | Intracellular amyloid deposition | Hippocampus |

# Amyloid Deposits: A Case of Mistaken Identity

When the Alzheimer's mouse model from the research team led by Dana Wirak and Axel Unterbeck fell, there wasn't so much as a hint of misconduct; the issue was simple—disappointing, but honest scientific error. Reporting in the 19 July 1991 issue of *Science*, the researchers, who work at the Miles Research Center in West Haven, Connecticut, thought they had shown that they could reproduce one of the major features of Alzheimer's pathology in transgenic mice. But then came a thoughtful critique from a pair of sharp-eyed neurobiologists working in Baltimore, and the Miles group found themselves having to take another look at their model. To no avail, though.

When the critique is published as a technical comment (along with a statement by Wirak and Unterbeck), in the 13 March issue of *Science*, the Alzheimer's research community will experience the loss of the second of the three mouse models for Alzheimer's that have been published since July of last year (see story opposite for the first). And that's a big problem. Says Alzheimer's expert Dennis Selkoe of Harvard Medical School: "We need these models."




**Artifact.** *These deposits* (arrows) *were supposed to be β-amyloid, resulting from activity of a human transgene, but they're not.*

The three groups who produced the mouse models all used similar approaches. Each introduced human gene sequences encoding a protein called β-amyloid into the animals. They chose that particular protein because it's a prominent component of the abnormal structures called plaques that stud Alzheimer's brains, and some researchers think the β-amyloid deposition may even cause the nerve cell degeneration of the disease. And each group hoped that if it could get the animal brains to make β-amyloid, they would develop plaques and the other pathological features of Alzheimer's, such as neurofibrillary tangles, abnormal structures consisting of protein filaments bundled together in the nerve cell cytoplasm.

And all three groups succeeded—or appeared to succeed—to varying degrees, thereby giving further credence to the idea that β-amyloid deposition is a cause of Alzheimer's. The other paper that's in trouble reported, for example, that all the major pathological features of Alzheimer's developed in the transgenic animals. The Wirak-Unterbeck model did not show such extensive pathology, but their transgenic animals did develop what the researchers thought were β-amyloid deposits in brain neurons, particularly in the hippocampus, one of the principal brain areas affected by Alzheimer's. But even that struck neurobiologists Mathias Jucker, who was then at the Gerontology Research Center (GRC) in Baltimore, and Lary Walker of Johns Hopkins University School of Medicine as dubious. To these two, the deposits looked too familiar.

The year before, Jucker and Walker had detected similar inclusions in hippocampal neurons from normal aged mice of the C57BL/6 strain—a strain that contributes to the genetic background of the animals the Wirak group used to create their transgenics. Their earlier finding raised the distinct possibility that the transgene had nothing at all to do with the deposits described in the *Science* paper, and the two researchers went to Donald Price, a Johns Hopkins neuropathologist who has worked on Alzheimer's for many years, with their concerns. Price contacted Wirak, and the two groups, the GRC-Johns Hopkins team on the one hand and the Wirak team on the other, immediately began working together to find out just what was going on in the transgenic animals. Indeed, throughout the project, Price says, "the interactions with their [Wirak's] group have been very collegial—just the way it should be done."

Collegial, correct, but the outcome has still not been a happy one for the Miles researchers. In their technical comment for *Science*, the GRC-Johns Hopkins team concludes that, as suspected, the deposits seen by the Wirak group develop normally in the mice. And in an even more disheartening development, the deposits may not even contain β-amyloid. In the original work, Wirak and his colleagues found that they are stained by antibodies to the protein. But the more recent work shows that they are also stained nonspecifically by several different antibody preparations, a result that indicates that the deposits have a high affinity for antibodies generally and provides little information about their composition. "Whatever the inclusions are, they may be age-related and have no relation to the transgene," says Price.

In a statement that will appear with the technical comment, the Wirak group will concede that point, stating that for now the data in their original paper "cannot be used to support the conclusion that a β/A4 [amyloid] transgene causes formation of amyloid-like deposits in mouse brain." Wirak says that his team missed the possibility that the deposits might develop normally in their mice because of a sampling problem when they chose their control animals. "I know for a fact that if we had done a larger sampling of the nontransgenic litter mates, we would have seen it."

The Miles researchers are not ready to give up on their model totally, however. There's a chance, Wirak says, that the transgene may have accelerated the formation of the deposits, and that will be investigated. The group will also do further work to see whether the deposits contain β-amyloid after all.

And as for the larger issue of what the demise of these two transgenic models means for the hypothesis that amyloid deposition contributes to Alzheimer's disease, it can only be said that the jury is still out. The transgenic models weren't the only evidence for that view, and in any event the third model, published in *Nature* last July by Barbara Cordell's group at California Biotechnology Inc. in Mountain View, California, is holding up. And all the researchers involved say they will continue to work on finding amyloid gene constructs that will yield Alzheimer's pathology. They particularly want to try a mutated form of the gene that was recently linked to the disease. As Price says, "I'm very optimistic that in the long run this [transgenic] strategy will provide a model for genetically determined Alzheimer's disease." And so, it's back to the bench. ■ **J.M.**

plains, that the micrographs were made at such a high magnification that they don't show the larger brain structures that would permit mouse brain to be readily distinguishable from human.

But the problems didn't stem solely from the published photos. Alzheimer's researchers who saw the original tissue samples about a month after the paper came out also became concerned that there might be a problem with them, according to George Martin, who as scientific director of the NIA is Higgins' boss. Those researchers include Selkoe, who visited Higgins' lab in mid-January, originally intending to set up a collaboration, and another long-time Alzheimer's researcher, neuropathologist Donald Price of Johns Hopkins University School of Medicine and his colleague Lary Walker, who looked at the samples at Higgins' request. The concerns expressed by those investigators led NIH officials to undertake the inquiry.

So now it will be up to the inquiry committee, which will be headed by neurologist Dale McFarlin of the National Institute of Neurological Diseases and Stroke to examine the evidence, including the original tissue samples, to determine whether the facts warrant a misconduct investigation. If such an investigation occurs, it would be carried out by the NIH Office of Scientific Integrity (presuming that office hasn't been supplanted—see "Sciencescope," p. 1199).

McFarlin estimates that the inquiry phase will take weeks. "NIH takes matters like this very seriously," he says. And with good reason. If misconduct should be found, the penalty could be as benign as a letter of reprimand. But it could also be as serious as a loss of a job.

Higgins says he welcomes the inquiry. "If there is an independent review, that's the way to handle the matter," he asserts. He is also insistent that no misconduct occurred. "I can assure them [the misconduct allegations] to be groundless," he says.

Higgins originally got involved in the transgenic mouse research, he says, back in the summer of 1991, when Gordon called and asked for his help with in situ hybridization studies for locating the areas of the mouse brains where the human amyloid gene was active. In addition, Higgins, who is a neuroanatomist and molecular biologist (not a pathologist as *Science* erroneously reported last week) did silver staining of the tissue samples with the aid of a poctdoc who is a neuropathologist. "We saw plaques and tangles in some pieces of tissue," he says. The results from two of these animals were described in the *Nature* paper.

But when Higgins began looking at brain tissue from additional transgenic animals of the same line, he did not see silver staining of plaques and tangles. That was even before the paper came out, and Higgins says he suggested to Gordon that they add a statement to the paper indicating that transgenic animals show varying degrees of the pathology. "My first concern was to notify the community to let them know about the variability," he says. (This doesn't jibe with Selkoe's memory of events, but by the time *Science* got the information from Selkoe, Higgins had left his lab for his family's vacation home in Vermont and could no longer be reached for comment.)

By Higgins' account, however, Gordon, who is the transgenic animal expert of the group, said it wasn't necessary to add a note on variability because people familiar with transgenic animals know how variable they are. When *Science* contacted Gordon and Mt. Sinai spokesman Mel Granick for a response to Higgins' assertion, both declined to comment at this time. "Our position is that we think it's inappropriate to comment pending the outcome of the inquiry," Granick says. And the retraction letter is not much help with regard to the question of when the researchers began finding that the histopathological results were not reproducible. It says simply they have not detected them in any of 12 "recently studied" transgenic animals and therefore believe that it is prudent to retract those results. Meanwhile, they say, the issue of whether their transgenic animals constitute a useful Alzheimer's model remains to be assessed by further study.

And there matters will rest, until the inquiry committee completes its deliberations. But at least for people familiar with the seemingly endless misconduct investigations that have made headlines in recent years, there may be one consolation. It should be somewhat easier to sort out the facts in this case than, say, to pin down the origins of an AIDS virus strain that was isolated 8 years ago. Even though human and mouse brain samples may be hard to distinguish visually, immunological and genetic techniques should be up to the job. ■ JEAN MARX

# Canadian Science Wins—and Loses

In the United States the budget deficit all but dominates the budget process, and many U.S. scientists have marveled at the annual funding hikes research (particularly National Science Foundation-sponsored work) has received through the 1980s and into the 1990s. If only it had been so in Canada. Little noticed among U.S. scientists, the Canadian budget deficit is actually larger on a per capita basis than the U.S. shortfall. And in the past few years, Canadian research funding has suffered. Not so this year. For the first time since 1984-85, real growth for university-based research in Canada could exceed the inflation rate. Says Minister of Science William Winegard, "We can be very optimistic."

This year's budget, announced by the government on 25 February, included increases for the Canadian university granting councils (which provide most funds for basic research) of 4% for fiscal year 1992-93. This translates into a hike for the Natural Sciences and Engineering Research Council (NSERC) of $17.2 million to $500.8 million.

Science's winners in this year's budget include the Canadian Space Agency, which will receive an increase of $110 million (36%)—$46 million for Canada's participation in the international space station and $32 million for new headquarters. And $230 million over the next 5 years will go to improve the administration of the government's generous—but complex—research and development tax credit system.

Not all the news in the budget was greeted by applause from the scientific community, though. One contentious move was elimination of the Science Council of Canada, an advisory agency on science and technology policy. The government maintains that elimination of the council, along with 45 other nonscience agencies, was needed to streamline government. Prime Minister Brian Mulroney has said his own blue-ribbon panel, the National Advisory Board on Science and Technology, can do the work of the council.

Some critics, however, including researchers, university administrators, and members of the Liberal Party, think the disappearance of the council was due to the Conservative government's discomfort with the only independent science policy agency in the country. Recently, for example, the NSERC publicly criticized the government's decision to contribute $236 million toward the planned KAON particle accelerator at the University of British Columbia, arguing that the benefits weren't proportional to the cost in this Canadian version of big science.

Disappointment over the science council aside, however, most proponents of Canadian science funding were pleased with this year's budget, because it suggests that, after years of stagnation, federal spending on science and technology programs may finally exceed inflation rates. ■ DOUG POWELL

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