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The relationship between environmental toxins and cancer has plagued scientists for more than a century. School of Public Health researcher Patricia Foster, Ph.D., is investigating how chemical carcinogens cause mutations in bacterial DNA. See story on page 3.



Jerome O. Klein, M.D., examines the ear of a young patient. Klein and his colleagues are looking for factors that may increase a child's risk of ear infections. (Photo by James P. Dwyer)

Long-term effects of middle ear infections explored by research team

Middle ear infections are tantamount to a price most of us pay for negotiating the first three years of life.

Jerome O. Klein, M.D., a professor of pediatrics at Boston University School of Medicine, and his associates, in a study several years ago of 2,500 Boston children, found that 71 percent of the youngsters suffered at least one such infection in the first 36 months of life. Nearly half that group, moreover, had three or more infections.

Similar figures probably apply nationally, said Klein, noting that

middle ear infections are the most common reason for doctors' visits among children three years of age and under.

Dealing with the infections themselves is seldom difficult. "With an appropriate antibiotic, children will have remission of their acute symptoms--mainly fever and earache--within two or three days," said Klein, who heads the Division of Pediatric Infectious Diseases at Boston City Hospital.

The infections, however, can leave behind fluid in the middle ear.

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New technique yields genetic link, diagnostic tests for Alzheimer's, Down

A genetically-related cause for Down syndrome (DS) and Alzheimer's disease (AD) has been postulated by scientists in the past but, until now, research in the area has received little support. Recent reports by National Institutes of Health researchers and others, however, have cited and upheld the hypothesis that was first suggested two years ago by Miriam Schweber, Ph.D., an assistant research professor of biochemistry at Boston University School of Medicine, that both Down and Alzheimer's are the result of a quantitative change in the DNA that normally exists on chromosome 21.

To show this connection, Schweber has invented a new method that accurately measures and compares minute amounts of genetic material from various tissues, including blood. Her technique and device for carrying it out may provide researchers with a reliable diagnostic test for DS very early in pregnancy. It offers a diagnostic test at present for Alzheimer's and may offer a presymptomatic test as well.

The technique of DNA measurement already has proved effective in detecting Down from non-Down tissues. In a similar way, she hopes

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the test will prove as accurate for people with Alzheimer's and, by extension, for any condition involving quantitative changes in DNA. For example, patients with problems resulting from extra sex chromosomes (XXY or XO) would be likely candidates for this sort of screening.

Individuals born with Down syndrome are characterized by mental deficiency and often abnormalities of other organs, especially the heart. Alzheimer's is a form of degenerative dementia in adults characterized by progressive memory loss and personality changes. It has been estimated that Alzheimer's affects more than three million people in the United States.

As a departure point for her studies, Schweber reviewed the reports of autopsies on 200 individuals over age 35 with Down syndrome and 600 under age 35. In every case, the studies revealed the same abnormal structural changes in the brain found in victims of Alzheimer's--the same so-called plaques and tangles--and also, although less conclusively, the same biochemical alterations.

"Laboratory findings have indicated that all individuals with Down syndrome eventually get Alzheimer's, although they don't always express it behaviorally," said Schweber. "This has led to the idea that the effect of aging in persons with Down syndrome produces the clinical phenomenon called Alzheimer's. To me, the evidence of neuropathology and biochemical studies meant that the two conditions could have a common cause."

However, two obvious questions arise from her assertion: If all persons with Down eventually get Alzheimer's, why aren't persons with Down syndrome all demented at the time of death, and why don't all Alzheimer's victims have Down as well?



Miriam Schweber, Ph.D., has found a genetic link between Alzheimer's disease and Down syndrome. (Photo by John Thompson)

In Schweber's opinion, it is irrelevant to apply traditional criteria for Alzheimer's-type dementia to Down syndrome because AD involves a loss of abilities that a person with Down never had. The progression of the dementia, therefore, usually is masked in DS. However, according to Schweber, about 25 percent of adults with Down syndrome actually are classified as demented at time of death, but they are only identified as such because the symptoms have become so severe that they qualify as the terminal stage of Alzheimer's, characterized by violent personality changes, incontinence, total disorientation and even the inability to move about.

The answer to why not all Alzheimer's victims have Down syndrome is the subject of Schweber's current studies. There are two types of Down syndrome, she explained: Trisomy Down, in which there are three full copies of chromosome 21 instead of the normal two; and translocation Down, in which there is a triplication of a limited section of genetic material from chromosome 21.

"I think that Alzheimer's is due to three copies of a small segment of DNA within that limited triplication. Therefore, all Down individuals would develop Alzheimer's but not the reverse. We have to look closer now to

see how big the area of triplication is and how it differs in different forms of Alzheimer's," she said.

Schweber has tested 15 Alzheimer's patients so far, including examples of early- and late-onset, sporadic and familial cases. Samples of tissue from these people show that in all categories there is triple the amount of DNA on chromosome 21 in a small section near the Down location.

What enables Schweber to get this "closer look" has been her occupation for much of the past five years. She calls the result, unveiled in April at the annual meeting of the American Academy of Neurology, "Miriam's Monstrosity." The device, which stands six feet high, enables Schweber to pre-image a standard of measurement onto film. The system has been patented by BUSM.

"Our technique allows one for the first time to look at an image of DNA on film and tell if its an accurate representation of the amount of material there," Schweber said.

Essentially, the method is a modification of the Southern Blot technique for measuring DNA. A sample of raw DNA from a particular tissue is chopped into pieces using enzymes of different sizes. The mixture is put onto a gel and subjected to electrophoresis, which causes the material to separate according to size. By next inserting pieces of the chopped up, or recombined, DNA into bits of bacterial DNA called plasmids, one can compare quantities of DNA on different chromosomes. Using blood samples obtained from veterans hospitals, Schweber has accurately distinguished individuals with Alzheimer's from normal patients.

The next step, said Schweber, is to continue testing several hundred more samples for the Alzheimer's defect, and to refine her design in order to make the test less cumbersome, more accessible and less costly. "I'd like it to be available to any physician who wants to do a

screening, although I don't think it will be appropriate in an office setting. The technique is rather sophisticated and requires more expertise than a normal Southern Blot," she said. Schweber is in the process of setting up programs in conjunction with several hospitals.

As the early stages of Alzheimer's are extremely difficult to diagnose, presenting physicians with a bewildering array of symptoms, it often takes several years to determine if the problem is really Alzheimer's or some other, treatable problem. Schweber's procedure has the potential to offer early diagnosis of people at risk for the illness. Physicians then will know if the disease is treatable and can plan treatment strategies at a time when they might do some good. "There are more than 100 forms of dementia, some treatable, some not. This test could be used to rule out Alzheimer's if nothing else," said Schweber. "Do families want to know? It's up to them, but at least it's available.

"Alzheimer's is clearly a complex disease," said Schweber. "Right now, a completely certain diagnosis can only be made with an autopsy, which doesn't help the sufferer much, nor is it useful in looking for treatments."

Schweber's work has been supported by a grant from the Retirement Research Foundation and an Allied-Signal Corp./Alzheimer's and Related Disorders Association Faculty Scholar Award. Her colleagues in the research include postdoctoral colleagues Ruth Shiloh, Ph.D., and Ziva Ben-Neriah, M.D., of Jerusalem, Israel, and laboratory technician Clare Tuson.

--Caroline H. Lupfer

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SPH researcher looking for clues to cancer in bacterial DNA

In 1775, British surgeon Percivall Pott discovered a high incidence of scrotal cancer in chimney sweeps, and identified the culprit as soot.

The short article he later wrote on the subject was one of the first attempts at linking the human disease with chemical carcinogens.

Since then, scientists have scrutinized the relationship between a myriad of suspected environmental toxins and cancer. One of these scientists is Patricia Foster, Ph.D., an assistant professor of environmental health at Boston University School of Public Health, who has spent the last six years looking for clues to the cause of cancer in the DNA of the bacterium *Escherichia coli*. According to Foster, understanding how a chemical carcinogen induces mutations (a single mutation can change a normal gene into its oncogenic derivative) may shed light on the etiology of cancer.

Two of the chemicals involved in Foster's research, the insecticide ethylene dibromide (EDB) and the chemotherapeutic agent *cis*-diamminedichloroplatinum-II (*cis*-Pt), are known carcinogens, and Foster is studying their effect on *E. coli* DNA. EDB also is used as an antiknock additive in leaded gasoline and as an industrial solvent. Her investigations focus on four main questions: What DNA lesions are induced? Which give rise to mutations? How are the lesions repaired? And what mutations result?

According to Foster, 80 to 90 percent of chemical carcinogens cause mutations in bacteria; therefore bacteria provide good model systems for her study. "My basic feeling is that the kinds of damage that arise in DNA are not specific to any particular organism," said Foster.

"There is quite a bit of experimental evidence indicating that the DNA is essentially the same and the kinds

of DNA damage you see in bacteria are exactly the same as those in higher organisms. So the research is aimed at understanding what damage there is and how that damage is repaired." This is important, Foster added, because much of the DNA repair activity is the same in bacteria as in mammals.

DNA lesions, like crosslinks and base deletions, arise from three pathways: direct interaction of the chemical with the DNA, the interaction of a metabolically activated chemical with the DNA and the repair of the initial lesion caused by the chemical. Each of these pathways leads to recognizable biological effects. Foster has developed what she calls a mutagenic "fingerprint" to identify the DNA lesions that give rise to mutations. She does so by exposing *E. coli* to a mutagen and determining the effects of specific repair defects on the mutations that result. She then can confirm the results biochemically. According to Foster, "This knowledge can be applied to higher organisms where the biological endpoint may not be the same, but the DNA lesion is likely to be."

Foster begins her research with a known carcinogen, like *cis*-Pt. The mechanism by which *cis*-Pt and many other chemotherapeutic agents work is by killing the cancer cells at a faster rate than the normal cells. But patients using these agents run the risk of developing second-site cancers. "The cancer cells are dividing faster, which means they are replicating their DNA faster," said Foster. "So if you use a DNA damaging agent, you can achieve that differential toxicity. But over time, you're causing DNA damage and that in itself is a carcinogenic process."

By using bacteria that are defective in repair enzymes--enzymes that can be eliminated genetically--Foster can see the changes that occur in the DNA when *cis*-Pt is introduced. Once you know how the *cis*-Pt molecule is interacting with the DNA, said Foster,

you can identify what types of mutations are induced by sequencing the DNA. "So now I have a good idea of how the chemical is interacting, what the lesion is and what the result is. The next step is to break them (the *E. coli*) open, get the DNA out and find the lesion," said Foster.

According to the scientist, cis-Pt is a bifunctional agent: It can link two DNA bases together either in the same strand or across the strands of the double helix. "My suspicion is that when it's linking in one strand, that's mutational and when it's linking across the strands, that's lethal," she said. "I want to find out what kind of mutations you get under those two different conditions," she said. In other words, Foster wants to know if, for example, two mutations occur because two bases are tied up or if base deletions occur when a DNA strand loops out.

"One would like to figure out if there's a way to decrease the toxicity and potential for causing second-site cancer but maintain the chemotherapeutic activity," she said.

Foster chooses the chemicals in her research for two reasons. One is

that many of them, like cis-Pt, cause "interesting types of damage." Another is that, like EDB, they are important environmental carcinogens. As a pesticide, EDB is used in grain storage and in orchards, and, according to Foster, has shown up in produce in grocery stores.

Foster's EDB research also is applicable to human cancers, especially where occupational hazards are concerned. EDB is a carcinogen in rats, which suggests that it causes cancer in humans as well. According to Foster, the compound is not active until it is metabolically processed, usually in the liver. There are two possible pathways in humans, each using different enzymes, by which EDB may be activated into what is called the "ultimate carcinogen," capable of directly interacting with the DNA. The other pathway may detoxify the chemical.

Using the bacteria, Foster can test which of these pathways processes the EDB to the active compound. If the bacteria has only one of the pathways and mutations arise when EDB is introduced, then it's likely that the same pathway in humans will activate the compound. "I've been working for four years to be able to do these analyses very rapidly and easily," said Foster.

It's important to know which pathway activates the EDB because certain drugs, like those used to curb extreme forms of alcohol abuse, depress one route, said Foster. Therefore, if this is the detoxifying pathway, then anyone working with the chemical should not be on the drug.

Foster believes she can extrapolate from her research because the repair processes are so similar in bacterial and human DNA. In fact, she said, "I think bacteria are more sophisticated than higher organisms in this particular aspect, because they're living in the fast lane. Things have got to go right; they have to repair their damage. They don't have compartments where cells can be

safely destroyed, because they are the cell. So, they've evolved a lot of capabilities that higher organisms farm out to different cell systems."

Foster emphasizes that hers is basic research, the primary goal of which is to understand just how the DNA damage and repair systems work. "Eventually you turn to mammalian cells and ask, 'are you getting the same type of damage and lesion and is it activating an oncogene?' That's the eventual goal of all of this--that you want the whole thing, from chemical to oncogene, to make sense."

The next step for Foster, whose research is funded by grants from the National Cancer Institute, is to study eukaryotic systems, particularly yeast. A lot of research has been done on yeast, an organism containing DNA structures that are absent in *E. coli*, such as chromosomes, histones and oncogenes. "If I think about what the ultimate application is," she said, "it would be to know, in the face of environmental insult, how animals are coping with DNA damage. And then to apply that knowledge to prevent or reverse the damage."

--Cynthia A. Koury



Patricia Foster, Ph.D., hopes to find clues to cancer in the DNA of *E. coli*. (Photo by Bradford F. Herzog)

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Middle ear infections...

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Though the fluid usually dissipates fairly rapidly, in some cases the fluid may last several months. When that occurs, added Klein, problems may ensue.

"Fluid in the middle ear reduces sound conduction, on average, by about 25 decibels," he said. "As a British investigator has described it, for a young child who's just starting to acquire language skills, that's equivalent to trying to learn a foreign language with your fingers in your ears."

There is evidence that such hearing impairments interfere with learning. Part of the data comes from follow-up studies of children who were involved in the original middle ear study by Klein and his associates.

Roughly 200 of those youngsters were tested at around the time of their third birthdays for language capabilities. "Many of the children who had had a lot of ear disease had significantly lower scores on selected tests, including those for vocabulary, speech production and speech comprehension," said the investigator. Especially affected were youngsters from higher-income families, and those who had infections during the first year of life.

When the same children were tested again at around their seventh birthdays, the investigators found that children who had suffered repeated ear infections in the first three years of life still scored lower than their peers even though few were affected by new infections after they reached age 3.

Though such findings strongly suggest a need to treat the persistent middle ear fluid, the most common approach to treatment is controversial. The approach involves making a tiny incision in the eardrum, which is part of the outer wall of the middle ear, and inserting a small, short plastic tube that allows the

fluid to drain.

"There are widely differing opinions about that procedure in the field," noted Klein. "Many otolaryngologists feel that since it is an operation, and involves anesthesia, it should be done sparingly." In fact, however, there are roughly 500,000 such procedures performed each year in the nation.

Klein and BUSM colleagues, David Teele, M.D., an associate professor of pediatrics, and Stephen Pelton, M.D., an associate professor of pediatrics, have joined with a group from Children's Hospital in Boston in a new study headed by Gerald B. Healy, M.D., a professor of otolaryngology, of 2,000 children that may help resolve the issue of when surgery is justified. The study is sponsored by the National Institute of Neurologic Diseases and Communicative Disorders and Stroke.

The investigators are concerned with whether it makes sense to start treating more youngsters during their first year of life. Relatively few in that age group currently undergo surgery related to ear infections.

The study, which began in early 1986, involves monitoring every child who visits one of four designated medical-care centers: a primary care center at Boston City Hospital, the East Boston Neighborhood Health Center, and private practices in Weston and Holliston, two relatively affluent suburban towns west of Boston.

The diversity of settings reflects an effort to explore the apparent connection between socio-economic factors, learning problems and middle ear infections.

"It may be that children from lower socio-economic backgrounds have a lot of different factors that are interfering with their learning, so the hearing loss is less significant overall than for children from more affluent backgrounds," explained Klein. "In our study, we hope to clarify whether that's really what's going on."

The study also is examining other issues related to the long-term effects of middle ear infections. Among them: What are the major factors that tend to

increase a child's risk of acquiring a middle ear infection? To what extent do the effects of the fluid persist after the fluid is gone? And, is it possible to reduce the risks of recurrent infections?

Klein's earlier study pointed to a number of factors that may increase a child's risk of ear infections.

One was the fact that susceptibility seems to run in families. Klein believes this probably reflects inherited anatomical, physiologic or immunologic characteristics. He noted that the passage between the nose and the middle ear is generally wide and comparatively flat in young children anyway, thus allowing infectious agents to travel with relative ease from the nose and upper respiratory tract into the middle ear. He suspects that some children inherit a configuration that makes that passageway even more conducive than usual to movement of viruses and bacteria.

Another factor that influenced risks of middle ear infections was breastfeeding. Children who had been breast-fed during their first year of life tended to have fewer middle ear infections during that period than those who were not breast-fed.

"This tells us that breast milk probably transmits some type of immunological substance from the mother to the child," said Klein. "The key point is that it seems to confer protection during the first year." The study also indicated that children who had an episode early in the first year of life were more likely than their peers to suffer multiple infections during early childhood.

In the new study, Klein and his associates are planning to explore other potential risk factors not fully explored in the earlier research. Specifically, they want to find out if youngsters in day-care settings have a higher risk of middle ear infections than those who are cared for at home, and also if exposure to tobacco smoke increases a child's infection risks.

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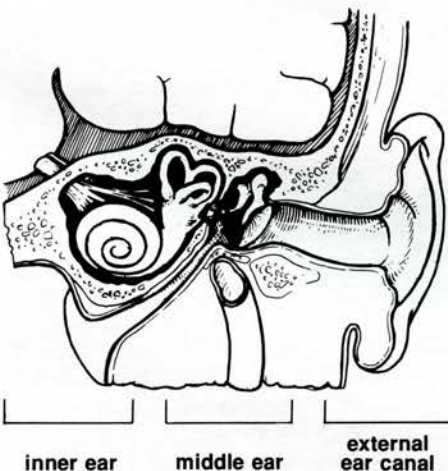
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"There's evidence that smoke, or at least some of its constituents, affects mucosa that lines the respiratory tract, including the middle ear, and may damage some of its clearance mechanisms," noted Klein. Among such mechanisms, he added, are the cilia, tiny hairs that can sweep away bacteria or viruses by means of a wave-like motion.

As the investigators seek to pinpoint additional risk factors, they also are testing a preventive therapy for children deemed at high risk for multiple infections.

The children involved are those who suffer an infection at any time during the first year of life. The drugs to be used, said Klein, are a sulfanamide (sulfisoxazole) and a penicillin (Ampicillin). If an infected child's parents consent, each antimicrobial drug

Klein believes that susceptibility to ear infection may reflect inherited anatomical characteristics. (Diagram courtesy of BUSM Educational Media)



will be administered in a modified dose to one-third of the children enrolled in the study for up to six months. The other third will serve as a control group, getting a placebo instead.

"We think we may be able to reduce the colonization rate of the infectious organisms in the respiratory tract, and by so doing, lower the risks facing these children," said Klein.

The third major aim of the study is to further investigate the effects of persistent middle ear fluid on learning. All of the youngsters will be given speech, language and cognitive tests on their first, second and third birthdays, and their performances will be considered in light of their disease patterns through that period. One goal, said Klein, is to see if there are critical periods when otitis media-related hearing problems have an especially serious impact on children's acquisition of language skills.

"If we find that infections in the first year of life don't have long-term consequences, that would suggest there is little need for an aggressive approach to treatment," noted Klein.

"On the other hand, if the infections during this period are associated with learning deficiencies in the early years of life, that might indicate that we should be aggressively treating infants with sustained middle ear effusion."

--Richard P. Anthony

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