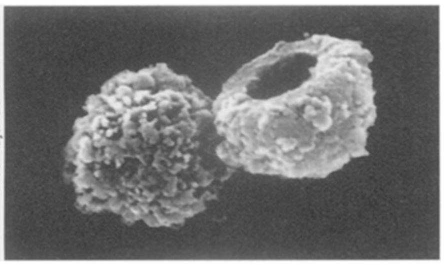
The Bacteria in the Stone

Extra-tiny microorganisms may lead to kidney stones and other diseases

By JOHN TRAVIS

In the fine tradition of transforming lemons into lemonade, here's a story of a laboratory annoyance and how it may lead researchers to a new therapy for one of the most painful maladies that people suffer.

Almost a decade ago, E. Olavi Kajander faced a problem that has frustrated many a biologist. The biochemist at the University of Kuopio in Finland was trying to culture mammalian cells, but they simply weren't thriving. The cells grew very slowly, if at all, and many contained abnormal bubbles, or vacuoles, within their cytoplasm.


Photos:Kajander *et al.*/PNAS

Electron micrograph of two mineralized shells, about 2 micrometers in diameter, created by nanobacteria.

Scientists usually grow mammalian cells in fetal bovine serum, made from the fluid part of cow blood. The serum is considered sterile, but viruses and mycoplasmas—small bacteria without rigid cell walls—sometimes contaminate it and cause problems. So Kajander and his colleagues examined their sickly cells with an electron microscope.

The investigators didn't find any viruses or mycoplasmas, but they did capture images of unusually small bacteria inside many of the cells. In the years since then, Kajander and his colleagues have isolated and characterized the tiny microorganisms, dubbing them nanobacteria because they range in diameter from 50 to 500 nanometers (see sidebar).

"They are as small as the largest viruses—and smaller," says Kajander.

Although his group's work suggested that nanobacteria often contaminate fetal bovine serum, and more rarely human blood, Kajander's research was largely ignored by his colleagues in Finland and the rest of the world.

Last month, however, nanobacteria stepped into the scientific spotlight. In the July 7 Proceedings of the National Academy of Sciences (PNAS), Kajander and his colleague Neva Çiftçioglu contend that nanobacteria live in urine and, by precipitating calcium and other minerals around themselves, induce the formation of kidney stones.

"We believe that they are the real starting point of most kidney stones," says Kajander.

That provocative claim, along with the possibility that antibiotics might help people who suffer from chronic stone formation, has caught the attention of some researchers. "It's exciting," says Leroy M. Nyberg, director of urology programs at the National Institute of Diabetes and Digestive and Kidney Diseases in Bethesda, Md. "I think it's the first real theory as to what is the nucleation factor for [kidney stones]."

"I'm sure there will be a lot of controversy around this, but I think controversy is good if it leads to experimentation," adds Dennis A. Carson of the University of California, San Diego, who published a commentary on the research in the same issue of PNAS.

Why have nanobacteria escaped the attention of microbiologists until recently? First, their small size makes them difficult, if not impossible, to see with traditional light microscopes, says Kajander. Moreover, many of the chemicals used to stain cell walls or other components of traditional bacteria fail to bind to nanobacteria.

Also, the microbes don't thrive on agar, the jellylike medium used to grow most bacteria. Finally, while many bacteria divide every hour or so, nanobacteria multiply very slowly, doubling in number about every 3 days. This slow growth makes it tough to study their metabolism, explains Kajander.

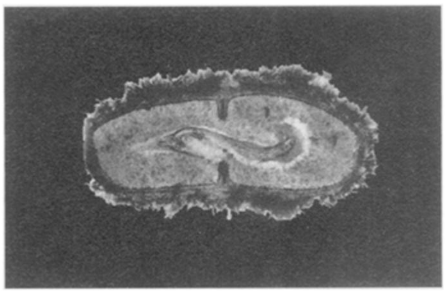
Despite such challenges, the Finnish researchers have put together a fair-size dossier on the microbes. When grown in serum, alone or with mammalian cells, they often form a white biofilm. The bacteria also appear able to trick cells that don't normally engulf microbes into incorporating them. In doing so, says Kajander, nanobacteria can trigger the cell suicide program known as apoptosis, which likely explains how nanobacteria stymie scientists trying to grow mammalian cells.

The investigators have been able to develop antibodies that bind to surface proteins of nanobacteria and have also isolated some of their DNA. In particular, they've sequenced the gene encoding a component of ribosomes, the protein-making machinery in all cells. By comparing that gene's sequence to those of the corresponding genes of other organisms, the Finnish scientists conclude that nanobacteria are closely related to *Brucella* and *Bartonella* bacteria. Some species of these bacteria are also known to infect the blood of animals and people.

Kajander and his colleagues have found that about 5 percent of Finnish people are, or have been, infected with nanobacteria. The scientists have detected nanobacterial proteins in human blood and also have grown the organisms from blood samples. Yet, blood may not be the primary habitat of these microbes. When injected into animals, the bacteria seem to move quickly to the kidneys and end up in the urine, says Kajander.

At least one other microbiologist has confirmed the existence of the nanobacteria. James W. Coulton of McGill University in Montreal, Quebec, who specializes in the surface proteins found on bacteria, has also isolated slow-growing microbes that build calcified shells. Coulton says his organisms are the same as those observed by the Finnish group and that they are indeed bacteria.

"We've isolated proteins from these organisms which are quite like the proteins of other bacteria," Coulton says. These proteins, called porins, are usually employed by bacteria to form channels that allow nutrients to enter through the cell wall.

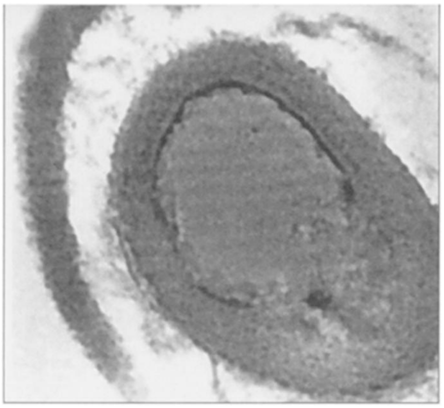


A nanobacterium, covered in hairlike apatite crystals, is caught in the act of dividing.

As Kajander and his colleagues learned more about nanobacteria, they grew increasingly curious as to whether the microbes were responsible for any human diseases, particularly ones not normally attributed to infectious agents. Such connections are not unprecedented. Take ulcers, for example. Though many physicians resisted the idea for years, most now agree that the bacterium *Helicobacter pylori* causes the majority of ulcers.

Although the evidence is not yet nearly as compelling, scientists have also built cases that illnesses as diverse as heart disease, diabetes, arthritis, and multiple sclerosis might in some cases stem from bacterial or viral infections.

An unusual feature of nanobacteria immediately suggested that the Finnish researchers look into kidney stones. Under certain growth conditions, including the acidity and mineral concentrations found in urine, the microbes somehow induce precipitation of dissolved calcium, phosphates, and other minerals into carbonate apatite, a major component of many kidney stones.



A nanobacterium found inside a partially dissolved kidney stone.

Through this still unexplained process, the nanobacteria build a mineralized shell around themselves, a structure large enough to be visible even with low-powered light microscopes. These "castles," as Kajander calls them, may be made by the microbes as a protective measure. Or they may simply result from the interaction of proteins on the nanobacterial surface with minerals dissolved in the surrounding fluid.

Whatever the explanation for the apatite-encrusted bacteria, the researchers began to wonder if such microbes provided the foundation for kidney stones, as grains of sand or other irritants provide the nucleus for pearls.

Kidney stones strike an estimated 10 percent of people in the United States at least once. Although scientists know that the painful stones form when minerals precipitate out of urine, they're unable to explain why this process begins.

"We still don't know what the nucleus is that starts the precipitation. We've never really been able to determine that," says Nyberg. "We've plateaued in stone research for almost the last decade. We really need something to open it up again."

Nanobacteria may offer that opening. Nyberg notes that some bacteria have already been linked to kidney stone formation, although the mechanism behind that connection is dramatically different. For the 5 to 15 percent of kidney stones formed largely of the mineral struvite, bacteria that produce an enzyme called urease are to blame. Urease makes urine more acidic, a condition under which mineral precipitation occurs more readily, and so indirectly encourages kidney stone formation.

Kajander and Çiftçioglu have linked nanobacteria more directly to kidney stones. In PNAS, they describe how they broke apart human kidney stones and then looked for evidence of the microbes.

In all 30 stones tested, antibodies that bind specifically to nanobacteria proteins attached to material derived from the stone. Electron microscope images also showed nanobacteria in samples of dissolved stones. Finally, the researchers were able to grow nanobacteria from some of the dissolved stones.

Kajander is confident enough of his group's result to suggest that physicians should begin testing whether antibiotics can help people with recurrent kidney stones. Nanobacteria are resistant to many antibiotics, perhaps because of their mineral shells, but tetracycline has proven effective against them in test-tube experiments, he notes. Because tetracycline accumulates on apatite, it may concentrate near nanobacteria, says Kajander.

The association with kidney stones should draw much more attention to nanobacteria, predict researchers. Coulton suggests that microbiologists may have so far shunned these microbes because

Nanobacteria by any other name

What's in a name? Controversy, sometimes. By calling their novel life-forms nanobacteria, E. Olavi Kajander and his colleagues from the University of Kuopio in Finland have landed themselves in a microbial minefield. The explosive nature of the name swirls around its previous use with a slight difference in spelling—nannobacteria—by Robert L. Folk, a geologist at the University of Texas at Austin. For almost two decades, Folk has riled microbiologists by claiming that bumps and knobs that he sees in electron microscope pictures of soils and rocks represent bacteria with diameters as small as 10 nanometers.

According to Folk, such nannobacteria are widespread and plentiful, and by precipitating various minerals, they may have had a major impact on geology. His speculations gained greater notoriety in 1996 with the discussion of possible evidence of extraterrestrial life in a meteorite from Mars. One group of scientists suggested that the so-called microfossils detected in that meteorite (SN: 12/14/96, p. 380) resemble Folk's nannobacteria.

Many microbiologists, however, argue that Folk offers no compelling evidence that his bumps and knobs are alive. These scientists remain intensely skeptical that bacteria that small can exist. "The Folk stuff really stretches the theoretical limits. . . . It doesn't just stretch them, it denies them," says Kenneth H. Nealson, a microbiologist at the NASA's Jet Propulsion Laboratory in Pasadena, Calif.

While bacteria generally have diameters of a micrometer (1000 nm) or more, biologists have for many years recognized the existence of tinier bacteria, whose diameters range from 50 to 200 nm. Below such sizes, Nealson and others claim, there's simply not enough room to contain the machinery of life as we know it.

Even a bacterium 50 nm in diameter—the smallest that Kajander describes—would be jam-packed, assuming its cell walls were about 10 nm thick and the microbe contained DNA and even one protein-making ribosome, which is 25 nm in diameter. "At 50 nm, one could imagine that things could stay alive. When you get much smaller than that, it's really hard to imagine," says Nealson.

Viruses can be much smaller, but they depend upon host cells for reproduction, so they are often regarded as not truly alive.

By calling their microorganisms nanobacteria and citing Folk's work in their paper in the July 7 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, the Finnish researchers give the geologist an undeserved legitimacy, contends Nealson. That association may also cause microbiologists to treat the research connecting nanobacteria to kidney stones with more than the usual skepticism, he suggests.

"One could have left out the word nanobacteria and had the paper be 10 times better," says Nealson.

To the microbiologist, the argument over how small life can go is not just theoretical musing. Nealson notes that NASA has planned missions to Mars that would return with soil and rocks (SN: 4/25/98, p. 265). To look for signs of life or to declare the samples sterile, biologists need to have some parameters on what they're searching for, he explains. "We need to know what the lower limit of life is," says Nealson. "If we bring back samples from Mars, do we have to search them at the 10-nanometer range? If we do, it's going to take years to say that there aren't bacteria in the samples and that they're safe."

—J.T.

they prefer to study bacteria, such as *Escherichia coli* or *Bacillus subtilis*, that grow quickly and can be genetically manipulated with ease.

"But because of the disease link, I think people are going to be a lot more open to the idea that [nanobacteria] warrant further study," he says.

The medical significance of nanobacteria may eventually extend beyond kidney stones. In a variety of human disorders, including dementias, atherosclerosis, cancers, and arthritis, unexplained calcium precipitation occurs in various tissues of the body, notes Carson in his commentary. And people who get frequent blood transfusions as part of dialysis treatment often develop dangerous calcium deposits, he adds.

Adding to the intrigue, Kajander and Çiftçioglu have conducted a preliminary study of Turkish people who are undergoing dialysis and found that 80 percent displayed evidence of nanobacteria in their blood.

"There is ample cause to investigate thoroughly the part that nanobacteria play not only in renal stone formation but also in the many perplexing diseases associated with pathological extraskeletal calcification," concludes Carson. □

Computers

Quick cracking of secret code

Cryptographers have dramatically reduced the time it takes to decode a message digitally encrypted by a widely used, government-approved method for scrambling sensitive data. Known as the Data Encryption Standard (DES), this method is used routinely by many banks and financial institutions to protect electronic funds transfers and credit-card transactions.

A custom-built computer costing less than $250,000 needed only 56 hours to identify the required numerical key—a particular sequence of 56 1s and 0s out of 72 quadrillion possibilities. That achievement shattered the previous record of 39 days, set by a network of thousands of computers communicating via the Internet.

"DES can be cracked quickly and inexpensively," says John Gilmore of the Electronic Frontier Foundation (EFF) in San Francisco, which funded the project to counter claims made by U.S. government officials that DES provides adequate protection of sensitive data. EFF announced the feat last month.

"It unambiguously demonstrates that DES is vulnerable, even to attackers with relatively modest resources," comments Matt Blaze of AT&T Labs Research in Florham Park, N.J.

Designed and built by Paul Kocher of Cryptography Research in San Francisco and his coworkers, the new code-cracking computer incorporates more than 1,000 integrated-circuit chips specifically configured for checking the strings of 56 binary digits that comprise DES encryption keys. In its record-breaking run, the computer tried about 25 percent of the possible combinations before finding the correct key to decode a message and win a contest sponsored by RSA Data Security in San Mateo, Calif. The message reads: "It's time for those 128-, 192-, and 256-bit keys."

Some businesses are already using a more robust variant of the DES method, known as Triple DES, which hasn't yet been broken. The U.S. government has also begun a competition to find a new encryption standard to replace DES. According to requirements established by the National Institute of Standards and Technology (NIST) in Gaithersburg, Md., the new method must allow encryption key sizes of up to 256 bits.

Groups from the United States, Australia, Canada, France, Germany, Japan, and Norway have submitted entries. NIST will announce this month which candidate algorithms meet all the requirements and are eligible for testing and review. —*I.P.*

Computers in the house

Survey data prepared for the National Science Foundation (NSF) show a substantial increase in U.S. household computer use in the past decade. According to the just released NSF report "Science and Engineering Indicators 1998," the proportion of households with computers increased from 8 percent in 1983 to 43 percent in 1997. At the end of that period, about 11 percent of the population reported more than one working computer at home, and one-third owned a computer with a modem. Nearly 9 million individuals had two or more e-mail addresses, and 16 percent of the population had access to the World Wide Web.

"Approximately 30 million adults had looked for specific information on the Web during the year preceding the 1997 interview," says Jon D. Miller of the International Center for the Advancement of Scientific Literacy in Chicago, who conducted the computer usage survey. This response indicates that many people are using the Web as a reference library. About a third of those searches involved information about a particular health problem.

The distribution of home computers is strongly related to level of education, the NSF report notes. Where nearly 90 percent of U.S. college graduates used a computer at home or work, only 21 percent of those who did not complete high school worked with computers (SN: 4/18/98, p. 247). —*I.P.*