ENHANCEMENT CHAPTER

Infectious Disease and Bioterrorism

(George Johnson, Washington University, St. Louis)

Concept Outline

13e.1 To defeat an infectious disease, you must control its transmission.

The Battle Against Infectious Disease. To control killer diseases like plague, typhus, malaria, and cholera, it is necessary to prevent their being communicated from infected people to healthy ones.

13e.2 Biological warfare programs open Pandora's box.

Bioweapons. Any effective bioweapon must be easy and safe to produce, practical to disperse, and do its job (that is, be lethal or incapacitating, depending on the bioweapon).

Germ Warfare. For several decades the United States and Russia carried out extensive bioweapons programs. The American program was discontinued in 1969, but the Russian bioweapons program continued for another two decades.

A Closer Look at Anthrax. Anthrax is a lethal infectious disease spread as spores. Weaponizing anthrax spores involves considerable technology.

A Closer Look at Smallpox. Smallpox, one of history's greatest killers, was eradicated from earth in 1980. However, there was extensive bioweapons development of smallpox in Russia in the 1980s, and samples kept for research preserve the threat of future release.

13e.3 Future threats may involve novel pathogens.

Declaring Biowar on Crop Plants. The spores of pathogenic fungi that attack corn or wheat might be effective bioweapons directed against key American crops.

The Nightmare of Gene-Modified Pathogens. Inserting human genes into infectious pathogens may produce lethal bioweapons against which there is no defense.

FIGURE 13e.1
The influenza epidemic of 1918 killed 20 million in just 18 months. With 25 million Americans alone infected during the influenza epidemic, it was hard to provide care for everyone. The Red Cross, seen here with masks over the faces of the nurses, often worked around the clock.
Source: Courtesy of the National Library Museum.

Humanity's ongoing battle with infectious disease stretches back as far as recorded history, and involves many kinds of protists, bacteria, and viruses. Often disease has had a major impact on history. The flu epidemic of 1918 (figure 13e.1) killed 20 million people worldwide, more than died in the World War which preceded it. With the success of antibiotics in treating bacterial killer diseases like typhus and cholera, many of us have been lulled into thinking that the battle against disease has been won. However, with the advent of antibiotic-resistant strains, many diseases like tuberculosis are making a comeback. In addition, the new century has seen the introduction of a new and more deadly way for disease to spread -- by the deliberate actions of terrorists. The anthrax attack on America in 2001 has left no doubt about the reality of the threat of bioterrorism. In this chapter we examine this harsh and regrettable reality.

13e.1 To defeat an infectious disease, you must control its vector.

The Battle Against Infectious Disease

Very few students reading this chapter have been seriously affected by infectious disease. One of the greatest triumphs of modern medicine has been the control of infectious disease. Particularly with the advent of antibiotics and immunization, it has become possible to eliminate or treat diseases which used to kill tens of thousands of Americans each year. Largely safe from the threat of dying due to infection, it is easy for us to forget that the infectious diseases that killed people in the past are perfectly capable of doing it again. In less fortunate countries lacking the modern medical care we take for granted, infectious disease remains a real danger. Nearly 2 million people will die of malaria this year, and some 3 million of tuberculosis (TB).

Much of our success in combating disease has come from understanding the transmission of particular diseases from one person to the next. Some disease agents pass directly from one individual to another; others are transmitted by living infectious agents, called **vectors**. A vector is a living agent that transmits a disease. One of the great lessons of the long battle against infectious disease, perhaps the greatest lesson, is that to control the spread of a disease, you must control its transmission.

As a way of understanding the problems posed by infectious disease, and the success we have had in combating it, it is useful to take a look at some of the big killers, their vectors, and the reservoirs of these vectors.

Rodents as Disease Reservoirs

A variety of serious human diseases are transmitted by flea vectors that reside on rodents (rats and mice), a reservoir found worldwide. Among the most important of these "rodent diseases" are bubonic plague and typhus.

Bubonic Plague. Plague is a deadly disease caused by the bacterium *Yersinia pestis*. The plague bacteria are carried from one person to the next by fleas on rats (also wild rodents and squirrels). Common in wild squirrel populations in the western United States today, plague killed one-fourth of the population of Europe in the 14th century. Plague is not the major killer it used to be, as its 14th century reservoir, rats, are not as numerous as they used to be and don't move about, carrying plague from one focus of infection to another in the way that used to spread the disease rapidly within human communities.

Typhus. Typhus is one of the greatest killers of people in recorded history. It strikes in times of crowding and poor sanitation. It is caused by a small kind of bacteria, *Rickettsia: R. typhi* is transmitted from one human to another by the bite of rat fleas, and *R. prowazekii* is transmitted from one person to another by human lice. Soon after infection, an acute fever develops, and a rash appears on the chest only on the 5th day. One of the most deadly of diseases, typhus has a peak untreated mortality rate of 70%—seven in ten people contracting typhus die of it. In the Crimean war (fought between a British/French/Turkish Coalition and Russia in 1854-1856), before the typhus vector and its reservoir was understood, this disease had a devastating impact:

war casualties	197,339	*typhus casualties*	767,411
war dead	63,261	*typhus dead*	104,494

Eighty percent of casualties and 62% of deaths in the Crimean War were due to typhus! The British, horrified, set out to understand typhus better, and learned that the disease passes from one human to another in two ways: on the lice that often inhabit an unbathed soldier's hair, and on fleas carried by rats from one person to another. To control the disease, they set out to eliminate the vector. Rats were ruthlessly exterminated in army camps, as were lice on soldiers' heads. Army camp sanitation and bathing were improved. In the first world war, the British had not one death due to typhus. The Russian army, which adopted none of these measures, lost over a million soldiers to typhus.

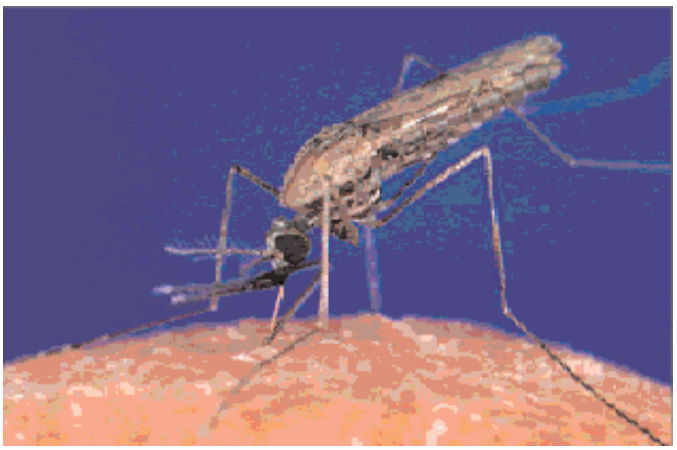

FIGURE 13e.2
The malaria vector. Control over malaria only became possible when a British doctor, Ronald Ross, discovered in 1897 that mosquitoes transmitted the disease from one person to the next. Source: Centers for Disease Control and Prevention courtesy of James Gathany.

Insects as Vectors

Among the most contagious and deadly of infectious diseases are those carried by arthropods—principally flying insects. The greatest killer among these diseases is malaria (its life cycle is described in chapter 14 on page 328).

Malaria. In 1941, more than 4,000 Americans died of malaria. In the year 2000, by contrast, only five people died of malaria in the United States. The key was a discovery made many years before in the summer of 1897 by an English physician, Ronald Ross, working in a remote field hospital in

Secunderabad, India (figure 13e.2). Malaria, which took more than a million lives in India that year, was known to be caused by a microscopic parasite called *Plasmodium*, which could be found in the blood of malaria victims. However, no one was sure how the parasite was transmitted. How did infected people acquire the parasite? Working alone, Ross discovered the answer to this key question.

Ross observed that patients in the field hospital who did not have malaria were more likely to develop the deadly disease in the open wards (those without screens or netting) than in wards with closed windows or screens. This suggested to Ross the hypothesis that in the open wards, mosquitos, a kind called *Anopheles*, were spreading the disease from patients with malaria to patients who did not have the disease. To test his hypothesis, Ross compared the blood of mosquitos that had fed on malaria patients with the blood of mosquitos which had fed on uninfected individuals. In the blood of mosquitos that had fed on malaria patients he found parasites; in that of mosquitos that has fed on uninfected individuals, he did not. He carefully dissected each mosquito's stomach and found that mosquitos that had fed on malaria patients contained living malaria parasites. By contrast, when he gathered newly-hatched mosquitos that had not yet eaten, fed them blood from people who did not have malaria, and examined their stomachs, he found no malaria parasites. Ross went on to show by careful dissection that the parasites spread through the mosquito's body to its salivary glands, passing in the saliva to anyone the mosquito bites. The idea that malaria epidemics could be prevented by combating the mosquito vector was first put forth in a letter written by Ross to the government of India in 1901. Before the end of that year, American army doctors had eliminated almost all malaria from Havana, Cuba—where malaria had been at an epidemic stage—by greatly reducing the mosquito population. Malaria was virtually eliminated in the United States when discovery of DDT and other insecticides made it possible to eliminate the *Anopheles* mosquito vector by spraying.

Yellow Fever. Yellow fever is caused by a flavivirus, and spreads from one human to another by the bite of mosquitos. Infection results in a high fever that is often fatal. If untreated, this disease has a peak mortality of 60%. During construction of the Panama Canal early in the last century, yellow fever killed in excess of 20,000 before American army doctors learned that mosquitos were the vector transmitting the virus. Strenuous programs to eliminate mosquitos quickly brought the yellow fever epidemic in the Canal Zone under control.

Human Reservoirs

Some of the most serious infectious diseases are transmitted directly from one person to another without a vector—in a very real sense, we ourselves are the reservoir. Among these diseases are influenza, one of the greatest killers of all time, killing 20 million in 18 months in 1918-19 (described in chapter 13 on page 308), smallpox (described later in this chapter), hemorrhagic fevers like Marburg and Ebola (described in chapter 13 on page 310), tuberculosis (described in chapter 13 on page 300), and cholera.

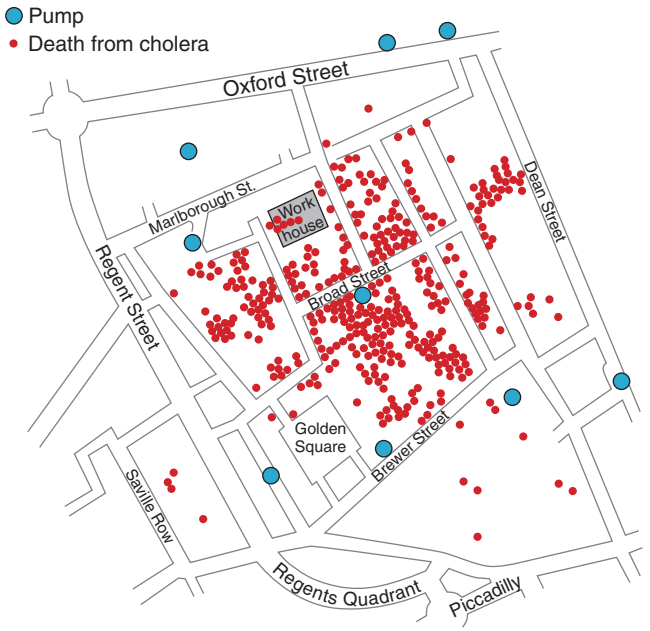

FIGURE 13e.3
Discovery of how cholera spreads. There have been six major pandemics of cholera in the last two centuries. During the 1850s London epidemic, John Snow made a map of cholera deaths in London, enabling him to pinpoint a feces-contaminated well on Broad Street as the source of the epidemic.

Cholera. Another of the great killers, cholera is a bacterial infection causing severe diarrhea that can lead to death by dehydration. Peak mortality is 50% if the disease goes untreated. Like typhus, cholera is a major killer in times of overcrowding and poor sanitation; over 100,000 died in Rwanda in 1994 during a cholera outbreak. There have been six major pandemics of cholera in the last two centuries. During the epidemic in London in the 1850s, John Snow linked the transmission of cholera to consumption of water contaminated with human wastes. In an attempt to understand the epidemic, he carefully mapped the location of each fatality. Snow's map showing cholera deaths in London in 1854 (figure 13e.3) enabled him to pinpoint a well on Broad Street as the source of the epidemic. The well was contaminated with human feces, suggesting for the first time that poor sanitation was the culprit. The disease is controlled by good sanitation, which prevents its spread from one individual to another. The infectious agent that causes this disease was not clearly recognized until Robert Koch discovered the causative bacterium, *Vibrio cholerae*, in 1883.

The "killer" infectious diseases are kept under control by controlling the transmission from one human to another.

13e.2 Biological warfare programs open Pandora's box.

Bioweapons

Starting in the 1950s, the United States and Russia began programs that turned the concept of controlling infectious diseases upside down. Instead of destroying disease vectors, military researchers developed technologies to make vectors and their toxins more effective. Cheaper and less destructive than hydrogen bombs, it was anticipated that "germ" weapons could achieve equally devastating effects .

What Is a Bioweapon ?

While humans are subject to a large number of infectious diseases, most of these diseases are not suitable for use as a bioweapon. To be effective, any bioweapon must meet three deceptively simple criteria:

1. The pathogen must be easy and safe to produce. One of the major perceived advantages of bioweapons over nuclear armaments was that they cost far less to manufacture. Because the pathogen will be required in large amounts—literally tons—it must be possible to scale up its production for reasonable cost, and to produce large amounts of it safely.

2. The pathogen must be hardy and practical to disperse. An infectious disease like typhus would make a poor bioweapon because it requires an insect vector—rat fleas—to spread it. An ideal pathogen would be composed of particles that are carried through the air like pollen, infecting those who breathe them. This suggests that the most effective bioweapons will be based on pathogens acquired through inhalation.

3. The pathogen must be effective. One use of a bioweapon is to incapacitate enemy troops on the battlefield. Soldiers that are violently ill rather than dead must be cared for, tying up the battlefield resources of your enemy. If this is the weapon's intent, then it is important that the pathogen not kill those infected by it. Rather, they should stay quite ill so as to require intensive care. Quite the reverse is true, however, if the goal of the bioweapon is simply to kill large numbers of enemy troops. Such a pathogen should be as lethal as possible.

Choosing a Bioweapons Agent

Incapacitating Bioweapons. For an incapacitating bioweapon, military bioweapons developers have focused on four pathogens which rarely kill but produce devastating illness (table 13e.1). One such bioweapon developed by the Russian military is based on *tularemia*, an animal disease that affects humans much like pneumonia. While severely debilitating, tularemia is rarely fatal. As a battlefield weapon, it has the disadvantage that symptoms do not appear until several days after exposure.

To create an incapacitating bioweapon, the American military took a different approach. They developed a "cocktail" of three pathogens, chosen so that one acted very quickly, one a little later, and the third after a while and for a long time. The fast-acting agent was not a pathogen at all, but rather a toxin produced by one, *staphylococcal enterotoxin B*. Within a few hours of inhaling small amounts of this toxin, people suddenly become violently ill, running a very high fever for several days. Then the second agent kicks in, a virus disease called *Venezuelan equine encephalitis* which continues the fever, and adds nausea, vomiting, and diarrhea. After 10-12 days, the third agent takes over, a bacterial disease called *Q fever* which produces respiratory distress, acute headaches, and high fever persisting for three weeks or more. While totally incapacitating, this mixed-pathogen bioweapon is not lethal, killing fewer than one in a hundred.

Lethal Non-Infectious Agents. For a lethal bioweapon that will kill anyone directly exposed to it, but no one else, there are really only two choices that satisfy all the requirements noted above. One is *botulinum toxin*, a highly poisonous protein manufactured by the bacteria responsible for food poisoning. This toxin is one of the most deadly chemicals known—a hundred millionth of a gram (a few molecules) is enough to kill you. As a bioweapon, it could be expected to kill large numbers of people, but would not be easy to administer on a battlefield without endangering one's own troops. It is an ideal bioweapon for a terrorist intent on attacking a city, however, as it could be added to a community water supply and reach large numbers of people.

Another lethal noninfectious bioweapon is pulmonary anthrax. Sometimes called "inhalational anthrax," this deadly disease results from the inhalation into the lungs of spores from the bacterium *Bacillus anthracis*. Because it is so deadly (40-80% of infected individuals die) and spores offer a ready means of disseminating the disease, anthrax is the bioweapon of choice for killing large numbers of people on a battlefield without setting off an epidemic that might spread elsewhere.

Lethal Infectious Agents. If the aim of a bioweapon is mass destruction—to kill very large numbers of people—then one of three infectious deadly pathogens are available. The least deadly, *smallpox*, is the weapon of choice. Smallpox would probably kill the greatest number of people, for while its mortality is only 30%, smallpox is highly infectious. *Hemorrhagic fevers* like Ebola have far higher mortality, sometimes exceeding 95%, but epidemics tend to spread only through half a dozen people before halting. *Plague* also has a high mortality, but is not easily spread from person to person.

Only a small number of pathogens fit the requirements for a bioweapon, but those that do are terrifyingly apt.

Table 13e.1 Bioweapons

These naturally-occurring pathogens are among those reported as having been developed into biological weapons by the United States or Russia.

INCAPACITATING

Q fever

agent: *Coxiella burnetii* (a rickettsia-like bacterium)

transmission: transmitted by ticks, by inhalation of the bacteria from animal hides, or by direct contact with infected farm animals.

symptoms: an acute respiratory illness; onset is abrupt 10-12 days after infection; chills, throbbing headaches, and high fever (up to 104 degrees) may persist for 3 weeks or more.

death: rarely fatal; peak mortality of untreated individuals is less than 1%.

tularemia

agent: *Francisella tularensis*

transmission: a disease of rabbits, deer, and other animals transmitted by deerflies and ticks, or by direct contact.

symptoms: ulcers and skin lesions, fever, and pneumonia appear 3-5 days after exposure.

death: disease is disabling but rarely fatal; fewer than 5% of untreated cases die.

horse encephalitis

agent: Venezuelan equine encephalitis virus (VEE)

transmission: transmitted from horses to humans, and among humans, by mosquitos.

symptoms: severe headache, high fevers, nausea, vomiting, cough, and diarrhea, lasting for several days, followed by weeks of weakness and lethargy.

death: in humans, not usually fatal; the peak mortality rate in untreated adults is less than 1%, in children 4%.

LETHAL NON-INFECTIOUS

pulmonary anthrax

agent: *Bacillus anthracis*

transmission: spores drifting in the air; a cattle disease transmitted to humans by spores; anthrax spores can be ingested, can infect the skin (cutaneous anthrax), or can be inhaled (pulmonary anthrax).

symptoms: fever, fatigue, flu-like symptoms at first, followed within 6 days of exposure by severe breathing difficulty and turning blue.

death: 24-36 hours after onset of severe symptoms. Peak untreated mortality: 40-80%. Treatable with antibiotics (penicillin, ciprofloxin, doxycycline) if given immediately after exposure.

LETHAL INFECTIOUS

pneumonic plague

agent: *Yersinia pestis* (bubonic plague)

Transmission: transmitted from one individual to another by rat fleas; in the absence of fleas, pulmonary form may be transmitted by exhaled air droplets to nearby individuals.

symptoms: high fever, chills, and headache begin within 6 days of exposure and progress quickly to severe breathing difficulty and coughing up blood.

death: within 2-4 days after onset of symptoms. Peak mortality if untreated approaches 95%.

smallpox

agent: *Variola* virus

transmission: transmitted via exhalation of tiny droplets containing the virus that are then inhaled by others; highly contagious in the days immediately aftere the onset of the rash.

symptoms: high fever, fatigue, headaches and backaches begin about 12 days after exposure, followed in 1-2 days by a rash and lesions on face, arms, and legs.

death: within 2 weeks after onset of symptoms; peak mortality 40%.

hemorrhagic fever

agent: Marburg/Ebola viruses

transmission: the natural reservoir of these Central African viruses are unknown; the virus is transmitted between individuals by contact with body fluids, and perhaps by respiratory transfer.

symptoms: the virus infects the connective tissue lining blood vessels; the infection produces high fever, muscle aches, chills, and diarrhea within a few days, followed by shock and often by extensive bleeding.

death: death occurs within a week of infection; peak mortality can be as high as 92%.

TOXINS

botulinum toxin

agent: *Clostridium botulinum*

symptoms: toxin attacks the cholinergic nervous system, causing death by paralysis.

death: a deadly biochemical; contact with minute amounts (as little as a millionth of a gram) of toxin is fatal.

staphylococcal enterotoxin B

agent: *Staphylococcus aureus*

symptoms: acts in 3-12 hours; produces chills, headache, and high fever (up to 106 degrees) for several days

death: incapacitating but rarely fatal.

Germ Warfare

Since the United States and Russia began large-scale bioweapons programs after World War II, the possibility of "germ warfare"—war using infectious disease as a weapon of mass destruction—has been a nightmare growing ever closer to reality.

The American Bioweapons Program Stops

In the summer of 1968 the nightmare achieved chilling closeness. Near a small atoll in the South Pacific a thousand miles southwest of Hawaii, American forces were in the midst of highly secret tests of germ warfare weapons. At sunset one quiet July day an armada of ships was positioned in the ocean waters around Johnston Atoll, upwind from a line of barges with hundreds of cages containing Rhesus monkeys on their decks (figure 13e.4). A lone Marine Phantom jet flew in low past the island, then shot off over the horizon. As it passed the island, a pod under one wing released a powder into the air, a long tendril of smoke that soon disappeared. Only a small amount of powder was released in the few minutes the plane shot across the several miles of this "line source laydown," and the wind soon carried the tiny particles out to sea. A thin, long curtain of powder swept past first one barge loaded with monkeys, then, at increasing intervals, another and another, finally passing the most distant barge nearly fifty miles away. Afterwards, the monkeys were taken back to Johnston Atoll. Over the next few days half of them died. Even the monkeys positioned fifty miles away from the laydown were not protected by distance. Anyone watching the test that day knew beyond any doubt that bioweapons really work, that germ warfare could be used to kill millions of people.

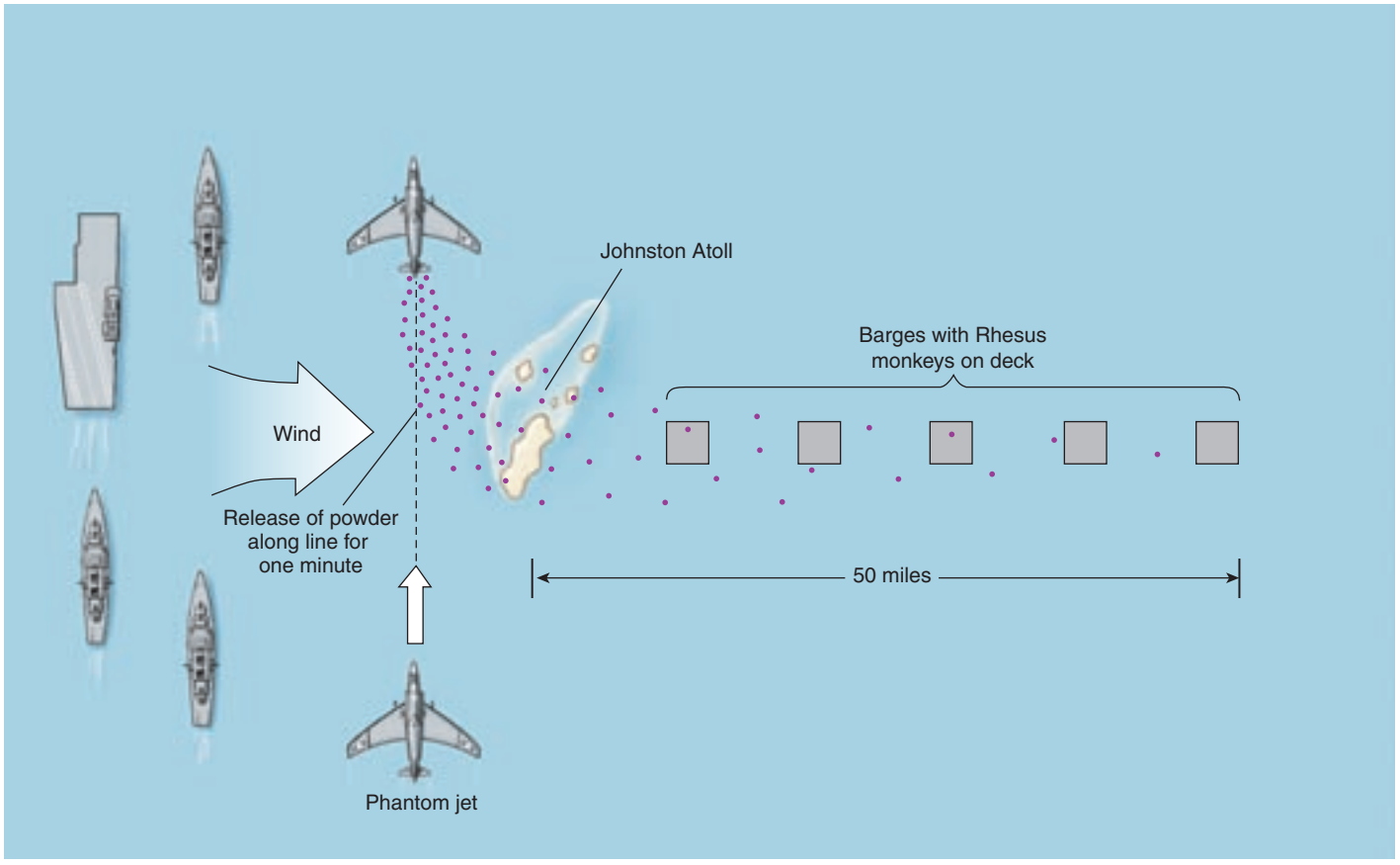

FIGURE 13e.4
Proving that bioweapons work. In this 1968 weapons field test near Johnston Atoll in the South Pacific, the bioweapon agent was released as a fine powder by a jet, and carried by the wind past a series of barges. Rhesus monkeys on the barges were severely affected for up to 50 miles.

The nature of the bioweapon tested at Johnston Atoll has never been confirmed by the U.S. military, but it seems likely to have been a weaponized form of anthrax. In 1969, one year after the secret Johnston Atoll tests, President Nixon renounced the use of bioweapons by the United States, and ordered all American bioweapons destroyed and American research into germ warfare halted. In 1972 the United States signed the Biological and Toxin Weapons Convention, along with over 140 other countries. The convention called for the destruction of all stocks of offensive bioweapons, and termination of all research on their development.

The Russian Bioweapons Program Does Not

Russia also signed the treaty, but interviews with defecting senior officials of its bioweapons program now reveal that Russia viewed America's retreat from bioweapons as an opportunity for them to gain a military advantage. The bioweapons treaty had no inspection provisions (It has been speculated that American pharmaceutical industries feared the loss of trade secrets), so no one outside of Russia knew at the time that, instead of stopping, the Russian military massively expanded their bioweapons program. Their focus was on anthrax, bubonic plague, and a lethal fever of horses called glanders caused by *Pseudomonas mallei* recently renamed *Burkholderia mallei*). Nor was the bioweapons program limited to research. At the height of the Russian bioweapons programs, the Soviet military was manufacturing more than a thousand metric tons of each of these agents each year (table 13e.2).

The cessation of American vaccination for smallpox in 1980 (the year after the disease was officially certified as eradicated by the World Health Organization) was viewed by the Russians as another strategic opportunity, and they began a program to "weaponize" the *variola* virus—to modify the virus so that particles of it can be efficiently disseminated in tiny aerosol droplets. They then set out to produce the weaponized smallpox virus on a very large scale. Ken Alibekov, former first deputy chief of research (that is, second in command) for the Soviet bioweapons program, who since defected to the United States, reports that by 1989 over 20 metric tons of weaponized smallpox had been produced, and tons of it loaded into missiles to be dispersed in bomblets.

The Continuing Threat

With the economic meltdown of the Soviet Union in the 1990s, the Russian bioweapons program ground to a halt. The bioweapons are said to have been destroyed, lest the treaty violation be proven. Substantial numbers of the scientists and technicians working in the large Soviet bioweapons programs found themselves without salary. Iran, Iraq, Syria, and North Korea actively recruited some of these workers (most apparently resisted the temptation to emigrate), and with the lax security of Russian research centers in recent years no one knows what knowledge or bioweapons samples might have found their way to countries sponsoring terrorism.

In the classical myth, Pandora's box, once opened, is not easily closed. That is the nightmare of bioterrorism we face—we may never be rid of the weapons we have created. Weaponized anthrax, smallpox, and other lethal bioweapons have not been destroyed (samples preserved in government laboratories in the United States and Russia may have escaped their confinement. We cannot rule out the possibility that rogue states may have acquired one or more of the bioweapons, and might at any time provide them to others. The largely ineffective anthrax attack launched through the mails against American news organizations and the United States Government in September, 2001 suggests the reality of the danger.

Table 13e.2 Bioweapon Production (metric tons)

UNITED STATES	
staphylococcal enterotoxin B	1.9
tularemia (*F. tularensis*)	1.6
Q fever (*C. burnetii*)	1.1
anthrax (*B. anthracis*)	0.9
Venezuelan equine encephalitis virus	0.8
botulinum	0.2
SOVIET UNION	
tularemia (*F. tularensis*)	1,500
anthrax (*B. anthracis*)	4,500
bubonic plague (*Y. pestis*)	1,500
smallpox (*variola* virus)	20
glanders (*P. mallei*)	2,000
hemorragic fever (Marberg virus)	50

While America and Russia have ceased their bioweapons programs, the weaponized agents and the knowledge of how to prepare and use them may have escaped control.

A Closer Look at Anthrax

Anthrax is a disease of cattle, goats, and sheep caused by a bacterium, *Bacillus anthracis*. It is rare for humans to be infected. Most infections that do occur are localized to small cuts in the skin whose edges turn black (hence the name "anthracis", after anthracite coal). The disease is deadly for humans because *B. anthracis* produces lethal toxins. Like other members of the *Bacillus* genus, *B. anthracis* produces endospores (figure 13e.5). An endospore is a tiny dormant cell, a tough package of DNA wrapped in protein that a bacterium makes when times are tough, sort of a "seed" that can persist for centuries, until times improve and the spore germinates to reestablish the anthrax population. The problem arises because humans can inhale these spores. If the strain is a virulent one (most are) and a person inhales a few thousand spores, the spores may establish themselves in that person's lungs, producing an infection that is often fatal. This form of infection, pulmonary anthrax, does not occur often—the last fatal case of pulmonary anthrax in the United States until the attack on America in 2001 was in 1976.

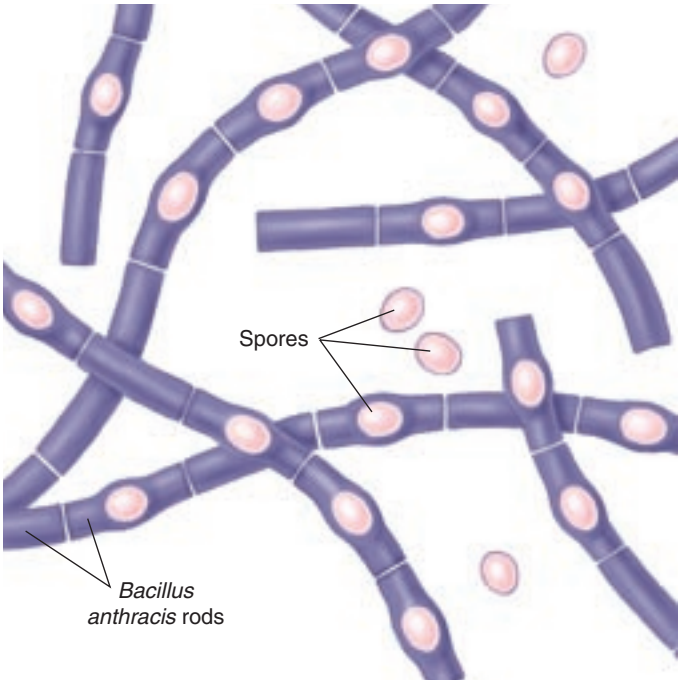

FIGURE 13e.5
Anthrax spores. *Bacillus anthracis*, like many other members of this genus of bacteria, forms tiny spores that can travel for considerable distances in the air. Breathing of these spores can lead to pulmonary anthrax.

How Anthrax Kills

Evolution has designed the anthrax spore as an efficient killing machine. The surface of the lungs are patrolled by scavenger cells called macrophages that engulf foreign matter and alert the immune system to infections. When a macrophage encounters an anthrax spore, it ingests it. Once inside the macrophage cell, the spore germinates into an anthrax bacterium, which starts to grow and divide. Soon a cluster of anthrax bacteria burst out of the macrophage into the bloodstream, and begin reproducing explosively.

Carbon dioxide in the blood is the trigger that begins the killing of the infected individual. The carbon dioxide activates the anthrax's toxin regulation control, a gene called *AtxA*. This gene in turn switches on three other genes, an intricate three-part mechanism to kill animal cells. The first of these three genes produces a protein called *protective antigen* (named before its role was understood) that is designed to dock onto the receptor proteins that stud the surface of macrophages (figure 13e.6). Proteins made by the other two genes stick onto the docked protective antigen protein like sticky balloons. Successful docking then triggers a process called "receptor-mediated endocytosis" that introduces the attack complex into the macrophage cell.

Now the protein made by the second gene, called *edema factor*, comes into play. It is an enzyme, and it begins to busily produce a molecule used by cells for internal communication. It produces so much of the signal that the immune system cells which should detect and remove the infected macrophage become confused and fail to do so. In effect, the excessive amount of signal disables the body's first line of defense against infection.

Now the stage is set for the protein made by the third gene, called lethal factor. This powerful toxin is also an enzyme. It causes the macrophages to start madly producing two powerful agents that provoke local inflammation. The two agents, tumor necrosis factor (TNF-alpha) and interleukin-1-beta, are natural parts of the immune response, but an excess of them produces quick death.

Treating Anthrax Infections

Like most Gram positive bacterial infections, anthrax can be treated effectively with antibiotics if administered early in the infection. Because of worries that an anthrax infection may involve weaponized anthrax that has been made resistant to penicillin, other antibiotics that works differently, ciprofloxacin (CIPRO), iprofloxacin, and doxycycline are the drugs of choice in treating anthrax infections.

An effective vaccine against anthrax was first produced by Louis Pasteur in 1880 using heat-inactivated bacteria. Today's vaccines are a complex broth of proteins filtered from a nonthreatening strain of anthrax. Shots must be repeated for several months to gain full protection. New alternative vaccines based on a genetically engineered version of a single key antigen are going into clinical trials, and are anticipated to produce 95% protection with a single shot.

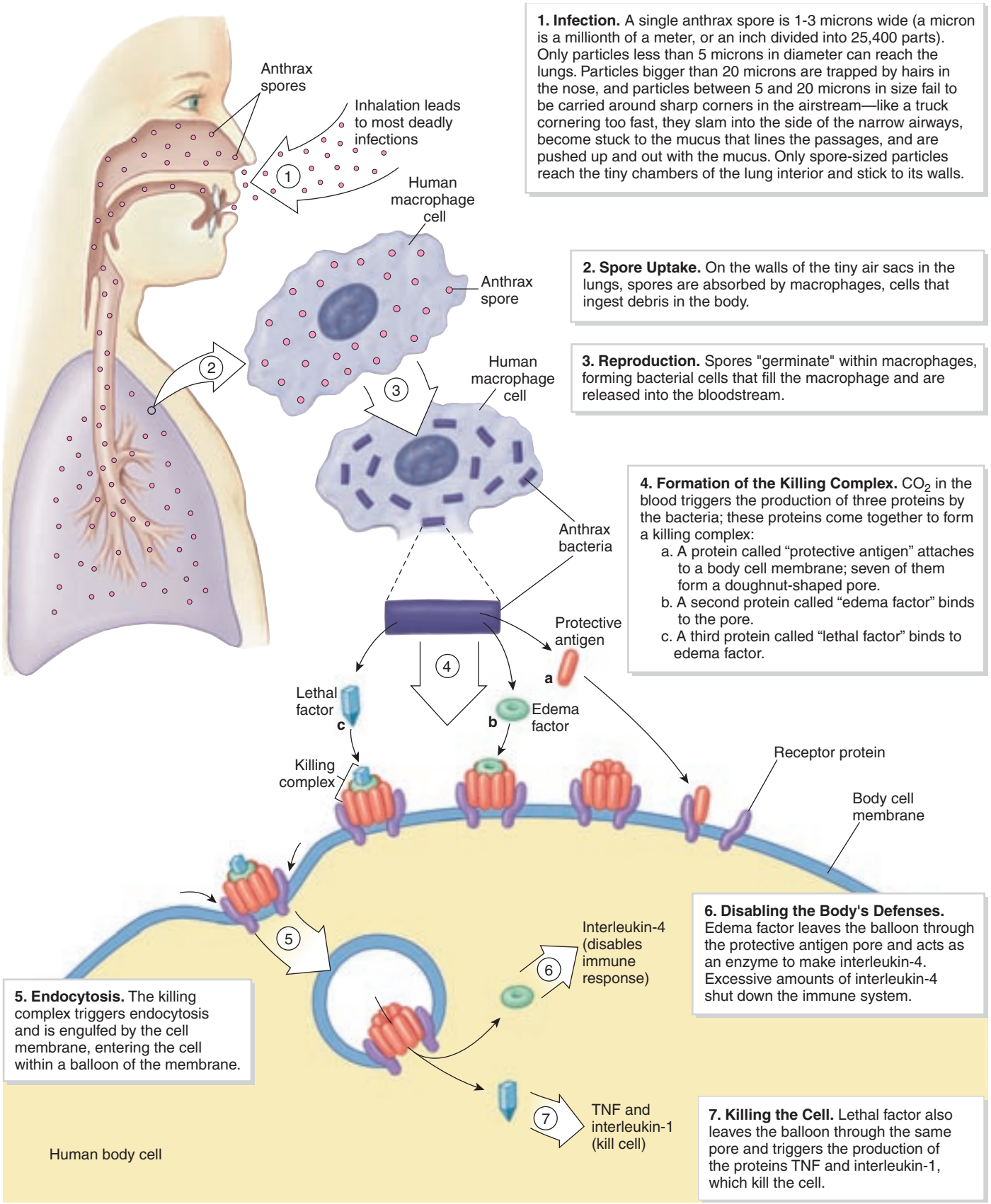

FIGURE 13e.6
Anthrax's deadly journey. How humans contract pulmonary (“inhalation”) anthrax.

Anthrax Has Been Used As a Bioweapon

Because it is deadly, noncontagious, and dispersed by spores, anthrax has always been considered a good candidate for a bioweapon (table 13e.3). Late in 2001, this possibility became a reality. Letters containing anthrax spores were sent to several news reporters and two United States Senators. Five people died of inhalational anthrax as a result of exposure to these spores.

A major investigation has been launched to identify the terrorist who sent the Anthrax Letters. Examination of the spores in the letters reveals that a great deal of sophisticated technology was used in producing them, the sort of technology found in a government bioweapons program:

1. A deadly strain. Many naturally occurring strains of anthrax are not virulent, and there is great variation in the potency of the strains that are. More than 90 distinct strains (subspecies) of anthrax are known worldwide. Examination of the spores in the Anthrax Letters revealed them to be of a particularly virulent strain called the Ames strain, developed by the U.S. bioweapons program.

2. Weaponized spores. The spores in the anthrax letters were particularly deadly because the particles had been processed to improve their dispersion through air. A dense solution of the spores was first converted to a powder by freeze-drying the spore solution—basically, the solution was frozen solid, then placed in a vacuum so that the water sublimated off. After freeze drying had removed the water, the solid block of spores was stable and dry at room temperature.

Spore particles in the anthrax letters were of a uniform 5 micron size, ideal for human inhalation (larger particles would be trapped by the hairs and mucus-lined walls of the nasal passage). To make 5 micron particles, it would have been necessary to "mill" the solid block of spores to obtain particles of just the right size. This could not have been done simply by grinding the freeze-dried block of spores, as rough grinding damages the spores being released from the block. In bioweapons laboratories, spores are released from the surface of the block by gentle milling, lubricated by clays such as bentonite, with dislodged 5 micron spores collected on a moving stream of air. Traces of lubricant were in the Anthrax Letters.

The surfaces of natural spore particles have electrostatic charges which cause individual spore particles to clump together. The spores in the Anthrax Letters did not clump. Their electrostatic charges had been neutralized. In bioweapons programs this is done, in effect, by adding soap. Detergent molecules bind to the surface charges, neutralizing them. The spores in the Anthrax Letters bear traces of specially-designed detergent additives developed by the U.S. bioweapons program.

3. No antibiotic resistance. To be maximally effective as a bioweapon, weaponized strains of anthrax are often genetically modified to be resistant to antibiotics. This was not true of the version of the Ames strain used to prepare the spores in the Anthrax Letters.

4. Delivering over a wide area. Military bioweapons programs have focused on aerosol sprays that deliver the spores over wide areas in cluster bombs, a very effective approach so long as the heat from the explosion of the bomblets does not inactivate the spores (tiny plastic spheres are mixed with the dry spores to absorb the heat). The terrorist who sent the Anthrax Letters chose a far simpler delivery system, the mail.

Because of the highly technological processing they had undergone, and its detailed similarity to procedures developed by the U.S. bioweapons program, it seems likely that the source of the spores used in the Anthrax Letters was one of the U.S. bioweapons laboratories.

Table 13e.3 Anthrax through the ages

1500 B.C. -- Fifth Egyptian plague, affecting livestock.

1600s -- "Black Bane," thought to be anthrax, kills 60,000 cattle in Europe.

1876 -- Robert Koch confirms bacterial origin of anthrax.

1880 -- First successful immunization of livestock against anthrax by Louis Pasteur.

1915 -- German agents acting in the United States believed to have injected horses, mules, and cattle with anthrax on their way to Europe in World War I.

1937 -- Japan starts biological warfare program in Manchuria, including tests involving anthrax.

1942 -- England experiments with anthrax at Gruinard Island off the coast of Scotland. The island has only recently been decontaminated.

1943 -- United States begins developing anthrax bioweapons.

1950s and '60s -- U.S. biological weapons program continues after World War II at Fort Detrick, Maryland.

1968 -- Anthrax bioweapon reported to have been successfully tested at Johnston Atoll in Pacific.

1969 -- President Richard Nixon ends United States' biological weapons program.

1972 -- International convention outlaws development or stockpiling of biological weapons. Russia signs the convention, then secretly undertakes massive expansion of its bioweapons program, making tons of smallpox and anthrax.

1979 -- Weaponized anthrax aerosol released accidently at a Russian military facility, killing about 68 people.

1990-93 -- The terrorist group, Aum Shinrikyo, releases anthrax from rooftops in Tokyo, but no one is injured.

1995 -- Iraq admits it produced 8,500 liters of concentrated anthrax as part of a bioweapons program.

2001 -- Letters containing milled anthrax are mailed to U.S. news organizations and Congress in the first use of bioweapons by terrorists.

A Closer Look at Smallpox

Smallpox has been one of the most deadly diseases in human history. Caused by a virus named *variola* (from the Latin word for "spotted"), smallpox is an ancient human disease. Introduced to the New World by one infected slave in Cortez's second voyage, smallpox had a devastating effect on the American Indian population, which had no native immunity. While there are no precise numbers, roughly 90% of the Indian population of Mexico and Central America died within 100 years, over 12 million people. Later, in New England, the Native American population went from 72,000 in 1600 to 8,600 in 1674, the deaths largely from smallpox. The Huron Indians lost two-thirds of their population in eight years! These numbers are of particular interest today, because the American smallpox vaccination program stopped in 1980, and the vaccine only protects effectively for 7-10 years. Americans today have never been exposed to smallpox, and are as vulnerable as the Indians who first met Cortez.

Smallpox is highly infectious, passing in the air within tiny droplets of moisture from infected individuals to others. For 12 days there are no symptoms, as the virus multiplies within an infected individual. For several days before onset of symptoms, virus levels are high enough that the person becomes infectious, spreading the virus to others by the simple act of breathing. On about the twelfth day a fever appears, soon followed by a rash and spots all over the body. Over a period of days the spots become disfiguring pustules and the fever continues. One in three infected individuals die.

The Eradication of Smallpox

Humans are the only hosts of the smallpox virus. No animal reservoirs exist. Fortunately, an effective vaccine exists. Indeed, vaccination was invented by Edward Jenner in the 1790s to combat smallpox by inoculating people with its harmless relative, *vaccinia* ("cowpox"). Thus if all susceptible people can be inoculated, it should be possible to eradicate the disease.

Officials of the World Health Organization of the United Nations reported in 1948 that widespread vaccination had eliminated smallpox from North America and Europe. By 1959 the disease had been eliminated throughout much of the Western Hemisphere, and an intensive worldwide campaign was initiated. As late as 1967 smallpox was still common in thirty three countries, with ten to fifteen million cases occurring that year.

Attention then switched from attempts at universal vaccination to a focus on individual outbreaks. Every time a case was reported, the sick individuals were isolated and everyone in the vicinity was vaccinated. Asia was clear of the disease by 1975. By 1977 Somalia, in Africa, was the last country on earth in which the scourge of smallpox persisted. A 23-year-old resident of Merka, Somalia named Ali Maow Maalin contracted the last known case of naturally-occurring smallpox anywhere in the world in 1977 (figure 13e.7).

FIGURE 13e.7
The last smallpox victim. Ali Maow Maalin of Somalia is the last known individual in the world to have contracted smallpox, which left permanent lesions on his chest.
Source: Centers for Disease Control and Prevention courtesy of the World Health Organization.

The Continuing Threat of Smallpox

Smallpox is an ideal bioweapon, if the object is mass destruction of life. Russia produced 20 metric tons of *variola* virus during the high-point of its bioweapons programs, only destroying this lethal harvest in the late 1980s as Russia dismantled its bioweapons effort.

There has not been a reported human death of smallpox since the death of a laboratory worker in 1978. Because the smallpox virus requires humans to spread, its total absence as a disease anywhere in the world ensures that it is extinct—except in two government research laboratories, one at the Centers for Disease Control and Prevention in Atlanta, and the other in a laboratory in Russian Siberia. The destruction of these last samples of the virus was delayed repeatedly as scientists studied them, each country feeling the need to develop better vaccines lest the other use the virus as a weapon. The possibility that another nation, or a terrorist group, has obtained the virus led the United States in 2001 to order the production of 300 million doses of smallpox vaccine, enough to vaccinate every American.

Smallpox has been eradicated as a disease, but there is fear that the virus may find its way into terrorist hands.

13e.3 Future threats may involve novel pathogens.

Declaring Biowar on Crops

While the development of bioweapons by the United States and Russia in past decades focused on human pathogens, there is another potential target which also represents a real danger—the crops we eat. Cereal grains feed most Americans, and most of the people on earth. Fully one half of the calories consumed by humans are obtained from wheat, rice, and corn. A bioweapon targeted at cereal grains could, if used successfully, have a staggering impact.

Plants are certainly subject to as many diseases as humans. Roughly one-eighth of crops worldwide are lost to disease each year. There are four major groups of plant pathogens that affect crop plants:

1. *Pseudomonads.* These soil bacteria cause most important plant diseases.
2. *Pathogenic fungi.* A variety of rusts and smuts attack cereal grains.
3. *Mycoplasmas.* Transmitted by insects, mycoplasmas infect corn and many kinds of citrus.
4. *Viruses.* Over 600 plant diseases are caused by viruses, often slowing growth rather than killing.

Designing a Crop Plant Bioweapon

For maximum impact in North America, a plant bioweapon should be directed against corn or wheat. Both of these cereal grain crops are subject to serious fungal diseases.

Corn. A smut caused by the pathogenic fungus *Ustilago maydis* infects the cells of growing corn plants, causing the infected cells to form large growths called galls. Serious infestations often lead to the total loss of ears, so that the corn plant produces no useful food. The fungus responsible for the disease grows in the soil during the winter; in the spring, its spores are spread by the wind. Landing on the leaves sheathing corn ears, the spores germinate and infect the plant.

Wheat. A rust caused by the pathogenic fungus *Puccinia graminis* attacks wheat, forming reddish lesions on the stem and leaves. *Puccinia* spores germinate on the leaves and form hyphae that enter the plant interior through stomata (tiny pores on the underside of leaves). As the fungus grows within the wheat plant, it erupts with the reddish lesions characteristic of rusts, releasing spores that can travel 100 miles to infect other wheat plants. *Puccinia* is a very damaging pathogen of commercial wheat in the United states. Over one million tons of wheat annually are lost to stem rust in the United States (figure 13e.8).

The spores of either *Ustilago* or *Puccinia* spread readily on the wind. Weaponizing the spores of either fungus would employ much the same technology as used with *Bacillus anthracis* spores. Techniques for propagating large cultures of the fungi would need to be developed, and ways found to induce massive spore formation. Spores could be converted into easily-dispersed particles by freeze-drying, milling, and treatment with charge-neutralizing detergents, just as has been done with anthrax.

Puccinia is a particularly dangerous bioweapon candidate, as it possesses the advantages of both anthrax (its spores are lethal to its target, and the spores are easy to convert into a stable dry powder) and smallpox (infections are self-propagating, spreading from a single focus of infection to epidemic proportions). Like anthrax, many subspecies of *Puccinia* are known—over 200 have been collected and described. This makes it particularly difficult to breed wheat that is *Puccinia*-resistant; resistance to one subspecies need not confer resistance to others.

The spores of pathogenic fungi that attack corn or wheat might be effective bioweapons directed against key American crops.

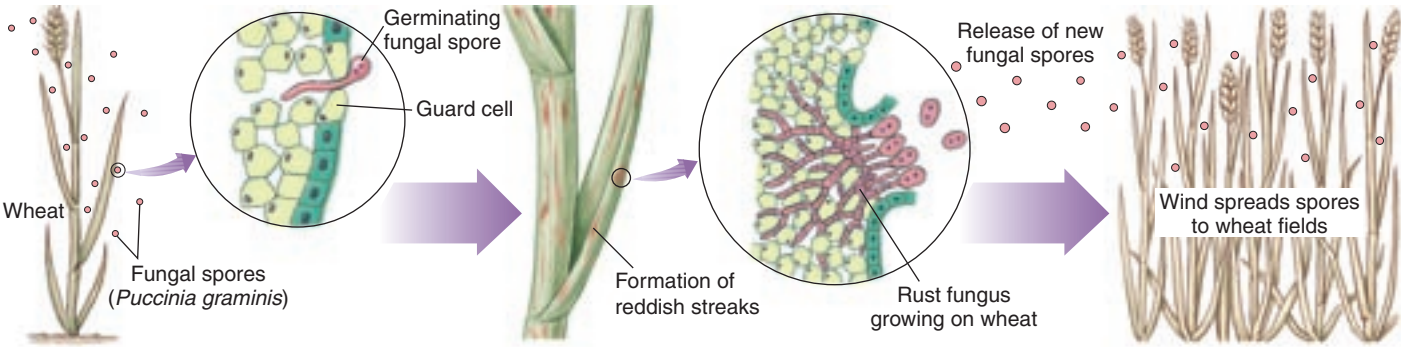

FIGURE 13e.8
How a *Puccinia* epidemic starts.

The Nightmare of Gene-Modified Pathogens

The Russian bioweapons programs were not limited to producing massive amounts of weaponized human pathogens. During the 1980s, scientists at their Biopreparat germ warfare laboratories began experimenting with a novel approach to biological weapons, one that involved using genetic engineering. The goal was to insert genes into infectious agents capable of turning the human body against itself.

GM Myelin Peptide Bioweapons

The general idea behind gene-modified (GM) weapon development programs is to trigger an autoimmune response in infected people. A strong autoimmune response can trigger anaphylactic shock and death. The Russian scientists set out to insert DNA fragments from the mouse myelin gene into an infectious agent. After infection, the production of myelin peptides might trigger an autoimmune response in the brain, the body's immune system attacking the myelin that sheaths the brain's nerve cells. In effect, what they sought to achieve was a sort of instant multiple sclerosis.

When the myelin gene fragments were inserted into *Legionella* bacteria (the cause of Legionnaires' disease, a troublesome but usually nonlethal pneumonia) and the GM *Legionella* allowed to infect guinea pigs, the animals at first exhibited a mild pneumonia, from which they soon recovered. Days after all signs of the infection were gone, the animals began to exhibit symptoms of brain damage. Paralysis and death followed. Mortality was nearly 100%.

Although the Russians never produced a GM myelin peptide bioweapon, the importance of this result cannot be overstated. Clearly, GM bioweapons would work.

GM Interleukin-4 Bioweapons

The magnitude of the threat posed by GM modified bioweapons only became evident in February of 2001, when Australian scientists reported some unanticipated results of what was intended to be a benign experiment. The Australians were involved in a pest control project, trying to find a way to control excessively large mouse populations. Their experimental goal was to render the female mice infertile by triggering an autoimmune response against their own eggs.

They used as a vector the mousepox virus, a relative of human smallpox. They inserted into the mousepox DNA a gene from mice that controls production of the molecule interleukin-4. Interleukin-4 is a powerful stimulator of the immune response, and the researchers hoped it might so stimulate the female's immune sensitivity that the female mouse would reject its own eggs as foreign.

That's not what happened. Instead, all the infected mice died.

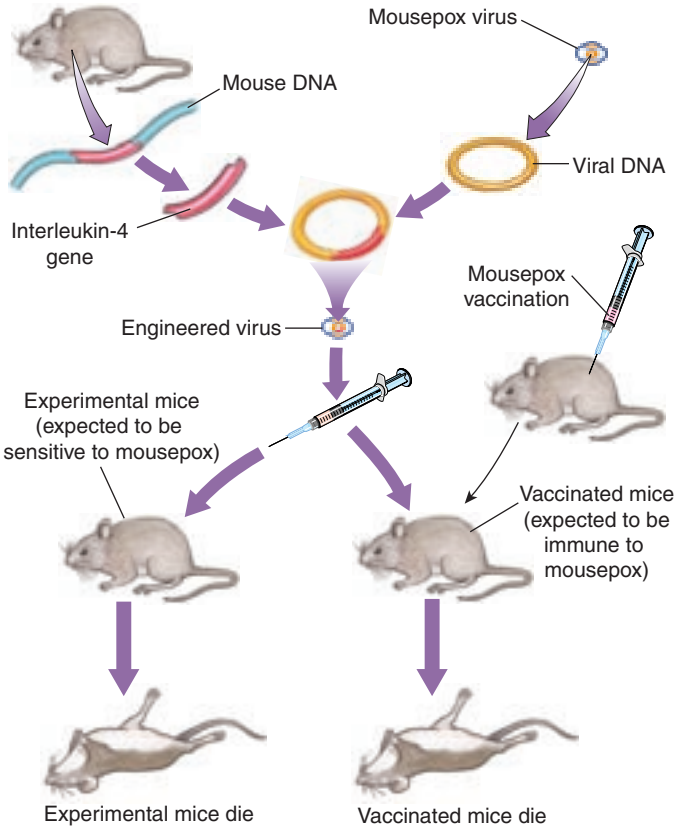

FIGURE 13e.9
The unintended invention of a terrifying GM pathogen. Insertion of the interleukin-4 gene into a pathogen disables immune defenses, rendering vaccines useless.

Something else happened, too, something unexpected and very troublesome. The control mice, which had been vaccinated against mousepox and which should have been immune to the infection, also died. Apparently the excess interleukin-4 had thrown the mice's immune response totally out of whack, so that immunity in these mice no longer works (figure 13e.9). Their bodies after infection with the GM interleukin-4 mousepox had no defense against the virus—they had totally lost their immunity.

The problem that this result presents is that it suggests that smallpox or influenza genetically modified to contain the human interleukin-4 gene would defeat any vaccine! No government would create such a bioweapon, as there could be no way to defend their own troops and people against it. To a terrorist without such scruples, it might seem the ideal bioweapon. The Australians published their result in the hope that governments around the world would see the need to regulate research into the genetic engineering of pathogens. Once produced, this sort of bioweapon might someday be used, and would lead to an epidemic beyond control.

Genetically modified bioweapons, were they ever to be produced, offer terrifying possibilities.

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