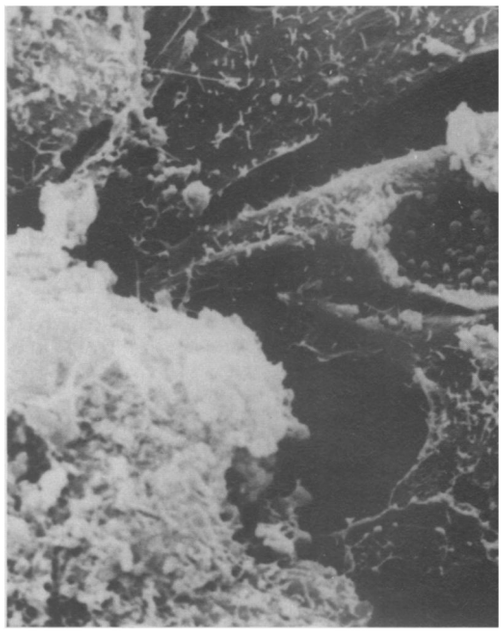
Clueing in on Chlamydia

Microbial stealth leads to reproductive ravages

By WENDY GIBBONS



It can be as sneaky as an ingrown toenail and as innocuous-seeming as the common cold. It infects more people in the United States than any other sexually transmitted disease, yet it ranks as one of the least familiar of those rarely divulged afflictions. It leads to infertility in more than 20,000 women in the United States each year, but many of them won't discover they have had the disease until they try to get pregnant.

The bacterium responsible for this trickery, *Chlamydia trachomatis,* has managed to escape much of the public recognition it deserves for its role in female infertility. Yet scientific studies conducted over the last decade have consistently shown that about 75 percent of women who cannot get pregnant because of a fallopian tube blockage also test positive for chlamydia antibodies, indicating past or present infection, notes H. Hunter Handsfield, an epidemiologist at the University of Washington in Seattle.

Scientists have also begun to blame chlamydia for a dramatic rise in ectopic, or tubal, pregnancies over the past two decades. This dangerous complication of early pregnancy occurs when the fertilized egg becomes wedged within a fallopian tube instead of making its way to the uterus, where it belongs. When the misplacement becomes apparent, physicians must remove the fetus and repair any damaged tissue. Otherwise, the growing fetus may burst through the fragile tube—an emergency that can cause massive blood loss and send the mother into physiological shock.

Last December, the Centers for Disease Control reported that more than 88,000 women in the United States developed ectopic pregnancies in 1987 — a nearly fourfold increase over the 1970 rate. Ectopic pregnancy now ranks as the leading cause of pregnancy-related death among women in the first trimester.

Although other sexually transmitted diseases can also cause ectopic pregnancy, the rising numbers appear "inextricably linked" with chlamydia, asserts A. Eugene Washington, an epidemologist and gynecologist at the University of California, San Francisco. Chlamydia may be responsible for as much as 50 to 80 percent of the increase in ectopic pregnancies, he says.

But scientists might avert the infection's tragic reproductive effects if new avenues of research live up to their early promise. Investigators have begun to unravel the basic biology of how chlamydia causes such lasting problems, and in doing so they have uncovered links to a class of proteins with mysterious connections to other diseases such as leprosy and tuberculosis. Eventually, such work may suggest ways to refine the clinical diagnosis of chlamydia, and may even yield the first effective vaccine against the disease.

Another facet of this research could produce a test that would offer women with a history of chlamydia a way to gauge their risk of infertility or ectopic pregnancy, and would assist physicians in determining which of these patients need more extensive testing to detect hidden damage resulting from the infection. Findings emerging from this work already offer clues to help explain why so many chlamydia-infected women experience no symptoms.

Physicians can diagnose an active infection by culturing *C. trachomatis* from cervical cells, and can detect a past infection by testing a blood sample for antibodies to the bacteria. But women who test positive for chlamydia antibodies have little way of knowing whether the infection has scarred their fallopian tubes. Physicians can look inside these delicate structures with an endoscope—a lighted instrument inserted through the abdomen—but most do not resort to such invasive diagnostic procedures until a woman reports difficulty getting pregnant.

C. trachomatis can live within the cells of a variety of mucosal tissues, including the cervix, the fallopian tubes, the urethra and the conjunctival membrane lining the eye. In men, the sexually transmitted infection targets the urethra, typically causing painful urination and/or a discharge. Occasionally, an untreated urethral infection will spread to the testes and cause infertility.

Men actually account for more than half the known chlamydia cases in the United States, but their outward symptoms increase the likelihood of prompt diagnosis and treatment.

In women, however, the infection strikes silently, beginning in the cervix. Antibiotic treatment at this initial stage can quench the disease and prevent fertility problems — but the absence of symptoms works against such early detection.

If untreated, the infection can go on to invade the uterus and fallopian tubes, causing a condition called pelvic inflammatory disease (PID). This intensive inflammation can result from any of several sexually transmitted diseases, but its potential for damage remains the same. In severe cases, the fallopian tubes — normally the diameter of a pea — can swell to that of a golf ball.

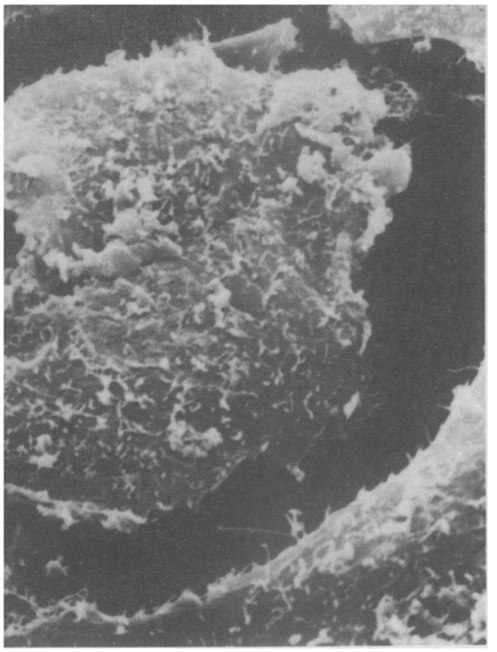
As PID sets in, most — though not all — women do experience symptoms, such as vaginal discharge or sharp pains in the abdomen. But even if a woman receives antibiotics immediately after these symptoms appear, she faces at least a 1 in 4 chance of ectopic pregnancy if she later conceives. (The overall U.S. rate is roughly 1 in 60 pregnancies.) And without antibiotic treatment, PID may produce enough scarring to completely block the fallopian tubes, leaving the woman infertile.

How can an initially "silent" infection trigger such devastating effects? The question has puzzled chlamydia researchers for years, but some tentative answers are beginning to emerge.

The first clue arose in the 1980s, when investigators began to observe that some women seem more susceptible than others both to chlamydia and to reproductive damage from the infection. Researchers reasoned that these women's immune systems might respond to the bacteria with a reaction similar to a delayed allergic response, says Richard S. Stephens of the University of California, San Francisco.

The human immune system launches its attack on *C. trachomatis* by producing antibodies that specifically recognize the invaders. In some women, however, the infection may spur a harmful immune response that could actually trigger the scarring, Stephens says.

The first strong evidence for an immune system role in chlamydia-associated fallopian scarring came in 1985 from Robert C. Brunham and his colleagues at



Scanning electron micrograph of a human cell bursting open to reveal Chlamydia trachomatis *bacteria within.*

Caldwell

the University of Manitoba in Winnipeg. The team studied a group of fertile and infertile women, all of whom tested positive for the standard chlamydia antibodies. They discovered that 11 of the 13 infertile women – but only one of the 11 fertile women – also had a particular antibody that zeroes in on a protein produced by *C. trachomatis.* This, Brunham says, suggested a connection between chlamydia's reproductive consequences and a specific type of immune reaction.

Although the researchers did not identify the protein, their findings hinted at the eventual possibility of using it in routine lab tests to detect the antibodies associated with fertility problems in women who have had chlamydia. A simple, reliable blood test for the protein-specific antibodies might help physicians fine-tune predictions of tubal pregnancy risk among such women and simplify the diagnosis of chlamydia-caused infertility, Stephens says.

But scientists needed a more detailed understanding of the mystery protein and how it might trigger inflammation before they could establish a rationale for developing such a test.

In 1987, experiments with guinea pigs yielded an intriguing discovery. At the National Institutes of Health's Rocky Mountain Laboratories in Hamilton, Mont., Harlan D. Caldwell and his colleagues started out with the observation that a rodent-specific chlamydia strain *(C. psittaci)* caused guinea pigs to develop an inflammatory eye disease – but only if the animals had already undergone an initial bout with the bacteria.

Then, using another group of guinea pigs that had recovered from an initial infection, the researchers flushed the animals' eyes with proteins washed off the outer surface of the disease-causing bacteria. The eyes swelled and eventually became scarred – indicating that the surface proteins themselves were enough to trigger disease symptoms in the previously infected animals.

Richard P. Morrison, also with the Rocky Mountain Lab, went on to direct a series of studies attempting to isolate from *C. psittaci* the specific protein or proteins that caused the eye inflammation. Again working with preinfected guinea pigs, he and his colleagues identified one candidate protein that brought on many of the symptoms. Using DNA sequencing, they unmasked the malefactor as HSP60 – a member of a distinctive class called stress proteins.

Scientists have found stress proteins in every organism they have examined, whether exotic or commonplace, simple or complex. Many different types of cells produce vast quantities of these proteins when they perceive an environmental stress such as a foreign organism or a change in acidity.

Stress proteins, also known as heat-shock proteins because researchers first identified them in bacteria exposed to heat, appear remarkably similar from one species to the next. Thus, a stress protein made by a simple bacterium resembles that made by a fruit fly or a human. One of these ubiquitous proteins is HSP60.

In 1988, scientists reported the surprising discovery that a number of stress proteins – including HSP60 – from the bacteria that cause leprosy and tuberculosis can trigger a strong immune response in humans. Other studies that same year implicated HSP60 from the tuberculosis bacterium in a type of rheumatoid arthritis, and scientists have since detected the human version of another stress protein in greater-than-normal amounts in aggressively malignant breast tumors.

Last August, Morrison's team took a crucial step from guinea pigs to humans by isolating HSP60 from *C. trachomatis* itself. Armed with copies of the HSP60 gene, researchers now have the basic ingredients to concoct a clinical test for severe complications of chlamydia, Stephens says.

With an eye toward such a test, Stephens and his colleagues had been in the process of repeating Brunham's search for a differential antibody response among chlamydia-infected women – but this time they looked specifically for the response to HSP60. In the October 1990 JOURNAL OF INFECTIOUS DISEASE, Stephens' group describes the results. Among the 21 women who had developed an ectopic pregnancy and who had a history of chlamydia infection, 17 showed antibodies to HSP60. In contrast, the marker antibodies showed up in only six of 19 women with chlamydia-caused PID who had not as yet shown signs of reproductive problems, the researchers report.

Even more promising, says Morrison, is Stephens' report in the January INFECTION AND IMMUNOLOGY, showing that women who had suffered ectopic pregnancies had antibodies that recognize a particular section of the protein. Stephens concludes that such specific recognition indicates a woman's infection has caused severe enough scarring to lead to ectopic pregnancy.

Researchers have yet to establish whether the *C. trachomatis* HSP60 can actually lead to inflammation and scarring in humans as *C. psittaci* does in guinea pigs, but Stephens suspects that it could. For one thing, he says, the bacteria "export" an abundance of the stress protein to their outer cell membrane. "They make a ton of it," Stephens says. "I like to think that this sort of unusual export would have a lot to do with the really excessive inflammation."

Because chlamydia HSP60 closely resembles human HSP60, the immune system may not recognize the chlamydia version as foreign during an initial infection, he explains. With continued exposure, however, immune cells called T-lymphocytes may eventually recognize the chlamydia stress protein. Unfortunately, these same T-cells may also target the look-alike human HSP60, launching an indiscriminate attack on healthy human cells as well on the protein-cloaked bacteria, Stephens suggests. The result: progressive inflammation and scarring characteristic of PID and trachoma.

Morrison questions this scenario. "Right now, it's speculation," he says. Human and chlamydia stress proteins, though similar, may not resemble each other closely enough to fool the immune system, he adds.

Morrison says his guinea pig experiments strongly support a different mechanism for the severe inflammation and scarring that develop in susceptible individuals. These symptoms, he suggests, reflect an allergic-type reaction to HSP60, primed by an earlier bout with the infection.

Stephens and Morrison agree on one point, however: A test for detecting HSP60 antibodies could help identify women at high risk of severe complications from chlamydia infection.

Stephens says he plans to expand his ectopic pregnancy study in order to examine in more detail how different degrees of fallopian scarring correlate with the immune response to HSP60. He also has begun to identify pieces of HSP60 that might give a better indication of scarring than the whole protein.

Devising a highly accurate, easy-to-use

test for the risk of ectopic pregnancy will take extensive research, Brunham and Morrison caution. And even if scientists perfect such a test, the risk assessment may have limited usefulness, says Nicholas R. Kadar, a epidemiologist at the Robert Wood Johnson Medical School in New Brunswick, N.J. Physicians can do little, he says, to restore scarred fallopian tissue to its former state.

However, says Kadar, if an HSP60 test could predict infertility as well as the risk of ectopic pregnancy, in many cases it could allow women to bypass the elaborate and costly techniques now used to diagnose infertility. "It would save a lot of grief," he asserts.

The new findings about chlamydia's progression to PID also have important implications for researchers seeking a vaccine against the infection. Given that HSP60 may lead to a damaging inflammatory response, these investigators must ensure that their vaccines do not contain it, and must find another major chlamydia-produced protein to use as an immunity-triggering antigen.

In separate efforts, Stephens' and Caldwell's groups — as well as several others — are focusing on a particularly promising protein called major outer membrane protein (MOMP). Like HSP60, MOMP is abundant on the bacterium's outer surface. Stephens and Caldwell have narrowed their searches by looking for pieces of MOMP that might serve as an more effective basis for a vaccine than the whole protein.

Last June, at an international chlamydia symposium in Harrison Hot Springs, British Columbia, researchers announced the first evidence that a MOMP vaccine could help protect guinea pigs against *C. psittaci* infection two weeks later. The vaccine provided only partial immunity, however, says study participant Roger G. Rank of the University of Arkansas in Little Rock. "The intensity of infection was depressed, but the length wasn't any shorter," he told SCIENCE NEWS.

Because chlamydia microbes congregate only in mucosal tissues, the traditional strategy of conferring general, systemic immunity may not provide complete protection against future infection, Caldwell suggests. This means that vaccine developers must find ways to enhance immunity within the mucosal membranes themselves, he says.

"The trick is how to target or deliver the MOMP," Caldwell says. "It's probably a very challenging goal."

Ultimately, investigations of chlamydial PID may have benefits that extend beyond the reproductive realm. A clearer understanding of how the infection leads to scar-producing inflammation might help researchers find a way to stop the spread of yet another disease caused by *C. trachomatis*. This highly contagious eye infection, called trachoma, is transmitted by flies as well as by direct contact among humans. A painful disease that eventually leads to blindness, trachoma currently afflicts millions of children in Third World countries. "These kids don't know what it's like not to feel like they have sand in their eyes," Stephens says.

The growing recognition of the chlamydia's reproductive ravages also raises the stakes for preventing the sexually transmitted infection, or at least halting its progression before it spreads beyond the cervix. The many women today who seek to overcome infertility by undergoing expensive and painful high-tech methods, such as *in vitro* fertilization, offer poignant testimony to chlamydia's effects on people's lives.

Until researchers develop a vaccine or discover other ways to protect individuals from the disease, efforts to curb chlamydia's consequences must focus on early detection and treatment, Handsfield says. He emphasizes the need for more frequent testing to identify ongoing infections, especially among young, sexually active women who face the greatest threat of the disease but might otherwise remain oblivious to its presence.

"We really need to control this from the start," Handsfield says. □