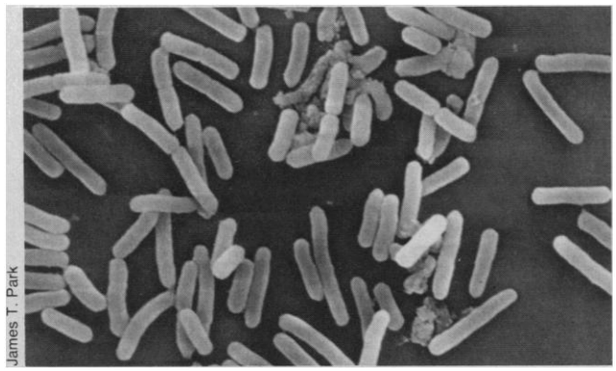
Bad News Bacteria

The complex genetics of increased virulence and increased resistance to antibiotics is turning certain bacterial strains into a deadly threat

MUPPETEER JIM HENSON'S sudden death in May shocked millions of children and adults who shared a special fondness for his creations. But to medical researchers Henson's death was unsettling on a different count. The bacterial infection to which he succumbed—a particularly virulent strain of group A streptococcus—causes a newly recognized kind of toxic shock, called toxic shock–like syndrome (TSLS), that can fell otherwise healthy people within hours of the onset of symptoms. The illness has recently surfaced in England, Scandinavia, Australia, East Germany, Canada, and New Zealand, as well as in the United States, and no one yet knows why the organism that gives rise to strep throat, impetigo, and scarlet and rheumatic fever in children is suddenly causing an acute, fatal disease in adults.



Harboring resistance. *Harmless bacteria (such as these* Escherichia coli*) serve as a pool of genes for antibiotic resistance—which can then be transferred to harmful strains.*

One theory holds that TSLS is due to the reappearance of the bacterial toxin that causes scarlet fever, a toxin that disappeared earlier in this century. But that's only one hypothesis, and the appearance of TSLS is confirmation of the fact that bacterial diseases are still far from being understood at the molecular level. The pacemaker for the cyclical variations in virulence, for example, has never been established. In recent years the tools of molecular biology have helped researchers pick apart some aspects of virulence, including some of the genetic "master switches" that control bacterial toxicity. Many of the protein products of those genes, however, are still shrouded in mystery.

In addition, the problem of treating bacterial infections is greatly complicated by another issue: resistance. The widespread use of antibiotics—in humans and in animal feed—has fostered the rise of drug resistance among harmless and harmful bacteria alike. Most strains are resistant to at least one antibiotic—and some of them are resistant to almost all the known antibacterial treatments. What is even more disturbing is that it is now clear that harmless bacteria can serve as a reservoir for resistance genes, which can then be transferred to virulent strains within an individual, spawning a microbial one-two punch: a highly virulent, resistant pathogen.

Some investigators hope they will eventually be able to disarm the virulent bacteria by attacking the harmful genes themselves rather than undermining the viability of the entire organism. By not killing the killers, this strategy would interrupt the Darwinian struggle that allows the resistant strains to proliferate. For such an approach to succeed, however, both the disease process and drug resistance would have to be understood in considerably more molecular detail than they now are.

The epidemic of TSLS indicates how difficult that search may be. TSLS can start with a mild skin infection or with a sore throat and a cough. Named after a similar syndrome that is commonly associated with staphylococcus infections that developed in some women who used particularly absorbent tampons, TSLS progresses rapidly to a high fever, a drop in blood pressure, and a loss of circulation that can result in death or necessitate amputation. "It's not a disease you're likely to forget," says Patrick M. Schlievert of the University of Minnesota Medical School.

Schlievert, an expert on the toxins produced by group A strep, is one of those who has suggested that there is a link between TSLS and the recent reappearance of strep toxin A, which launched scarlet fever epidemics earlier in this century. Group A strep stopped making toxin A in the 1940s, and with that the cases of scarlet fever vanished. "Why it disappeared over the past 30 years isn't clear," says Vincent A. Fischetti of Rockefeller University. "But it's come back."

Some investigators think TSLS is simply scarlet fever returning in a more severe form. They view its sudden onslaught as part of a general surge in the virulence of group A strep evidenced by a recent increase in the incidence of rheumatic fever in children.

Others disagree. Ben Schwartz, an epidemiologist at the Centers for Disease Control, says TSLS doesn't look like scarlet fever to him. "This is not just a repeat of scarlet fever, only worse," Schwartz says. "My reading of the medical literature is that this disease has never been seen before." Schwartz thinks the strep could be manufacturing an entirely new toxin or a mutated version of an old one that causes a novel clinical syndrome. "How can toxin A come back and not cause the same disease that it used to?" he wonders.

More data might clear up the dispute. Last fall the CDC began working with state and local health departments in six states—Maryland, Alabama, Ohio, Colorado, Arizona, and California—to provide clinical and epidemiological information and isolates from all patients with group A strep invasive disease. The states were selected based on their geographical distribution and willingness to cooperate in the project, not because of any evidence of increased infection, Schwartz notes. In all, between 10 and 15 million people will come under surveillance.

The difficulty of pinning the blame for TSLS on any one mechanism typifies the uncertainty surrounding the etiology and expression of bacterial disease. The same species of bacteria can produce different disease determinants—in this case, toxins—and the same toxins seem to have different effects on various populations at different times. Fischetti offers the example of a strain of streptococcus that caused an outbreak of rheumatic fever in one community while coexisting peacefully with the human popu-

lation of another.

Sometimes it is not even clear which traits are essential for an organism merely to survive and proliferate and which are actually responsible for causing disease. The fuzzy boundaries between the two sets of traits are due partly to the complexity of the system responsible for pathogenicity. Molecular studies carried out over the past decade have shown that pathogenicity is multifactorial: it depends on the action of many different genes and gene products acting at different times during infection.

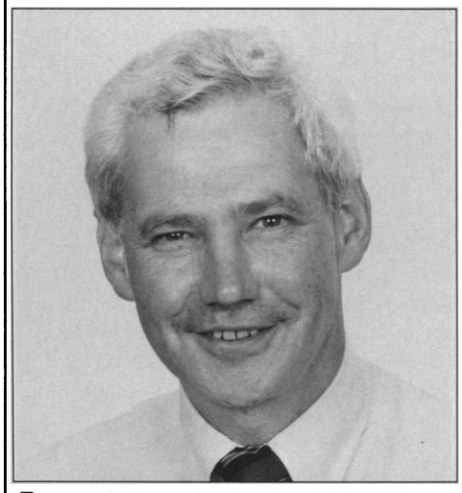
Investigators have had some success in identifying the master switches of virulence, which coordinate expression of many other genes in response to environmental conditions such as temperature, osmolarity, and pH. For example, the *toxR* gene of *Vibrio cholerae*, which causes cholera, regulates at least 14 other genes. In *Staphylococcus aureus*, the *agr* locus controls the expression of at least 12 different proteins.

But understanding the genetic switches that turn virulence on and off doesn't guarantee an understanding of virulence itself, which clearly relies on a complex array of other genes and their products. Eduardo A. Groisman of the University of California at San Diego studies the *phoP* regulatory locus of *Salmonella typhimurium*, which causes a typhoid fever–like illness in mice. Even with the benefit of an animal model—which many infectious diseases lack—the critical gene products that give rise to the symptoms of the illness have been impossible to pinpoint. "We know more about the regulator than the genes that it regulates," laments Groisman.

Indeed, for *Salmonella*, "the question of what are the virulence genes . . . is nowhere near being answered," says Robert Tauxe of the CDC. "Some people say there might be as many as 100 or 200. What 'virulence' means in *Salmonella* is sort of like the blind men describing different parts of the elephant."

The other side of the picture in evaluating bacterial diseases has to do with human beings—both the individual patient and the society in which that patient lives. One of the people who has given the most thought to these factors is Stanley Falkow, a microbial geneticist from Stanford who thinks the status of the person who catches the bug has been a neglected element in many studies of infectious disease. Falkow, who titled an introduction to the molecular basis of pathogenicity, "The 'Zen' of bacterial pathogenicity," also thinks that many current bacterial diseases are "essentially diseases of human progress." He cites outbreaks of salmonellosis propagated by mass preparation of food and the menstrual toxic shock syndrome that resulted from more absorbent tampons.

In some respects the spread of bacterial resistance can also be seen as a plague of progress, Falkow argues. For example, the routine use of antibiotics in animal feed began with the aim of preventing disease in farm animals and promoting their growth (see box on next page). Yet the use of antibiotics may have become a kind of "poi-



Zen and the art of microbial maintenance. *Stanley Falkow of Stanford University.*

son pill"—by applying tremendous selective pressure in favor of the resistant strains.

It has been known for some time that the genes for resistance can be carried by commensals (the normal human microbial flora) such as nonpathogenic forms of *Escherichia coli*. In fact, it seems such normal flora have become a reservoir of resistance determinants that pathogenic bacteria can tap. As an example, the resistance to ampicillin that arose in *Haemophilus influenzae* and then in *Neisseria gonorrhoeae* in the 1970s came from *E. coli*. "There's a pool of resistance genes ready to jump over into the pathogenic organism," says CDC's Tauxe.

And some of these strains carry genes for resistance not just to one but to many therapeutic agents. Tauxe and his colleagues recently published a chilling account of an outbreak of a multiply resistant strain of *Shigella* on a Native American reservation in Arizona in 1983. More important than its vividness is the fact that their report was one of the first to document the transfer of resistance traits from *E. coli* to a pathogen within one individual.

The individual who triggered the epidemic was a 57-year-old Hopi woman with a 5-year history of urinary tract infections treated with trimethoprim and sulfamethoxazole (TMP/SMZ). In September 1983, the woman was hospitalized with dysentery caused by a strain of *Shigella* that was resistant not only to TMP/SMZ but also to a big part of the antibiotic arsenal: ampicillin, carbenicilin, streptomycin, sulfisoxazole, and tetracycline. Such a multiply resistant *Shigella* strain had not been recognized before.

But within 3 weeks, *Shigella* isolates from six other people on the reservation were reported to show the same pattern of resistance. The resistant strain had spread from a 2-year-old boy who visited the woman to his 7-month-old cousin, his mother, his cousin's father, and two friends of the family.

Tauxe and his colleagues examined several of the *Shigella* isolates as well as a sample of the *E. coli* from the 57-year-old woman's urinary tract. They found similar if not identical resistance plasmids in all isolates and concluded that the original plasmid had been transferred from the *E. coli* in the woman's digestive tract to the invading *Shigella*.

Between 1983 and 1985 shigellosis resistant to TMP/SMZ appeared on the larger Navajo reservation that surrounds the Hopi site. And in those 2 years, the incidence of resistance increased from 3% to 21%. Although among the Navajo the resistance seemed to be conferred by a number of different plasmids, Tauxe and his colleagues presented evidence that, once again, commensal bacteria were acting as a reservoir of the highly mobile genetic elements—such as plasmids—that carry the genes for resistance. "The plasmids are playing their own game," says Tauxe. "And it's a separate game from the one the bacteria are playing."

In light of his experiences, Tauxe says it might make sense for clinicians to get a sample of a patient's normal microbial flora and test it for resistance before prescribing treatment. In one of the first studies of its kind, Marilyn C. Roberts and her colleagues at the University of Washington School of Public Health in Seattle evaluated the mechanism of tetracycline resistance in the urogenital flora of individuals who were neither sick nor taking antibiotics. Between 30 and 90% of the hundreds of isolates they evaluated carried genes for resistance to tetracycline that had been picked up from commensals, pathogens, or both.

The conventional wisdom is that the mechanisms of antibiotic resistance are more one dimensional, in a genetic sense, than the determinants of pathogenicity. But that may change—as bacteria begin mobilizing more intricate defenses to match their elaborate offensive genetic strategies. Alexander Tomasz, a colleague of Fischetti's at Rockefeller, has pointed out that the mechanisms of antibiotic resistance are bound to appear "crude and simplistic" compared to the specialized bacterial functions that mediate

pathogenicity. After all, Tomasz notes, the interactions between host and pathogen have evolved on an evolutionary time scale, whereas "the history of antibiotics and antibiotic resistance can be measured in decades."

But in a paper prepared for a 1989 workshop on the microbial determinants of virulence and host response, Tomasz argues that "the deployment of antibiotics in the clinical environment which began in earnest in the late 1940s may indeed be considered a kind of modern, nondiscriminating 'host response.' " He goes on to describe the strategies of resistance that pneumococci and staphylococci are elaborating in response to the beta-lactam drugs, which are currently the most important class of antimicrobials. These defensive strategies include the restructuring and even novel synthesis of enzymes for making cell walls and the activation of a phalanx of auxiliary genes.

Tomasz concludes that "in complexity and in the apparent integration of a multiplicity of bacterial functions and genes for an essentially adaptive task . . . the story of antibiotic resistance begins to resemble that of bacterial virulence genes."

Barbara E. Murray of the University of Texas Medical School in Houston concurs. She notes that strains of enterococci, a common cause of hospital infections, have recently been isolated that are resistant to the antibiotic vancomycin—which means that enterococci resistant to every commonly used antibacterial have now been found. The enterococci could spread vancomycin resistance to staph and strep as well. When bacterial pathogens such as the enterococci become unassailable by antibiotics, she says, "resistance is functionally equivalent to a virulence trait."

Thus, it is clear that resistance has begun to rival virulence in its complexity and in its dimension as a medical problem. In addition, as the experience of the Hopi and Navajo reservations show, although resistance and virulence have no a priori relationship, they are clearly becoming overlapping problems in epidemiology.

How can these twin threats be contained? There may not be any easy answer. As in many other problems related to progress, the clock cannot be turned back. An outright ban on the use of antibiotics is clearly not a practical solution. Stuart B. Levy of the Tufts University Medical School and the New England Medical Center has called for stepping up research aimed at finding new antibiotics for which resistance determinants have not been found and agents that could interfere with the mechanisms of resistance already established. Yet he admits that such drugs would just be another assault in a perpetual war with the spread of resistance.

Falkow has, typically, preached a more Zen-like approach. In order to defeat infectious disease, he says, you have to "think like a microorganism." Disease, after all, isn't what microbes want—because disease elicits bodily defenses designed to destroy the invaders. The most effective treatment for bacterial infection, he claims, would be one that allows the bacteria to persist in the body while it dismantles the genetic systems that cause the host distress. Such an approach is not inconceivable. But it will require a great deal more progress in research, relying as it does on an understanding of disease that even nature herself might envy.

■ KAREN WRIGHT

Karen Wright is a free-lance science writer based in New York.

The Policy Response: In Limbo

Many researchers believe that widespread use of antibiotics in animal feed has led to development of drug resistance in bacteria—and ultimately affected human health. The policy response to this problem, however, has been anything but brisk. Indeed if one word could be chosen to characterize policy-making in this area over the past 13 years, the word would be "limbo."

Farm animals are the target of nearly half the antibiotics produced in the United States, mostly in the form of low or subtherapeutic doses aimed at preventing disease or promoting growth. Most are broad-spectrum antibiotics such as penicillin or tetracycline. Although it's difficult to prove an association between antibiotic use and resistance, there is strong indirect evidence, including a landmark 1986 Centers for Disease Control report correlating an increase in drug resistance among strains of *Salmonella* with drugs in animal feed.

In 1977 the Food and Drug Administration took a tentative first step toward banning low doses of penicillin and tetracycline in feed when it published a notice of opportunity for hearing in the *Federal Register*. Such notices give interested parties a chance to convince the agency that further evidence needs to be aired before a regulation is instituted. The agency then decides whether or not a hearing is justified.

But in this case the FDA never made a decision. "It's very frustrating," says Gary Dykstra of the agency's Center for Veterinary Medicine. "Every time we try to do something definitive, Congress pokes its head in."

Congress first poked its head in in 1978, when it directed the FDA to evaluate more rigorously the health risks of using antibiotics in feed before it took action. The FDA complied by requesting a report from the National Research Council of the National Academy of Sciences, a report duly issued in 1980.

In its fiscal 1981 appropriations hearings, Congress butted in again, asking the FDA to delay a decision on the hearing until some of the research recommended in the NRC report could be carried out. It attached a carrier to the agency's funding stipulating that the agency spend $1.5 million on such research.

The FDA contracted for a study on resistance in *Salmonella* and *Campylobacter* from the King County Department of Public Health in Washington State. That study appeared in 1984, the year the National Resources Defense Council petitioned the Department of Health and Human Services to suspend approval of penicillins and the tetracyclines in subtherapeutic doses in animal feeds on the grounds that their use constituted an imminent hazard to human health.

The FDA determined that there was insufficient evidence to back the NRDC's charges, and the petition was rejected. The issue languished until 1987, when commissioner Frank Young requested yet another study—this time a predictive risk evaluation—from the NAS's Institute of Medicine.

When the IOM study came out at the end of 1988, the director of the Center for Veterinary Medicine, Gerald B. Guest, asked Dykstra to form a working group to review the issues raised by the study. The group recently concluded its review and is moving to present its recommendations to acting commissioner James Benson in July.

"People have spent an enormous amount of time on these reports and they've been left dangling," says Stuart B. Levy of Tufts University Medical School and the New England Medical Center. "I think the data are sufficient to warrant substituting broad-spectrum antibiotics with narrow-spectrum antibiotics or other therapeutics that wouldn't result in resistance."

"We're hoping to have some decisions made by the end of the summer," says Dykstra. But he doesn't sound as if he's holding his breath. ■ K.W.