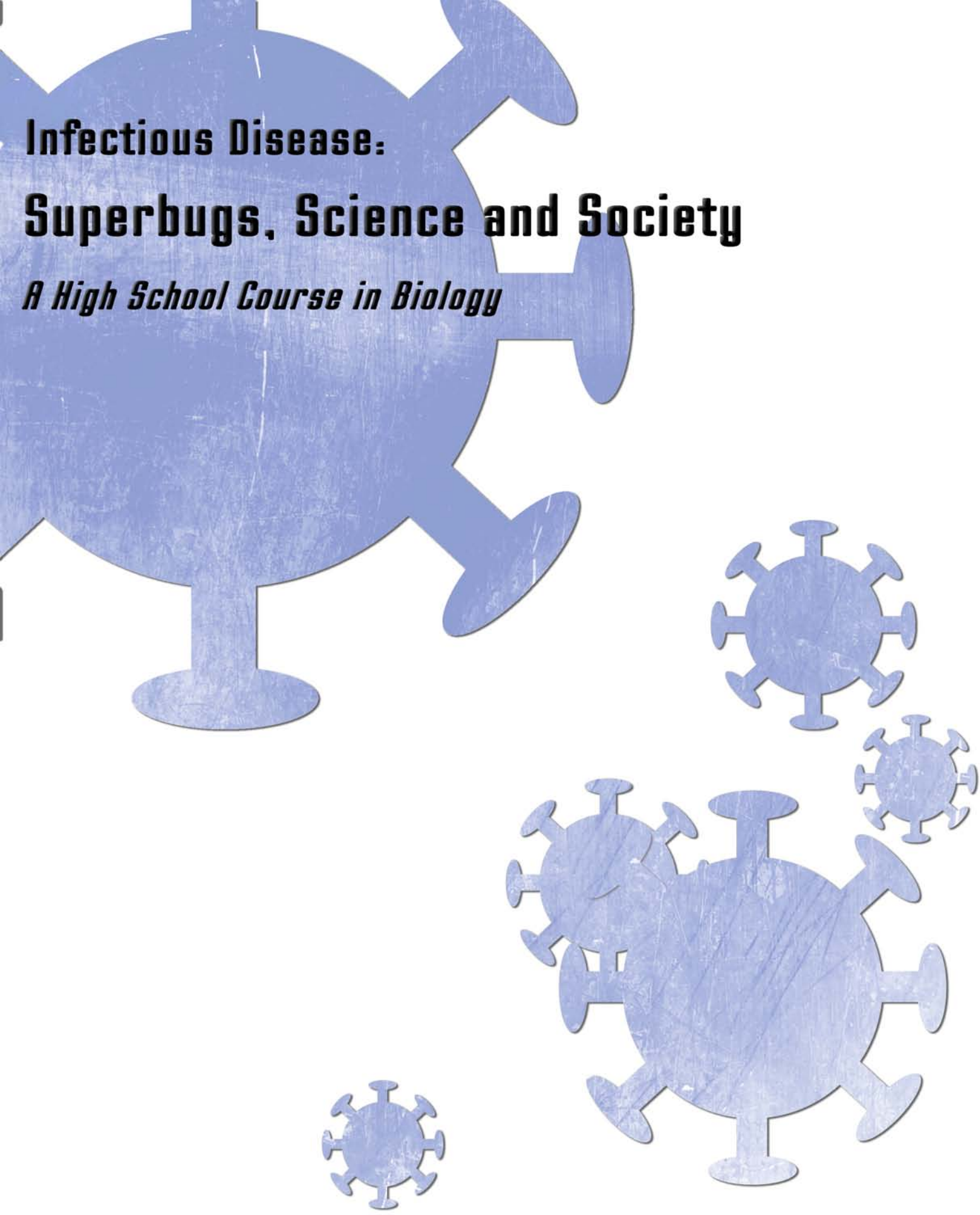
Infectious Disease:
Superbugs, Science and Society

A High School Course in Biology



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A High School Course in Biology

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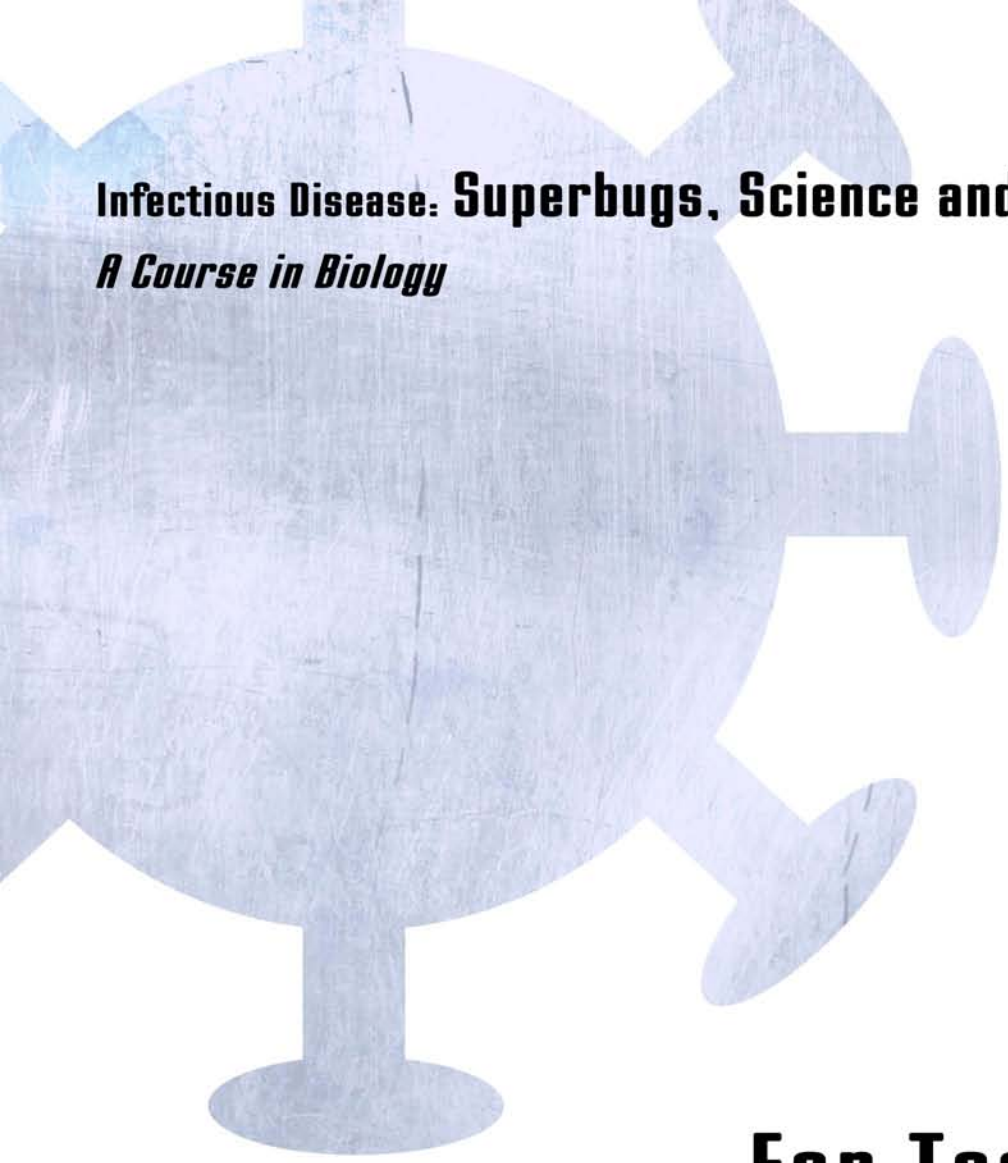

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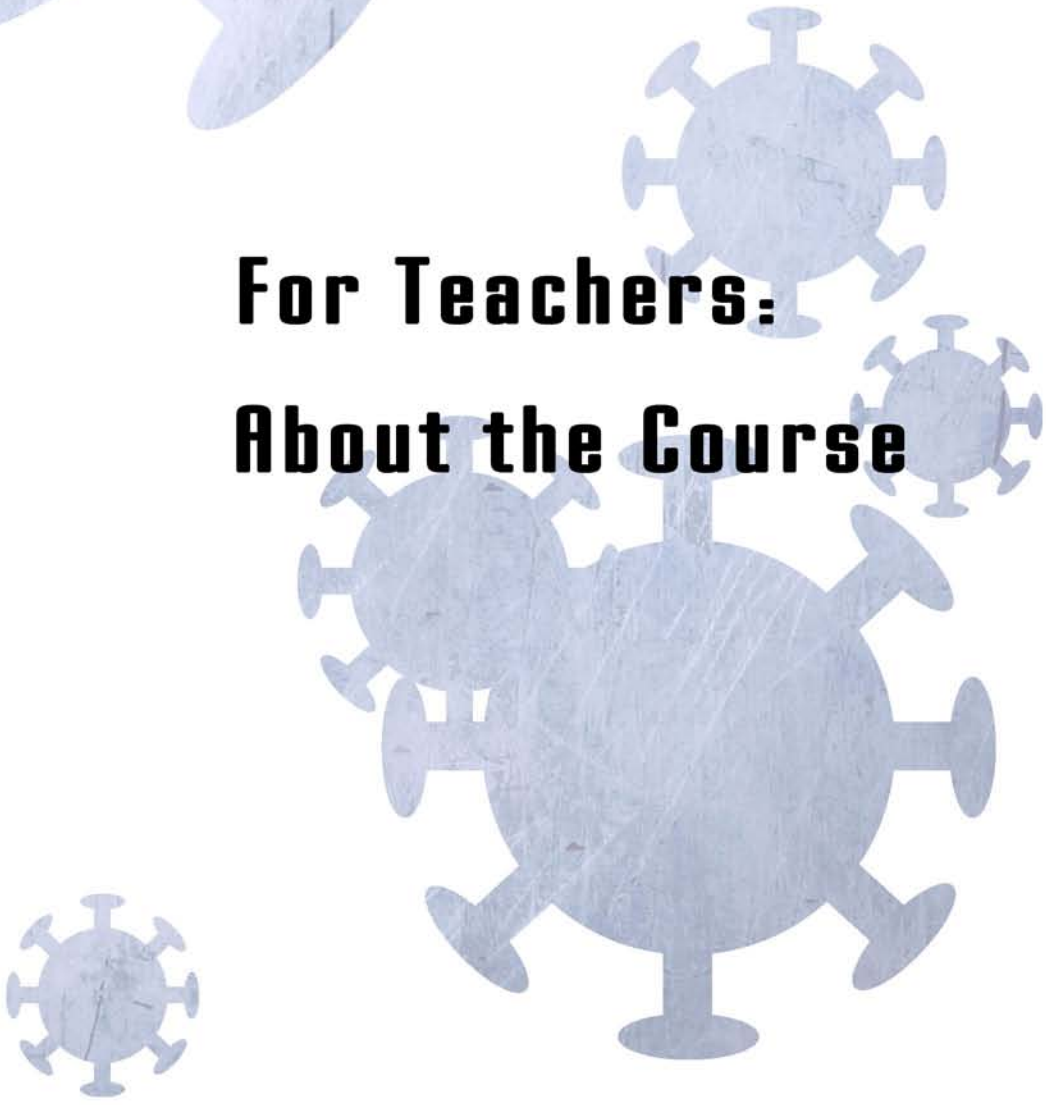
About the Course



Introduction

Effectively treating infectious disease requires more than just an understanding of science. While biology may dictate how the human body responds to infection, it doesn't do justice to the larger victim of disease: society. The fabric of economy, ethics, politics, and culture that compose our world determine how diseases are spread, who gets treated – and why. Throughout this course, we hope to emphasize that disease is as much a social phenomenon as a biological one with ramifications for both the individual and their family, neighbors, and fellow citizens. Through this larger perspective, we hope to illuminate disease in a way that relates directly to student's lives, showing them that biology operates both under a microscope and in their daily interactions with the world.

The SENCER Approach

The interconnectedness of science and the surrounding world is the motivating philosophy of the Science Education for New Civic Engagements and Responsibilities (SENCER) program. This National Science Foundation (NSF) initiative aims to change the undergraduate science curriculum by examining science disciplines through the lens of larger, immediately relevant public issues. Through this interdisciplinary approach, complex science is rendered in an exciting, relevant, and complete format. According to their "Ideals", SENCER:

- robustly connects science and civic engagement by teaching "through" complex, contested, capacious, current, and unresolved public issues "to" basic science.
- invites students to put scientific knowledge and scientific method to immediate use on matters of immediate interest to students.
- reveals the limits of science by identifying the elements of public issues where science doesn't help us decide what to do.
- shows the power of science by identifying the dimensions of a public issue that can be better understood with certain mathematical and scientific ways of knowing.
- conceives the intellectual project as practical and engaged from the start, as opposed to science education models that view the mind as a kind of "storage shed" where abstract knowledge may be secreted for vague potential uses.
- seeks to extract from the immediate issues, the larger, common lessons about scientific processes and methods.
- locates the responsibility (the burdens and the pleasures) of discovery as the work of the student.
- encourages student engagement with "multidisciplinary trouble" and with civic questions that require attention now. By doing so, SENCER hopes to help students overcome both unfounded fears and unquestioning awe of science.

As educators in Duke's RISE (Raising Interest in Science Education) office in the Department of Pharmacology and Cancer Biology, we wondered why such an approach should be confined to undergraduate education. High school students need innovative science courses just as much – or maybe more – than their collegiate peers. Research has certainly reported the view that teaching students through the SENCER approach leads to improved results in the classroom (http://www.sencer.net/assessment/independentevaluation.cfm).

The Course Structure

With these principles in mind, we developed *Infectious Disease: Superbugs, Science, and Society* as a high school elective course, particularly for specialized health sciences high schools. In the interest of covering a distinct variety of biological disease and social issues, the course is divided into six units: HIV/AIDS, Tuberculosis, Malaria, Bioterrorism (i.e. Anthrax, Smallpox), Avian Flu, and Prion Disease. Though each unit considers a distinct type of infectious agent, unifying themes include treatment/maintenance approaches, resistance challenges, and historical/ethical considerations.

This course is designed to place the students in the active role. Although the format is flexible, lecture time should ideally be kept to a minimum. Further, each module opens with a case study that directs the students in their investigation into the material. Embedded in the fictional narrative of a group of high school students investigating infectious disease, these case studies should provoke student-driven inquiry into the content. Also included in each module is a list of suggested supplementary classroom activities.

The course has been thoroughly reviewed by high school teachers serving as members of our advisory committee. They provided useful advice concerning the relevance, clarity, interest, and feasibility of the course. We welcome any additional advice and feedback as you incorporate this kind of course in your teaching. We hope you and your students find this course to be a rewarding experience.

The Superbugs RISE Team

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RISE
Raising Interest in Science Education

Course Themes

Introduction: “Hi, I’m a Microbe”

The first week of the fall semester at Fairview is interrupted by construction, as a work crew jackhammers a water line through the concrete base on the eastern wing. Sometime over the summer, the system had gotten clogged by mineral deposits. The small blockage had quickly become an immense problem when slurry of art supplies had been poured down the drain and become trapped at the bottleneck created by the accumulated salt. Since the system had been covered over by concrete during a poorly-planned former renovation, the entire area was now torn up for the reconstruction. The teachers, driven mad by the constant noise, had cancelled several classes already. Mrs. K, with her sensitive ears, was no exception.

As a result, the second week of school is well under way before the four classmates experience a full period of instruction, rather than long reading assignments in their (numerous) textbooks. On this day, Mrs. K (late again) begins talking non-stop as she enters, throwing her bag down quickly on the desk.

“Good afternoon, did everyone get the assignment for today?”

They nod, and Angelica and Maxine exchange hesitant looks – they’re not quite sure what they think of today’s activity, but if past experience is any indication, it’s probably going to be 1) embarrassing and 2) not particularly educational. Mrs. K turns to the class.

“Who wants to go first?”

Their assignment had been to look up information on a specific microbe and present it to the class in an “interview”; they would pretend to be the organism, and their classmates would ask questions about it.

No one raises their hand.

“Fallon,” says Mrs. K, hoping to break the awkward silence, “why don’t you begin?”

Sensing that fate has not been kind to him today, Fallon slowly pulls out his scribbled notes on tuberculosis, trying to run through the fact in his mind one more time. Having retrieved his outline, he slouches back in his chair, waiting for the questions to begin.

“Would you mind coming to the front of the room, so we can all see you?” asks Mrs. K. Fallon quickly complies, growing more nervous as he turns to face his (clearly skeptical) classmates.

“And who are you?” asks Mrs. K.

“Oh, hi, I’m Fallon,” he answers.

“No . . .,” she says, “what *microbe* are you.”

The other students giggle, clearly unimpressed with his introduction. “Hi, I’m Lang – I don’t believe we’ve met,” whispers Lang, drawing more laughter from the two girls. Fallon sighs, sensing this isn’t going to be pleasant.

“Hi, I’m tuberculosis, and I’m a pathogen.”

“What to do you mean, you’re a *pathogen*?” asks Mrs. K. Fallon stares blankly at her, trying to think of an answer.

“Wikipedia says I’m a pathogen.”

The laughter from the other students is louder this time, causing Mrs. K to glare in their direction. They quiet, as she turns again to Fallon. “So what *is* a pathogen? Why aren’t you just a microorganism? Are all microbes pathogens?”

Fallon, finally understanding, answers, “oh, because I can make people sick.” This, too, his classmates find quite hilarious. “I’m contagious,” he adds, which doesn’t quiet their laughter.

"Right," says Mrs. K, "a pathogen is a microbe that can cause disease. There are a lot of tiny organisms that don't – in fact, most don't. It's just the ones that can harm us that are called pathogens."

"Why do you make people sick?" Angelica asks Fallon, trying to keep a straight face.

"Yes," says Mrs. K, "good question. Why do you cause an *infection*?" Fallon tries to remember what Wikipedia said about this, and pulls blank. Maxine raises her hand to answer, and despite the fact that this is his presentation, Mrs. K calls on her – no point in prolonging this, after all.

"Yes, Maxine?"

"The tuberculosis *bacterium*," she starts, correcting Fallon's mistake, "can cause an infection because it can get inside the human body and grow, if the environment is right – I think the tuberculosis bacterium usually grows in the lungs."

"Good, says Mrs. K." Maxine continues.

"But the bacterium doesn't cause the *disease* tuberculosis unless the fact that is growing actually harms the body. Even Wikipedia," she smirks, "would probably tell you that there are a lot of people with the bacterium growing inside of them who never develop the disease."

"Very good," says Mrs. K. "So an *infectious disease* is . . ."

"A disease caused by microorganisms invading the human body and damaging it by growing and killing cells and tissues in that body," answers Angelica. Now Fallon is mad – her answer *was* on Wikipedia, he remembers the exact line.

"Lang," asks Mrs. K, "would you like to go next."

"I don't understand the point of this assignment," he answers.

"Well, by role-playing different microbes," she replies, "you're supposed to all look up the information that you're supposed to be reading. Plus, this is interactive, so you learn it again in a different way."

"Sure," he replies, "but microbes don't talk. What is the point of me playing a talking virus when talking viruses don't exist?"

She starts to think of a reply, but stops, deciding to end this experiment for the day. "Very well, I think we've had enough acting for today. Everyone just turn in a three page report on your pathogen next time. Class dismissed."

The Major Themes of this Course:

Throughout *Superbugs, Science, & Society*, we will revisit the same concepts again and again. Sometimes the specific details of a given topic may seem overwhelming, but they should be clearer if you keep a few basic principles about infectious disease in mind:

1. Pathogens are Unfriendly Microbes

It's a testament to the diversity of life that a single drop of lake water or a handful of dirt can contain a rich ecosystem of tiny organisms. Most of these are harmless to us, and many are actually helpful, as you'll find out in our discussion of bacteria. The fact that we don't get sick every time we go outside should be proof that only a limited range of microbes can cause disease: we call these pathogens. Pathogenic microbes include bacteria, viruses, fungi, protozoa (single-celled organisms like the trypanosomes we'll discuss later), prions, and helminths (multi-

cellular organisms). We'll see all but the last group in this course, and provide more information as it's necessary.

How do these pathogens cause disease? Like any organism, microbes want to grow, multiplying and spreading out into new places. They are infectious if the human body is one of the places they can grow; however, they only cause disease if they are destructive to their home, killing the tissues they inhabit.

2. How do we know a Pathogen Causes a Disease?

In the days before modern science, people used to think diseases were caused by all sorts of strange things – swamp gas, bad blood, or evil spirits. The idea that tiny creatures were actually responsible was a major breakthrough. The proof is summarized by "Koch's Postulates," a set of four rules that establish how a microorganism can be nailed as the culprit of a disease.

1. The microbe is found in the tissues of anyone with the disease.
2. The microbe can also be isolated from the infected host and grown in pure culture.
3. A healthy host will develop the disease if injected with this pure culture of the microbe.
4. The microbe must also be able to be isolated from this second, experimentally infected host.

We'll return to these rules many times, and even look at some situations where they don't apply. In general, though, they serve as a good rule of thumb.

3. Pathogens have Multiple Ways to Jump Ship

How does a microbe get inside our bodies to begin with? In general, pathogens can be acquired by direct, indirect, or vertical transmission.

Direct transmission is probably most familiar to us. Whether a fellow patient sneezes on you at the doctor's office, or, in a more extreme case, if you are bitten by a rapid dog, disease-causing microbes directly enter the body through saliva, blood, mucus, or skin contact. We say this is direct transmission because the microbe has directly moved between its former location – the "reservoir" – to the new one – you.

Indirect transmission occurs when there is an extra step between the reservoir and the host. An example of this would be picking up a cold by touching the tissue that your fellow patient had sneezed on, rather than being sneezed on personally. Similarly, some microbes (like anthrax, which we'll learn about later) can hang around in the environment for extended periods – picking them up from wood or other natural sources is another example of indirect transmission.

Both direct and indirect transmission are called horizontal forms of transmission, which is different than vertical transmission. Vertical transmission involves pathogens that are passed between generations – the best example of this is mother-to-child transmission of the HIV virus through maternal blood.

Admittedly, this idea of a "generation" might seem tricky when trying to distinguish vertical from horizontal transmission. If an adult passes a disease to a child by sneezing on them, is this direct or vertical transmission? It's direct transmission – the reservoir and the host may be in different generations, but the transmission of the microbe is not a *result of* this generation gap.

In other words, we only use the term vertical transmission when the pathogen is spread through things related to birth, like sperm cells, eggs, maternal blood in the womb, or during birth.

4. Lots of Fences for Bad Neighbors

With all these ways that microbes can get inside our bodies, what tools do we have to we have to stay healthy? Many are probably already familiar to you, such as drugs and vaccines, but others you may not think about as much.

What is the difference between a drug and a vaccine? A drug is, generally, a particular chemical compound that kills a microbe. It could cause a bacterial cell wall to rupture, or prevent a virus from replicating. Either way, it is an active response to an infection, after the microbe has already invaded the body.

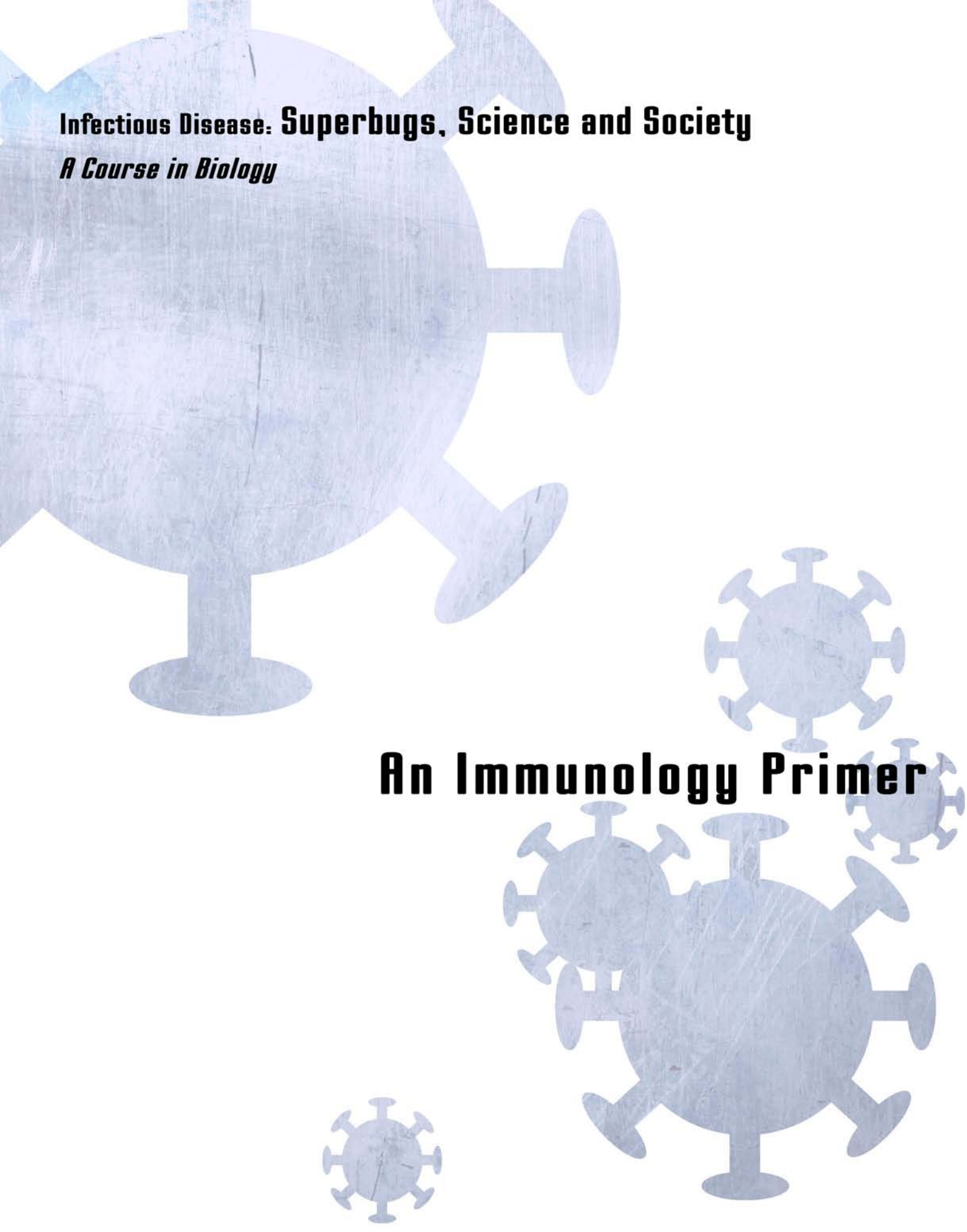
In contrast, a vaccine is a pre-emptive strike, an attempt to prevent the microbe from invading in the first place. Vaccines are effectively early warnings: a doctor injects a patient with a particle that is somehow related to the microbe. It could be a molecule contained inside (like a distinctive piece of RNA or protein), or small amounts of the microbe itself. The body has an army of white blood cells specially trained to recognize messages like this, but their ability to respond is limited by what "warnings" they've received. Vaccines prevent infection because these white blood cells receive the message and generate chemical defenses specially suited to fighting off a particular pathogen. Thus, when they encounter this microbe, they are already prepared to destroy it before it infects the body.

There is also a third class of treatments that may not be as obvious, called control measures. These can be public health projects like chemical spraying to get rid of mosquitoes, or a screen on your own porch to keep out these pests and the disease-causing microbes that live in their saliva. The important point is that control measures try to prevent the microbe from spreading in the environment, so we have less chance of coming into contact with it and getting sick.

We hope this introduction has been informative, and, with these few basic points in mind, you enjoy your further explorations into the sometimes confusing, often terrifying, but always fascinating world of infectious diseases. Enjoy!

Infectious Disease: Superbugs, Science and Society

A Course in Biology



An Immunology Primer

The Immune System: One Pathogen's Journey into the Unknown

Your Body is (mostly) Not You

It's true: even though 10^{13} cells compose your organs and tissues, there are 10^{14} extra cells[1], from bacteria to eukaryotic parasites, who are just along for the ride. Thus, for every one of your cells, there are ten miniscule hitchhikers – and this doesn't even account for non-cellular guests like viruses. Luckily for us, most of these co-inhabitants of your body aren't harmful, and peacefully cover areas such as the inside of your digestive track and mouth. How is it possible for you and so many microscopic neighbors to live side-by-side? The answer is the innate immune system.

Slushy Fences and Watchdogs: The Innate Immune System

While human skin may seem fragile, able to bruise or be cut in everyday activities, it is a remarkable barrier against many inadvertent or intentional invaders. The layer of cells forming human skin is enough to keep out many pests, and the harmless ones don't usually cause trouble unless they fall through a gap in this wall.

Another of the body's first-line defenses isn't so visible, coating the insides of your digestive and respiratory system: mucus. By coating these cells, this slimy substance prevents invaders from attaching and growing in places that otherwise present a tempting home.

However, even if an invader does get inside, there are additional defenses. Let's follow the path of one such unwanted visitor, who we'll call Pathogen X. Pathogen X, unlike most of the body's cellular hijackers, is not content to simply co-exist with skin cells and muscle cells: it seeks to actively invade the host. Moving along the body's surface (in whatever way it does), it finds a crack in the skin, mucus, or both that allows it to slip past these first lines of defense.

Fortunately though, the immune system has a few tricks left of its own. One is a set of large cells called *phagocytes*, which means "eaters." This is an apt description of what they do, engulfing any unfamiliar intruders in the areas they patrol (they're not particularly picky diners). One class of these eaters which we'll encounter many times in this course are *macrophages* – their name literally means "big eaters." Like a not-particularly bright watchdog, they devour invading pathogens, digesting them with harmful oxygen-based molecules. However, another class of phagocytes, called *dendritic cells*, takes a slightly more sophisticated approach, engulfing their prey but also keeping a record of what they've found. They do this by displaying a piece of Pathogen X on their outer membrane, and, like a watchdog playing fetch, return to other members of the immune system to demonstrate their find.

Metal Detectors for Molecular Fingerprints: The Adaptive Immune System

Retreating deeper within the body, the dendritic cell shows the fragment of Pathogen X it has acquired to its cellular cousins, the more intelligent *lymphocytes*. These cells won't go after just any prey: they have to be shown a target. This is the purpose of the Pathogen X fragment: to act as a special kind of molecule called an *antigen*. This stands for *antibody-generating*

compound, and in practice it serves as a kind of molecular "fingerprint" so the lymphocytes know what to look for. From here, the two classes of lymphocytes differ in how they react to the knowledge that Pathogen X is invading.

The first class of lymphocytes are called *B-cells.* The B is short for bone marrow, which is where these cells are formed, but you can also think of them as antiBody-cells, because their response to infection is to produce antibodies to respond to the antigen signal. An antibody is a "Y" shaped molecule that functions as a kind of "metal detector" for particular molecules. Just as one end of a metal detector is used to sense the presence of metal, the antibody helps lead other members of the immune system to the site of infection by recognizing the "fingerprint" – the antigen – used to generate it. This recognition occurs because the inside of "prong" in the antibody is very specially made so that it clings to the antigen more tightly than other molecules: the amino acids are just the right shape and composition so that the two surfaces interlock. Once it attaches to an antigen, or, more specifically, a certain recognition site on the antigen called an *epitope,* the antibody directs both T-cells (see below), phagocytes, and destructive proteins called *complement* to cells infected with Pathogen X, which display the Pathogen X antigen on their surface, or to free-floating copies of Pathogen X. Complement can poke holes in the membrane of a bacterium or cause damage to a viral coat, the phagocytes can engulf the pathogen, and T-cells secrete harmful chemicals to destroy cells infected with the pathogen (see below).

Each B-cell produces a unique antibody, so your body is constantly generating new B-cells so that it has a chance of making ones suitable for combating any given pathogen the immune system might encounter. How the B-cell uses the antibodies to respond to an infection depends on how developed they are. The younger, less developed B-cells have their antibodies protruding from their plasma membranes, and use them to sense the presence of antigens. Others, the older B-cells, are also called *plasma cells*. They are larger, and secrete large quantities of antibodies into the bloodstream. Plasma cells are usually only produced after the body has responded to a pathogen, since they must develop from the younger class of B-cells.

However, some lymphocytes don't use antibodies. These are called *T-cells*. As before, the T is an indication of the origin of these cells, an organ called the thymus, but you can also think of them as Tracker-cells or Translator-cells. The first kind, the trackers, are called "killer" T-cells, and roam throughout the body looking for the antigen for Pathogen X. As mentioned above, cells infected with Pathogen X will display its antigen on their surface, and when the killer T-cells find it, they latch on, secreting harmful chemicals that destroy the pathogen-infected cell. The other kind of T-cells, the translators, are also called "helper" T-cells. While both classes of T-cells physically sense the antigen with a receptor on their surface, the helper "translators" relay the message to the B-cells. Additionally, the helpers secrete other chemicals that help B-cells produce antibodies, and mobilize killer T-cells to more effectively destroy the target pathogen.

The beauty of this system is that lymphocytes have extraordinary memories: once they have "seen" a particular antigen, they never forget it. Thus, on Pathogen X's first visit, it will be finished off by the lymphocytes if the phagocytes don't completely destroy it. Subsequently, if Pathogen X invades the body, there will already be a group of lymphocytes waiting that "know" exactly how to kill it, having already generated antibodies or acquired other means to specifically

combat this foe. At this point, your body is said to be immune to the pathogen. This is how natural immunity works, which is different that the artificial immunity caused by vaccines, as detailed below.

False Infections: How Vaccines Work

The above scenario is essentially how flu shots or other vaccines function. These preventative measures contain either an individual antigen, or the antigen attached to the whole pathogen. However, whole pathogens are either weakened or deactivated. For example, a virus might replicate slowly, or a bacterium might be completely dead in such a sample. Confronting these individual antigens or crippled pathogens, the immune system easily overcomes the intruders, but not before generating an adaptive arsenal of lymphocytes in preparation of future attacks. Thus, when the full-strength pathogen invades, there is already a carefully developed defense specifically targeted against it waiting in the immune system[2]. Vaccines are also helpful because the sooner the pathogen is destroyed the less likely you are to pass it on to those near you. In fact, if enough members of a community are vaccinated, the rate of transmission of a pathogen is low enough to eventually wipe it out. This is called *herd* immunity, and is what allowed the World Health Organization (WHO) to destroy smallpox earlier in this century with limited vaccine stocks: not everyone had to be vaccinated to prevent the spread of the virus, just enough people in a given area so that its transmission rate eventually dropped to nothing.

There are several ways to stimulate the immune response through a vaccine, as summarized in the following table.

Vaccine Types-Name	Disease(s)	Advantages	Disadvantages
Live, attenuated	Measles, polio	Strong immune response, lifelong immunity	Possibility of reversion to virulent form, must be refrigerated.
Inactivated/killed	Influenza, plague	Safer, more stable than live, easy to transport because it doesn't have to be kept cold.	Weaker immune response than live, requires booster shots.
Toxoid (e.g., targets bacterial secretion)	Diphtheria, tetanus	Immune response to bacterial toxins	
Subunit	Hepatitis B, pertussis	Targets components of microbe (specific), fewer antigens means less chance of adverse reaction.	Hard to identify best subunits to use.
Conjugate (e.g., targets sugars or proteins attached to pathogen membrane)	Influenza type B	Can allow infant immune systems to recognize some bacteria.	
DNA	Under development	Strong antibody and cellular (T cell) immune response, easy to make and inexpensive	Still being tested.
Recombinant (e.g., insert component of pathogen genome into a harmless microbe genome and use the mutant to stimulate an immune response).	Under development	Mimics natural infection, strong immune response.	Still being tested.

Burning Up: Inflammation

What does an immune response look like to the naked eye? This is actually a trickier question than one might think, as it is easy to confuse a visible symptom that is caused by a pathogen, or one that results from the immune reaction itself. For example, consider the two following symptoms:

A: After contracting a bacterium, a patient develops ulcers.

B: In response to a viral infection, a patient runs a fever.

The first is an example of a symptom caused by the pathogen: the ulcer is due to the bacterium destroying stomach lining tissue. In contrast, a fever is a reaction by the immune system to the viral infection. This second case is part of a larger phenomenon called *inflammation*, from the latin word for "flame" or "burning." The classic signs of an *inflammatory reaction* are probably familiar to you: they include 1) increased body temperature, 2) redness at the site of infection, 3) increased fluid production or swelling at the site of infection (as in a blister) and 4) pain. Thus, when you see the word *inflammation* in the rest of this course, you should know that it simply means any of the four symptoms listed above, caused by the immune system responding to a microbial invasion.

References:

[1] B Alberts, A Johnson, J Lewis et al., *Molecular Biology of the Cell*, 4th ed. (Garland Science, New York, 2002).

[2] NIAID, 2003; NIAID, 2003.

Prologue: School's In

"This strange disease of modern life, with its sick hurry, its divided aims."

-Matthew Arnold, *The Scholar Gypsy*

Prologue: "School's In"

Fall has come again to North Carolina, with its leaves fiery red and muted golden, and ever-earlier twilight hours. Pulled – some more regretfully than others – from the carefree days of summer, the students of Fairview High School shuffle onto yellow buses bound for the steel and glass polished by off-season touch-ups. Soon, the bell rings, a shrill sound summoning the sleep-deprived through the tiled corridors, towards numbered doors where a teacher is already rasping chalk along the sheer, dark board.

Towards the end of the west annex, the door to room 412 opens hesitantly. A navy baseball cap, followed by a pair of green eyes and dirty blonde hair, poke inside, surveying the mostly empty desks. The instructor hasn't arrived yet, and only three seats among twenty are occupied. Sitting beside the window, a thin boy wearing black and grey contemplates the athletic field outside – or perhaps stares through it. His shoe, rythmically tapping against the inactive radiator, proclaims his absorption elsewhere. Unlike the two girls seated in the second row, he doesn't turn at the entrance of the newcomer.

At first glance, they look like they could be sisters, twin pairs of hazel-brown shooting towards the door as the hinge creaks inward. Despite their equally curly locks, intuition denies any familial relation. One, dressed in green, loops a strand of hair around her index finger as she looks up from the calculator's faintly glowing screen. The other, lacking her friend's waist-length cut, rests a red-clothed arm over the back of her chair, a pencil propped thoughtfully against one cheek. A passing car outside catches the sun, illuminating the slight variation in shade between the stares, a difference already confirmed by their posture and more subtle hints.

"Are you here for Biology 301?" asks Fallon, still standing in the doorway.

"Yeah," answers Maxine, releasing the strand from her grip. "We were wondering who the last person was. Mrs. K hasn't showed up yet."

"She left a note on the board," adds short-haired Angelica, "apparently the summer assignment isn't due until next week." Rolling her eyes, she turns, grumbling. "I'm so glad I spent last weekend doing my paper while my family was at the mall."

Fallon steps inside, scanning the available desks. Normally, he would sit at the back, but that somehow seems inappropriate in a four-person group. There's a gap of two rows between the two girls and the boy gazing out the window. Fallon's memories of AP English with the quiet Lang suggest that the toe-tapping boy doesn't think much of his soccer-playing classmate, and he quickly pulls a chair from the third row on the other side of Maxine and Angelica. An awkward silence settles over the room as the four wait for the arrival of their instructor.

* * *

It had happened during course sign-ups the previous spring – a special flyer in the syllabus, announcing an "experimental course" on disease biology. Starting in the upcoming year, the science department of Fairview would begin developing this advanced curriculum, part of larger educational initiative by nearby Pierpont Pharmaceuticals of Research Triangle Park.

Because it was a trial-run, only four students would initially participate, with selection based on interest and teacher recommendations.

Initially, Fallon had been uneasy about signing up for such a class. His last experience with "experimental teaching" had been in AP Psychology, where the teacher had demonstrated hypnosis on him. Why he had volunteered he couldn't remember, but it probably had something to do with making up for the quiz he had bombed the previous week through participation. Other students claimed he had performed several embarassing acts during the demonstration, though he couldn't remember the incident at all. Behind his back, his classmates suspected this had more to do with the number of times Fallon's head collided with soccer balls during his team's season than the intrinsic power of hypnosis. However, his psychology teacher had pulled him aside after class during course signup and almost forced him to put his name down for the new class. Unlike most of the student body at Fairview, Fallon's standout performance on the statewide aptitude testing was not a secret to Mr. H. The wiry old man suspected that if the outgoing soccer player could achieve the school's third highest score on the exam, Fallon was capable of more than was indicated by his test grades in class. The boy just needed to be pushed. So, deferring to his instructor's wishes, Fallon had placed the course on his sheet, and dropped the sealed envelope containing Mr. H's letter of recommendation at the guidance office.

A week before final exams, he received a letter at home notifying him that he was one of the four students chosen to participate in the new class, accompanied by a list of six books on which he had to write an essay over the summer. Despite his best efforts to procrastinate, he had actually finished the assignment, though the identity of his three future classmates remained mysterious.

* * *

Taking a pen from the front pocket of his faded yellow backpack, Fallon regarded the hastily scrawled message on the blackboard.

Summer assignment due next week on Tuesday. Welcome back!

"That was Mrs. K's car that just went by," says Lang, eyes turning towards his classmates. "She must be running late today."

"How long do we technically have to wait before we can leave?" asks Maxine, looking at the minute hand of the featureless white clock above the door. "It's been almost fifteen minutes."

As Maxine turns toward him to study the clock face, Fallon leans over again quickly to fish for a notebook inside his cluttered bag, afraid that he'll get nervous and blush under her gaze. Though he isn't sure what the math whiz thought of him before the hypnosis incident, he's fairly certain that it didn't improve his image in her eyes. As far as he knows, she's unaware of his attraction to her, and plans to keep it that way for now.

"Well since now we *know* she's not absent, we don't really have much choice I think," says Angelica. "At least class will be short today."

* * *

Maxine hadn't been surprised when she received her acceptance letter for Biology 301. Mrs. K, her AP Biology instructor, had been asked her several times for help on a mathematical

model of disease propogation, which Maxine guessed had something to do with the new course – why else would a biology teacher need to know so much about math? Not that it was unusual for her to be consulted on such problems; as the captain of the math team and the best student in every mathematics course she'd taken since kindergarten, many of the teachers at Fairview sought Maxine for help. Given that she had helped refine part of the curriculum, she suspected that there was a good chance there would be a place for her in the fall.

* * *

Lang, like Fallon, hadn't initially considered even putting his name down for the new class. However, unlike his classmate, whose hypnosis session he vividly remembers from last year, it wasn't because of the workload, but the subject matter. Though perfectly capable of plugging numbers into rate equations or reciting the components of a eukaryotic cell, Lang had always preferred literature and history to science and math. Now, seated across the room from the soccer player who had spoken only three times last year in AP English – each time to ask about the late homework policy – he wonders whether enrolling was wise.

Ironically, it was his writing that got him into this science course. As part of a poetry unit in AP English last year, Lang had written a long essay about the work of Tory Dent, a contemporary poet whose pieces often concern her life with AIDS. Expanding on the social and ethical context of the author's disease in his composition, Lang's essay had piqued the attention of his instructor, who had passed it along to Mrs. K, the biology teacher who would be undertaking the new class in the fall. He had received a note from Mrs. K, whom he hadn't had as a teacher since his freshman year, requesting that he put his name down for her new course. She explained that she had read his essay, and thought he could contribute a lot to the humanist component of her class, since Pierpont's curriculum was based on the social and ethical factors affecting disease along with the requisite biology. On a whim he had consented, but not thought much about his decision, even after being chosen for the class – until now.

Sitting in the empty room, with only a soccer player and two science-heads to keep him company, he starts to follow the minute hand of the clock, ticking away the hours and days until this course will be mercifully concluded. If he were every cheerful, his lack of enthusiasm might be apparent to his classmates. As it is, Lang's reserved silence strikes none of them as out of the ordinary.

* * *

Angelica signed up for Mrs. K's class out of necessity. Having already exhausted all the science courses offered at Fairview, including the pseudo-sciences like psychology, she needed *something* to take next year. Looking through the course catalog, she was initially excited about Biology 301, before discovering that it included non-scientific material such as ethics and history. She still put her name down, though not without cringing. Based on her experience with the theatrics of AP Psychology – the hypnosis incident remains prominent in her memory – she had hoped to avoid any further coursework beyond the simplicity of molecules and cells. At the microscopic level, everything made beautiful sense; add in anything more human, and it was messy, lacking the symmetry and logic of chemical reactions. That human element had produced the hypnosis incident, but also the books Angelica bought summaries for in english.

She had been accepted into the class – she didn't see how she couldn't, since there wasn't anything left for her to take in the science department. But Fallon was here, so perhaps the Fairview faculty were less rational than she had thought.

* * *

Mrs. K finally sweeps through the door, seventeen minutes late.

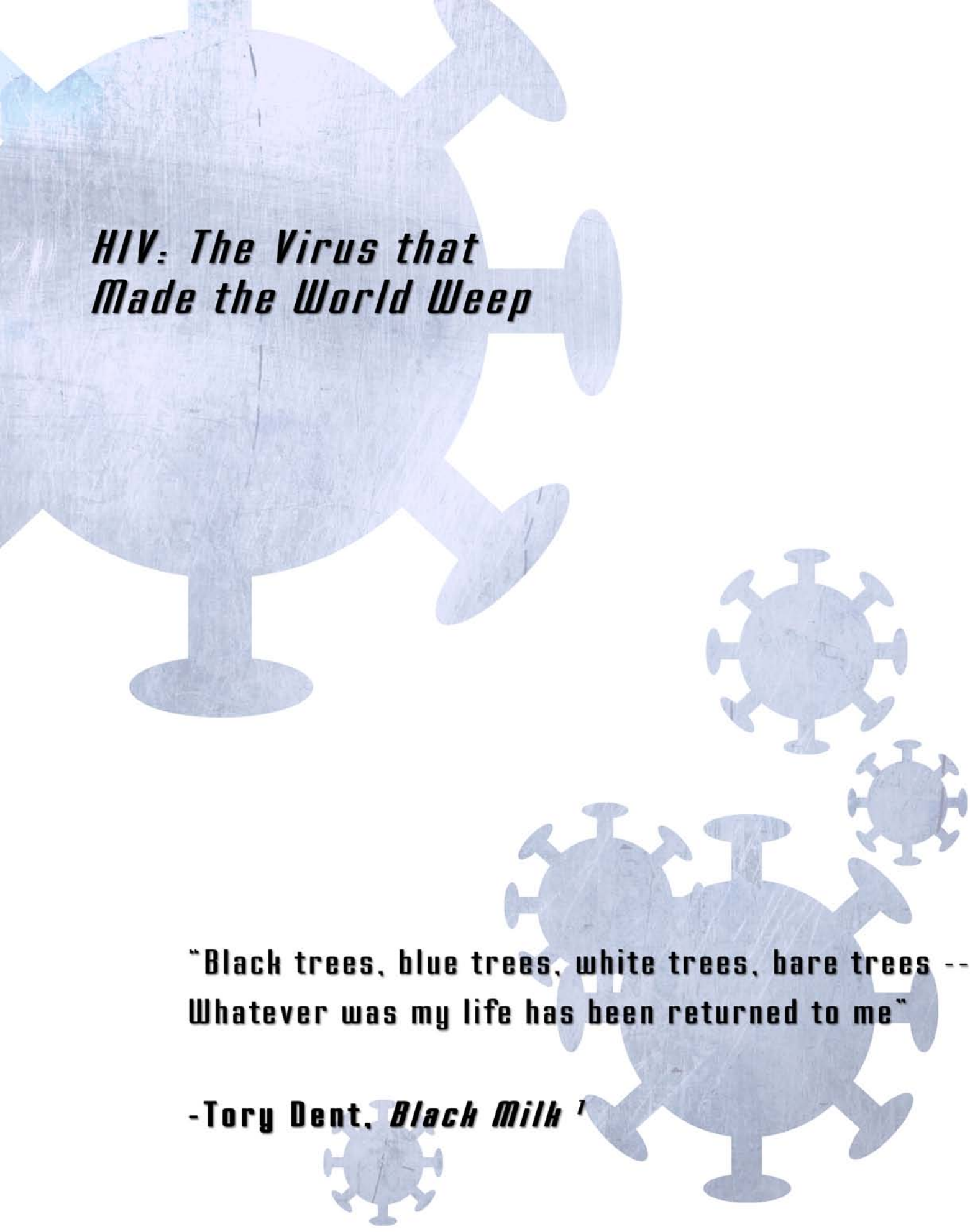
"Sorry for the wait," she says breathlessly, "I accidentally left your coursepacks at home, and a lane was blocked off on Griffon for repair work." She stops for a moment, resting her hands on the desk long enough to steady her pulse. Once she has collected herself, she continues, turning to the scrawled message on the blackboard.

"As you can see, mercy smiles on you today – though I assume you all had your assignments complete anyway, didn't you?" She looks over the class, receiving nods from everyone but Fallon, who is again digging in his backpack. Pulling out the eraser he had been looking for, he also places his essay on his desk, supposing that Mrs. K's question is directed specifically at him.

"Any questions?" Receiving no responses, Mrs. K begins passing out the thick, stapled bulk of her coursepacks, emblazoned across the cover with the logo of Pierpont Pharmaceuticals. Afterwards, she distributes a brief syllabus to each student before returning to the blackboard to erase her note.

"Okay then ... let's begin."

HIV: The Virus that Made the World Weep



"Black trees, blue trees, white trees, bare trees --
Whatever was my life has been returned to me"

-Tory Dent, *Black Milk* [1]

“First Lesson”

The clouds moving over the West annex today seem as tired as the students in Biology 301; they seem to creep rather than float, shuffling across the blue behind them. Lang watches them, wondering if that blue tint is the same the world over, or whether it’s unique to North Carolina. Such thoughts have been brewing within him for weeks, ever since the class started their correspondence with students from Pierpont Pharmaceutical’s charter school in Malawi.

* * *

It had begun several Thursdays ago, with an odd question.

“Who thinks they’re good at geography?”

Unsure whether it was a rhetorical question to which the answer was understood to be “no one smart,” none of the four had responded to Mrs. K’s question.

“Let’s try an example,” she continued, pulling down a global atlas over the smeared chalkboard. “Where is North Carolina on this map?”

Reluctantly, Fallon ultimately pointed the state out on the eastern seaboard, rather than endure another awkward silence.

“Right,” she said, “and . . . France anyone?”

Fallon had again volunteered, before Mrs. K had finally come to the point of this exercise – which was, as they had guessed, not to test their knowledge of Western geography.

“And where is Malawi?”

As she had expected, blank stares proved the only reply from her students.

“Strange, isn’t it, how we can talk about disease in developing countries and have no idea where they are?”

They would have been more embarrassed if it hadn’t been obvious that this was the point of her demonstration. Nevertheless, Angelica had offered that it “was somewhere in Africa.”

Mrs. K had turned back to the map, looking at the size of the continent and replying, “and North Carolina is somewhere in North America.” Pausing a moment, she continued. “Pierpont has arranged a very unique opportunity for you all to find out where Malawi is – the charter school the company has constructed there has agreed to engage in some cross-cultural exchange.”

“What does that mean?” Maxine had asked.

“Letters.”

* * *

Though he had initially been skeptical, in retrospect Lang thought the envelopes he had dropped in the school mailbox were the most enlightening part of the class so far. It was one thing to study how all these horrible illnesses affected the developing world, but another to receive notes from other students looking at the lazy clouds outside.

As the class soon learned, the sliver of eastern African land containing Malawi was not an easy place to live. The students who wrote back to them described an

instructor in his mid-fifties as astonishingly old – with malnutrition and AIDS, the nation's life expectancy had dropped steeply. It became clear, as well, that most of their correspondents knew friends or family members infected with HIV. With a term paper already drawing near, Lang had a thesis in mind: "The first lesson in understanding disease is to know the human face behind the disease."

Unlike the clouds drifting over the West annex, the political situation in the area around Pierpont's charter institute was far from placid. Local elections were on the horizon and, as Lang and his classmates had learned, the result could have a major impact on the AIDS crisis in the area. One of the letters had arrived, describing:

> *. . . the younger brother of our history teacher . . . he finished studying surgery abroad, and has already become a local hero after performing a risky heart surgery on a newborn. He's promised that if he gets elected he'll end some of the ridiculous policies that have allowed so many people to get sick . . . he says that knowledge is one of the most powerful medicines.*

Maxine had been immediately charmed by the idea of an educated physician moving back to his home to make a difference, leading Fallon to talk noticeably for a week about wanting to do something similar – despite the fact that health crises were few and far between in Fairview.

"I wonder, though," Lang says, turning from the window, "whether most of this guy's voters even know how bad things are – I certainly wouldn't have learned all the material about AIDS on my own. Maybe some of them just think life is hard."

"I never thought of it that way," replies Angelica, uncharacteristically affected by his observation. "It's just become . . . normal."

"However," says Lang, "I guess it's not all that different from what goes on in the US, too – I mean, if everyone our age actually understood everything we know about transmission, do you think there'd be so many infections in the US?"

"It's almost the opposite problem," Maxine thinks aloud. "Everyone in Malawi thinks it's normal; everyone in the US thinks it's abnormal enough that it couldn't possibly happen to them."

"That's what they thought in the eighties, too," says Angelica. "It was just a 'gay' disease. Then it became everyone's disease. Now it's the third world's disease – just that it's not really, people just think that way."

"What could convince them otherwise?" Lang wonders.

Questions:

1. **What are some reasons AIDS might not be discussed in public in other countries?**

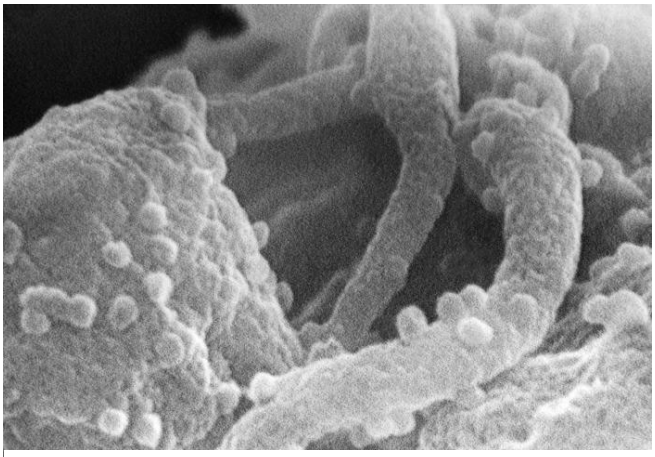
2. **How would you go about describing AIDS to someone who knew nothing about viruses? What are the most important features of the virus and the disease?**

3. **What hygiene and public health recommendations would be important to impart to that individual? How would your suggestions differ between Africa and the US?**

4. **What might be some challenges to implementing those recommendations here and abroad?**

Introduction

In the course of **Superbugs, Science, and Society**, *we'll encounter many health threats that are dark clouds on the horizon, not current thunderstorms. Avian flu, anthrax – these occupy the realm of fear, not public health reality. In other cases, such as malaria, diseases exert a much heavier burden on the developing world than America. However, none of these is the case with HIV and the disease it causes: AIDS. Even though the AIDS epidemic in Africa may have been ignored by Western policy makers, its impact in the US is unmistakable. From the earliest hints of this new killer in the 1980s, to its undeniable influence on modern sex education and public health, AIDS has had a profound effect on US culture. It is a health crisis shared across the globe, uniting America with Sub-Saharan Africa and other struggling regions.*



Electron micrograph of HIV virions on the surface of an immune cell.

Goals:

By the end of this unit, students should be able to:

1. Analyze the likelihood of infection with HIV through different transmission routes.
2. Synthesize knowledge of the biology and history of the virus to evaluate different theories of its origin as a human pathogen.
3. Evaluate how a particular public health measure for HIV/AIDS might differ in effectiveness between the US and Africa.
4. Based on historical evidence, predict how a funding proposal for HIV/AIDS research might be treated differently in the 1980s and the present day.
5. Propose a new antiretroviral compound or HIV vaccine based on current knowledge of the virus' mode of action.

A Word on Layout

Our discussion of HIV/AIDS, like the other courses in this unit, follows a general format. We begin by describing the history of the disease, following the pathogen's course through human history. Having illustrated this larger-scale picture, we narrow down to focus on the molecular biology of the disease, its transmission, and it treatment. Finally, in cases such as AIDS where public health measures are as much a part of treatment as the medicine itself, we survey current initiatives – and ethical quandaries that may arise from them.

AIDS: A Dual History

More so than the other historical summaries in this course, defining a history of AIDS is a difficult task. Epidemics of the disease have taken different forms based on their geography, and the human elements of this story are inseparable from the scientific search for a cure. What follows are two stories from two continents: one is the spread of AIDS in Sub-Saharan Africa and policy measures to confront it. The second is the development of the AIDS epidemic in the US. Together, intermingled, these histories comprise at least a partial image of the much larger story of AIDS.

Origins of the HIV Virus

Where did HIV come from? The answer to this question was long unknown. While one of the first recorded cases of HIV infection occurred in 1959 in Kinshasa, Democratic Republic of Congo, the virus' ultimate source remained mysterious[7]. Scientists now believe that they have traced the origin of HIV-1 to chimpanzees in Western and Central Africa[8]. While strains of Simian Immunodeficiency Virus (SIV) were previously discovered in primates, these pathogens were not particularly similar to HIV. However, a SIV strain much closer to HIV was found in a species of chimpanzee that lives primarily in Western and Central Africa – the place where human cases of HIV infection were first reported[8]. Thus, HIV appears to have begun in an animal reservoir from which it jumped to infect humans.

However, the mechanism by which the virus transferred to humans remains unknown. One explanation is that an infected chimpanzee was consumed by a hunter, who subsequently became infected with the virus himself: this first human case allowed HIV to then be transmitted between people[9]. On the other hand, another hypothesis asserts that the conditions of African colonialism – the economic and political system under which many European powers divided up the African continent into territories under their control – favored the species jump between humans and chimpanzees[10]. One piece of evidence for this theory is that the institution of labor camps, where malnourished Africans would work for long periods, coincided with the rise of HIV. Perhaps the weakened immune systems of these abused workers made it easier for the virus to infect humans.

In addition to these suggestions, inaccurate and/or scientifically refuted explanations have been offered. One such hypothesis proposed that a native ritual involving human consumption of ape blood caused the virus to infect humans[11]. Anthropology scholars have pointed out that this idea is based on a prejudiced view of Africa as a culturally backward nation. There has never been any evidence that rituals of this kind actually existed. Another persistent myth suggests that HIV is a biological weapon designed by the US Government to wipe out homosexuals and African Americans[12]. Yet another inaccurate hypothesis asserts that infected chimpanzee cells were used in preparations of polio vaccine, thereby spreading the virus to polio-vaccinated patients. Incidentally, this claim was later refuted through laboratory analysis of the vaccine preparations[13].

The story is further complicated by scientific evidence that HIV may have been present even earlier than 1959 in the human population. Examples of HIV-like infection crop up as early as 1939 in Germany[14]. Even earlier cases may have occurred in West Africa in 1930[12]. Though such early instances are not plentiful, each possible "first case" inevitably complicates the exact mechanism by which HIV became transmissible within the human population.

The Sails Come In: 1976

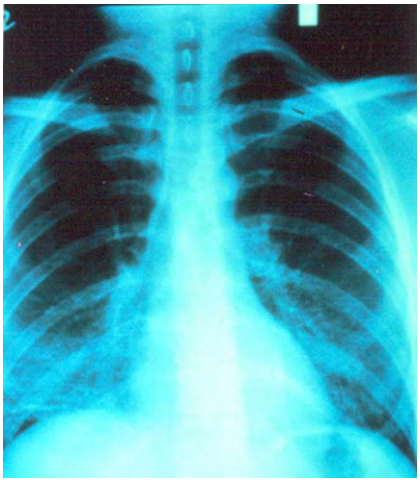
Whatever the ultimate origins of the virus, HIV's introduction to the US might have coincided with July 4th Celebrations in New York City during the bicentennial [15]. While epidemiologists certainly have not resolved the issue, the conjunction of people from all over the world might well have allowed HIV to enter the US population. Years later, evidence of this new pathogen would begin showing up in clinics from New York to San Francisco Bay.

Strange Purple Spots: 1981

St. Francis Hospital physicians were surprised to see the symptoms. The patient, a 37-year old homosexual resident of San Francisco, had suffered from mild stomach problems for two years before the purple splotches began appearing on his skin. Finding that the patient's lymph nodes were swollen, a biopsy was sent to a pathologist at UCSF, who provided the diagnosis of Kaposi's Sarcoma (KS), an extremely rare form of cancer typical of much older patients. This was the first diagnosis of KS in San Francisco. Meanwhile, the patient was worsening, and tests revealed that his headaches were caused by *Cryptococcus*, a common (and normally harmless) fungus found in pigeon droppings. Something odd was going on, but physicians at St. Francis could not tell what it was.

PCP at UCLA: 1981

Among the first hints of the coming epidemic were the increasing number of homosexual patients with *Pneumocystis carinii* pneumonia (PCP) seen in UCLA in early 1981. PCP was a highly rare infection, making the volume of cases peculiar. Initially, high levels of cytomegalovirus (CMV) in the blood of these patients made doctors think that the virus was somehow involved, though it was proving more harmful than usual. As additional cases from the county Public Health department were announced, doctors at UCLA decided to publish a report about these findings. They initially approached the *New England Journal of Medicine*, but after learning of the slow turn-around time, opted instead for the *Morbidity and Mortality Weekly Report*, a publication delivered to thousands of hospitals and other health care providers. The report, published on June 5, 1981 by the CDC, would later become a well-known starting point of the AIDS epidem



Lung X-ray of patient shows infection with *Pneumocystis carinii* pneumonia.

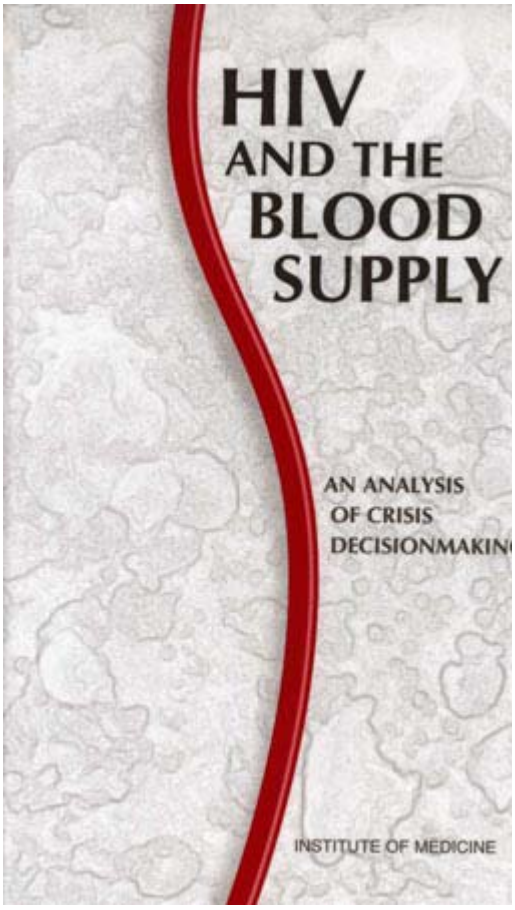
Patient Zero

Ultimately, many of the early HIV victims were revealed to have been partners of Gaetan Dugas, an airline steward from Quebec who frequently traveled between NYC, San Francisco, and other cities. He became known as "Patient Zero" in reference to an epidemiological starting point of the AIDS epidemic. Of course, the disease predated him, but Dugas has nevertheless acquired historical importance as the common relation between many infected with HIV during the early years of the epidemic. He could ultimately be connected to 9 of the 19 cases in Los Angeles, 22 in NYC, and 9 cases in 8 other cities in the US. As a result of this analysis, it became clear that the disease was transmissible. However, the many routes of infection were only hinted at by clinical observations at the time.

Bad Blood

The parents of a baby boy who had received a recent blood transfusion at UCSF were dismayed. After receiving the transfusion, the child was now suffering from what appeared to be an immune dysfunction. Medical records revealed that a 47-year-old man with swollen lymph nodes had donated recently, and that whatever had infected the boy probably originated in this older patient. The potential contamination of the blood supply was just becoming clear, and new safety precautions would have to be taken following the rise of HIV in the US population. At the height of the scare, 194 such cases were reported across 30 states, with infants accounting for 10% of incidents[16].

Perhaps the most famous victim of infection through blood transfusion was Ryan White, a teenaged hemophiliac who was diagnosed with AIDS in 1984[17]. Attempting to return to school after receiving this news, Ryan continually confronted fear and misunderstanding – his school system initially wished to prevent him from attending, and many of his classmates chose to become homeschooled rather than share a classroom with an AIDS patient[17]. Ryan ended up switching to a more tolerant school system, and began appearing on national news programs to promote understanding about the disease[17]. Dying at age 18, his funeral was attended by



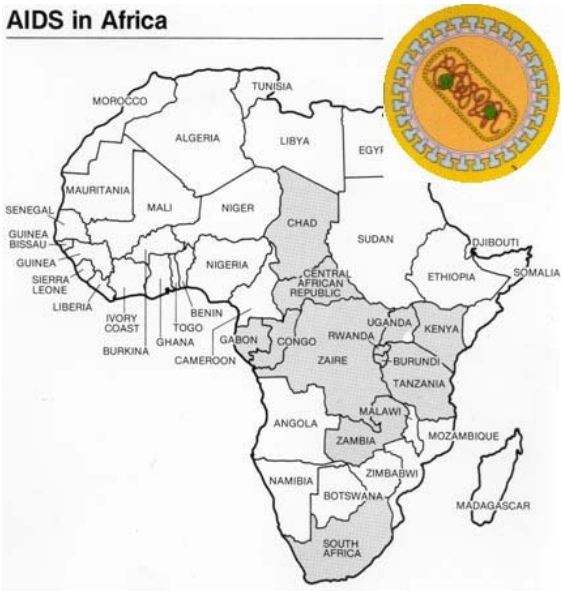
Upon recognizing that HIV could contaminate the blood supply, government scientists sought ways to keep it safe.

many celebrities, including Elton John, who dedicated the song "Candle in the Wind" to the deceased teen[17]. A national AIDS care program now bears the teenager's name, the Ryan White Care Act[17].

As physicians became aware of this route for HIV transmission, donors were more carefully screened before their blood was added to the transfusion pool. Doctors began performing laboratory tests to check for evidence of HIV in samples from potential donors, and the procedures for such tests improved over time. A retrospective study indicated that these measures have been effective: the estimated risk of being infected with HIV through a blood transfusion is 1 in 677,000 units of blood[18]. Further, routine testing of first-time donors over the interval 1991-1996 suggests that these individuals have a much lower prevalence of HIV infection than the general population[19]. What is more, that prevalence declined further over the five years considered in the report[19]. However, there is still cause for concern: research has also demonstrated that routine blood tests can sometimes miss viruses present in blood samples if the amount of virus is too small[20], which can occur with HIV when the virus has not yet begun replicating at full capacity inside its host. Clearly, vigilance is still necessary.

Theories of Transmission 1981-82

Initially, it was unclear exactly how this new disease was transmitted between patients; the initial article in *MMWR* merely mentioned a correlation between homosexual lifestyle and the new outbreak of PCP. The pieces of the puzzle began to fall into place at San Francisco's Public Health Department when reports accumulated showing a pattern of infected lovers, roommates and friends. Though lacking hard proof, scientists began to believe that the condition was probably transmitted through sexual contact. At nearby UCSF, researchers had similar thoughts, suspecting that the label the disease had acquired – Gay-Related Immune Deficiency (GRID) – was misleading. For the time being, Washington wasn't funding research into the new epidemic, even though, as an angry newspaper in San Francisco pointed out, millions had been spent only years earlier on the relatively rare Legionnaire's disease. This theme would continue throughout the early years of the AIDS epidemic, with the virulence of HIV compounded by the stigmatism initially attached to the illness.



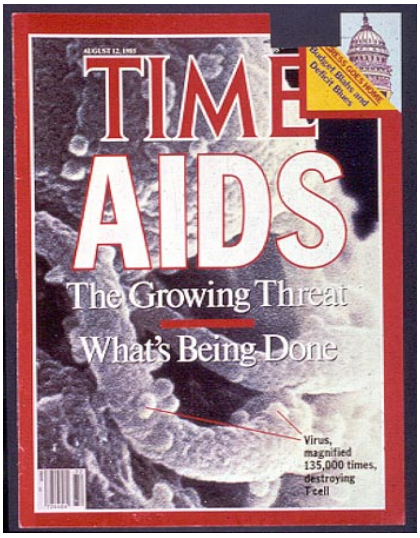
As the epidemic expanded, a map showed the first African countries affected by HIV/AIDS.

1982-83: A Storm Brews

Through 1981, HIV infection remained a gay-associated disease in the US. Meanwhile, unnoticed by much of the Western world, the first hints of a new African medical epidemic emerged

in 1982. Doctors in Uganda reported a new disease they named "slim," due to the way in which its victims wasted away, losing body weight and, towards the terminal phase, struggling to move[21]. Though the symptoms of "slim" bore similarity to HIV-infected patients in the US, the African cases fell into none of the "high-risk" categories identified in the Western World such as homosexuality and drug use [22]. The CDC learned of these cases in 1983, and, guessing that these patients represented only a fraction of the infected population in Africa, quickly convened a team to carry out a field study in Zaire[23]. Upon arrival, even a cursory examination of the under-equipped hospitals in the region revealed the growing devastation. Dying patients, many with the same Kaposi's sarcoma seen among homosexuals infected with HIV in the US, lined the infirmaries.

After blood samples confirmed that these patients were suffering from the same immunodeficiency syndrome as "GRID" victims, the CDC team returned to the US with the unprecedented report that the disease was spreading through heterosexual contact. Indeed, the incidence of "slim" among men and women appeared approximately equal, with the main route of transmission being prostitution, a growing problem in the economically disheveled region[24] [25]. However, the *New England Journal of Medicine*, a premier medical journal, rejected their findings as the editors remained unwilling to believe that the disease was not specific to the gay population. The data from Zaire would not be printed until 1984.



An article in Time reflected the growing threat of AIDS and described the government's response.

This kind of reaction would characterize much of the early work in international AIDS issues. The US was dealing with its own crisis, and many, like the editors of the *NEJM*, still believed the disease to be unique to the gay population. The true dimensions of the AIDS crisis in Africa would only become apparent later, after it was too late to save millions of victims.

AIDS activists organized to spur AIDS research and to make experimental treatments more widely available.

Garnering (Lack of) Support

Though the CDC's report on Africa was ignored, increasing numbers of KS and PCP cases in cities across the US were not so easily dismissed, and scientists began seeking funding to address the new crisis. However, in these early years they would frequently find the road for

support a frustrating and difficult path. Economic policies at the time meant that the medical research budget was just ahead of inflation with no support allocated at all to GRID/AIDS. Anyone who wanted to study the disease would have to find support through odds and ends. At the meeting, speakers described the stigmatism attached to the disease because of its association with homosexuality and pointed out that tens of thousands of dollars were spent on three cases, a cost that would only grow with time. Problems accumulated: in addition to the paltry $1 million offered by the National Cancer Institute (NCI) for KS research, the Hollywood conference was not even picked up by the media, further underscoring the combination of scientific and public policy issues that would reign during the early years of the AIDS epidemic.

Expanding Risk

Clues from African epidemiology notwithstanding, data from other parts of the country began to reveal the larger scope of the AIDS epidemic. By early 1983, for example, gay patients were only a minority of the AIDS cases in New Jersey with intravenous drug users and immigrant Haitians making up large proportions of the incidences. Indeed, AIDS was becoming a disease of many marginalized groups as epidemiologists documented its spread among black and Hispanic residents of the low-income areas around New York.

Candlelight March

Confronted by a growing health crisis, activists in the US were challenged to put a human face on the tragedy, and so force the government and public to finally accept the magnitude of the growing epidemic. A major moment in this effort occurred on May 2, 1983 when a crowd gathered on Castro Street in San Francisco and carried candles in a mile-long line in remembrance of lost friends. The event garnered immediate media attention, and similar marches were planned in other US cities.

Policing the Baths

Activism led to increasing awareness of the AIDS crisis, with the response of public health officials not always welcomed by lobbyist groups. In 1983, San Francisco's government attempted to place restrictions on the city's bathhouses, the clubs and exercise facilities where the city's homosexual population met to socialize. Many feared that such places had helped the initial spread of AIDS. Some wished to entirely shut down the facilities, while the mayor and her allies took a more moderate approach, advocating that the bathhouses provide safe-sex information to customers. Though supposedly a health issue, the debate was fueled equally by concerns over whether closing the bathhouses was actually policing gay sexuality. Initially, rather than closing the facilities, high-risk sexual activity within them was banned in 1984, a move that raised objections from both sides of the debate. By October, many baths were officially ordered to shut down, and over the following months, most would close of their own accord due to declining business.

A House for AIDS

Bathhouses were not the only establishments facing restrictions, as hospitals for AIDS patients also encountered roadblocks. Due to objections from university officials,

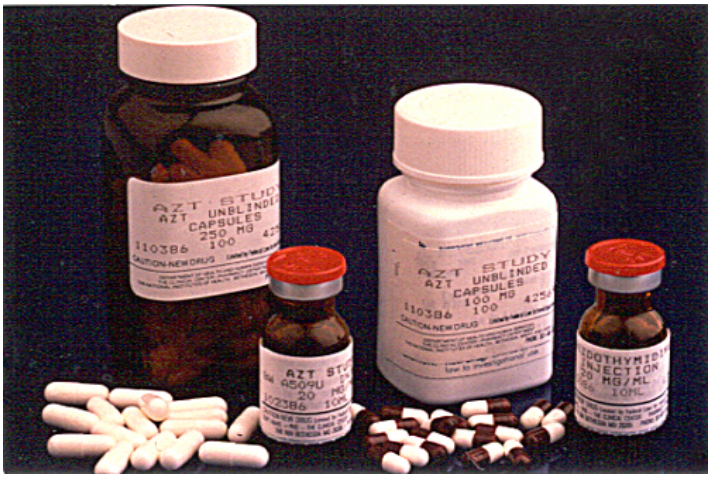
initial efforts to establish an AIDS clinic at UCSF were unsuccessful, and the unit was ultimately located at San Francisco General Hospital. It was the first of its kind and therefore became a focus of AIDS treatment (and not always in a completely humanitarian way). For example, in 1983 an AIDS patient was sent from a Florida hospital to the San Francisco facility because the original health center estimated that it would cost less money to fly the man to California than to care for him during the terminal phase of the disease.

Rock Hudson

A major turning point in the public perception of AIDS occurred on July 23, 1985, when actor Rock Hudson, recognized as a dashing leading man in television and film, was revealed to be ailing from AIDS-related liver cancer. Suddenly, major networks were all covering the story, and AIDS had become not just the disease of the dispossessed but the affliction of America's leading man. The physicians, who had been battling for such recognition of the disease for years, recognized the announcement as a major change, a fact that would be confirmed by a fundraiser in Los Angeles that made $630,000 in a single day. Michael Gottlieb, the man who had originally reported AIDS in *MMWR* years earlier, delivered Hudson's official diagnosis of AIDS to a media conference after seeing the actor at UCLA.

1985: The First International AIDS Conference and False Statistics

A similarly important press conference that year was the first International AIDS Conference convened by the CDC in Atlanta, Georgia. At this event, many leading scientists presented alarming statistics about the spread of the disease in Africa, claiming that over 50% of certain demographics (e.g. children, prostitutes) were infected in some countries. However, many of the figures were unintentionally inflated due to problems with the blood testing methods, since concurrent patient infections could elicit "false positives". These methodological errors would undermine the credibility of African AIDS epidemiology for years to come, leading US policy makers to think that the problem was less severe than it actually was.



Zidovudine, better known as AZT, was the first antiviral shown to be effective against AIDS.

Funding paralleled this false notion: by the end of 1986, when almost 4 million people globally were infected with HIV and a quarter million had actually died of AIDS, the US had donated a grand total of $2 million to relief efforts internationally [26]. This money was used to begin a global AIDS program under the auspices of USAID, an organization created by the Kennedy administration to assist the development of needy

foreign nations. The program faced an uphill battle, as the disease was still highly stigmatized in the US. Activists had their hands full trying to secure relief for patients in San Francisco and New York City, let alone Africa. Next to domestic issues, global AIDS did not register on the national radar.

A Cure? ARVs and the Global Pandemic

Through the end of the G.H.W. Bush administration and Clinton's first term, little progress had occurred in the treatment of Africa's growing AIDS epidemic. Political disasters, such as the capture of US Rangers in Mogadishu, Somalia, made African interventions seem less attractive. Congressional focus remained directed inwards towards domestic policy issues, and even as funding for homeland AIDS programs such as the Ryan White CARE act increased, the US hardly supported the global AIDS efforts.

Against this backdrop, the Eleventh International AIDS Conference was convened in Vancouver in 1996. During the event came the historic announcement of the development of antiretroviral, or ARV, therapy, a drug regime that promised to suppress the virus' effects. Suddenly, AIDS seemed treatable. Deaths from the disease dropped precipitously in the next two years, and many major newspapers ran articles declaring the end of AIDS. The problem seemed to be solved for many Americans, even though access to the promising antiretroviral therapy would remain an impossibility for Africa for years to come.

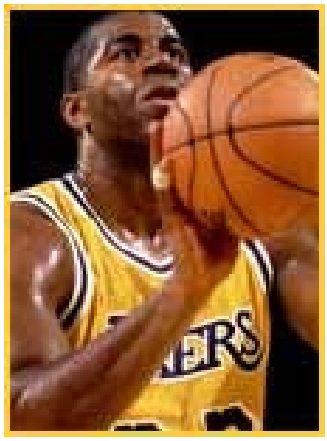
South African Crisis

In February 1990, only three months after the Berlin Wall came down, Nelson Mandela was released from twenty-seven years of imprisonment in South Africa. Apartheid was being dismantled, and it appeared that a new era was beginning for the beleaguered nation. However, the rise of the AIDS epidemic would parallel the country's new freedom. The roots of the epidemic had been laid in the 70's and 80's, when apartheid drove South African freedom fighters into neighboring countries where they contracted the virus. This system of racial segregation also produced a vast migratory labor population who, removed from their families, were drawn to mining towns where prostitution – and HIV – spread rampantly. All these factors combined for imminent disaster when the freedom fighters and laborers returned home after the end of apartheid when they transmitted the virus to their wives (and indirectly, children).

Nelson Mandela, freed as his homeland became trapped by an epidemic.

Statistics reinforced the escalating crisis. In 1990, only 1 percent of South Africa's pregnant women were infected with HIV; only 4 years later, this figure had risen to 8 percent [27]. Early efforts to raise public awareness about the issue had failed, and Mandela hardly addressed the matter even after assuming the presidency. In 2000, South Africa led the world in most HIV prevalence, topping 5 million HIV-infected individuals[28].

Magic Johnson



A world away from South Africa, the LA Lakers were dealing with their own crisis. In an announcement with as much social impact as Rock Hudson's diagnosis in 1985, NBA star Magic Johnson revealed that he was HIV-positive to a press conference at the Laker's stadium in 1991[29]. Johnson had learned of his infection during a routine blood test and believed he had contracted the virus from a female lover during his promiscuous early years. Retiring from the NBA, Johnson became a leading advocate for HIV/AIDS prevention and treatment through the charitable foundation he created, using his celebrity status to reach vulnerable sections of the US population such as inner city youth.

AIDS in the Real World: Pedro Zamora

Eventually, HIV/AIDS would appear not just on the silver screen in the form of Rock Hudson, or on the basketball court in the case of Magic Johnson: it struck every media, entering America's households weekly during the second season of MTV's hit reality show *The Real World*. The 1993-94 season, filmed, appropriately perhaps, in San Francisco, featured a gay Cuban immigrant among its cast, who revealed both his sexual orientation and HIV-positive status to his housemates[30]. Pedro Zamora had decided to be a part of the show in order to raise awareness about the disease[31], and in addition to the regular broadcasts toured high schools to promote greater public visibility for the AIDS epidemic. He became a spokesperson for those afflicted with the virus, speaking before the US congress as well as on prominent news programs[32]. He died in 1994, shortly after the filming of *The Real World* had wrapped for the season, but Zamora remains an important figure in the history of AIDS activism[33].

AIDS activist and *The Real World* cast member Pedro Zamora.

Pharmaceutical Debate

In 1997, as Johnson encouraged support for AIDS research in the US, Vice President Gore was encouraging South Africa to address their own health epidemic. South Africa responded almost too well, beginning to import antiretroviral drugs from countries where the cocktails were cheaper than in the US. As a result, many major drug

development firms filed suits against the patent-defying nation. Gore, who was depending upon financial support from the pharmaceutical industry in the next Presidential election, urged South African leaders to reconsider their decision. Soon after, South Africa appeared on the "watch list" of the US Trade Representative, jeopardizing the nation's ability to attract foreign investment because of the risk implied by this designation. Ultimately, a deal was brokered in which South Africa was taken off the "watch list," in exchange for reversing their anti-pharmaceutical policies. However, members of the South African government also began seeking independence from Western economies, to the point of adopting the use of toxic drugs created in Africa over effective antiretroviral therapies. Even when President Clinton announced that the US would not take punitive action against other nations for importing less expensive antiretroviral drugs in 2000, many South African leaders had already shunned Western medicines.

President George W. Bush announces a new Mother and Child HIV Prevention Initiative. Standing by the President from, left to right, are Secretary of Treasury Paul O'Neill, Secretary of Health and Human Services Tommy Thompson and Secretary of State Colin Powell.

Growing Crisis: The Expansion of AIDS in Africa

By the time the Eleventh International Conference on AIDS came to Lusaka, Zambia in 1999, AIDS had already claimed 11 million victims in Africa, while 23 million were infected with HIV [23]. By the late 1990s, almost ten times as many Africans were dying of AIDS than war, a sobering figure in the midst of war-torn continent [34]. In 1999, leaders of several southern African nations, including Zimbabwe, Namibia, and Kenya, finally acknowledged that AIDS was a dire issue. Why had it taken so long for the crisis to be publicly recognized? Even moreso than the US, sexuality remained a taboo issue in many of these nations. Additionally, leaders did not wish to acknowledge that practices such as prostitution existed within their borders. The leaders of many nations were in their sixties and seventies - elderly and religious men who did not wish to confront the implications of the disease. Further, many feared that admitting the devastation wrought by AIDS might have adverse effects on foreign economics interests or their perceived strength among rival countries. Still, many of these leaders continued to spend more money on defense than public health.

A Global Fund and a New Pledge

The 2001 African Summit on HIV/AIDS, Tuberculosis and Other Infectious Diseases proved a momentous event as UN Secretary General Kofi Annan revealed his plans for a Global Fund to battle these illnesses, based on financial counsel from leading US economists. Though Annan had asked for billions in contributions to the endeavor,

the US fell short in these expectations, delivering an initial investment of only $200 million. Ultimately, the first substantial pledge of aid to the global AIDS crisis would come in the 2003 State of the Union address, when Bush declared that the US would commit $15 billion over five years to AIDS relief in Africa and the Caribbean. The impact of this Emergency Plan for AIDS Relief remains to be seen.

Comprehension Questions:

1. From which continent did HIV originate? What is a plausible theory for its emergence?
2. What was the primary clinical indication that HIV had come to San Francisco? In what group was it seen most prevalently?
3. What are some reasons that AIDS patients were stigmatized during the early years of the epidemic?
4. What are some issues surrounding the distribution of AIDS drugs in South Africa?
5. What celebrities have been infected with HIV? What effect did their infection have on US perception of AIDS?
6. What is the current US plan to confront the AIDS epidemic in Africa?

HIV/AIDS: The Biology

Clearly, HIV/AIDS has had a significant impact on human history in the last three decades. But what do we know about the culprit, the virus itself? How does it kill? How is transmitted? What can we do to stop it? In the following sections, we introduce the biology of the virus responsible for AIDS, which will provide a basis for our discussions of viral replication in later units.

What is HIV? What is AIDS?

As in the case of pathogens discussed in later units, HIV/AIDS terminology is often confused in everyday conversation. HIV and AIDS have become almost synonymous, even though from a biological perspective, they are far from the same. **Human Immunodeficiency Virus (HIV)** is the infectious agent *responsible for* **Acquired Immune Deficiency Syndrome (AIDS).** "HIV positive" does not necessarily mean that someone also has AIDS- just that s/he will potentially develop the illness.

Incidentally, the term "syndrome" is actually outdated. Typically, a "syndrome" is a set of clinical symptoms for which the cause is unknown. In the early 1980s, when scientists hadn't yet identified HIV as the cause of AIDS, physicians could only observe the effects of HIV infection, not the transmission of the virus. As a result, they called it a syndrome, because they didn't know that all the various infections they observed in AIDS patients were caused by a common virus.[35] Now that the details of the pathology of the HIV virus are known, AIDS can be labeled as a disease. Indeed, a better name for AIDS might be "HIV Viral Disease."[35]

"No One Dies of AIDS"[36]

This quote, taken from the pages of the *New York Times*, isn't just journalistic exaggeration. As will be illustrated below, AIDS doesn't actually kill its victims, but

merely leaves them helpless to ward off other opportunistic infections by organisms that wouldn't ordinarily cause disease but do so when an individual's immune system is too weak to fight pathogens.[37] However, before we launch into further detail, it is useful to keep the following principles in mind.

1. **One disease, many treatments**: Even though the HIV virus itself cannot be entirely removed from a patient's body as yet, the prevention of opportunistic infections can greatly extend the lifespan of those afflicted with AIDS. The use of an entire "pathogen arsenal" - antibacterial, antifungal, antiparasitic drugs - may improve a patient's ability to resist other opportunistic infections, and, as a result, prolong life.

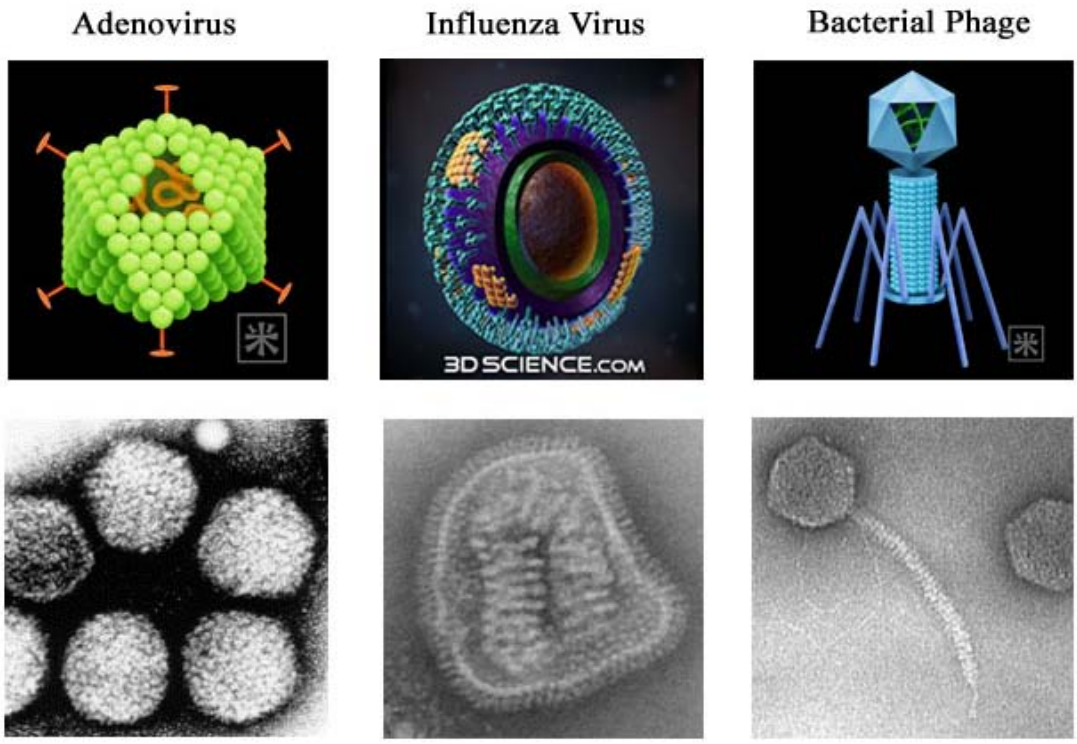
2. **AIDS can "interact" with other diseases**: This was demonstrated by the concurrence of the AIDS epidemic with the emergence of drug-resistant strains of tuberculosis in the early 1990s.[38] Strategies to combat AIDS are necessary not just for their own sake, but also because immune-compromised patients may effectively serve as "reservoirs" for other pathogens to propagate.

3. **Each AIDS patient may have different symptoms**: Related to the point above, each patient, though they are infected with the same virus, may experience different symptoms based on the opportunistic pathogens that colonize their body. Thus, each case is in some sense an individual disease.

Keep these in mind when thinking about the biology of HIV/AIDS, and you should have a better appreciation for how treatment is practiced. First, though, it is necessary to understand the pathogen responsible for this disease: the HIV virus.

A Virus

Viewed under a powerful microscope, a virus doesn't seem like much: just a tiny shell of protein covering the nucleic acid of its genome. A genome, in turn, is just the entire genetic sequence of an organism encoded in DNA or RNA. The smallest of all pathogens, they are measured in fractions of a micron [39]. Yet, despite their size, viruses actually constitute the largest biomass on the planet [40]. Additionally appearances can be deceiving since even this minute clump of protein and nucleic acid can ravage the human body, slipping inside cells and replicating at a frightening rate.



The many shapes and shades of viruses. Top: Artistic render, Bottom: Electron micrograph. Top left, right: Original artwork by Senmaio Zhan.

The small size and relative simplicity of viruses raises an important question: are they alive? Like higher organisms, viruses have DNA or RNA genomes. In other aspects though, viruses deviate wildly from what is traditionally regarded as "life" [41]. They don't grow. They don't carry out metabolic activities like glycolysis or respiration. They don't respond to environmental stimuli. They don't have limbs or flagella to propel them through their environment. In fact, they are not even composed of cells! Yet, viruses *can* replicate, doing so by hijacking the molecular machinery of the host cell [39]. So are they alive? By traditional definitions, no – but these criteria were based on the characteristics of larger organisms. If instead the criteria for life are based on the *smallest* known life forms (like viruses), then the only real requirement becomes replication, with viruses forming a class of "acellular" organisms.

Viruses are found in many different shapes and sizes. Classes of different viruses can be characterized by identifying factors including their genome type (i.e. single- or double-stranded RNA or DNA), mode of replication, and whether it is typically surrounded by a plasma membrane derived from its host cell. Specifically, the HIV virus possesses a single-stranded RNA genome and replicates through a DNA intermediate through the actions of the enzyme reverse transcriptase. Because of these properties, HIV is also known as a retrovirus. Other classes include viruses with double-stranded DNA

genomes (e.g. smallpox and herpes virus) and viruses with single-stranded RNA genomes (Ebola and influenza).

A virus exists in two forms: one, in which a mobile virion (single virus) drifts outside a host cell and the second, in which the virus invades a host cell and replicates [42]. For HIV, this time inside a host cell is further divided into the latent and lytic stages. During the latent stage, the viral genome remains unreplicated inside the host – in the case of HIV, for up to a decade[43]. While it isn't replicating, it is invisible to the immune system[43]. This situation is reversed during the lytic stage when the virus replicates, overwhelming the host cell and causing the cell to burst. This cell lysis releases a surge of virions that can go on to infect other host cells[44]. Together, these phases form a carefully orchestrated set of biochemical events, not unlike a symphony with two movements. At each stage, HIV acts as a conductor, directing its host cell in the complex task of hiding and then replicating the tiny invader.

Comprehension Questions:

1. What shapes can a virus have?
2. What does it mean to be an obligate intracellular pathogen?
3. What characteristics are used to classify a virus?
4. What are the general characteristics of the "viral life cycle"?

"The HIV Symphony": An Analogy

In order to understand the molecular processes by which HIV infiltrates a human cell and replicates, consider the following:

*Franz Schroeder is an out-of-work conductor living in Vienna. He's just finished his latest masterpiece, but all the orchestras in Vienna are booked for the current season. However, looking in the newspaper one day he sees a notice: "*Royal Symphony Orchestra of Copenhagen seeks orchestra master. Auditions ongoing." *Overjoyed, Franz immediately boards a plane to Denmark, carrying the newspaper clipping with him to identify the Royal Symphony Orchestra Hall. After hours of searching the streets of Copenhagen, he finds an imposing building at the corner of the marketplace. He checks the name on the billboard outside against his clipping: they are the same.*

However he finds that the building is locked. Inquiring with the doorman, Franz learns that he needs to get a key from the bank across the street, which is helping to organize the search for an orchestra master for the Royal Symphony. Obtaining the key from the bank, Franz finally enters the symphony hall and, when greeted by the musicians, realizes he has a large problem: they speak Danish, and he only knows German! The sheet music itself may be universal, but Franz's liner notes are in his native script.

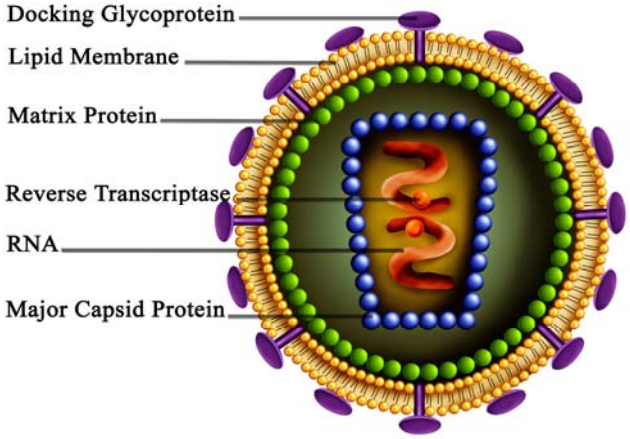
Returning to the bank across the street, the frustrated conductor manages to get the address of a translator, who returns with him to the symphony hall. After a few hours of work, she is able to transcribe his liner notes into Danish, and musicians begin playing Franz's latest work. They are impressed, and decide to hire him, inserting his piece into

their current repertoire. However, the old walls of the Royal Symphony Hall are fragile, having been disturbed by an earthquake a few years back. While this hasn't been problem so far, Franz's symphony happens to contain the exact vibrational frequencies that will cause the building to collapse, which it does after the first few performances of the season.

So how does the story of this disastrous performance relate to viral replication? Let's start with the conductor.

The Conductor: HIV

The conductor of our symphony is the HIV virus. Only about a ten-thousandth of a millimeter across, the HIV virion appears as a spherical plasma membrane, or viral envelope, encasing an inner core of viral proteins and RNA[43]. Like most biological membranes, the viral envelope of HIV consists of a lipid bilayer punctuated by spines of glycoproteins or protein molecules that combine carbohydrates in their structures[43]. Underneath this bristling exterior is a layer called the matrix, a type of "inner envelope" composed of only proteins[43]. If the envelope is the "skin" of the virion, then this inner layer of protein forms a "skeleton." Finally, encircled by the matrix is the capsid, a projectile-shaped mass of protein[43]. The contents of this protein bullet are the HIV's RNA genome and the three enzymes it needs to replicate it: protease, integrase, and reverse transcriptase[43]. While the protease clips viral proteins into their final, functional form, and integrase allows the viral genome to be integrated into its host's genome, reverse transcriptase is perhaps the most important of the three: by translating RNA instructions into DNA language, it allows HIV to replicate using it host's machinery[43]. This is how the HIV "conductor" translates its "sheet music."



Deadly message in an envelope: the architecture of the HIV virion. Original artwork by Senmaio Zhan.

The Sheet Music: the HIV genome

If the immense human genome is a symphony, then HIV has only a few lines of music by comparison. However, even these nine RNA genes are sufficient for the virus' deadly activity[45]. These nine genes, when produced by the cell's protein-making machinery, are divided into three functional classes:[*]

Structural Proteins: These are the proteins found in viral envelope[46].

Regulatory Proteins: These proteins ensure that the viral RNA is copied correctly, during infection[46].

Accessory Proteins: These four proteins determine the effectiveness with which HIV colonizes a cell[46].

How the Conductor Moves: HIV Transmission

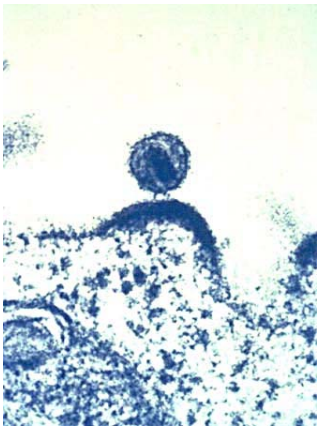
Clearly, unlike our conductor, the virus can't move between bodies by boarding a plane. So how does HIV actually move between human bodies and into the blood stream where it causes so much damage? The most common means of transmission is unprotected sex of any sort – vaginal, oral, or anal. The virus enters the body through the mucosal lining of any of these organs[47].

Routes of HIV transmission. Original artwork by Senmaio Zhan.

In addition, there are also a number of non-sexual means of transmission to keep in mind as well[47]. Since the virus is present in the serum of infected individuals, exchanging needles (a common practice among intravenous drug users) can serve as a means of infection[47]. Similarly, there have been documented cases in which AIDS was contracted through a blood transfusion though transmission through this particular route is almost non-existent with the development of routine HIV testing[47]. Blood or breast milk can also facilitate transmission of the virus between mother and child, a phenomenon responsible for a proportion of HIV proliferation in sub-Saharan Africa[47,48].

The Symphony Hall: Human Immune Cells

Once inside the human body – whether it is carried in by blood or other bodily fluids – the HIV conductor looks for the right home, using its glycoprotein spines to prod adjacent cell surfaces. What it is looking for is the protein **CD4**, a protein found jutting from the membranes of two kinds of white blood cells: **"helper" T cells** and **macrophages**[43]. **Helper T Cells** excrete chemicals that help fight off invading pathogens; **macrophages** directly engulf these intruders[43,†]. Thus, like our conductor, the virus locates the



The HIV virus is shown budding out of a human immune cell, which the virus infects and uses to replicate.

[†] See the Immunology Appendix for more specific information on classes of lymphocytes.

right place to play its RNA notes.

However, even having located the CD4 “sign”, the virus still needs a key to enter. This key is the **co-receptor**, a second molecule that completes the link between the virion and the cell. This link allows HIV to be absorbed in an envelope of plasma membrane[43]. The specific co-receptor molecule employed by the virion varies based on how far HIV infection has progressed[43]. For example, during the early stages of infection the co-receptor CCR5 is the virion’s exclusive target[43]. However, over time a wide array of molecules can be utilized as secondary attachment points by HIV[43].

Translating the Sheet Music: Reverse Transcriptase

Having entered a white blood cell in a pocket of plasma membrane, HIV uses reverse transcriptase to copy its RNA genome into DNA[43]. The integrase enzyme then cuts and pastes the DNA into the host genome[43]. At this point, the virus has two options: it can either hide from the immune system in its untranslated form, or forge ahead with the creation of new copies of itself.

This first possibility accounts for the frequent gap between HIV infection and the appearance of AIDS symptoms. While on a microscopic scale HIV’s replication is dramatic, it is only slowly recognizable in the human scale. In fact, it rightfully earns its title as a lentivirus, a class of viruses sharing an extended period from the point of infection to the first clinical signs of disease[43]. For HIV, this “incubation period” can last 10 or more years, a pause explained on a cellular level by the initially aggressive response to infection by the body’s immune system[43]. However, the high number of cells where the virus is replicating coupled with rapid mutations that hides it from the body’s homing systems, ensures that not every virus is destroyed[43]. Also, the viral genome sequence can remain invisible to the immune system by remaining latent (though integrated) in the host genome[43]. Even the trace amounts of virus remaining are responsible for the devastating clinical symptoms that ultimately result[43].

Alternatively, the virus borrows its host’s enzymes, and it’s a simple matter of the cell carrying out its normal transcription and translation processes on this foreign DNA sequence, producing the proteins and RNA strands necessary to build a new virus. Both these components, once they are relocated from the nucleus to the cytoplasm, are bundled into a pocket of plasma membrane[43]. However, the new virus must use the enzyme protease to finish assembly, since the proteins that compose its inner core have to be cut to a specific size before the component can be turned into a fully operational virus[43]. Like the orchestra hall weakened by the music performed within, the cell begins to crumble after repeated viral replications, unleashing new virions into the body.

Precisely how the HIV destroys its host cell, though, has not yet been entirely resolved[43]. One theory is that the high volume of viral replication within the host simply overwhelms it, preventing its internal machinery from functioning[43]. The virus may also signal the cell to kill itself, or other blood cells to engulf the host[43,47].

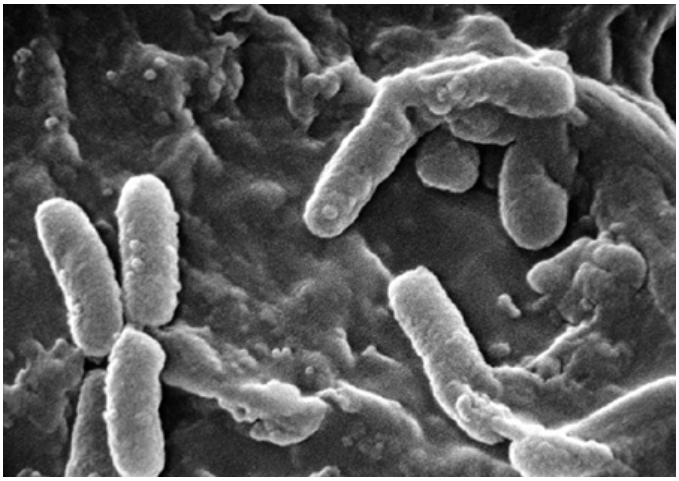
Comprehension Questions:

1. What are the components of the HIV virion?
2. What are the stages of its replication?
3. What enzyme does the virus use to replicate its RNA genome?
4. What molecule allows the HIV virion to stick to its target cell?

A Wilting Defense Against Unwanted Visitors: Immunodeficiency

Once HIV has had enough time to wear away at the immune system by steadily destroying the cells that once protected the body from invasion, symptoms of AIDS begin to appear. Indeed, the disease is officially defined by particularly low counts of CD4-expressing T-cells in a patient's blood[47]. As a result of this decreased arsenal of immune cells, a plethora of normally harmless bacteria, fungi, and other microorganisms begin to invade the body. These infections, called opportunistic because they don't infect individuals with healthy immune systems, require the "opportunity" offered when HIV destroys T cells to enter a human body. In this sense, HIV doesn't actually kill a person; it merely opens the way for an army of other threats to colonize its immunodeficient victim including bacteria, fungi, other viruses, and parasites[49].

Bacteria: A good example of an opportunistic bacterial infection is *Pseudomonas aeruginosa*, a microbe found throughout the natural world, including the human body. Normally harmless, the bacteria grows to unhealthy numbers in AIDS patients, causing pneumonia, ulcers, dermatitis (e.g. skin inflammation) and other reactions[50]. This diversity of symptoms is a reminder that each AIDS patient may have an entirely different reaction to the disease.



Electron micrograph of Pseudomonas bacteria.

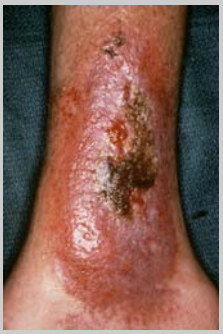
Fungal: The most common cause of fungal pneumonia among AIDS patients is *Cryptococcus neoformans*, a type of yeast that occurs naturally in soil samples and bird excrement [51]. It can also cause meningitis, as it has a tendency to colonize the central nervous system (CNS) in immunodepressed individuals such as AIDS patients.

Viral: Just because a patient has HIV doesn't preclude infection with other viruses; in fact, it may actually make it easier for other viruses to invade, such as Cytomegalovirus (CMV), one of the major causes of mortality during the early US AIDS epidemic [52]. CMV causes a number of symptoms, from retinal lesions to CNS disorders[53].

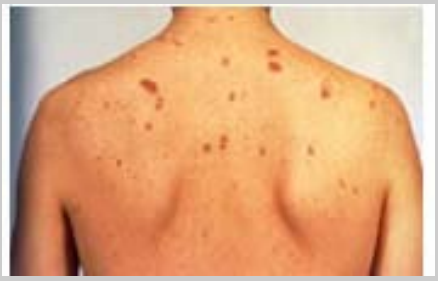
Parasites: Toxoplasma gondii is an example of a parasite that preys upon AIDS patients; it is normally encountered in uncooked meat or vegetables [54]. In AIDS patients, *Toxoplasma* colonization often causes sores in the brain, called *lesions*.[55] This condition, called *toxoplasmic encephalitis*, can produce neurological symptoms such as seizures and movement disorders, though it isn't usually fatal[56,57].

Kaposi's Sarcoma

An early sign of the AIDS epidemic in the US was the unusual number of cases of Kaposi's Sarcoma (KS) showing up in San Francisco area hospitals. Kaposi's Sarcoma is a rare form of cancer that develops in connective tissue (like muscle and fat) or fibrous tissue (like tendons), causing red-purple blotches in the affected area [58]. While these can be painful when they form on the skin, they are usually harmless. However, if the sores appear on internal organs such as the lung or intestines, they can cause life-threatening injury[58]. For example, a KS lesion in the intestine will often cause massive internal bleeding because the growth disrupts and opens the numerous blood vessels in this tissue. Before AIDS entered the US in the 1980s, KS was extremely rare, affecting mainly elderly males of European (primarily Mediterranean) origin[58]. However, the rash of cases among young homosexual men in San Francisco was one of the first clues that a new disease had entered the US population.



Above: Kaposi's sarcoma of the leg
Right: KS along back,



Comprehension Questions:

1. What does it mean to be "immunodeficient"?
2. How do pathogens that infect immunodeficient patients differ from regular pathogens?

Diagnosis

The immune system can be compromised by other factors than AIDS. So how do doctors tell for sure if a patient has the virus? There are actually a number of common blood tests for HIV infection, and a combination of several positive results is used to verify the diagnosis. With names such as *ELISA*, *western blot*, and *Immunofluorescence*, all of these different tests basically boil down to a simple principle:

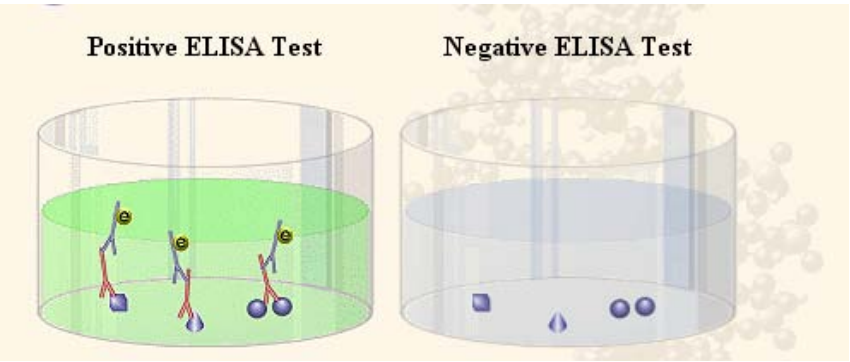
1. Take an antibody against the HIV virus. This is a "Y-shaped" molecule produced by white blood cells that allows the immune system to generate responses to specific pathogens. The "cleft" in the "Y" is what allows this recognition, since

the chemical makeup of its surface allows it to recognize a molecular "fingerprint" called an epitope. While the body naturally generates antibodies, they can also be generated through artificial means through advanced laboratory procedures: this is how scientists get the antibodies for HIV.

2. Apply the antibody to a treated sample of blood, in which a chemical/physical process has been carried out to purify contaminants from the crude sample taken from the patient's vein. Just because the antibody is *supposed* to recognize the HIV "fingerprint" doesn't mean it *can't* bind to other molecules. Indeed, many artificially generated antibodies have limited specificity, sometimes attaching to targets other than the one they were created to recognize. To lessen this problem, the blood sample from the patient has to be purified, which is usually done by spinning it at high velocity to remove the larger components from the mixture. These heavy components, called platelets, are what allow scabs to form, but can also complicate tests because antibodies tend to attach non-specifically to such large molecules.

3. Wash away unbound antibody. Because the antibody will theoretically bind tighter to the particular "fingerprint" it recognizes than other molecules, washing the sample with a gentle solution will both remove unbound molecules and get rid of many non-specifically bound antibodies. If left in the sample, the unbound molecule would also "light up", making the result impossible to read – the antibody could have bound to nothing, and it would still look positive!

4. Indicate where the antibody has bound through a color or light signal. Usually, the "light" molecule attaches to the end of the antibody (the "stem"), and is activated by a particular solution being poured over it.



A nice animation of the ELISA test in HIV diagnosis can be viewed at: http://www.biology.arizona.edu/IMMUNOLOGY/activities/elisa/main.html.

These tests tend to be very specific, because antibodies are naturally generated by the body to recognize extremely specific protein surfaces or other biological elements. Thus, when used in laboratory tests against an artificial target, false-negative results are quite uncommon[59].

Treatments

While there is not currently a cure for AIDS, a number of treatments exist for those afflicted.

Antiretrovirals:

These compounds interfere with HIV's fundamental biochemical processes. Many times, like antibiotics, these drugs are given in "cocktails" containing several different drugs. The simultaneous use of multiple drugs counteracts the virus' ability to evolve resistance to the medicine, an important concern in light of HIV's rapid replication.[60] Indeed, as discussed below, this rapid mutation has greatly inhibited attempts to generate an AIDS vaccine. There are currently two broad classes of antiretrovirals available:

1. *Reverse transcriptase inhibitors (RTIs)*

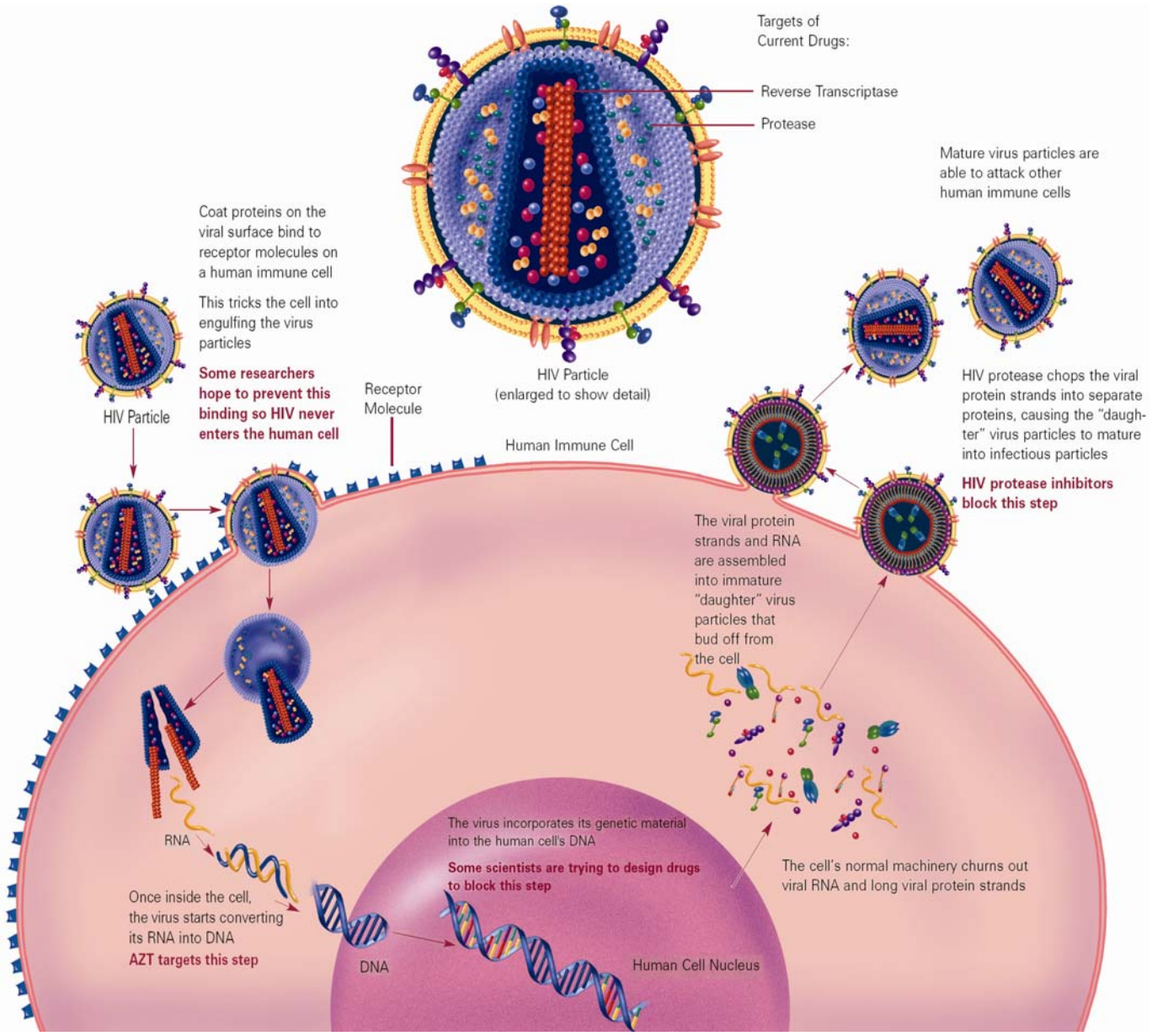
A common target of HIV therapy is the reverse transcriptase enzyme. Remember that reverse transcriptase allows HIV to copy its RNA genome into a DNA strand that is inserted into the host cell's genome.[43] Some RTIs are chemically similar to the building blocks of DNA (e.g. the A,T,G, and C used to build a DNA strand), but once the RTI is added to a growing DNA strand, it doesn't have the right "connector" to allow further elongation [61]. In essence, it's like adding a caboose in the wrong place in a train: the line of cars is terminated, even if all the passenger cabs aren't yet attached. Without a complete copy of the HIV genome, the DNA version will be defective, unable to produce full viral particles to destroy the host cell. A well-known RTI is azidodeoxythymidine (AZT), the first approved AIDS drug[62].

2. *Protease Inhibitors*

When the HIV genome is copied by the cell's translational machinery, it is not fully-formed. The proteins that make up the viral coat have to be "cut" by proteases into their correct length[43]. As an example, think of making a three dimensional object out of paper; the sheet has to be cut in the correct places to make the folds possible. Protease inhibitors effectively disable the protease "scissors" used for this purpose, thereby preventing the HIV proteins from folding into their correct conformation [61]. However, protease inhibitors often require large doses, which can be unpleasant considering their usually foul taste. Like other antiretrovirals, they can also have adverse reactions with other drugs.

Antiretrovirals: Tailoring the Treatment

In clinical trials, reverse transcriptase inhibitors have proven more clinically effective than protease inhibitors[2]. Further, antiretrovirals have proven effective even when combined with antibiotic treatment, a regimen sometimes instituted for immunodeficient patients suffering from TB[3]. However, the saying "every patient is different" is certainly true in the case of antiretroviral therapy for AIDS. Not only the mode of action of the drug itself, but also variations between patients, can alter the performance of these compounds. Differences in the genetic sequences of an individual's enzymes, along with variables such as gender, race, and weight, can drastically affect the effectiveness of HIV treatment [4]. For example, there is documented evidence that the RTI drug efavirnez is metabolized (or broken down into inactive components) more slowly by blacks and Hispanics than Caucasians [5]. In this case, the difference is caused by genetic variations in the liver enzyme responsible for breaking down efavirnez in the body [6]. Blacks and Hispanics usually have the less active form of the enzyme, meaning the drug is broken down more slowly [6]. Consequently, lower doses of efavirnez are needed to treat black and Hispanics than Caucasians. Outside genetic differences among patients, geography can also impact treatment. For example, in areas where other epidemics are also prevalent (e.g., malaria in Africa, or herpes virus in the US), the interaction of drugs for each illness must be taken into consideration when deciding on treatments.



The HIV life cycle and points of drug intervention.

Vaccines:

Over the years, researchers have taken a number of approaches to developing HIV vaccines, using different molecular targets including:

1. Subunits of the virus: By training the immune system to target the sticky glycoprotein "spines" on the surface of the HIV envelope, a patient might be immunized by exposing them to just pieces of the intact virus – a safer strategy than using inactive forms of the complete virus[63]. However, this approach is

impeded because the body does not seem to respond equally to free-floating and envelope-embedded forms of these glycoproteins.

2. Hybrid viruses: By fusing components of HIV into a non-HIV pathogen (such as poxvirus or salmonella), the immune system can be trained to recognize HIV components on a different virus[64]. Clearly, one disadvantage of this approach is that the recombinant virus can itself cause disease in the host. Further, if the patient has already been immunized against the virus used to house the foreign HIV particles, than the vaccine probably will not work because the body is already trained to destroy the very system used to introduce HIV particles. The immune system thus never gets a chance to "train" itself on the HIV components.

3. Live, inactivated HIV: The most effective vaccines thus far use live HIV that is inactive; this clearly has safety concerns, but fail-safes can be incorporated into the viral genome that, for example, make the live strain more susceptible to antiviral compounds [65].

Reshuffling the viral deck: Resistance to drugs and vaccines

However, despite over a decade of research, an actual cure remains an unmet challenge in the battle against AIDS. Many factors make vaccine and drug development difficult, including the virus' rapid replication rate [60]. Compared to the replication of the human genome during cell division, RNA viruses, like HIV, copy their genetic material in an extremely error-prone manner. In fact, it is faulty enough that one mutation can occur each time the virus replicates[66]. One mutation may not seem like much, but since HIV can produce 1-10 billion new virions a day, favorable mutations aren't very hard to achieve statistically in its small genome[67]. In fact, these numbers mean that every base pair in the viral genome can be mutated in a day. Further, any treatment can exert selective pressure on the viral population, pruning away weaker variants and leaving only the hardiest viruses left over to replicate further[67]. Given all of this, it's easy to see that making a vaccine or drug would be difficult, since HIV's genetic code is so inherently unstable.

A second impediment to vaccine development, similar to mutation, is that different HIV strains can recombine to further increase the variability of their genetic makeup[60,68]. This happens because HIV, unlike other viruses, has *two* copies of its RNA genome[69]. Normally they are identical, but if a cell is infected simultaneously by more than one HIV, then some virions can actually inherit two RNA strands that originally come from *different* viruses. When these recombined viruses then replicate, their reverse transcriptase can skip between the two strands when making its DNA copy, allowing genetic sequences from two viruses to be recombined into a single strand[70]. Since antibodies are usually specific to a given genetic sequence, either source of variation can quickly eliminate the single site to which an antibody would bind, rendering a vaccine useless.

Another obstacle is that scientists still do not fully understand what kind of immune responses might limit the virus' growth. Clearly, one way to answer this question might be to study immune responses in individuals who remain HIV-negative even after being exposed to the virus many times (see more on this below) [60]. However, even in these cases it is often unclear whether these immune responses are actually preventing infection or if they are the left-over reactions to viral infections that did not take hold[60]. In other words, it's hard to know whether the virus failed to successfully infect the body, or the immune system actively prevented it from doing so. Additionally, the fact that the virus can integrate itself into its host cell's genome (where it consists of just a DNA sequence, without translated protein) means that it can avoid antibodies directed at viral proteins[60]. As mentioned above, the virus is invisible in this form to the immune system[60].

Natural Immunity?

A glimmer of hope in the effort to develop an HIV vaccine came with the discovery of a group of prostitutes in Nairobi, Kenya, who, despite repeated exposure to HIV through their work remained uninfected[71]. Scientists guessed that this was due to a large number of "anti-HIV" immune cells stimulated by repeated exposure to the virus[71]. Thus, trials began to develop "multi-vaccine" treatments that mimicked repeated exposure to HIV by "boosting" the initial vaccine with a second injection at a later date [72]. However, the ultimate results in clinical trails reported in 2004 proved disappointing [73]. Additionally, some of these immune prostitutes have developed AIDS upon leaving the sex-trade industry, leading to further uncertainty about this phenomenon [71-73].

Comprehension Questions:

1. What makes an antiretroviral different from a vaccine?
2. What is one way in which an antiretroviral compound can inhibit HIV, on the molecular level?
3. What components of the HIV virus can be used as a vaccine? Why might an un-enveloped virus not be as effective as an enveloped virus for this application?

Policy Quandaries

How Large is the Problem?: Global Statistics

While one never wishes to reduce the human dimension of health epidemics with an over-emphasis on statistics, a few numbers may be helpful to emphasize the extent of the AIDS crisis. According to the December 2010 report of the WHO and UNAIDS, 33.3 million people worldwide are currently afflicted with AIDS[74]. The geographical distribution of AIDS victims is also telling: the report also states that 22.5 million of these are from Sub-Saharan Africa[74].

Policy Initiatives in Africa

As former UN Secretary General Kofi Annan announced in 2000, AIDS has killed more of Africa's people than all the continent's wars combined[75]. Other statistics are similarly horrifying: 2 million died of AIDS in 2005 in Sub-Saharan Africa, and an estimated 24.5 million are thought to be infected[76]. The enormity of this crisis, however, has not correlated with a strong response by Western countries. Funding to study the African AIDS epidemic was unavailable in the late 80s, and by the 90s the US had already found ways to control the spread of AIDS within its own borders[77]. Thus, AIDS relief measures at home and abroad became a low priority for one of the few nations with the financial resources to improve the situation in Africa[77]. Even when aid is given, economic interests are never far behind. Indeed, three countries refused a loan from the United States in 2000 on the basis that they did not want to become further in debt to the US[78].

Pharmaceutical companies, like foreign governments, have also been unresponsive to the epidemic. The UK-based charity Oxfam has even accused several major drug companies of violating human rights by trying to prevent access to needed HIV treatments, a situation highlighted by a 2001 case in which several major firms attempted to sue South Africa for generating generic versions of patented antiretrovirals[79]. While public outcry led to the charges being dropped, such issues will surely be an integral consideration in any future policy solution.

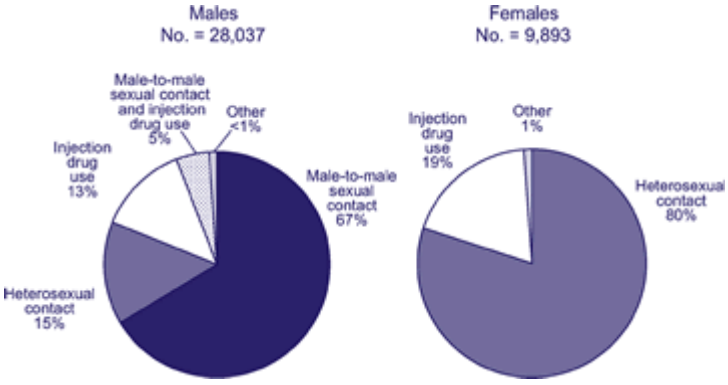
Foreign entities alone, though, are not the sole cause of this dire situation. The widespread poverty in Africa is also a major hindrance to effective public health measures, leading to AIDS being termed a "disease of poverty"[80]. A powerful social stigma against AIDS patients also impedes treatment in some countries. For example, in Malawi, the disease is not openly discussed, with doctors recording AIDS deaths as "fever" on medical forms[81].

Not surprisingly, AIDS has taken an immense toll on the African continent's economic infrastructure. Current estimates indicate that between 1970 and 1990, Africa's share of world exports dropped 60% - a loss of a fifth of the continent's total output[14]. The number of orphaned children also increased during this period, leading to unstable communities of parentless children who, without education and guidance, may become a generation unable to sustain the disintegrating societies around them[14].

Policy Issues in the US

While it is not as severe a crisis in America as in Africa, AIDS is still a major public concern for the US, causing over 13,000 deaths in 2007 according to the latest CDC report[82]. Transmission statistics demonstrate several trends. For example, the highest proportion of HIV/AIDS diagnoses was for male-male sexual infections[83]. Similarly, males account for almost 75% of diagnoses[83]. The next highest percentage of diagnoses were for heterosexual infections among both adults and adolescents[83].

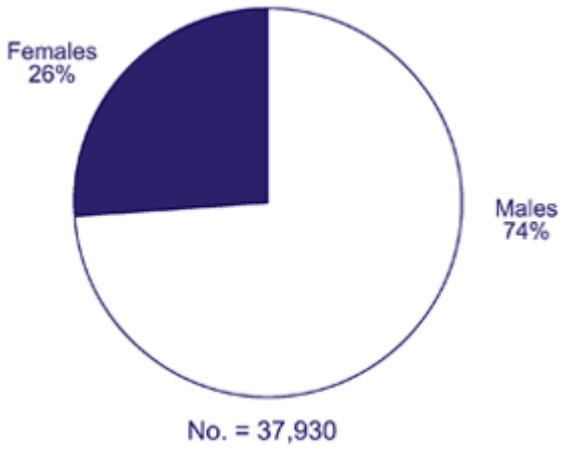
Transmission categories of adults and adolescents with HIV/AIDS diagnosed during 2005



Note. Based on data from 33 states with long-term, confidential name-based HIV reporting.

*The term HIV/AIDS refers to 3 categories of diagnoses collectively: (1) a diagnosis of HIV infection (not AIDS), (2) a diagnosis of HIV infection with a later diagnosis of AIDS, and (3) concurrent diagnoses of HIV infection and AIDS.

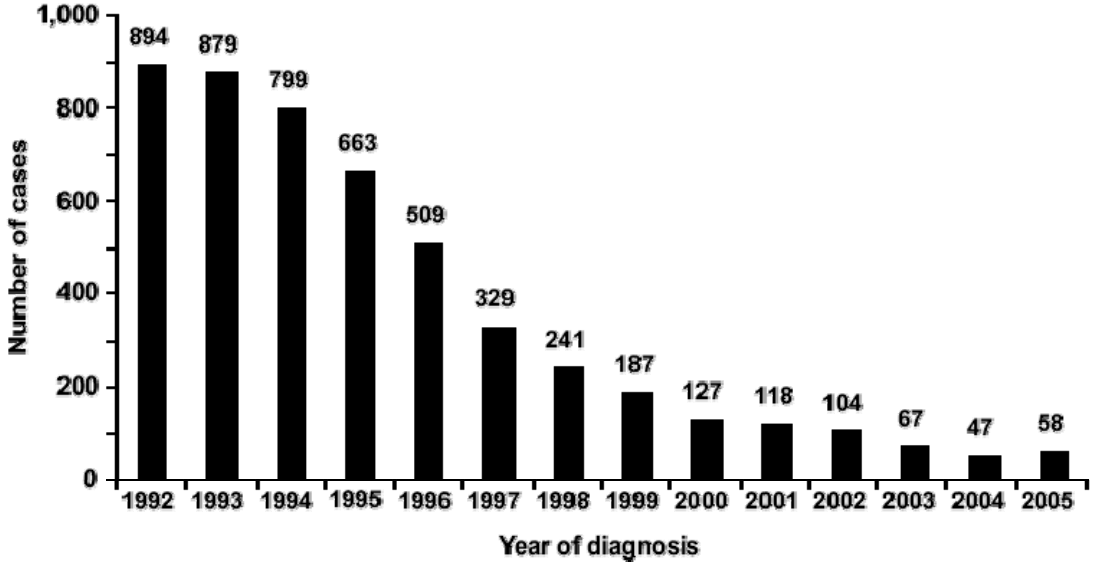
Sex of adults and adolescents with HIV/AIDS diagnosed during 2005



Note. Based on data from 33 states with long-term, confidential name-based HIV reporting.

By age group, a decreasing number of diagnoses were among children less than 13 from 1992-2005[83]. However, the CDC report also suggests that such young patients do not comprise a very large percentage of overall infections[83].

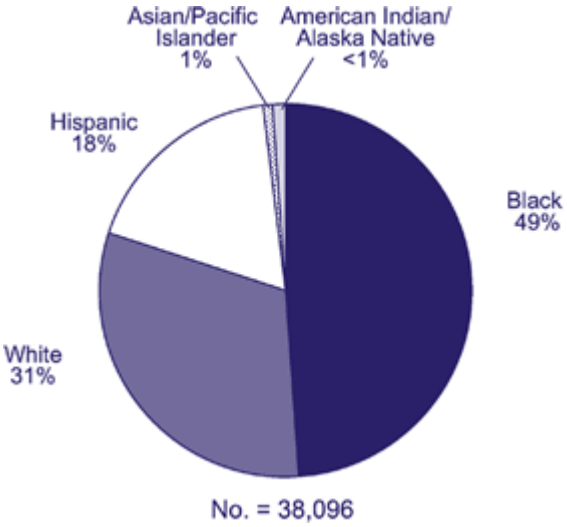
Estimated numbers of AIDS cases in children <13 years of age, by year of diagnosis, 1992–2005—50 states and the District of Columbia



Note. These numbers do not represent reported case counts. Rather, these numbers are point estimates, which result from adjustments of reported case counts. The reported case counts have been adjusted for reporting delays, but not for incomplete reporting.

Along racial lines, the numbers are striking. Black Americans constitute almost 50% of the diagnosed HIV/AIDS cases, even though this group accounts for only 12% of the overall population[83]. Such statistics suggest the socio-economic inequalities that continue to affect AIDS treatment in the US.

Race/ethnicity of persons (including children) with HIV/AIDS diagnosed during 2005



Note. Based on data from 33 states with long-term, confidential name-based HIV reporting.

The Federal Government continues to pursue AIDS initiatives in several areas, funding scientific research on HIV as well as health care for those already infected with the disease. The latter is accomplished in part through the Ryan White CARE Act, a program instituted in 1990 through which many innovative treatments are funded[84]. Among the current reforms in AIDS policy suggested by the White House are greater flexibility in distribution of funds, emphasis on life-extending treatments, and the incorporation of all available service providers (including religious organizations and others) into AIDS treatment[85]. In related areas, policy initiatives also seek to increase access to prescription drugs, promote HIV testing (particularly for prisoners and intravenous drug users), and incorporate faith-based organizations in awareness campaigns among high-risk communities such as underrepresented minority groups[85].

In a smaller forum, California lawmakers are currently voting on several AIDS initiatives, including programs to increase the availability of sterile syringes, legalize assisted suicide for terminally ill patients, facilitate condom distribution and HIV testing in prisons, increase funding for testing and counseling services, and reduce the abuse of methamphetamines[86].

Activity 1: Does HIV Cause AIDS?

Dr. Peter Duesberg seems to enjoy courting controversy. His current research seeks to explain cancer not as unchecked cell growth, but as the result of chromosomal abnormalities.[87] *In the late 1980s, he took on a similarly contentious stance, one that has still not been accepted by the larger scientific community: that the HIV virus does not cause AIDS.*[88] *Rather, Dr. Duesberg thinks that drug use and the toxic effects of antiretroviral compounds are the primary cause of the disease.*[88] *While, Dr. Duesberg's hypothesis seems unlikely, given almost two decades of contrary evidence, it does raise an important question: when is a theory strong enough to support through clinical practice?*

Students will read "HIV is not the cause AIDS" (Science, Vol. 241, pp. 514-517, July 29, 1988: available online at http://www.duesberg.com/papers/ch2.html). Before you consult any other sources, quickly jot down what the assumptions of Duesberg's nine points of contention are. Can you see any possible errors in reasoning? If necessary, look up terminology in a virology textbook.

Each group (or students, depending upon class size) will take one of Duesberg's nine points and research his conclusion. Is it valid? What literature supports/denies his conclusion? Each group/students then prepares a 1-2 page summary of their findings to present to the class, concluding either "yes" or "no" for Duesberg's proposition.

Activity 2/Civic Engagement Exercise: Designing AIDS Education Information

Based on the questions they consider in the case study at the beginning of this unit, students are commissioned to develop a resource about HIV/AIDS for a secondary school in Africa.

Part 1: The students should work together in groups to collect the following information:

1. What information do their peers abroad already know about HIV/AIDS?
2. What are the most important things for someone living in a HIV-prevalent country to know to prevent infection?
3. What are common myths about HIV/AIDS that might need to be debunked?

Ideally each answer should be about a paragraph, with sources listed.

Part 2: Students create informational brochures, posters, etc.

Using their answers from Part I, students compose a pamphlet about HIV/AIDS. Among the things they should consider in the design are:

1. How would the information be best organized?
2. What design would likely catch the attention of a reader?
3. How might the information be distributed?

Activity 3: Pharmaceuticals for the Developing World?

Students will read the article "Ethics and AIDS Drugs" from TIME Magazine, then consider the following question:

"Should HIV/AIDS drug patents be enforced in the developing world?"

Students divide into two groups: one representing pharmaceutical companies and one representing developing countries. Optionally, a third group representing the US government may also be included. Each group should formulate their group's answer to this question, and find 3-5 articles sources supporting their view.

Each group then presents their arguments. These presentations are followed by a rebuttal period and summary statements.

Activity 4: The Case of the AIDS Respirator

As a possible introduction to this unit, students will read Chapter 1 of Abraham Verghese's book "My Own Country," then consider the following questions:

1. Can AIDS actually be transmitted through a respirator? (This should give the chance to introduce some details about transmission). Was the hospital staff's reaction to the respirator appropriate?

2. What are some ways that AIDS could be transmitted in a hospital setting? (See the chapter of this same book dealing with a blood transfusion case at Duke Hospital).

Activity 5: Movie Possibilities – Delivering the Goods; And the Band Played On

Delivering the Goods

(1 hr 52 min)

"At the dawn of the 21st century, we can prevent, treat or cure most of the deadliest diseases known to humankind—and yet millions die needlessly every year because the benefits of modern medicine and public health fail to reach them. What are the obstacles to providing care to populations in need? From the villages of the Gambia to the cities

and towns of Thailand, from the sun-scorched refugee camps of Chad to the teeming streets of Bangladesh—this episode chronicles innovative health programs and charismatic leaders who, against all odds, are *Delivering the Goods* to millions of individuals—and inspiring a new vision for the future of global health."

And the Band Played On

(2 hrs 20 min, rated PG-13)

"A real-life drama about the tragic, time-consuming battles among government agencies, gay groups and scientists that impeded the discovery of, and research on, the AIDS virus. Gay leader Bill Kraus is dying -- and no doctor can name the disease that's killing him. All anyone knows is that some epidemic is attacking homosexual men. But rather than get down to serious experimentation and study, the Center for Disease Control stonewalls any effort to prove that the disease is transmitted through the blood; French and American scientists squabble about who should get credit for discovering the virus; and the gay community, sensitive about criticism of their lifestyle, refuses to admit that their own actions could make a difference in the spread of AIDS. Meanwhile, the death toll mounts... and mounts... and mounts..."

Activity 6: Webquest – "In Their Own Words" (http://aidshistory.nih.gov/)

How can you tell that a patient has a previously unknown disease? How do you care for someone with an unknown illness – and protect yourself as well? How do you investigate the cause of a new disease, and find ways to treat and prevent it? *In Their Own Words* documents how NIH researchers answered such questions when asked to recall the early days of HIV/AIDS. Students will navigate this site where NIH scientists discuss the initial days of the current HIV epidemic and their efforts to understand what was happening. Video and audio accompany.

Activity 7: Disease Transmission Simulation

Students will model the spread of HIV (and other infectious diseases) through "fluid exchange". Each student will transfer the contents of their water cup to other students' water cups. One cup will be "contaminated" with baking soda (for a basic pH). Indication of the pathogen will be visualized by phenolphthalein color change. Students will see how quickly a disease can spread and determine the growth model of the transmission.

Activity 8: Pathogen Size Modeling

For a long time, people did not know that viruses caused sickness because the virus particles were too small to be seen with a microscope. As we developed stronger and stronger microscopes we began to see the unusual shapes now known to be viruses. So just how small are they?

Students will use basic math to create size relationships between viruses and bacteria and to determine the number of viruses that could fit on the head of a pin.

Activity 9: A Poster from the Past

Take a look at the posters on the Handout provided, and try to answer the following questions.

- What is this poster about? What message is it trying to get across? How might it reflect the prejudices of the 1980s?
- How might poster be the same or different if it was made today?

Activity 10: AIDS Myths

Students will examine the AIDS Myths provided in Handout: they are mixed in with real facts. Based on their knowledge, have them research and explain why a particular fact is true or a hoax.

Activity 11: Celebrities Living with and Dying from AIDS

We've discussed Ryan White, Rock Hudson, and Magic Johnson in this unit, but there are more details to their individual stories, as well as other famous HIV positive celebrities. Have students research one, and prepare a short presentation on this person's life history and how they dealt with the disease. Multimedia is encouraged (e.g. playing "Candle in the Wind" as part of a presentation on Ryan White.)

Suggestions: Tory Dent, the poet whose work opens this unit, or Pedro Zamora, cast member on the second season of MTV's *The Real World*.

Activity 12: HIV, Before and After

Have students pick a country in sub-Saharan Africa and research the country's demographics before and after the emergence of AIDS. How has the death age changed, for example? How do these compare with the same statistics in areas of the US?

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Tuberculosis: The White Plague

"I know the colour of that blood; it is arterial blood. I cannot be deceived in that colour. That drop of blood is my dead warrant. I must die."

-John Keats (Tuberculosis Victim)[1]

“All Hallows”

Angelica pulls the plastic fangs from her mouth, rubbing at the sore spot they’ve made on her gums.

“I really don’t think these are actually ‘one size fits all’,” she grumbles.

The sky over historic Jamestown is growing dark, with torches fired up along the street competing with electric lights indoors. Though it is October, a few lingering insects still meander through the dusk. The class had arrived early that morning, waking up two hours before school began to meet the charter bus for the long ride. They had slept most of the way there, and spent the morning roaming the streets with Mrs. K. After lunch, their teacher had gone to an educational workshop, while they were left on their own until it was time to leave.

Angelica hands the fangs back to Fallon, adding “I can’t believe you scared that little kid earlier – it looked like he almost had a heart attack.” Earlier in the day, Fallon has surprised a younger tourist with his costume, a joke he had found quite amusing until he realized that Maxine was horrified. Since then, he had been sulking.

“Please . . . let’s just forget it, all right?” he murmurs.

“I still don’t understand why a colonial history site is selling Halloween costumes,” says Lang, who had been irked by the gift shop earlier. “Why are we here, again?”

“It was a historical comparison,” says Maxine, looking up from the brochure towards the nearest intersection. “We’re supposed to be learning about how diseases were treated in the past, not just modern medicine.”

In the morning, they had gone to a presentation on 17th Century medicine during which Lang had unsuccessfully tried to stay awake – at least, he thought, he never snored. From what he had gathered from the others, medicine was pretty frightening hundreds of years ago, with leeches and amputations and other things he didn’t want to think about. That and the moist environment around Jamestown had ensured that the settlers were constantly plagued by mosquitoes carrying nasty diseases.

A gnat lands on Lang’s forehead, which he swats quickly. “Do you know where we are?” he asks Maxine. “I thought the entrance was just up here.”

“That’s what I *thought*,” she says, adjusting her glasses and turning back to the map, “but we’re definitely lost. We were supposed to meet Mrs. K half an hour ago – I wonder if the bus is here yet.”

Angelica looks back the way they came, towards the bend in the road leading to graveyard. A line of trees obstructs her view of the cemetery, and she’s partially thankful for it. Walking through the lines of moldering stones that morning, Maxine had started calculating from the legible inscriptions how long the residents of Jamestown had typically lived, arriving at an average in the thirties. At that rate, most of them would already be having mid-life crises in the colonial era – that is, if they had survived birth, which a display they had seen about midwifery suggested was a dangerous point in anyone’s life at that time. The idea of people dying so young was unsettling, and Angelica had been glad to leave the tombstones while Maxine was still calculating the average.

“Fallon, what are those?” says Mrs. K, walking up behind the group and eyeing the white plastic fangs.

"A souvenir?" he replies weakly, hoping one of his classmates won't bring up his earlier antics. Maxine appears noticeably relieved by the sight of their instructor.

"I think we took a wrong turn about ten minutes ago," she says. "The buildings all look the same, and the names on the map don't always match the signs – if there are signs."

"I figured you'd all gotten lost, or were stalling on purpose," Mrs. K answers. "Either way, the bus is waiting, and I don't think the driver is getting any more patient."

* * *

Though many had intended to sleep the entire trip back, Mrs. K proved determined to make productive use of the time.

"So," she started, addressing the four pairs of sagging eyes in front of her, "what did you learn today? What did colonial settlers have to worry about most? What medical improvements separate us from them?"

"They didn't have insecticide," offers Maxine, "so all those mosquito-borne illnesses were a bigger problem."

"Right," says Mrs. K, "they probably didn't even know that they could get sick from mosquitoes – but we'll talk about this more during the malaria unit." She turns to Fallon. "What did you think of the surgical equipment?" she asks.

He glances up, realizing that he had been sleeping. "I . . . I'm glad we have real anesthesia," he says, remembering the leather gag which, with a shot of rum, was the only thing dulling the pain for his colonial forebears.

"They probably didn't disinfect their instruments," says Angelica. "I wonder how many deaths were caused by bacterial infection, instead of the wound."

"Very astute observation," says Mrs. K. She looks again at Fallon, who is fidgeting with his plastic fangs. "Speaking of bacteria," she says, "does anyone know where vampire legends in America came from."

Fallon, realizing that Mrs. K is watching him, looks up. Four sets of blank stares confirm that none of them have completed the reading assignment from last night, and she sighs. "Well, perhaps when you get back, you can finish the homework you were supposed to do last night, and this time write a three page paper to prove you did it!"

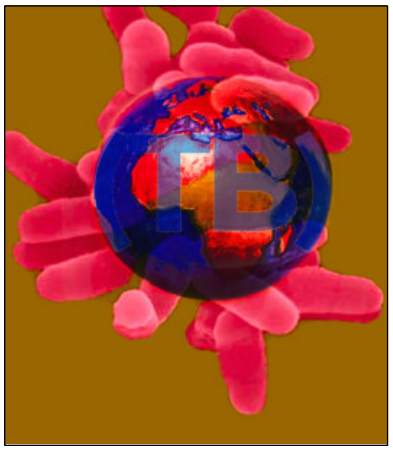
The classmates groan, realizing that a very long day is about to become an even longer night.

1. **What might have been some factors that increased the deadliness of tuberculosis in the past? What inventions might have made a difference?**
2. **What advice would they give colonial settlers on how to prevent infection? What might be some risks of modern medicine (think resistance)?**
3. **What strategies would you recommend to prevent drug-resistant bacteria from developing in a colonial community with seasonal infections?**

The White Plague: Tuberculosis and Other Topics in Bacteriology

We're not alone.

That's right; use a Q-tip to swab your hand, or the inside of your mouth, and streak the residue on an agar plate. If you incubate it for a day, you will see that there is a veritable world of tiny organisms crawling over the gelatin. Some of them are benign hangers-on, using us as a habitat and source for the dead skin flakes they eat. Others are more insidious, making us sick when they infiltrate our immune system and colonize our bodies. This unit is about the second group, the bacteria responsible for illnesses ranging from mild colds to life-threatening infections.



Our focus will be on a forerunner in the war between humans and bacteria, the leading agent of bacterial death the world over: Mycobacterium tuberculosis, the cause of the disease tuberculosis or TB. In contrast to AIDS, this disease was well known to our ancestors, and for many centuries was a major health threat to the world's population. Advances in bacteriology made more effective treatment of the so-called "White Plague" possible, and TB was initially thought to be conquered earlier in this century. However, the disease has experienced a revival in recent years, rearing its head once again as a potent health epidemic.

Goals:

By the end of this unit, students should be able to:

1. Given a scenario, predict the risk and likelihood of TB infection among a group of people in a certain environment.
2. Assess the risk of a new drug in terms of antibiotic resistance.
3. Analyze how tuberculosis might have affected society during another period of history, using evidence from literature and visual art.
4. Predict whether a given case is likely to result in drug resistant tuberculosis, and suggest ways in which such risks can be minimized in a given clinical environment.

T.B. Harlem: A Portrait of a Disease

In her 1940 painting "T.B. Harlem," painter Alice Neel portrays a tuberculosis victim of Spanish Harlem. The subject – her lover's brother Carlos, who actually lived near Neel – is shrouded in white, pointing plaintively to the bandage that covers his surgical wound. This incision, made to drain Carlos' lung of fluid, warped his spine, producing the painfully contorted figure depicted in Neel's portrait[2].

It is significant that the artist connected the disease with its location in her title. In the remainder of this unit, students should think not just about the biological mechanisms of tuberculosis, but the social conditions that have made it such a threat.

Humanity's Unwelcome Friend: A History of Tuberculosis and its Victims

We've been observing tuberculosis infections for almost as long as we've been recording history. Every step of human civilization, the bacterium has followed: perhaps it's a bit ironic that modern medicine has been accompanied by the rise of multi-drug resistant TB, a similar "technological leap" on the part of our microbial nemeses. Here, then, is the story of mankind's unwanted partner in history.

Ancient History:

Tuberculosis is a very old disease, with remains from Egyptian mummies showing evidence of decay from the illness[3]. It was also known to the ancient Greeks, as the term consumption was first used by Hippocrates in 460 BC to describe the way in which victims seemed to be consumed by the disease, rapidly growing thinner and wasting away[3]. Later, during the Roman Empire, a variety of bizarre cures were suggested for the disease, including eating wolf's livers, drinking animal blood, or even bathing in urine[3]. However, Roman physicians were not entirely misguided when they recommended fresh air, a concept utilized hundreds of years later in the sanatoria of the 17th and 18th centuries[3].

The White Plague:

Tuberculosis became an immense threat in Europe during the late 18th and early 19th centuries, overshadowing other illnesses such as cholera[4]. Due to limited medical technology, the disease was usually fatal, and may have accounted for as many as a third of all deaths in the 19th Century[21]. The list of authors claimed by the disease reads like a who's who of the time, and consumption showed up frequently in their works as well. However, most at the time believed the illness was hereditary not infectious. The insight that it was caused by a small organism was supplied by English physician Benjamin Marten in 1720, and confirmed in 1865 by a series of experiments in which French doctor Jean-Antoine Villemin demonstrated the transmission of the bacteria between cows, rabbits, and humans[5]. However, he never observed the organisms responsible under a microscope – this would be Koch's contribution (see below).

Vampirism in the Colonies:

Likely introduced from the West by Columbus and other explorers, TB took hold in the new world and was a scourge of the New England settlers in the 19th Century. While in Europe, the image of the consumption victim was often romanticized in art. In New England, however, it became the basis for vampire legends[6]. Colonists observing pale TB victims and the subsequent wasting away of the victim's families believed they were witnessing a vampire preying upon its kin[6].

A particularly famous case was that of Mercy Brown, a young girl who died of tuberculosis in 1891 in Rhode Island after her mother and older sister had already fallen

to the disease[7]. When her brother Edwin also displayed symptoms of consumption, Mercy's former neighbors believed this indicated that one deceased member of the Brown family must be a vampire preying on the boy[7]. Persuading her father to exhume his wife and two daughters, the townspeople of Exeter discovered that Mercy's body was remarkably well-preserved in comparison to the others[7]. Taking this as a sign that she was rising as a vampire to feed on Edwin (instead of the remarkable ability of cold New England soil to preserve human bodies), Mercy's father was persuaded to remove the corpse's heart, burnt it, and give the ashes mixed with water to his ailing son[7]. Though this was meant to end the vampire's feasting, the bacteria actually responsible for Edwin's illness were not susceptible to such rituals, and the boy soon followed his other family members to the grave[7].

The Development of Sanatoria

To cope with the TB epidemic, some European physicians decided that fresh air was the best treatment and set about institutionalizing this approach. The first such treatment facility, which came to be known as sanatoria, was opened in Germany in 1854[8]. They were introduced to the US in 1885 with an isolated facility in the Adirondacks of New York State[9]. Sanatoria soon became popular across the US, and even if they did not directly cure TB, they may have ultimately aided its treatment. Indeed, Koch mentions in his Nobel lecture that his observations in the sanatoria helped guide his research: the hygienic procedures in these facilities led him to speculate about the transmission of the disease which was much more prevalent in close quarters[10].

In 1884 in New York, "Little Red", the first TB sanatorium in the country was opened. Left, exterior of Little Red; Right, interior of Little Red.

Keats, Physician and Poet:

> *'Beauty is truth, truth beauty,' - that is all*
> *Ye know on earth, and all ye need to know.*
> -"Ode on a Grecian Urn"

One of the more famous victims of tuberculosis was John Keats, the English poet whose mournful verses are a hallmark of Romantic Era. Interestingly, he studied as a surgeon before forsaking medicine for poetry[11]. Perhaps he might have lived longer if he had stuck with the former, for, when he sought relief from his tuberculosis in Italy in

1820, his physician did not believe he had the disease, and ordered vigorous exercise that probably hastened Keats to his grave at the young age of 25[11, 12].

Koch's Revelation:

On March 24, 1882, German Physician Robert Koch presented his discovery of the tuberculosis-causing bacteria to a room of stunned onlookers[13]. This address, considered one of the most important lectures in the history of medicine, laid out the germ basis of disease that would come to define modern medical research[13]. By demonstrating how these microorganisms could cause infection in a pool of laboratory guinea pigs, Koch gave inscrutable evidence that tuberculosis really was an infectious malady[13]. No wonder one of the audience members described the talk as "the most important experience of my scientific life[13]". For his seminal contribution, Koch would be awarded the Nobel Prize in 1905[13].

Koch's Postulates

Though he worked specifically on TB, Koch conceived a number of general rules through this research related to bacterial transmission of disease that are known collectively as "Koch's Postulates." By these rules, a disease is caused by a particular species of bacteria if:

1. The bacteria are found in any given case of the disease.
2. The bacteria can also be isolated from the infected host and grown in pure culture.
3. A healthy host will develop the disease if injected with this pure culture of bacteria. The bacteria must also be able to be isolated from this second, experimentally infected host.

TB Returns: "Ebola on Wings"[14]

With the discovery of streptomycin and other antibiotics that effectively treated tuberculosis, it seemed in the middle years of the 20th century that TB would soon become a thing of the past. However, that optimistic vision has been shattered in recent years by the emergence of Multi-Drug Resistant Tuberculosis (MDR-TB), which is strictly defined by the World Health Organization as a strain of *M. tuberculosis* that is resistant to both isoniazid and rifampin, two commonly prescribed antibiotics for TB[15]. However, sometimes the infection is resistant to *any* drug, and certain strains resistant to any single antibiotic have been found[16]. One of the worst things about this particular epidemic is that it might have been prevented decades ago when tuberculosis was almost eliminated. Even though US government was warned in the 1970s about the necessity for TB funding during the crucial years when the disease was almost eliminated domestically, no action was taken to combat remaining hot spots in areas like Harlem, New York. Ultimately the necessary funding for the final step in eliminating TB never materialized.

Mixing Maladies:

Early signs of trouble visited Harlem Hospital in 1981 when doctors began to observe a proliferation of unusual TB cases[14]. Usually the disease is confined to the lungs, but it was appearing in brain abscesses, wrists, spines, and other peculiar internal organs[14]. While all the patients were immune-compromised, AIDS had not yet been

definitively described nor had the interaction between AIDS and TB fathomed. The first indication of the true magnitude of the reinvigorated tuberculosis threat came in 1991, when 12 inmates and a guard at a Syracuse, NY prison died of what turned out to be a drug-resistant strain of the mycobacterium[14]. The guard was undergoing chemotherapy; the inmates were HIV-positive[14]. Both immunosuppressed conditions made the victims more susceptive to tuberculosis. In the coming weeks, more cases were reported across the US.

Soviet Collapse: TB in the Gulag

Meanwhile, the Soviet Union fell, causing an economic downturn that would send tuberculosis cases soaring in the troubled region[17]. Here, as was the case in Harlem in 1970s, social conditions helped the disease flourish. As the economy soured, more of the Russian citizenry became malnourished. They consequently contracted the disease more easily and spread it among members of crowded urban living environments. Similarly, these economic troubles led to greater petty crime and more imprisonments. Over time, the Russian penal system proved an immense harbor for the spread of tuberculosis, with almost 1 in 10 inmates infected with the mycobacteria[14]. In a crowded prison cell, the disease transmits easily between inmates, and every year the release of inmates from incarceration unleashes tuberculosis onto the rest of the population (in addition to the prison staff carrying the bacteria with them when they leave work)[14].

Overcrowded prisons, joined with poor medical care, can cause devastating TB epidemics.

Besides crime, Russia's economic problems have also correlated with increased incidence of prostitution and intravenous drug use – consequently, HIV/AIDS is also on the rise in the former Soviet Union, further boosting the spread of tuberculosis. Given all this, it seems no wonder that the average age of the Russian population is falling precipitously[14].

Improving Treatments:

How exactly did tuberculosis go from being almost exterminated to proliferating in resistant forms? Much of the problem has to do with how doctors prescribe treatments and see patients through the necessary process of recovery. As we will see in this unit, tuberculosis usually exists in a latent stage. Because of this, it is possible for a patient to

take antibiotics and relieve their symptoms by destroying *most* of the bacteria making them sick[18]. However, a small portion will be left over, encased in a tubercule behind a wall of macrophages. These remaining bacteria are more resistant to the antibiotic treatment and, further, can spontaneously evolve drug-resistant mutations while they remain in the patient's body[19]. In addition, there is evidence that many antibiotics are more effective against actively replicating bacteria than the slow-dividing cells in a tubercule, leading to a situation of slow and continual exposure to the antibiotic: this situation also favors the development of drug resistance[20]. These hardier bacteria will not cause immediate symptoms because they are trapped in the tubercule in a period of latency, but months or years after the patient has ceased their treatment, they can overwhelm the body's immune system, resulting in a new case of tuberculosis that is more resistant to the antibiotic treatments used to treat the original infection.

In the U.S., this scenario played out frequently in large urban hospitals in New York in the 1980s, where tuberculosis patients would leave, not finish their prescribed medicines after feeling better, and ultimately spread their disease to others. If they survived, they might arrive back at the hospital months later bearing a resistant strain of *M. tuberculosis*. Similarly, much of the current epidemic in Russia is the result of the poor medical system in the nation's prisons. Prisoners infected with TB are given few and insufficient medications, which cures their symptoms but does not destroy the entire bacterial population in their bodies. Thus, like the economically disadvantaged citizens of New York City, the closely packed inmates of the Russian gulag harbor resistant bacteria that then spreads quickly among their cellmates; the result is the same[14].

There have been advances, though, in combating this new threat. During the early 1990s in New York City, when the threat of MDR-TB epidemic loomed fearsomely, Directly Observed Treatment (DOT) became the new standard of tuberculosis therapy, in which physicians followed up with their patients and tried to ensure that those taking antibiotics for tuberculosis finished their full regimen of drugs[14]. While this kind of therapy has not been so effectively instituted in Russia, a modified version has been proposed in which prisoners are observed *and* their treatment is tailored to the specific strain of MDR-TB from which they are suffering[16]. The cost of the current crisis is immense, in both lives and money – it has been estimated that a single case of MDR-TB can cost almost $1.3 million to treat[21].

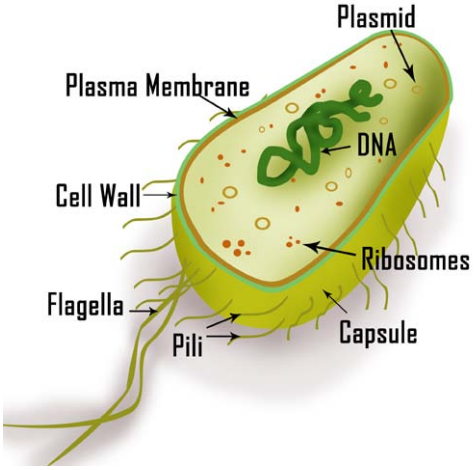
Though the threat of tuberculosis appears larger than ever, there is hope: new methods of rapid diagnosis using sophisticated genetic screens coupled with drug development based on the TB genome will increase the effectiveness of anti-drug resistance strategies[22]. DNA chip technology – where the genetic material from a sputum sample could be analyzed for a number of bacterial drug resistance genes – could significantly accelerate diagnosis of particular strains of *M. tuberculosis*. Further knowledge of the mycobacterium's unique genome should help identify novel drug targets, eventually increasing the number of antibiotics in the anti-tuberculosis arsenal.

Biology of the White Plague

Now that the historical significance of this killer has been elaborated, how does it actually work? How does it become resistant to standard antibiotics? Such questions are our next topic in this unit.

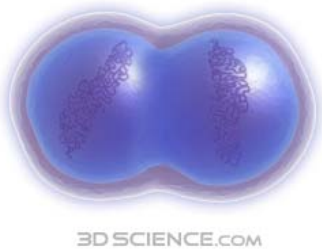
The Basics: What *are* Bacteria?

Bacteria are a diverse set of microscopic organisms, exhibiting a staggering range of sizes, shapes, and behavior. However, they all share a few common traits: all bacteria are single celled, or unicellular**,** and usually replicate by binary fission, splitting into two identical copies of themselves when they reach a certain critical size[23,24]. Other methods of reproduction are also possible, including conjugation (in which one bacteria gives another a piece of DNA through a tentacle) and spore formation, in which the bacteria enters an inactive phase until they find a host to infect[23].



Bacterial structure. Original arwork by Senmiao Zhan.

Unlike our own cells, bacteria have no nucleus, and their genetic material is contained in a circular chromosome of DNA[24]. Some bacteria have additional genetic material contained in plasmids, rings of DNA that are separate from the organism's chromosome and capable of replicating independently[25]. Because they lack many familiar organelles, including the mitochondria and endoplasmic reticulum, bacteria can be quite small, a trait that often aids their invasion of the body's recesses[24]. However, they do usually have a cell wall, a layer outside the plasma membrane that helps bacteria maintain structural rigidity and determines their sensitivity to anti-biotic agents[24].

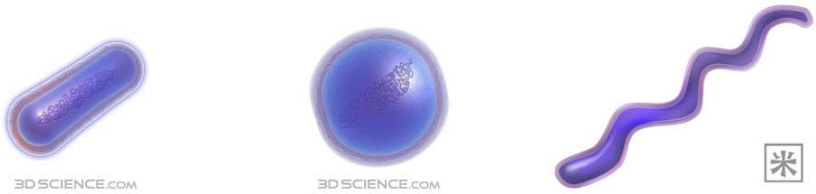


One bacterium becomes two through binary fission.

Bacteria swap DNA through conjugation.

Bacteria take on many shapes, including rods, cones, spheres, "commas", branched cells, and spirals[24]. Many times bacteria are classified according to their shape. For example, Bacillus bacteria are rod-shaped, Cocci refers to a spherical bacterial cell, and Spirochetes assume a sprial-form (such as that of Syphilis bacterium)[24,26]. Additionally, bacteria are often classified based on their reaction to a chemical test developed by Hans Christian Gram, a 19th century microbiologist who discovered that some bacterial walls absorb a dye (crystal violet)

better than others[27]. This test – named the Gram Test in his honor – is often used as a method of classifying bacteria[27].

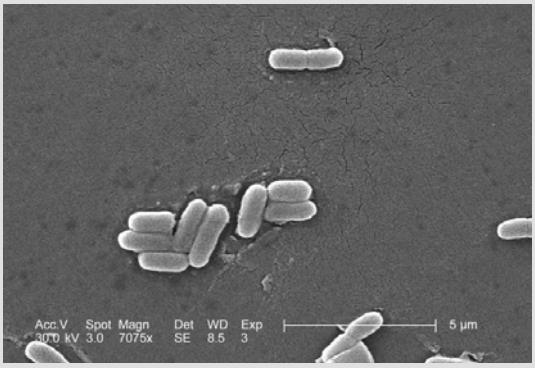


The many shapes of bacteria. From left to right: bacillus (rod), coccus (sphere), spirochete (spiral). Right: Original artwork by Senmiao Zhan

Not all bacteria have a cell wall. For those that do, the wall is often a collection of porous sugar-protein molecules called peptidoglycans, which, unlike the cell membrane, is permeable to most substances in the surrounding environment[28]. These bacteria are positive for the Gram test[29]. However, if the peptidoglycans are surrounded by an extra layer of fat molecules, they are negative for the Gram test[29]. Some even more complex cell wall structures are possible, including that of *M. tuberculosis* (as described below)[29,30].

Helpful Bacteria:

Not all bacteria are bad. In fact, they often form an essential part of the microscopic ecosystem, helping to recycle waste materials or utilize energy sources inaccessible to other creatures. For example, nitrogen is recycled through the environment through bacteria's breakdown of decaying organic matter. Besides natural debris, bacteria have also been engineered by scientists to clean up human spills including oil slicks by inserting genes for enzymes that are able to degrade fuel molecules[31]. In the laboratory, the bacterium *E. coli* has become a staple of molecular biology, as its genome is easy to modify for expression of a large array of proteins. For diabetics, bacteria can be a lifesaver, since the tiny organisms are used as a major source of insulin[32]. Finally, we encounter bacteria regularly through the fermentation process used for cheese, and - once we eat the cheese - in our bodies, where multiple layers of bacteria cover the gut[33]. However, our stomachs are not nearly as amazing as those of termites, who have a special bacteria to help them digest the tough fibers in wood[34].



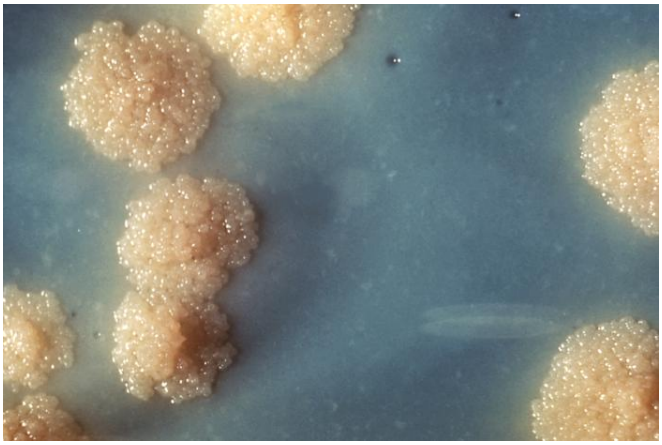
Under magnification, this scanning electron micrograph (SEM) depicts a number of Gram-negative *Escherichia coli* bacteria of the strain O157:H7, which is one of hundreds of strains of this bacterium. Although most strains are harmless, and live in the intestines of healthy humans and animals, this strain produces a powerful toxin, which can cause severe illness.

Comprehension Questions:

1. What are some different shapes bacteria can have?
2. What are the components of the bacterial cell wall? How does this affect the Gram Test?
3. What are some non-pathogenic bacteria?

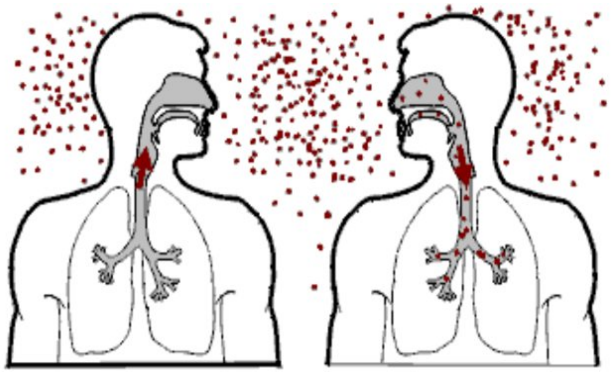
The Sleeping Killer: Acquiring and Waking Tuberculosis

The specific bacterium responsible for the development of tuberculosis is *Mycobacterium tuberculosis*. What exactly does this name mean? The term Mycobacterium refers to the genera of this bacterium, a name based on a substance called mycolic acid present in the bacterium's outer wall[35]. The species name – tuberculosis – refers to the clumps of white blood cells called tubercles that isolate the bacteria from rest of the body during infection[35]. The tubercles are visible in x-rays, appearing as dark spots inside the lungs[36]. Under the microscope, the *M. tuberculosis* bacteria appear as tiny rods, usually clumped inside the human cell they are invading. They usually test negative for the Gram test, largely because their cell walls are so thick – the strength of this barrier is but one astounding property of *M. tuberculosis* that we will touch on in this unit[35].



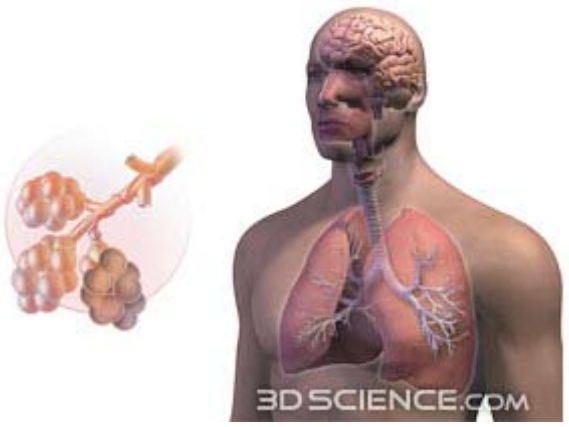
A close-up of a *Mycobacterium tuberculosis* culture reveals this organism's colonial morphology.

Typically, *M. tuberculosis* is transmitted by aerosol, with an infected individual coughing or sneezing clumps of bacteria into the air[35]. Those nearby can easily inhale these clumps, introducing the bacteria into a new environment. Once they enter the body, the bacteria are targeted by a class of white blood cells known as macrophages. The job of these cells is to devour invaders like *M. tuberculosis* and digest them. In most parts of the body, the macrophages do their job, using harmful oxygen molecules to destroy the bacteria before it can cause TB. The lungs, however, can offer a safe harbor for the bacteria. As air enters the lungs through the trachea, it follows increasingly smaller ducts that spread out like the branches of a tree[37]. At the very end of these branches are



Aerosolized bacteria can be transmitted by coughing.

tiny sacs called alveoli, which are surrounded by blood vessels where oxygen passes into the bloodstream and carbon dioxide is expelled[37]. Inside these alveoli are macrophages that have not yet developed the ability to destroy invading bacteria[35]. They are thus called immature, because they have not matured enough to become "microbe-eating machines". If the *M. tuberculosis* bacteria travel into the alveoli, these immature macrophages will engulf the invader, but will not be able to digest them.



The grape-like alveoli terminate in the air passageways inside the lungs.

To survive inside these immature cells, the bacterium has developed a protective cell wall that can only be described as truly extraordinary. Robert Koch noted the peculiar difficulty of staining *M. tuberculosis* back in 1882 and speculated that they were surrounded by a "special wall" with "unusual properties"[30]. However it is only with the tools of modern science that the exact nature of this barrier is finally being understood. The cell wall is organized into a four tiered sandwich, with a layer of peptidoglycan "chicken wire" overlaid by sugar and fat molecules[30]. The entire cell wall is then wrapped in a layer of wax that makes the bacterium nearly impenetrable[30]. This formidable wall prevents the bacterium from being digested by the immature macrophages, allowing the bacteria to begin multiplying within its host and steadily growing in number[30,35].

Breaking Down the (Cell) Walls

As bacteria numbers exponentially increase in the immature macrophages, the macrophage eventually bursts, releasing a swarm of bacteria into the alveoli [38]. The sudden surge of free bacteria in the lungs sends out a warning signal to macrophages in nearby alveoli, attracting them to the lysis site [38]. Once there, the immature cells once again engulf the bacteria, which then replicate and destroy the immature cells as before, forming a vicious cycle[38]. The destruction of the immature macrophages cannot continue forever though, since, as they die, the cells send out a chemical distress signal to the other members of the body's immune system, alerting them to the invaders[38]. Eventually, these additional blood cells of the body's defense system arrive to attack the bacteria, and bring with them a return message: a chemical that causes the immature cells to finally become full-fledged macrophages[35]. These adult macrophages then form clumps surrounding the invader, attempting to imprison the bacteria in the alveoli and prevent them from spreading to the rest of the body[38]. Such collections of macrophages surrounding the bacteria are the tubercules from which the disease gets its name.

At this point, the macrophages surrounding the bacteria try to ingest the invaders, while the bacteria continue to divide and attempt to kill the macrophages[35]. If the scales in this contest tip towards the immune system's favor, the bacteria will be devoured and

destroyed by the macrophages surrounding them in the tubercule[35]. While the tubercles can wall off the bacteria from the rest of the lung, this defense is a two-edged sword. The macrophages walling off the bacteria secrete chemicals that also kill the lung cells in that area, turning that spot in the alveolus into a cheese-like pus[35]. As the macrophages continue to cause damage to the lungs, the tubercule grows larger[35]. The destruction of lung tissue makes it difficult for the infected individual to breath, leading to the characteristic coughing and bloody phlegm that is characteristic of the disease[35]. On the other hand, the bacterial population may eventually overwhelm the mature macrophages, at which point a patient is said to have the clinical, infectious version of tuberculosis[35]. It is at this stage – when other immune cells enter the lung and begin fighting the bacteria – that pneumonia occurs[35]. However, it is important to keep in mind that pneumonia is a generalized term for any inflammatory reaction in the lungs, which can be caused by many kinds of bacteria, viruses, and even pieces of particulate matter like inhaled coal particles[39].

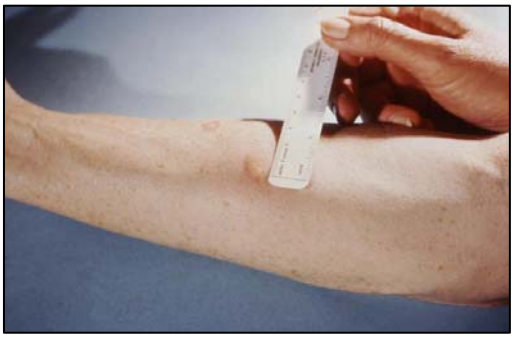
Between the two extremes is a stage called latency**,** in which the macrophages and the bacteria are in stalemate[40]. This latency period accounts for the third of the world's population being infected with the TB-causing mycobacteria yet not actually having the disease itself. This population of people serve as a large reservoir of mycobacteria in which the disease may develop in the future[40].

HIV and TB

If AIDS and TB were not bad enough by themselves, the two diseases have been found to form a lethal combination. Because their immune systems are weakened by infection with HIV, AIDS patients are more likely to develop tuberculosis[41]. Further, TB has been shown to make AIDS worse by further decreasing CD4 T cell counts in the blood[42]. Furthermore, drug-resistant tuberculosis is almost always fatal in AIDS patients, and has been called the "most malignant opportunistic infection yet associated with HIV-infection"[41,43].

Diagnosis:

The usual symptoms of tuberculosis include fever, sweating, weight loss, coughing, exhaustion and appetite loss – in other words, signs associated with many ailments including the common cold[35]. To specifically determine if a patient has TB, a physician usually administers a tuberculin skin test by injecting boiled bacteria under a patient's arm and observing if a welt forms or not[35]. Swelling results when a group of T and B Cells specifically formed to defend against the mycobacterium migrate to the site of injection, releasing chemicals that cause a typical inflammatory reaction. The very existence of this anti-TB group of lymphocytes indicates that the individual is infected with the



This patient presented with a positive reaction to the tuberculin skin test as indicated by the welt.

mycobacterium, which has previously stimulated development of this specialized immune arsenal[35].

While this is a fairly quick test, further confirmation can be achieved by examining the patient's lungs by X-rays. In contrast to healthy lungs, those with TB may have holes in them (from the destruction of the tubercles described above) or be filled with fluid (a common reaction to an infection)[36]. Looking at lung-rays can help corroborate evidence from a skin test, but it is important to remember that it does not prove that a patient has TB, since, as noted above, pneumonia can have many causes[36]. In addition to chest x-rays, it may be helpful for a physician to examine a patient's sputum, allowing them to effectively peek inside the lung's cellular environment by testing for the presence of the *M. tuberculosis* bacteria.

Comprehension Questions:

1. How does M. tuberculosis usually enter the human body?
2. How can some people test positive for the skin prick test but have no symptoms?
3. What are the major symptoms of tuberculosis?

Treatments

Vaccines

In many non-US countries, TB vaccination is quite common. The vaccine is called BCG (Bacillus of Calmette and Guerin, named after its inventors), and uses an attenuated strain of cow-infecting bacterium called *Mycobacterium bovis*[35]. However, in the US the vaccine is not routinely used for several reasons[35]. For one, the vaccine cannot prevent disease from being reactivated in patients already infected with the latent form of TB[35]. Thus, unless administered at birth, it has limited effectiveness. A related concern is that the vaccine does not prevent infection, thus the entire population would have to be vaccinated for the treatment to be uniformly effective[35]. Finally, the vaccine would complicate the previously described diagnostic tests, making it harder to read the skin test because the attenuated *Mycobacterium* would show up as a false positive[35].

The Rise of Antibiotics

With the knowledge that bacteria were disease vectors came a consequent desire by the scientific world to find ways to combat these harmful microorganisms. The first isolation of an antibiotic (e.g. antibacterial) substance was accomplished in 1889 by E. de Freudenreich, a German scientist who discovered a bacterial pigment capable of arresting the division of other bacteria[44]. Though effective, the pigment proved toxic to humans so could not be used in drugs[44]. An important moment in the fight against bacterial infections occurred in 1928, when British scientist Alexander Fleming discovered a mold contaminant growing in his bacterial cultures[44]. Amazingly, the mold seemed to lyse the bacteria, an effect Fleming had previously observed when human tears contacted certain bacterial cells[44]. Since the antibacterial substance had been produced by the *Penicillium* mold, the resulting drug was named penicillin. However, Fleming was unable to purify much of this compound himself, leaving most work on penicillin to be done by his scientific descendents[44]. Ultimately, it became one of the most widely used agents to treat

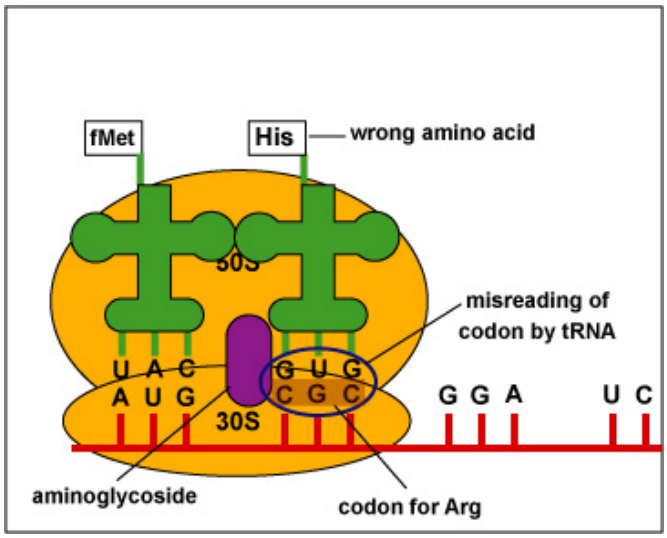
allied soldiers during WWII, with the US partnering with the economically deprived Britain to generate supplies of penicillin[45,46]. The public first became aware of the drug after it was used to treat burn victims of a Boston club fire at Massachusetts General Hospital, and by 1946 it was widely available[45].

Penicillin, like other antibiotics, works by blocking key processes in the bacterial cell – in this case the synthesis of the bacterial cell wall[46]. By preventing the cross-linking of peptidoglycans in the bacterial wall**,** penicillin prevents new bacterial cells from assembling a cell wall rigid enough to resist osmotic pressure. Normally, the connections between the components of the cell wall form a kind of "safety-net" for the plasma membrane. Even if water pushes on membrane from the inside, expanding it, the cell wall will keep the bilayer from actually bursting. However, with the cell wall weakened by penicillin, bacteria may well burst when placed in a solution of pure water[46]. However, it turns out that this same cell wall was the major determinant of penicillin's effectiveness, as it can only treat Gram positive bacterial infections. *M. tuberculosis* however is Gram negative[30]. Thus, a cure was still missing, though researchers had a promising lead.

Fleming's history-making fungus: the penicillin mold.

Until the 1940s, there was no cure for TB. However, a breakthrough came in 1943, when Selman Waksman of the University of California identified a potent antibiotic called streptomycin that showed effectiveness against *M. tuberculosis* [47]. So how does streptomycin work? By binding to the ribosome – a complex of protein and RNA responsible for synthesizing proteins from mRNA instructions – streptomycin shuts down the production of proteins in bacterial cells by ensuring that RNA messages are either terminated at too short a length or formed with the wrong amino acid components[48]. Without this translation, the cell cannot make enzymes, cofactors, structural proteins, or any of the other components it requires to function.

Many other antibiotics work on similar principles, shutting down biochemical pathways essential for life, be it protein synthesis, DNA replication, or the generation of cell wall components[49]. Alternately, the antibiotic might change the bacterial membrane's permeability, making it

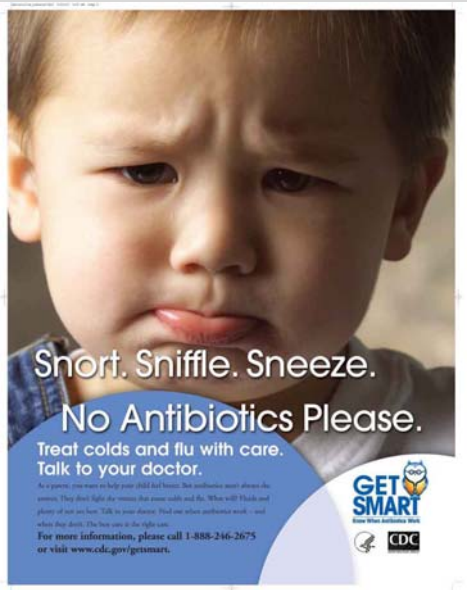


Streptomycin (aminoglycoside in this image) binds to the 30S subunit of bacterial ribosomes, leading to the misreading of the codons or premature termination of protein synthesis.

impossible for it to maintain the carefully balanced chemical conditions normally present in the cytoplasm[49].

Patients are not usually treated with just one antibiotic at one time. Instead, they are given a "cocktail" of different antibiotics which lowers the risk of encouraging drug resistance in the bacteria, an issue addressed in depth below[35]. By using multi-drug therapy, the bacteria is less likely to develop resistance [35]. Thus, even if the bacteria do acquire resistance to one antibiotic, another compound in the mixture will destroy it and prevent the resistance mutation from passing to the next generation of bacteria.

Treating Viral Infections with Antibiotics? No!



Given the ability of antibiotics to kill many kinds of bacteria, it might seem natural to assume that these drugs should be used for a virus-mediated ailment such as the common cold. The average U.S. teenager would probably agree with this conclusion, as a recent poll showed that 6 in 10 believe viral infections can be treated with antibiotics[50]. However, viruses and bacteria are vastly different kinds of organisms. As discussed in the introduction above, bacterial cells are like our own, carrying out many necessary life processes that can be interrupted by an antibiotic shutting down one of these essential pathways. In contrast, as we saw in the previous unit, viruses are usually nothing more than a strand of RNA or DNA wrapped in protein to protect it from the environment. They cannot replicate without hijacking a cell's existing machinery and do not have complex metabolic processes that can be disturbed by an antibiotic compound. What's more, using antibiotics on a viral infection is not just unhelpful, it can actually be harmful. Every time bacteria are exposed to antibiotics, there is a chance that they may develop antibiotic resistance. A patient with a viral infection who uses antibiotics may incidentally stimulate bacteria in their body to acquire drug resistance, leading to a worse problem than the original illness.

The Fall of Antibiotics: The Looming Threat of Drug Resistance

Alexander Fleming would be shocked if he knew that Staphylococcus aureus, the first bacterium to be effectively treated with penicillin, is once again a major health threat. In fact, the medical community now routinely deals with antibiotic-resistant bacteria, particularly in an inconvenient setting: the hospital! It happens like this: a patient checks into a hospital with a normal illness. During their stay, they become infected with bacteria lingering in the hospital, and, when the staff attempts to treat them, they find the infection is resistant to standard antibiotics. The consequences can be dire; these nosocomial infections can be more life-threatening than the surgery or treatment for which a patient is admitted.

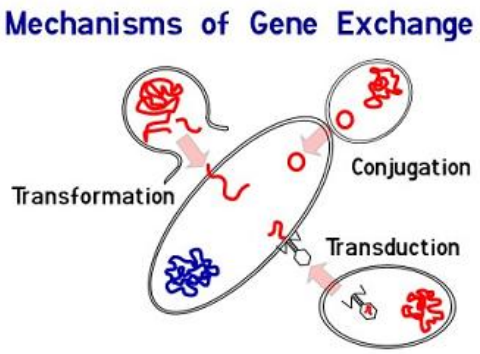
How could this happen? Like any organism, bacteria evolve or change over time. Given their quick reproduction time (mere hours in some species), any advantageous mutations they acquire can be speedily propagated to millions of individuals. As we will see below, bacteria also communicate with each other, swapping DNA (intentionally or unintentionally) that can contain genes for drug resistance.

The Evolutionary Waltz and the Plasmid Shuffle: How Resistance Dances Between Bacteria

During a bacterium's rapid replication of its DNA – sometimes every few hours – mistakes can be made. An incorrect base pair may be inserted within a codon, sometimes resulting in a different amino acid to be incorporated during protein synthesis. This change may make the protein nonfunctional or have incidentally have no effect at all. Every so often though – about once in every billion new bacteria – a beneficial change will occur, perhaps changing the bacterium's metabolic processes for cell wall synthesis, protein generation, or other essential pathways so that these biochemical pathways are no longer inhibited by an antibiotic.

When this effect is isolated to a single bacterium, the effect is small. However, if this bacterium is in a population exposed to an antibiotic, the rest of the population will die, leaving only the mutated bacterium as a survivor. Once it replicates, the new population of bacteria will *all* be resistant, as the antibiotic has become a force of natural selection. The fit survive, and the offspring are similarly hardy: they are officially termed antibiotic-resistant. This scenario – where bacteria acquire resistant genes from their progenitors – is known as Vertical Gene Transfer (VGT)[51]. The reason it is called vertical is clear if bacterial generations are thought of as a ladder, with each generation on a different rung. Passing a resistant gene to an offspring, antibiotic resistance moves vertically down the ladder of the bacterial lineage. That this is possible would be frightening enough if it were the only way for bacteria to acquire drug resistance: but it's not.

The action on the ladder of bacterial generations does not just happen vertically. When that activity involves the exchange of genetic information between bacteria, it is called Horizontal Gene Transfer (HGT) because genes are being passed back and forth among bacteria in the same generational rung. There are several ways that this exchange can take place:



Mechanisms of bacterial gene exchange.

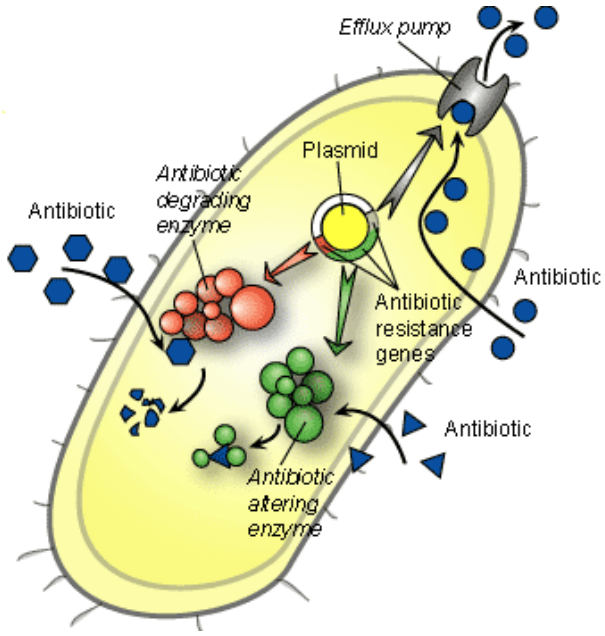
1: Bacteria, like humans, are preyed upon by organisms. In fact, bacteria have a class of infectious predators all their own: small viruses called *bacteriophages*. In some cases, bacteriophages are harmful to the bacteria (just as viral infections harm human cells). However, bacteriophages can repackage bacterial DNA and shuttle it to other bacterial cells[51]. Consequently, a

bacteriophage can transmit drug resistance genes between individuals through this process known as *transduction*[51].

2: As mentioned earlier, bacteria can have independently replicating plasmids in addition to their chromosome. Just like the chromosome, the plasmid can be mutated and acquire drug-resistance genes[51]. Unlike the chromosome however, the plasmid can jump between bacterial cells with ease. In some cases, this occurs through a process called conjugation, in which two bacteria exchange a plasmid through direct cellular contact[51]. This process, in fact, is thought to be the main culprit behind drug resistance. A second possibility is that a plasmid escapes the cell membrane of its original bacterium (this can happen, for example, when the first bacterium dies) and is taken up by another cell in a process called *transformation*[51]. The plasmid begins replicating in its new home, and the host cell acquires any benefits of its augmented genetic information, including drug resistance.

Terminating the Antibiotic

How exactly does a mutation make a bacterium resistant to an antibiotic? There are many ways to turn off an antibiotic. A common mutation alters a bacterium's existing enzymes so that it now turns the formerly lethal antibiotic into a harmless molecule[52]. Another, related strategy is to interfere with the antibiotic's target: whatever site in an essential enzyme was originally blocked by the antibiotic compound is altered so that the drug no longer binds, preventing its activity[52]. Lastly, the bacteria can simply expel the drug before it has a chance to work through an advantageous mutation in a pump bridging the cell membrane[52].



Mechanisms of antibiotic resistance.

How did this Resistance Epidemic Happen?

The drug development process itself is to blame on at least two counts. During the 1980s, too little attention was given to the development of new antibiotics. Indeed, developing new drugs has become an exceedingly high-risk and high-cost endeavor, with new products on average requiring 14 or more years of testing before the FDA will allow them to be commercially distributed[53]. Secondly, even though there is a diversity of antibiotics on the market, many of them are very similar in function. This is in part because developing new drugs is expensive, leading pharmaceutical companies to create variations on an old compound instead of pursuing the expensive venture of creating a new one from scratch. However, because these antibiotics work through similar

mechanisms, a bacterium can develop resistance to a whole class of antibiotics with a single mutation[51].

Social conditions have also contributed to the rise of antibiotic resistance. Increased infections mean more antibiotic prescriptions, so situations that facilitate the transmission of bacteria contribute to the problem. For example, studies have correlated the rise of ear infections in preschoolers over the last 30 years with the rising prevalence of daycare. Children are crowded into public spaces more often in this system, giving greater opportunity to pass infections between each other. Another example is homelessness whereby the presence of more unsheltered individuals on U.S. streets (usually without adequate access to health care) leads to more urban congestion in general, and specifically a population who, when in poor health, can serve as a ready means for an infection to spread through a city[54].

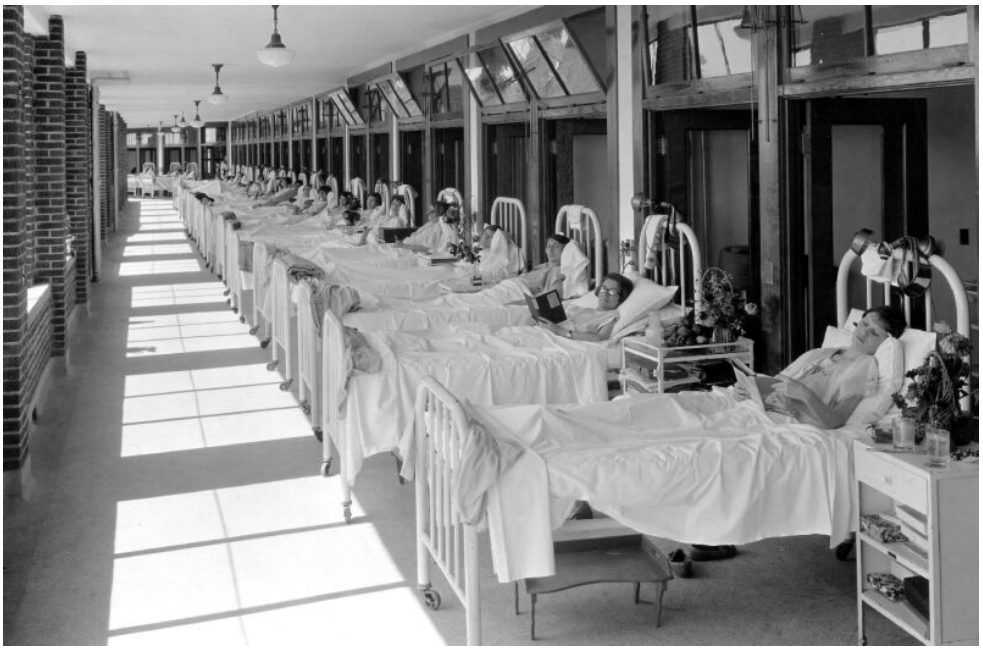
Another major cause of the resistance dilemma is the overuse of antibiotics. For example, in order to keep slaughter animals healthy in unsanitary conditions, antibiotics are used in massive amounts in livestock feed, effectively cultivating antibiotic-resistant bacterial strains. Antibiotics are also often overused in clinical settings as a treatment for viral infections (for which they are useless). Ironically, improvements in medical treatment may be to blame as well. The antibiotic overprescription is, at least in part, due to the increasing number of patients that can be kept alive in the hospital. The elderly and the immune-compromised now stand a much better chance of survival but as a result require antibiotics to stave off infections in their weakened immune systems. Consequently, more antibiotics are being used than ever before, and patients tend to expect them immediately as a treatment for bacterial illnesses[54].

Scrubbing Up:
Another recent controversy in the "superbug" debate concerns antibacterial soap. Many dermatologists have suggested that the use of antibiotics in hand soap creates an environment that is selective for antibiotic resistance in common bacteria[55]. In contrast, normal soap merely breaks up hydrophobic material and solubilizes it in water, allowing the grime to be washed away[56]. Indeed, one might argue that bacteria fall under the same category as other substances emulsified by soap in water, and that the antibiotics are unnecessary to its effectiveness. On the other hand, recent experiments have failed to demonstrate a significant link between antibacterial soap and the development of antibiotic-resistant bacteria[57]. In conclusion, the jury is still out on this debate.

Comprehension Questions:

1. What are two ways in which bacteria may acquire drug resistance?
2. How does the drug streptomycin work? How is this different from how penicillin works?
3. Why is an antibiotic "cocktail" usually more effective than any single antibiotic compound?
4. What is in a TB vaccine, and how does it prevent infection?

Activity 1: Sanatoria of Today?



Fresh air was a part of the regimen to battle tuberculosis. The above photo is of the patients on the sun porch at Waverly Tuberculosis Hospital in Louisville, Kentucky.

Is the hospital in your community equipped to deal with a multi-drug resistant tuberculosis outbreak? For this assignment, students should locate a convenient facility (e.g. the VA Hospital at Duke Medical Center) and address some of the following questions:

1. How many patients at this facility contract TB every year? Are there statistics? Are there any cases of multi-drug resistant TB?
2. Are there any predisposing factors for development of tuberculosis? (Do not just think about biology – what kind of patients frequent this hospital? Are there social factors that might be involved?)
3. How would a physician at this facility treat a patient who developed tuberculosis? Do they observe DOT guidelines?

Based on what they find, the students should either (1) make a presentation on a plan of action if a case of MDR-TB arose in this hospital, (2) make a pamphlet for the waiting room about their findings, phrased as things patients should be aware of, or (3) draft a memo for the hospital's administration.

Activity 2: TB Among the Literary Greats

John Keats is not the only literary figure that died of TB – for this assignment, students should each find a famous writer or poet who died of TB and present a brief report about their subject. Things to include:

1. Did TB kill this person at a particularly young age?
2. Do any of this author's characters die of TB?
3. Did anything about this author's lifestyle make them more or less likely to develop TB?
4. The class might keep a running tally of famous TB victims, updating each week of the unit as students find more.
5. (Optional). Students can undertake creative projects, such as a story in an author's style about TB, a poem about TB, a picture like Alice Neel, or any other artistic representation of the disease.

Activity 3: Art analysis – T.B. Harlem

Tuberculosis was examined not only in the laboratory and doctor's office but also in the world of art. Here, students will examine Alice Neel's TB Harlem, her 1940s painting depicting the disease before treatments existed. They will align Neel's portrayal with the science of TB.

Activity 4: Movie - Rise of the Superbugs

It is difficult to imagine a world without medicines—and yet, before the twentieth century there were not any. The discovery of the very first antibiotic, penicillin, and the subsequent development of more "wonder drugs" transformed the face of modern medicine. Rise of the Superbugs chronicles these historic successes, as well as the growing threat posed by new strains of germs, such as tuberculosis and staph, that are resistant to our best antibiotics. Are our strongest medicines becoming obsolete, and can we develop new drugs in time to replace them?

Activity 5: MDR Tuberculosis Case Study

Students will investigate many facets of TB including its relationship to HIV/AIDS, immigration, and public policy. Teacher and student guide provided.

Activity 6: Have TB, Will Travel

Students will read and discuss media articles about the recent international traveler with tuberculosis. Based on their knowledge of TB transmission, did the authorities act correctly? What would be a good protocol for controlling TB transmission across borders? http://topics.cnn.com/topics/tuberculosis

Activity 7: Sentenced to Tuberculosis

Students will read: "Russia: Prisoners Sentenced to TB" and then determine the best ways to reform the Russian prison system. Could this situation occur in the US penal system? Please refer to the "Why does evolution matter now?" video available at http://www.pbs.org/wgbh/evolution/educators/lessons/lesson6/act1.html.

Activity 8: Service-learning project – Antibiotic Resistance in Your Neighborhood

Adapted from http://www.pbs.org/wgbh/evolution/educators/lessons/lesson6/act1.html. (Part B). Students will assess the prevalence of antibiotic use in their communities by surveying. They will then develop public information items to educate the general population about antibiotic resistance.

Activity 9: Superbugs: An Evolving Concern

Source: Emerging and Re-Emerging Infections Disease (curriculum supplement available free from NIH Office of Science Education). In this activity, students learn that the evolution of antibiotic resistance among bacteria observed in laboratory experiments occurs in the natural environment as well, and that such evolution has serious consequences for the effectiveness of treatments for some diseases.

Activity 10: Educational game – TB

Nicely illustrates Koch's discovery of the TB-causing mycobacteria.
http://nobelprize.org/educational_games/medicine/tuberculosis/.

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Malaria:
Scourge of the Developing World

"The green and stagnant waters lick his feet,
And from their filmy, iridescent scum
Clouds of mosquitoes, gauzy in the heat,
Rise with His gifts: Death and Delirium."

-Malaria, Indian Poem[2]

“Chrysalis”

Though the sunlight is filtered by the gray clouds gathering over the western end of the school, it is enough to reveal the hardened insect shell hanging high on the window frame. It seems to defy gravity, stuck against the sill by some strange insect glue. Past this odd sight, the sun passes through the adjacent window, falling across the empty desk between Fallon and Lang. The map is out, and both boys suspect that Mrs. K. has planned another geography lesson. Their teacher turns towards them.

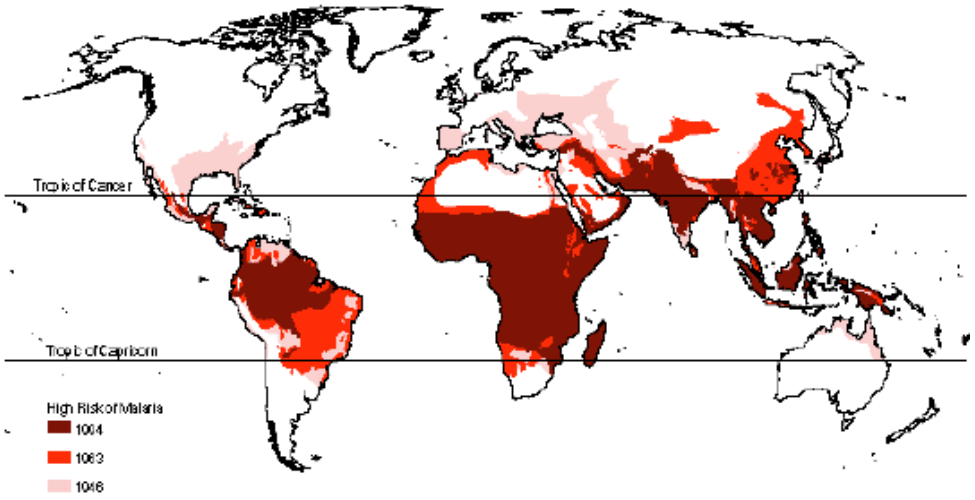
“Don’t worry, no geography today – well at least not in terms of names.” Maxine and Angelica share a look of relief, having feared that they would have to recall the location of some obscure African country they had studied the week before. The topic of today’s lecture appears in bold lettering on the overhead projector: *Malaria*.

“Before we start, does anyone know anything about malaria?” asks Mrs. K. Breaking up the silence that begins to settle between the four students Maxine raises her hand.

“It causes fever . . . people get it in the tropics.”

“Good,” continues her teacher, “and how convenient you should mention the tropics.” Mrs. K clicks a button on her remote, sending a pattern of red splotches shining from the projector over the world map now on the board.

“Here is the distribution of malaria cases around the world in 2003. The darker the color, the higher number of cases….”



Fallon breathes out, thankful that his instructor has – probably unintentionally – reminded him of the subject of today’s class. In theory, the students are all supposed to complete a set of assigned readings before each unit and be prepared to discuss them on the day the class begins covering that section. However, Fallon had not had time to do the reading during last weekend’s out-of-state soccer match. Racking his brain, he tries to remember anything about malaria . . . he thinks it has something to do with insects, or insecticides. Maybe both – or perhaps he has it confused with tuberculosis.

“Does anyone have any observations about this distribution?” Mrs. K. turns, expectantly, towards the four students. Wishing to break the awkward silence as soon as possible, Maxine raises her hand.

"The darker bands are all around the tropics," she says.

"Correct," notes Mrs. K., "but why might *that* have an effect on malaria cases? Also, notice that temperature and malaria distributions don't always coincide – so there's clearly another factor."

Fallon decides to risk embarrassment, and raises his hand.

"Yes, Fallon?"

"Mosquitoes survive in warmer climates and . . .," he looks quickly at the board, trying to determine if his guess is accurate, "they have to be climates that are warm and *damp*. That's why malaria is higher in those bands. Some of those countries also don't have good access to insecticides or medicine like the US does."

"Very insightful," says Mrs. K., smiling. The other three glare at Fallon when their teacher turns her back. They know he has just guessed and resent the praise he often seems to receive for reciting the few sentences of the text he happened to read. Fallon has become used to such reactions from Lang and Angelica but squirms a bit under Maxine's unpleasant gaze. He wonders if they're still thinking about the hypnotism incident.

"You're actually lucky though," their teacher continues. "A hundred years ago, and the dark red you see in Africa was also in the Southern US. The difference, these days, is . . . a 'small' difference, on a large scale." Her students look puzzled. "Let me show you."

A click of a button brings a new projector image onto the whiteboard, this one of a mosquito with its proboscis cleanly piercing the skin of an anonymous human snack. There's something strange, Lang thinks, about this arrangement. The mosquito lives by blood-feeding that also spreads malaria. There always seem to be payoffs in nature – species usually seem to harm each other in some way, rather than beneficially sharing the environment.

"Meet the tiny creature responsible for spreading malaria throughout the developing world," continued Mrs. K. "The *Anopheles* mosquito injects malarial parasites into the bloodstream of its prey where they cause the symptoms you read about last night in your book." She pauses. "Which I *assume* you read about in the book. I hope we don't need another surprise essay assignment."

The four all vigorously shake their heads, not wanting to repeat their experience last month during the tuberculosis unit.

"Since you all seem so well-versed, here's another question: if they spread malaria, why don't we simply kill all the mosquitoes?"

Angelica raises her hand in response. "Some of the insecticides are almost as bad as malaria – at least that's what Rachel Carson wrote about DDT."

"So you *did* do the reading this time . . . good," says Mrs. K.

"It's kind of like chemotherapy," adds Maxine. "The cure can be almost as bad as the cause."

"Hold on to that thought," Mrs. K. replies, "it may be helpful when we talk more about vaccines in a few months. For now, though, does anyone see any parallels between malaria treatments and AIDS?"

"In both cases," Lang answers, speaking for the first time today, "the treatments are cheaply available in the Western world but too expensive for most developing countries to afford. It's not a question of the treatments not existing but of economic

incentives. There's no financial benefit for pharmaceutical companies to generate drugs for poor countries."

"Very true," says Mrs. K. "Which raises another important question: how do you best treat a disease when a country can't afford the drugs that are so readily available in the US?" She walks the short distance to the desk, picking up a set of papers. "These articles should help you think about that problem. Since we're almost out of time for today, you can begin looking them over, and we'll begin our discussion again next time based on what you've learned." She stops. "So *please* do the reading."

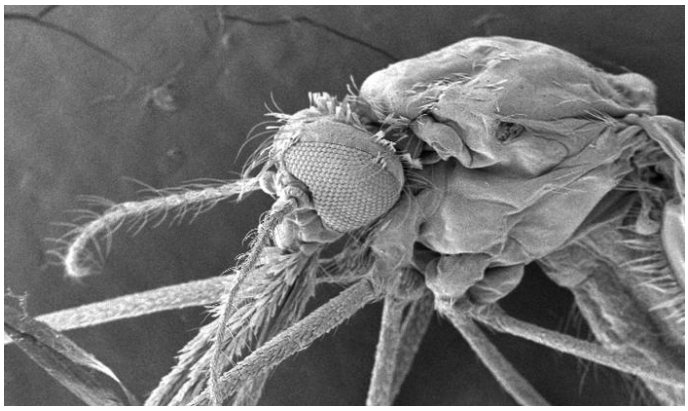
The four comply, beginning to busily leaf through the packet as Mrs. K. distributes them. As if the sound of rustling pages were enough to awaken its occupant, the cocoon outside begins to shudder, and a sliver of yellow appears in a crack between the enclosing fibers of musty brown. The period bell rings just as the sleeper emerges – but it's not a disease-spreading mosquito, just a harmless butterfly grown out of season, left to fend against an increasingly chilly sky.

Questions:

1. **What is malaria? Why does it show a unique global distribution?**
2. **Do we need a new policy to distribute malarial drugs in the developing world?**
3. **Is DDT an effective control option for malaria?**
4. **What are some non-insecticide, non-drug possibilities for malarial control? (Think public health measures.)**
5. **Describe the relationship between a country's economics and malaria prevalence.**

Introduction:

Humans and insects have a difficult relationship. On one hand, the world's six-legged denizens are a fundamental part of the ecological balance, working to recycle waste products left by larger organisms. However, they can also be humanity's bane, transmitting harmful pathogens to those on whom they feed. The mosquito is guiltier in this respect than most, harboring one of the world's leading pathogens: the malarial parasite. The disease affects three to five hundred million victims a year worldwide, ultimately causing almost a million deaths[3]. Even with US health care resources, approximately 1300 American cases occurred in 2004, consisting primarily of travelers returning from tropical regions. Malaria is truly a global disease[4].



The mosquito: vector of deadly parasites.

However, while it is described as a singular disease, malaria can actually result from infection by any of four species of parasites of genus Plasmodium. Which parasitic strain is contracted determines the severity and symptoms of a patient's reaction. Further, the varying geographic prevalence of each strain guides different anti-malarial strategies in, for example, Thailand versus Sub-Saharan Africa. In other words, the biology of malaria infection is not simply a matter of scientific nuance – they determine how health organizations decide to treat patients and what precautions they should take in the process. Similarly, the ways in which malarial parasites are transmitted between species is also vitally important to treatment efforts. Mosquitoes are thus necessarily key players in this story. However, before launching into these specifics, we'll begin with the most important aspect of malaria for us: the disease as it exists in humans.

Goals:

By the completion of this unit, students should be able to do the following:

1. Given geographical and socio-economic data about a region, estimate the risk posed by malaria.
2. Given information about several anti-malarial strategies (e.g., drugs, insecticide, etc.), compare and contrast the pros and cons of each option and propose an optimal solution.
3. Given an anti-malarial compounds properties and mode of action, anticipate possible side effects and the risk posed by drug resistance.
4. Analyze ethical arguments for and against a given anti-malarial strategy.

A Brief History of Malaria and Its Treatment

Ever since the early days of human civilization, people have sought to combat malaria around the world. From ancient remedies to modern pharmaceutical agents (and their noteworthy discoverers), the history of malaria and its treatment is a rich one.

History of Antimalarial Treatments

Malaria has afflicted humans for thousands of years. The "Father of Medicine," Hippocrates, described the disease in a medical text in the 4th or 5th Century BC. Even great warriors were no match for the tiny parasites as Alexander the Great may have died of a malaria infection at age 30 [5]. However, it was not until 1718 that the term malaria (from Italian *mala aria,* or "bad air") was coined by Italian physician Francisco Torti , a title stemming from the belief perpetuated by Roman physicians that the disease was called by malignancies in the swamp air[6-8].

The Beginnings of a Mosquito-Transmitted Malaria

For centuries after the Romans initially proposed the idea, it was widely believed that malaria was caused by something in the air rising from swamplands, and that contact with these fumes was a risk factor for the disease[8]. Though the notion of swamp-gas infecting travelers with malaria seems preposterous now, it was not immediately discounted by 18th century Italian physician Giovanni Maria Lancisi who gained great acclaim by observing black pigmentation in the organs of malaria victims[8].

The swamp-gas theory deteriorated over time, particularly once scientists correctly identified an animal culprit for infection[8].The concept of a mosquito-born illness was endorsed during an 1882 meeting of the Philosophical Society of Washington. Though the speaker's suggestion that a giant net be placed over the city to control the mosquito population was met with ridicule, the fact remained that many prominent scientists, including Robert Koch and Alphonse Laveran (see below), suspected that the bloodsucking insects were the root cause of infection[8].

Laveran Discovers the Malaria Parasite

Alphonse Laveran.

Even into the 19th Century, the means by which malaria was transmitted were still unclear. The tiny world of microorganisms and the role these life forms played in the spread of disease remained mysterious. The transmission of malaria was unraveled in 1880 by the French surgeon Alphonse Laveran, who, while stationed at a hospital in Algiers as a military surgeon, observed a parasite moving within a red blood cell from a malarial patient. For his discovery, Laveran was awarded the Nobel Prize in Medicine in 1907[8].

Identification and Naming of the Malarial Parasites

Italian neurophysiologist Camillo Golgi was the first to describe different species of malarial parasite (based on the frequency of attacks they caused and the number of parasites released once the red blood cells containing them ruptured), work for which he was awarded a Nobel Prize in 1906[8]. Italian researchers Giovanni Grassi and Raimondo Filetti first put a name to these different species, classifying *P. vivax* and *P. malariae*[8]. Americans William Welch and John Stephens later contributed, respectively, the names *P. falciparum* and *P. ovale*[8].

Camillo Golgi.

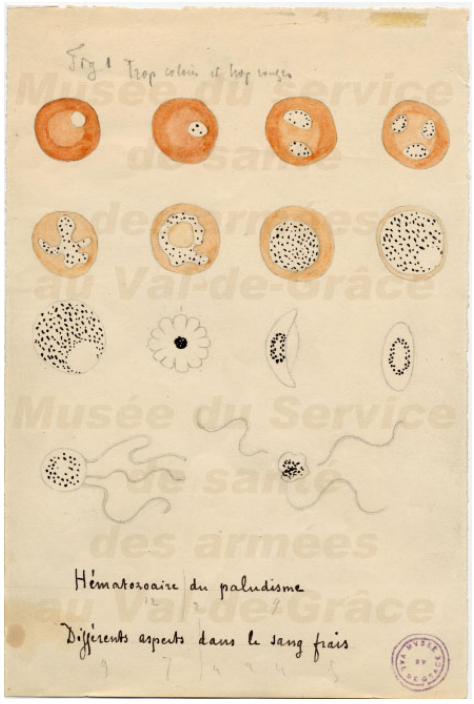
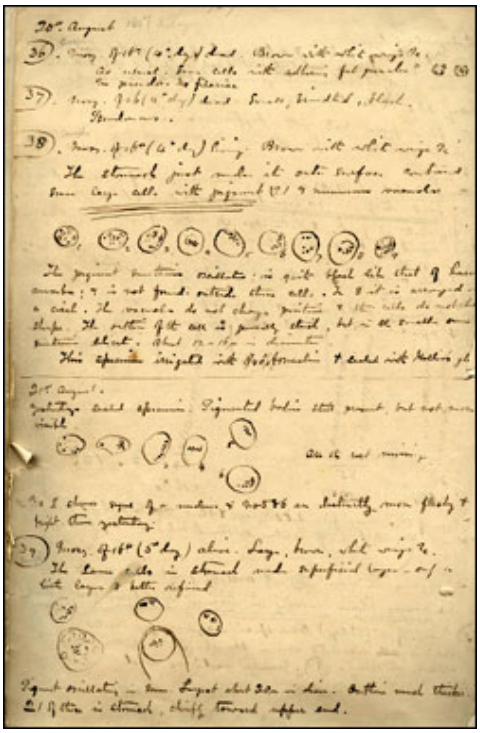


Illustration drawn by Laveran of various stages of malaria parasites as seen on fresh blood. Dark pigment granules are present in most stages. The bottom row shows an exflagellating male gametocyte, which "… move with great vivacity..."



Page from notebook where Sir Ronald Ross records his discovery of the mosquito transmission of malaria, 20 August 1897.

Discovering Malarial Transmission

The description of how malarial parasites move among different organisms was accomplished in two major steps. The first was English physician Sir Ronald Ross' painstaking efforts to show the complex life cycle of the malarial parasite. In his Nobel Prize acceptance speech from 1902, Ross describes his search for both the species of mosquito responsible for transmission and the location of the parasites within the insect's tissue[9]. While initially using many subjects from the native Indian population in his experiments (allowing him to show that mosquitoes feeding on malaria victims contained parasites in their tissues), his later breakthrough came when lack of human participants forced Ross to employ birds[9]. He was ultimately able to observe not only the female and male versions of the malarial parasite in avian hosts but also the transmission of fertilized parasites from birds to the mosquitoes that fed upon them[9]. Interestingly, Ross was not a trained scientist, but received considerable guidance from another prominent malaria researcher[9].

The second revelation that mosquitoes could also pass the disease between human hosts was shown by Giovanni Grassi and his team of Italian investigators in the late 19th Century[8]. This was done by shuttling willing hospital patients in a room with *Anopheles* and observing the development and progression of malaria in the subject, a protocol many of Grassi's contemporaries found exploitative[8].

The History of Antimalarials

Unrefined natural products served as the first antimalarial agents. In the 2nd century BCE, Chinese physicians identified the wormwood plant as an effective treatment[8]. The knowledge of this remedy was lost for thousands of years, while the Western world, coping with the seemingly insoluble problem of malaria, relied mainly on strategies such as DDT spraying into the 1950s[8]. With a shift in politics in the East came medical innovations. Following the Cultural Revolution, Chairman Mao's distrust of Western medicine led to a search for effective remedies documented in China's ancient medicinal texts[8]. One of these compounds was artemisinin, which soon gained great popularity worldwide[10].

Plate from "Quinologie", Paris, 1854, showing bark of Quinquina calisaya (from Bolivia).

In a similar scenario in early Latin America, native Peruvians recognized the beneficial properties of the cinchona tree long before quinine was identified in its bark. With the discovery of the Americas by Europe, an increasing flood of Spanish missionaries entered Latin America at the end of the 15th Century. In the early 1600s, these newcomers learned of the medicinal properties of the cinchona tree, which was used to cure colonists such as the Viceroy of Peru's wife (The countess of Chichon, from which the tree takes its name)[8]. The bark of the

tree was first introduced to Europe around 1640, where it spread from England to Spain as a popular antimalarial compound. Even when botanists finally classified the plant in the 1700s, it was still known colloquially as the cinchona tree[8]. However, the active chemical components of the cinchona plant were not isolated by chemists until 1920[11]. By the 20th Century, the main supply of cinchona trees had shifted to plantations in the Dutch East Indies, a geographical displacement that would cause problems for America in WWII (see below)[8]. Racing to develop antimalarial compounds at this time, German chemists developed a drug named Resochin that would late be known as the popular pharmacologic agent chloroquine[8].

World War II: Quinine Shortage and Wartime Research

As previously noted, the major source of cinchona trees had moved to the Dutch East Indies by the early 20th century. With the expansion of the Japanese Empire during WWII, Americans suffered from a lack of antimalarial drugs while fighting in the South Pacific, a region in which the disease was a major threat[12]. To combat this shortage, a campaign to collect quinine supplies scattered around the United States began in 1942. This period was also notable for the emergency-prompted bolstering of research on antimalarial compounds. Spurred by government support and a sense of national crisis during the war, many advances were made in the biological, chemical, and immunological understanding of the disease as well as methods to treat it, Among the discoveries from this period were alkaloid compounds, including the hydrangea extract febrifuge (which unfortunately proved far too toxic in clinical trials to be used as a treatment)[12]. Another was the identification of the insecticidal properties of DDT (a compound first synthesized in 1874) in 1939 by Paul Muller, a contribution for which he was awarded the 1948 Nobel Prize in Medicine[12].

The Birth of the CDC and the Worldwide Campaign Against Malaria

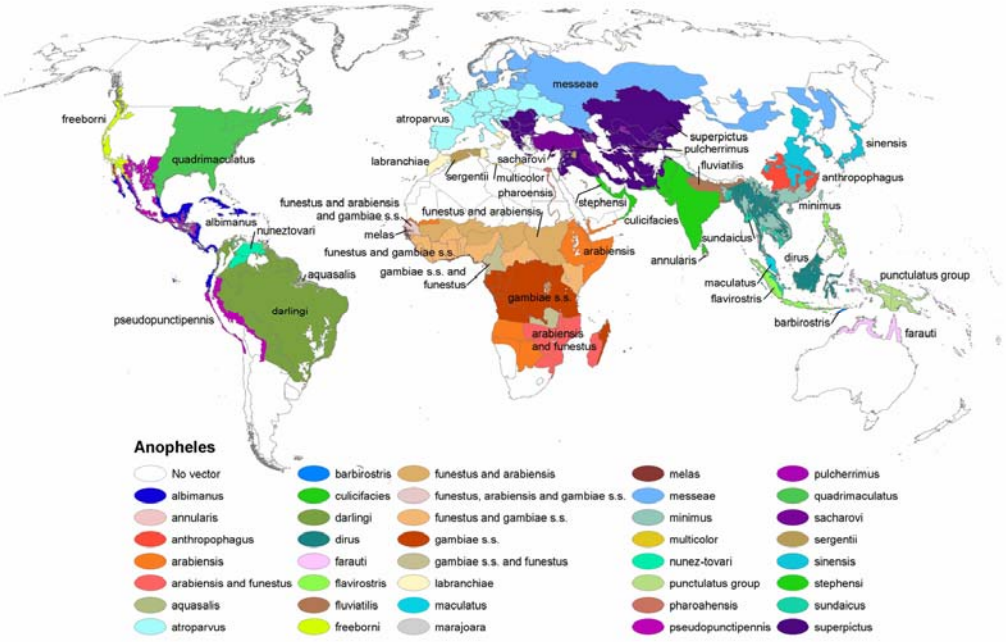
During its expansion into Cuba and the construction of the Panama Canal, the US Government took an active interest in controlling malaria outbreaks. The US Public Health Service (USPHS) obtained funding in the early 20th century to combat malaria within the United States itself. Additionally, North Carolina's Cape Fear was known as a malarial hotspot, which, along with the perilous offshore waters, may explain the region's ominous name[12,13]. On July 1st, 1946, the Center for Communicable Diseases was formed. This center, which would eventually become the modern CDC, dedicated itself to the eradication of malaria in the US, a goal that was accomplished by 1951[12]. Among the strategies used in this campaign were improved drainage to remove mosquito breeding sites and large-scale insecticide spraying over affected areas[14].

With this task completed, it turned its attention to the global issues of malaria treatment, the continuing focus of the present-day CDC's malaria research branch[12]. Following the CDC's campaign in the United States, the World Health Organization (WHO) began a program in 1955 to eliminate malaria globally, utilizing the advent of new antimalarial compounds and DDT in its mission[12]. While some countries, such as India, benefited remarkably from the WHO's efforts, others, such as sub-Saharan Africa, remained largely unaffected[12]. Difficulties such as drug-resistant strains of malarial parasites have ultimately made the WHO's original mission unfeasible, necessitating its transition to a mission of control rather than eradication[12,15].

Economics, Ecology, and Etiology: Geographical Pressures on Malarial Parasites

Looking at a map of the globe highlighting malarial "hotspots," a few primary themes begin to emerge. Malaria prevalence overlaps the habitats of the *Anopheles* mosquitoes, shown in the boxed diagram[16, 1]. However, as you can see, these insects are found around the globe, while incidents of malaria are concentrated in the tropics. Even if more *Anopheles* are found in the tropics, due to their faster development in temperate water, this still does not fully explain historical accounts in which malaria is reported in some regions earlier in more ancient time than others.

These differences might be explained if the disease arose in one particular place – the current theory is that Africa was the continent of origin[6]. After this beginning, malaria spread, the parasites either flourishing or declining based on the new climate[6]. For example, Native Americans may have been rendered malaria-free by their migration to North America during the ice age, entering a zone unfavorable to the life cycle of the mosquito vector [6,17]. More recent historical events that may have spread the parasites include the African slave trade of the 16th through 18th centuries and foreign travelers in ancient Greece[6]. Thus, the success of the parasite's adaptation to new climates, in addition to the fitness of their *Anopheles* carriers, may explain the distribution of malaria as humans spread across the globe[6].



Global distribution of Anopheles mosquitoes.

While this paradigm of environmental adaptation is plausible, factors outside the world of scientific theory may also help explain the geographical distribution of malaria; in fact, economics may play a pivotal role. The link between geography and economic prosperity was noted in the 18th century by economic pioneer Adam Smith in *The Wealth of Nations*[18]. Simply put, coastal regions have better access to shipping routes and thus outperform inland nations[18]. In the case of malaria, these economic and epidemiological factors are reciprocal: on the one hand, the geography of the interior tropics limits economic development, leading to fewer health care resources and ability to combat malaria[18]. Conversely, the disease retards economic growth, inasmuch as high infant mortality results in less investment in education and the market potentials enabled by educated individuals[18] Thus, the "vicious cycle" of disease and economic underdevelopment makes treatment of malaria in the tropics an appreciably difficult task[18].

Epidemiological figures underscore the disparity of the malarial burden between the developed and developing worlds. In 2002, there were 8 malarial deaths reported in the US, while some areas of Africa had 2700 deaths a day in 1995 from the disease – that is 2 deaths a minute[19]. The disease's impact on child mortality is also profound, causing 10.7% of all children's deaths in developing countries (the fourth highest cause)[19].

Comprehension Questions:

1. Why might coastal regions be more prosperous than inland ones?
2. Why might it be economically significant that malaria is a major cause of child mortality?

The Biology of Malaria: The Flu that Isn't

Imagine this scene: a patient in Africa, South America, or Asia comes into a regional clinic complaining of headache, chills, or weakness. They may have a fever and complain of aches and exhaustion. In the U.S., a doctor might interpret these as symptoms of the seasonal flu. However, in the tropics, the diagnosis might very well be malaria, especially if the patient is a child less than five years old (see table below: malaria is a major cause of childhood mortality). Economically, it has been estimated that countries with endemic malaria have 1.3% lower growth each year than those without[20].

Leading Causes of Death in Children Under Five Years of Age, Estimates for 2000-2003 (Source: World Health Organization, The World Health Report 2005)			
Rank	**Cause**	**Numbers (thousands per year)**	**% of all deaths**
1	Neonatal causes	3,910	37
2	Acute respiratory infections	2,027	19
3	Diarrheal diseases	1,762	17
4	Malaria	853	8
5	Measles	395	4
6	HIV/AIDS	321	3
7	Injuries	305	3
	Other causes	1,022	10
	Total	10,596	100.0

According to the World Health Organization's World Malaria Report 2005:

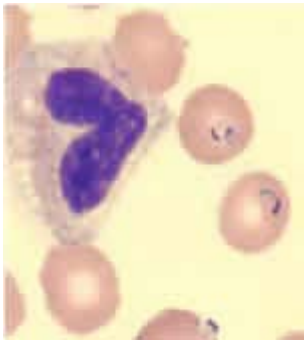
However, the mosquito bite that caused the illness might have occurred weeks before, because malaria-causing parasites typically do not begin multiplying immediately after flowing from a mosquito's sucker to the blood vessel of the human prey. In fact, it can take weeks for any sign of infection to appear[21]. Further, some drugs can extend this period of inactivity by weeks or months[21]. This is the reason American travelers returning from regions where malaria is common are often misdiagnosed when they develop the symptoms described above: it takes a knowledgeable physician to tell the difference between this dangerous infection and a cold picked up on a return plane ride[21].

When the signs of infection do manifest themselves, they usually take either an uncomplicated or severe form[21]:

Uncomplicated: Also known as a "classical malaria attack," this form of malaria consists of three events that occur in a cycle[21]: chills, fever, and finally massive sweating. Each of these three stages usually lasts 6-10 hours, and the entire cycle repeats every two or three days[21].

Severe: One of the four malarial parasites (*P. falciparum* – see later for more details) causes an even worse sickness, in which the victim's blood fails to form scabs when they are cut[21]. This is because the malaria parasites destroy the body's blood cells, preventing the normal buildup of cells needed to repair breaks in the skin. The parasites do not just cause damage by killing blood cells, though; by lurking inside, the parasites can cause normally healthy blood cells to get stuck to vessel walls that feed the brain with life-giving oxygen[21]. This buildup can be fatal if it progresses too far, cutting off oxygen to the brain in much the same way as a severe stroke.

The Signs of the Parasite



Parasite-infected red blood cells (indicated by purple stain).

Returning to our scene in the tropical clinic, the doctor strongly suspects that malaria is causing their patient's ailment, but how can they make a definitive diagnosis? A blood smear is one common method and the so-called "gold standard" of malaria laboratory diagnosis[22]. In this test, a sample of the patient's blood is stained using special chemical procedures and examined under a microscope in order to see the parasites, if they are there, within the blood cells (see image at right for an example)[22].

The Blood's Intruders: Malarial Parasites

Now, with this blood smear, our doctor has a picture under the microscope – but what does it mean? What exactly are those tiny specks squirming within a patient's red blood cells? These are the microorganisms that cause malaria: *Plasmodia*. Because they are single-celled but do have nuclei and organelles, *Plasmodia* are considered eukaryotes. They are further classified as members of the group *Protozoa*, single-celled animals lacking chlorophyll (the molecule that traps light during photosynthesis) that usually move with the aid of cilia or other protrusions[23]. There are over 100 kinds of *Plasmodia* known, but only four infect humans – these are the ones that cause malaria[24]. The two most prominent important species are:

P. falciparum, which is found globally in tropical and subtropical regions, is the only kind that can cause fatal malaria[24]. It has had a particularly large effect in Africa, where it is the most common kind of human-infecting *Plasmodia*, but it is also found in Southeast Asia[24].

P. vivax, while it is also found in Africa, predominates in Latin America and Asia[24]. Though it does not cause fatal forms of malaria, it can still cause extremely harmful infections[24]. It is also special because of its ability (like *ovale*, see below) to hide inside human liver cells for months, lying inactive until favorable conditions lead it to revive and cause the cycle of flu-like symptoms mentioned above[24].

There also two less common species of *Plasmodia*:

P. ovale is very similar to *P. vivax* but is found only in Africa (primarily West Africa) and the islands of the Western Pacific[24].

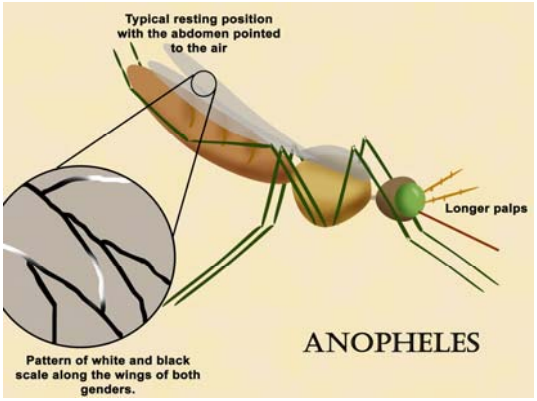
P. malariae, in contrast, is found worldwide and is notable for being the only species causing malarial attacks (e.g., flu-like symptoms) every three days instead of every two[24]. Additionally, it can remain inside a victim's body for a life time, causing long-term disease[24].

Before launching into treatments, it seems worthwhile to back up a bit and explain how *Plasmodia* infect humans in the first place. The biology of these microorganisms is closely linked to their carrier: the *Anopheles* mosquito.

Anopheles:

Of the 2,600 species of mosquito worldwide, only about 40 species of the *Anopheles* are responsible for spreading malaria[25, 26]. These are found in every continent except Antarctica, and are distinguished from other kinds of mosquitoes by the following features:

1. The palps (sensory appendages protruding from the head) are as long as the proboscis (the feeding tube by which the mosquito extracts blood from its victim)[26].

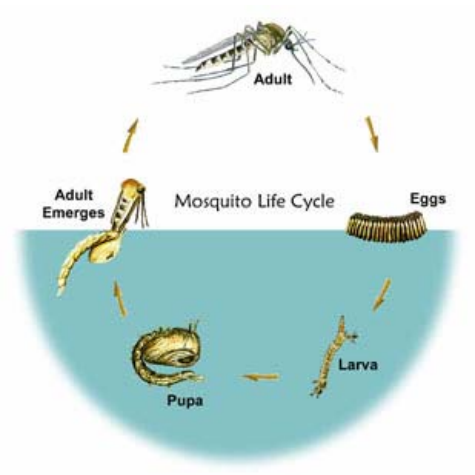


Original art by Senmaio Zhan.

2. A pattern of white and black scales appear on the wings of both genders[26].

3. The typical resting position of the male and female *Anopheles* is with the abdomen (anterior portion of the insect's body) pointed upwards, unlike other mosquitoes, who position their bodies parallel to the surface on which they are resting[26].

The *Anopheles* rest either indoors or outdoors after feeding on blood. In areas with indoor-resting mosquito species, insecticide-laced bed nets and spraying interior surfaces with DDT (see more information later) have proven effective at preventing the spread of malaria[26]. Conversely, outdoor mosquitoes are controlled by destroying the sites where they breed, particularly areas of clean, unpolluted standing water[26]. o



The mosquito life cycle: from pond to pest.

Why would a mosquito, an airborne organism, need water to reproduce? The answer is given by the image to the right, showing the life cycle of the mosquito. Water is an excellent incubator for mosquito eggs. In warm environments, the floating eggs will hatch in 2-3 days versus 2-3 weeks in cooler zones (the large number of *Anopheles* in Africa and Asia makes sense now, doesn't it?)[26]. The larva that emerge from these eggs float near the top of the water until they metamorphose into pupa that also cling near the water's surface[26]. The final stage of development into an

adult mosquito occurs 10-14 days after birth in the tropics. The females then seek blood meals, and the cycle begins anew during their 1-2 week lifespan[26].

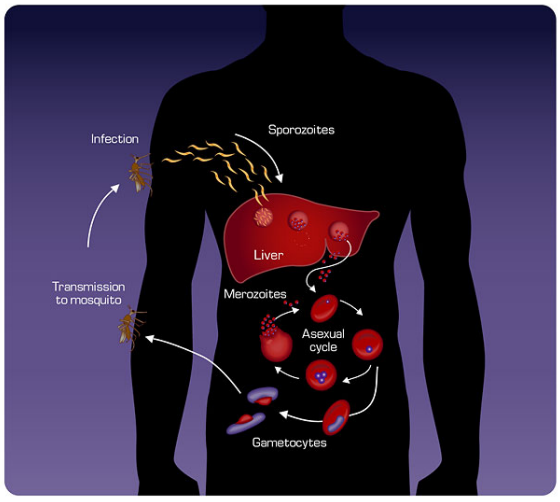
Larvae of *Anopheles gambiae*, the major malaria vector in Africa, can breed in very diverse habitats. Three habitats are shown from left to right: tire tracks, rice fields, and irrigation water.

From Mosquito to Human

Once the female *Anopheles* begins feeding, it has the potential to become infected with *Plasmodia* by human with malaria. In fact, statistical research has suggested that malaria infection may actually make human targets more attractive to the *Anopheles*[27]. As it turns out, the process by which the malarial *Plasmodia* gets from mosquito back into an uninfected human is more complicated than simply getting passed through insect saliva into the victim's bloodstream. For more detail, the reader is encouraged to view a nice animation at:

http://www.sumanasinc.com/scienceinfocus/sif_malaria.html

Inside the mosquito, the *Plasmodia* begin in either a female or male sexual form known as a gametocyte. When a male and female gametocyte merge during reproduction within the mosquito's body, they create cells called sporozoites that squirm their way into the inviting comfort of the mosquito's digestive system[28]. Here, the sporozoites stop and grow in cells lining the mosquito gut. Once they have finished their growth, the sporozoites burst out of the gut cell and migrates to the mosquito's salivary glands[28]. It is at this stage that the mosquito becomes infectious, as the sporozoites can exit through the saliva passing through the proboscis (feeding tube) of the mosquito[28]. From here, the sporozoites can pass into a human host the next time the mosquito takes a blood meal[28]. Upon entering the human bloodstream, the sporozoites initially travel to the liver and take up residence within its cells: here they can survive for years in a dormant (not growing) state or quickly mature[28]. Upon maturation, the parasites rupture the liver cell, just as they burst the mosquito gut cell, releasing them to begin infecting red blood



Life cycle of Plasmodia.

cells[28]. The red-blood cell infecting form is called the merozoite[28]. At this point, the parasite has two options:

1. Continue in its current (asexual) merozoite form, continually infecting and bursting red blood cells in a manner similar to its incubation in human liver cells or mosquito gut cells[28].

2. Differentiate into male and female sexual forms (gametocytes) that can return to a mosquito when it feed, traveling through the blood sucked up during the insect's feeding[28]. From here, the cycle begins again, with the gametocytes merging as sporozoites, and so forth[28].

The clinical symptoms of malaria are primarily caused by the frequent rupture (also known as lysis) of red blood cells during the merozoite phase. As described above, this rupture impairs the body's ability to transport oxygen, thinning the blood through the loss of cells in a condition known as anemia. This thinning will eventually cause death, because there are no longer enough blood cells to carry the required load of oxygen to all the places in the body that require this vital molecule.

Comprehension Questions:
1. Why does malaria cause anemia (blood thinning?)
2. What cells do trypanosomes (parasitic protozoans) colonize in humans? In mosquitoes?
3. Why do mosquito larvae favor a wet environment?

Other Mosquito-Borne Illnesses:

Malaria isn't the only disease transmitted from mosquitoes to humans. These insects seem to have a penchant for harboring nasty diseases that they pass to humans through saliva injected during a blood meal[1]. Other mosquito-borne illnesses include: Arboviral Enchephalitides (a class of illnesses including West Nile) that causes brain inflammation, Dengue Fever, Rift Valley Fever, and Yellow Fever.

Treatments:

A number of treatments are currently used to combat malaria. Since the history and ethical issues surrounding these various agents will be covered later, what follows is simply a brief summary of the activity of each compound.

Malaria Drug Treatments

With the genomes of both the Anopheles mosquito[30] and the Plasmodium parasite *P. falciparum* now completely sequenced[31], scientists are now working to develop more sophisticated medicines that target specific molecules (i.e. molecular medicine).These include DNA vaccines for particular components of *Plasmodia* genome[32]. However, given the complex life cycle of the organism, finding the best target gene is tricky[33]. Whether such techniques will prove successful, remains to be seen, but in the meantime, conventional pharmacological treatments include the following drugs.

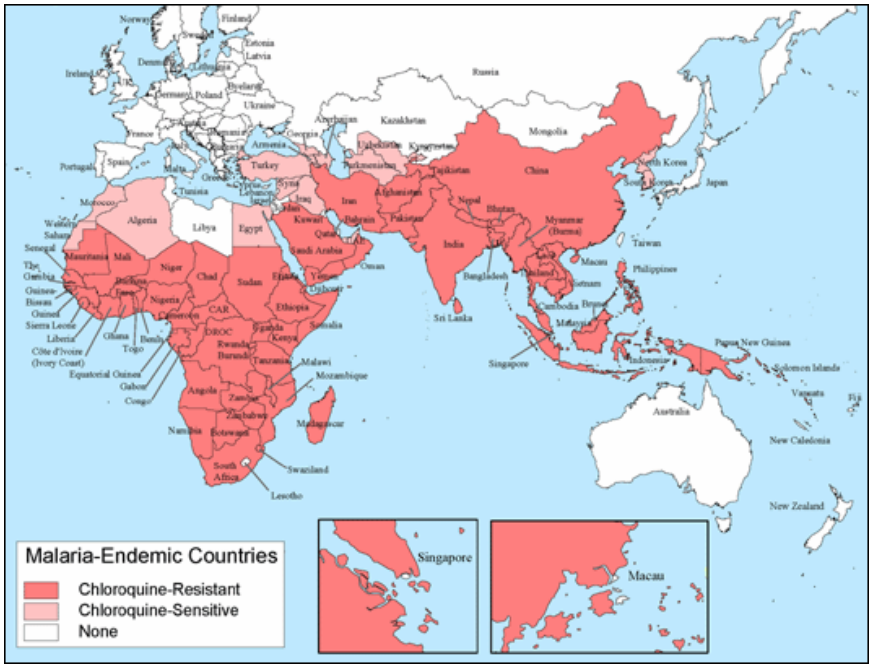
Quinolines (such as quinine and chloroquine) are thought to kill parasites by inhibiting the activity of heme polymerase, an enzyme responsible for degrading the heme ring of hemoglobin (which holds oxygen molecules) into harmless byproducts[34]. The parasites need this enzyme because, unlike human red blood cells, they are unable to bury the harmful heme ring within a hemoglobin molecule[34]. Left free within the Plasmodium cell, the iron-containing heme is toxic to the parasites, generating harmful oxygen species that damage the cell membrane and other sensitive structures[29]. Normally, the parasite will safely store the heme in a molecule called hemozoin[34,35]. Chloroquine, according to this hypothesis, damages the parasite by preventing the formation of this chain, leading to oxidative damage (see sidebar)[34,35].

Oxidative Stress: When a Good Molecule Goes Bad

Oxygen is usually beneficial to a cell, allowing it to carry on many of its most important energetic and metabolic processes. In these reactions, the end-product is usually harmless water. Why is water safe? When an oxygen atom is in water, its outmost electrons are used to bind to two hydrogens, a stable arrangement because the two electrons (one from each hydrogen) fill out oxygen's outermost shell, making it a full 8. As molecular (diatomic) oxygen, things are not quite as neat. Usually, the two oxygen molecules will form a double bond between them to maintain 8 electrons in each of their outer orbitals. However, because the electrons on each oxygen would prefer not to be right next to each other, since they are all negatively charged, it is not difficult for this double bond to change to a single bond. However, with only one bond connecting them, the outermost shell of each oxygen atom isn't full, meaning it will easily acquire an additional electron to make the shell of one of these oxygens full, giving the overall molecule a (-1) charge.

However, this leaves a single unpaired electron on the other oxygen, an arrangement known as a free radical. Because it is unpaired, the electron is effectively "looking" for something to react with. Inside the cell, what it usually does is react with bonds between lipids, proteins, or nucleic acids, causing structural damage[29].

Among the quinoline compounds, chloroquine is particularly effective because it has been shown to concentrate itself within red blood cells to levels many times higher than it exists in the free plasma[36]. Unfortunately *Plasmodia,* like most pathogens, have become resistant to chloroquine treatment. Many times, the parasites evade the drug's action by generating molecular pumps that quickly expel chloroquine[37]



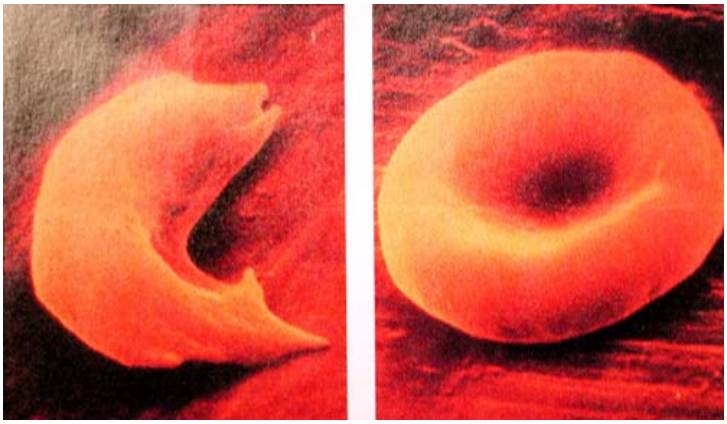
Chloroquine-resistance among malaria-endemic countries. Figure credit: CDC.

Artemisinin, another pharmaceutical agent, impairs parasite development by inhibiting the action of a calcium transporter in the membrane of the rough endoplasmic reticulum of the trypanosome[38]. Disrupting the transport of calcium ions disturbs the delicate balance of ions that create favorable chemical conditions inside the parasite cytoplasm and, in severe instances, trigger cell death[39]. The drug is also thought to inhibit digestive pathways in the parasite, and, like chloroquine, to cause the formation of free-floating heme and the resulting harmful oxygen species[40]. However, in the case of artemisinin, this is because the drug prevents the formation of hemoglobin molecules[40].

Vaccines:

As with other diseases covered in this course, vaccinations against malaria are also being researched. These treatments can target many different stages of the malaria parasite's life cycle, and are being developed in a number of forms. On the crudest level, inactive parasites or blood components from a person with natural malarial immunity can be used as a vaccine[41]. Additionally, research has focused on more refined methods. One is the development of a recombinant vaccine that would target several stages of the parasite's life cycle by training the body to recognize artificially generated *Plasmodia* proteins. This form has had promising results through trials conducted by The Walter Reed Army Institute of Research and SmithKline Beecham Biologicals[42]. Scientists have also explored generating *Plasmodia* proteins in the milk of genetically modified mice, an approach for which studies are still being conducted[43]. Yet another strategy is to vaccinate using just a circle of DNA containing the genes for *Plasmodia* proteins, which is then replicated by the human body to train the immune system to attack these molecules[44]. Finally, the ongoing sequencing of *Plasmodia* genomes has opened the possibility of testing large numbers of parasite proteins to see which one is most effective at generating an immune response from the human body[45].

Sickle Cell Disease: Nature's Defense to Malaria?



Normal (right) and sickled red blood cell (left).

Though not a treatment, another molecular-level defense against malaria is worth mentioning: sickle cell anemia. It occupies a special place in medical history as it was the first disease attributed to an abnormal protein, a fact determined in 1948[46]. A single mutation in the gene sequence for the hemoglobin molecule causes red blood cells to assume a distorted three-dimensional conformation. The mutated residue creates a "sticky" surface that causes affected hemoglobins to aggregate, resulting in "sickled" cells. When the mutation is homozygous (i.e., there are two copies of the defective gene, as described in the genetics sidebar), it causes the painful accumulation of red blood cells in small blood vessels, blocking blood flow[47]. The warped blood cells also have a shorter lifespan than normal, decreasing the number of cells that can carry oxygen to all the places it is needed in the body. However, those who possess only one copy of the sickle cell mutation (or heterozygotes) benefit from increased resistance to malaria[47]. In fact, for this reason, sickle cell anemia is common among people of African or Mediterranean descent, both regions where malaria has been prevalent.

For example a study of *P. falciparum* infection in children on the Kenyan coast revealed that heterozygotes enjoyed 50-90% fewer cases of malaria, depending upon the severity of infection (i.e., the most severe infections experienced the greatest percentile reduction)[48]. Interestingly, a second study found that the benefit was mainly conferred during ages 2-16 months, where earlier infection is combated my fetal immune factors, and later infections are repelled by acquired immunity or the fact that the more "susceptible" individuals in a population have already died[49].

Why this single mutant allele is beneficial in terms of malaria is still not completely understood though there are a number of possible reasons. One is offered by the observation that malarial parasites cannot survive in red blood cells that have sickled due to oxygen deprivation[50]. Another theory is that the generation of oxygen radicals within sickled red blood cells harms the parasite[51]. A final theory is that sickled cells may generate high amounts of sickled hemoglobin polymer, a molecule that can also damage malarial parasites[52].

The Genetic and Molecular Basis of Sickle-Cell

Sickle-cell disease is an autosomal disorder, meaning that it is a mutation carried on any chromosome but the sex chromosomes[47]. The disease is caused whether there are one or two copies of the mutated hemoglobin gene. This is because the mutated and normal forms of hemoglobin are codominant, meaning that neither form of the hemoglobin (gene) masks the other, thus the amount of defective hemoglobin caused by one mutated copy is enough to cause sickle-cell because the affected individual has a blend of mutant and non-mutant hemoglobin molecules in their red blood cells. Because it is not linked to the sex chromosomes, parents who have the mutation for sickle cell (either a single or double copy) can pass the condition to their children of either gender with equal frequency[47].

To understand how a single amino acid mutation can change the shape of a protein, it is necessary to understand a little bit about how proteins fold into their unique shapes. First, the linear sequence of amino acids, based on the chemical character of their side chains (such as charge, size, polarity), adopts either a helical or sheet-like pattern (a secondary structure). The protein is then folded into more complex shapes (tertiary structure) based on the chemical interactions between the individual peptide chains of the protein or between the surrounding media (for example, water) and the strands of the protein. Interactions can also exist among many individual proteins, producing multi-subunit molecules (quaternary structure) [53]. Sickle-cell anemia, therefore, is caused by a mutation in a single amino acid that, because of its involvement in multiple layers of folding, disrupts the three-dimensional structure of the protein.

Comprehension Questions:

1. *What molecules generated by quinines and artemesinin cause structural damage to a cell?*
2. *Why is making a vaccine against a trypanosome difficult?*
3. *What is the molecular difference between normal and sickle-celled hemoglobin? How does this change the protein?*

Insecticides and Other Mosquito Control Methods:

In the 1960s, DDT was a commonly used insecticide used to control malaria by targeting the mosquitoes that harbor the *Plasmodia* parasites. It functions by causing the insect nerve cells to repetitively fire, making them unable to activate specifically in response to signals. It does this by binding to a channel protein responsible for transporting the sodium ion (Na+) into the cell, which serves as the basis for signals to travel along neurons[54]. In a normal nervous system, a signal travels because the sodium channels are opened and closed at a specific time. DDT disrupts this rhythm, and the insect consequently dies due to the malfunction of its nervous system[55]. Significantly, studies suggest that the molecular structure of vertebrate and insect sodium channels are

different enough that some insecticides might be insect-specific and unable to affect vertebrate channels[54]. Whether this might be the case with DDT is not clear, but it is probably not completely exclusive to insects.

A Stearman bi-plane is spraying an insecticide during malaria control operations in Savannah, GA. Insecticides are important in disease prevention through vector control.

Usually DDT is applied to the walls of homes in areas with a high prevalence of malaria or is used to coat bed nets that also serve to physically keep mosquitoes away[55,56]. However, 1962 spelled the end of the era of DDT, with the publication of Rachel Carson's monumental *Silent Spring*[57]. While the contents of the book were perceived as somewhat alarmist at points, it led to wider awareness of pesticide toxicity. Besides DDT's toxic properties, Carson also highlighted the growing numbers of insecticide-resistant bugs in response to larger-scale spraying with increasingly toxic compounds[57]. Here is Darwinian natural selection on a time scale humans can appreciate: those insects that are naturally hardier will survive the insecticide to reproduce, leading to increasingly resistant populations. The more deadly the toxin, the tougher the bug must be to survive it. Thus, the problem only escalates as more "effective" pesticides are introduced. What causes this resistance? While Carson does note one instance of a molecular explanation – an insect enzyme degrading DDT to the less harmful compound DDE – in general the reasons have not been determined[57].

Besides insecticides, mosquito levels can also be regulated by destroying larva before they hatch. A number of strategies have been developed to do this: one is to use

This 1920s photograph, taken somewhere in the southern United States, shows workers practicing "vector control" by digging a drainage ditch in order to help disperse standing water that was acting as a popular breeding ground for a population of Anopheles mosquitoes. Vector control aims to decrease contacts between humans and vectors of human disease. Control of mosquitoes may prevent malaria as well as several other mosquito-borne diseases such as West Nile virus, St. Louis encephalitis, and Dengue fever.

bio-degradable oils to suffocate developing mosquitoes in their watery habitat[56]. Another is to introduce into these environments bacteria or fungi that only infect mosquitoes[56]. Finally, mosquito-eating fish have proven effective at reducing the number of larvae in particularly large breeding grounds such as lakes[56].

Beyond insecticides, scientists are now developing genetically-modified mosquitoes that can no longer effectively harbor malarial parasites. This is done by introducing foreign genes into the mosquito genome. Two examples of such genes are (1): a gene whose protein targets the parasites in the mosquito salivary gland, and (2): a gene whose protein prevents the *plasmodia* from adhering to the wall of the mosquito's digestive system[58].

Insecticide-treated bednets are now a major intervention for malaria control.

Comprehension Questions:

1. *What are the advantages of bed nets over other uses of insecticide?*
2. *What features of an environment are important for supporting mosquitoes?*
3. *Why might DTT spraying be cause for concern? For agriculture? For residential areas?*

Activity 1:
Ethical Issues of Malaria (Opportunities for Debate)

Introduction:

Just because a cure for a disease exists, does not mean that it will be delivered to those who need it or used effectively. This lesson is clearly embodied by the current global crisis in treating malaria: the essential philanthropic aims of medicine are at constant odds with market forces and the economic logistics of pharmaceutical development and distribution. Additionally, even the most altruistic of aims are sometimes opposed as in the debate over the use of DDT. Do the health risks of the insecticide outweigh its benefits in killing malaria-bearing mosquitoes? The following section summarizes some of the current debates on some of these ethical issues.

Chloroquine, ACT, and Vaccines: Supplying Drugs to Those in Need

As with antibiotic-resistant strains of bacteria, malarial *Plasmodia* that are resistant to traditional treatments such as chloroquine are becoming more prevalent in malaria-ravaged Africa. Indeed, drug resistance is a monumental problem as chloroquine-resistant *Plasmodia* strains have been documented along with strains of quinine and artemisinin-resistant parasites [37]. The more widespread a drug is, the greater the likelihood that parasites will develop resistance to it through spontaneous mutations that are passed on to the parasite's progeny.

Certainly, artemisinin "cocktail" therapies (ACT), in which more than one drug is mixed together to prevent the parasite from developing resistance to any one treatment, is a far more effective therapy than pure chloroquine. However, ACTs cost 10 times more than mono-drug therapy [59]. Since pharmaceutical companies stand to gain little financially from ACT sales in the developing world, their use remains low as no incentive structure exists to encourage their wider distribution[59]. Consequently, chloroquine continues to be prescribed throughout Africa despite the obviously rising ineffectiveness of this drug[60].

Many have suggested that large subsidies be given by governments to offset the cost of artemisinin supplies, allowing them to be distributed at prices that African nations can afford[61]. Whether such plans will reach fruition remains to be seen. Such economics concerns have also spelled trouble in the area of vaccine development. Given the choice of vaccines to pursue, world leaders recently placed malaria on a lower funding bracket due to the lower financial demand[62]. Further, because many African nations have poorly developed scientific research infrastructures, they often find themselves at the mercy of international colleagues in the vaccine development game, who do not listen to the concerns of African researchers about these projects[61,62]. Thus, the problem is not merely one of resources, but also, perhapsof autonomy.

Discussion Questions:

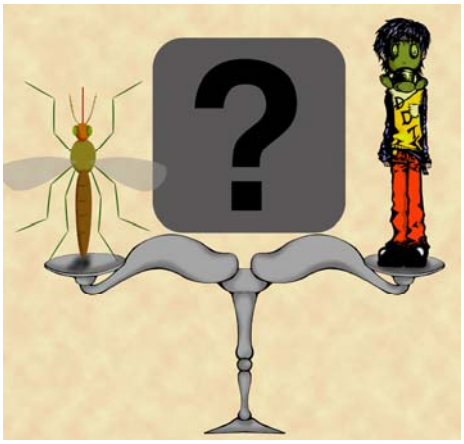
1. What is the best way to create incentive for the development and distribution of antimalarial compounds to the developing world where they are needed?

2. Do governments in the first world have a responsibility to less fortunate nations in distributing these kinds of supplies?
3. To what extent are such activities the responsibility of a government, and what aspects should be handled by non-government organizations (NGOs) or other philanthropic groups?
4. Do citizens in the developed world have caused to be concerned about these issues (ethically, medically, or otherwise)?

Background reading (for the teacher) provided: "Making Antimalarial Agents Available in Africa" and "Why the World Needs Another Malaria Initiative".

DDT: Insecticide or Homicide?

The insecticide DDT has certainly proven effective in reducing instances of malaria infection in many countries where the disease is endemic. In Sri Lanka and India, cases dropped by 99%, and two strains of malarial mosquitoes were eliminated in South Africa[63,64]. Also, as mentioned earlier, insecticides such as DDT were instrumental in eliminating malaria in the US[14]. DDT is usually applied in only small amounts on the interior surfaces of homes, as opposed to the mass spraying that caused earlier environmental concerns (see above). Thus, it operates on the principle of "consecutive probability" –the spray will prevent a proportion of mosquitoes from entering a home, deter a large percent that enter from biting before they leave, and kill a portion of the remaining insects before they can cause harm[64]. A nice simulation of this is given at



Original artwork by Senmiao Zhan.

http://www.malaria.org/teachingmodules/ddt-malaria-container.html.

Many, however, have expressed concerns that DDT disrupts hormone function, and animal tests have revealed some deleterious effects though the veracity of such studies is contested. One notable example of such dissension is Rachel Carson's seminal environmental text *Silent Spring*, which outlined how DDT made its way from insects through the food chain, accumulating in the fatty tissues of animals consumed by humans and potentially causing cancer and genetic damage[57]. The fundamental question, perhaps, is whether the health hazard of DDT outweighs the need to eradicate malaria-causing mosquitoes: which is the greater risk?

Discussion Questions:

1. Is it okay to use a treatment for one illness that might cause other harmful effects? How does one go about weighing the risks?
2. What measures should be taken to determine the safety of DDT? What should be done during the process?

3. Should DDT's low cost be a factor in weighing its efficacy?
4. What guidelines should be placed on the use of DDT (e.g. residential vs. agricultural use)?

An optional case study (To Spray or Not to Spray: A Debate Over Malaria and DDT) and accompanying teacher's notes are provided.

Cultural Sensitivity in Research

Since malaria is most prevalent in some of the less developed sections of the globe, a frequent concern is how to conduct field research on the disease while remaining sensitive to the particular cultural dynamics in these areas. One example is obtaining consent from tribal communities in which it is often necessary to approach community leaders first in order to effectively negotiate a research protocol[65]. In general, medical ethics is predicated on the notion of individuality - of single patients making decisions about their participation in treatment or research. Indeed, "informed consent" is one of the three primary components of the modern biomedical ethics triumvirate (the others being justice and the Hippocratic ideal to "do no harm"). However, this individualized notion of consent may not fully apply to communal societies, and imposing an outside system of ethics is arguably insensitive to such cultural differences.

Discussion Questions:

1. What barriers to medical research might exist in a different culture?
2. What are some things that doctors can do to overcome these issues?
3. Is treating a patient more important than respecting their culture?
4. What possibilities for abuse exist when doctors do research on subjects from a different culture than their own?
5. What might the subjects do to protect themselves against such abuse?

Activity 2: DDT resistance

Students will read Rachel Carson's *Silent Spring* chapter (provided) on insecticide resistance and the commentary in *Silent Spring Revisited* (provided). Then, students will compare the two perspectives, and write an opinion about whether DDT should be used or not (further research may be required on their part).

Activity 3: Multiple factors - Geography, Economics, and Malaria

Using a map of Africa, each student will choose a country to research. They should be able to answer the following questions:

A: What is that nation's climate?

B: What is the general economic stability of the country?

C: How prevalent is malaria?

D: Does there seem to be correlation between these factors? If yes, why? If no, why not?

Activity 4: Movie – Deadly Messengers

No one likes getting bitten by mosquitoes. But few people in the United States realize that the mosquito is the most dangerous spreader of disease on Earth. From malaria to yellow fever to West Nile virus, mosquito-borne diseases strike millions of people around the world. Malaria alone is estimated to kill one million children under the age of five each year. Yet, mosquito control is possible, and public health workers are working hard to get the upper hand in the battle against mosquitoes and the diseases they carry.

Students will role-play to examine how a vector transmits a disease. Next teams of students will research mosquito-control techniques. The class will then discuss which combination of techniques might work best to reduce the number of malaria-related deaths around the world. Include a recent strategy whereby researchers are genetically modifying mosquitoes to become resistant to infection with the malarial parasite (see "Malaria Researchers Target Mosquitoes").

Activity 5: "DDT for malaria control" teaching module

Interactive website teaching the biology of the parasite and how DDT fights this disease by killing mosquitoes. www.malaria.org/teachingmodules/ddt.html.

Activity 6: Educational game

Nobelprize.org/educational_games/medicine/malaria

Play the mosquito: Take control of a mosquito and try to find a human to bite and draw blood.

Play the parasite: Take control of a parasite, try to find your way inside a human being, and multiply as fast as possible.

Activity 7: Service learning – Bednet fundraisers

Students will develop ideas to raise money to buy bednets for children in Africa. Ideas (from http://malarianomore.org/; showcased on American Idol) include:

1. Host a bake sale
2. Sell homemade "Bednet Certificates" for $10
3. Host a garage or book sale
4. Host a film screening
5. Partner with a local athletic team
6. Host a car wash
7. Host a bike-, run-, walk-, or swim-a-thon
8. Dress up like a mosquito and tell people why

9. Create a YouTube! video
10. Throw a bednet birthday party
11. Create a MySpace or Facebook page
12. Make T-Shirts
13. Host a lemonade stand
14. Host an expert (scientist, development worker, etc...)
15. Ask your teacher if you can do sidewalk chalk drawings about malaria
16. Host a talent show
17. Team up with a youth group, dance team, or scout troop
18. Purchase a bed net on someone's behalf for their birthday or a holiday
19. Host an auction or raffle
20. Host a basketball, golf, hockey, volleyball, or tennis tournament
21. Find a corporate sponsor
22. Host an art exhibition

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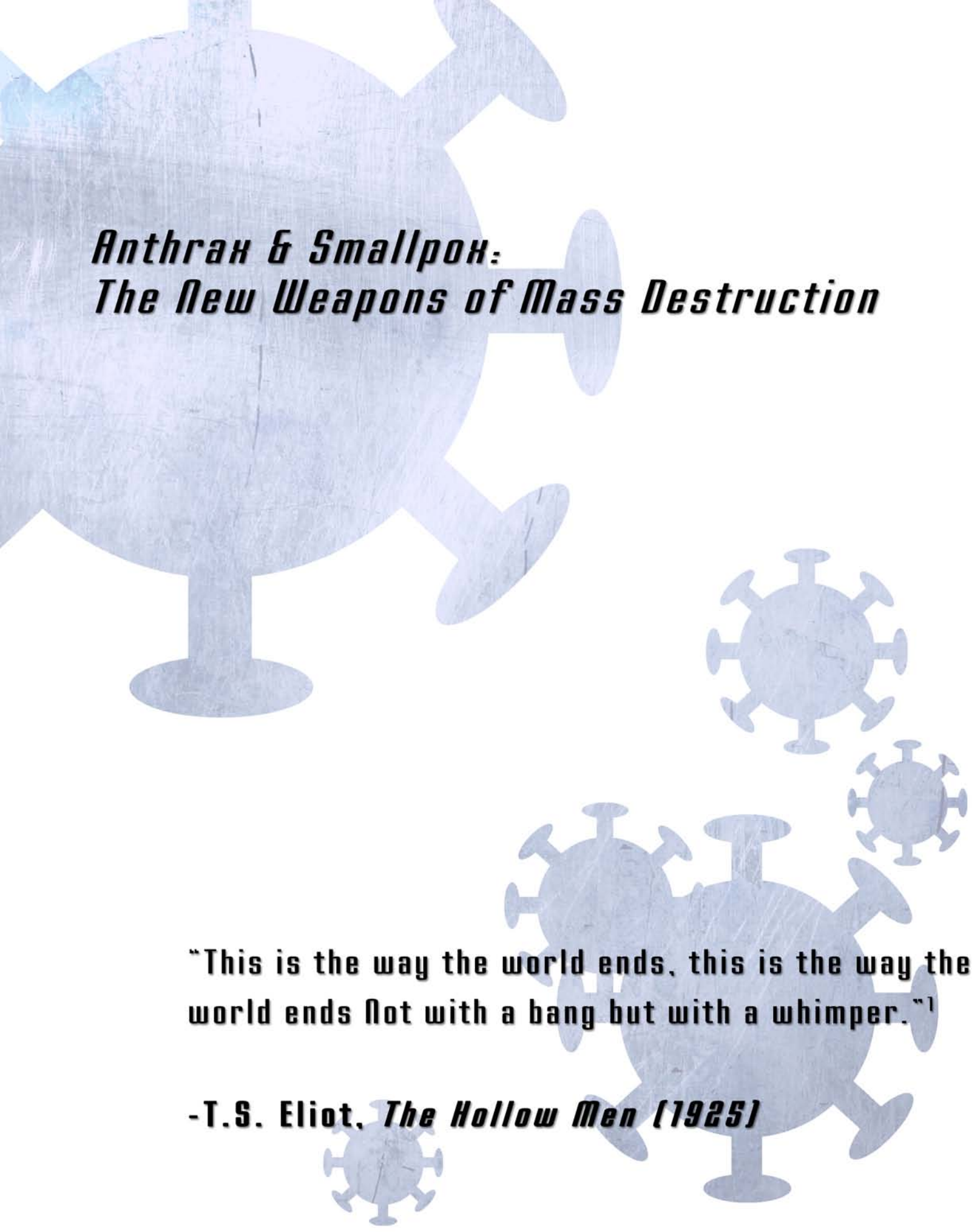
Anthrax & Smallpox: The New Weapons of Mass Destruction



"This is the way the world ends, this is the way the world ends Not with a bang but with a whimper."[1]

-T.S. Eliot, *The Hollow Men (1925)*

“Five Tickets to Nowhere”

The jet bridge whines softly as it retracts. Inside the aircraft, the hush of the ventilation system whispers through the confines of the coach section where Mrs. K’s students sit in a row just past the wing.

“You’d think if they could pay for this trip, they could at least put us in first class,” grumbles Angelica. The four had initially been excited when their teacher told them about the international field trip Pierpont was sponsoring for them over spring break in conjunction with their class. On the company’s vast budget, they would be attending a World Health Organization conference at the Institut Pasteur in Paris, where the latest updates on avian flu, AIDS, and other current crises were on the agenda. Experts in infectious disease from around the globe were set to attend. Maxine in particularly seems thrilled at the idea of meeting some of the scientists whose research they had studied in class.

“*Please* stop kicking,” hisses Maxine, at which Fallon’s twitching foot immediately freezes. Trying to hide his reddening face, he pushes up the window shade to stare at the blank tarmac of Raleigh-Durham International Airport. The sun rose only an hour ago for this early morning flight, and the sky still bears a few streaks of pre-dawn grey.

He begins to think that sitting next to Maxine wasn’t such a good idea. However, judging by the way Angelica and Lang quickly chose the seats not adjacent to his own, he suspects he actually didn’t have any other option. Regardless, this has the potential to be a very long flight. If only he could just get through it without embarrassing himself further….

Angelica leans back in her chair, just in time for the flight attendant to chime in with an entirely too-cheerful reminder: “Please return seat backs and tray tables to the upright position and prepare for takeoff.” Rolling her eyes, she presses the button returning her seat to its previously uncomfortable position. Lang, sitting beside her, a paperback clasped between his hands, focuses intently on the page.

“What are you reading?” asks Angelica, leaning over to discern the title above the top margin.

“*Paradise Lost*,” he replies, not bothering to look up. The red-head’s shadow blocks his light, and he winces imperceptibly at the disruption.

“I think we talked about that in AP English,” Angelica says, sitting back in her own chair as the plane’s engine drones louder, accompanied by the grind of retracting landing gear. “It’s about the garden of Eden or something, right?”

Lang turns the page quickly before answering, licking his finger to separate the sheets. “Something like that.”

“What part are you on?” She unlocks the tray table, but quickly refastens it as the flight attendant passes through the cabin. The woman shoots her a slightly unpleasant look, and Angelica has the feeling she and the flight attendant are not going to be getting along over the next eight hours.

“Lucifer is flying through the underworld,” says Lang and, as if on cue, begins reading aloud.

As when far off at Sea a Fleet descri'd

Hangs in the Clouds, by Equinoctal Winds
Close sailing from Bengala, or the Isles
Of Ternate and Tidore, whence Merchants bring
Their spicy Drugs: they on the Trading Flood
Through the wide Ethiopian to the Cape
Ply stemming nightly toward the Pole. So seem'd
Far off the flying Fiend.

The whine of the engine picks up as the plane jolts from the ground, and Lang decides not to compete further with the rising din. Angelica peers down the aisle, to where Mrs. K sits quietly, dozing beneath a sleep mask. Even though they had all risen at five that morning to make the flight, their teacher seems to be the only one who is tired.

"Good grief, do you *ever* sit still!" exclaims Maxine, as Fallon's fingers immediately cease their rapping on the armrest between them. Folding his arms tightly, he sighs and pulls the visor of his baseball cap further over his eyes. Kicking his protruding backpack further under the seat in front of him, Fallon recalls the delay caused by his luggage that morning.

Moving through the heightened security checkpoints around the international terminal, the four students and their teacher had been pulled over as part of the random screening process. Unfortunately, Fallon hadn't thought to run his laundry last night, and the guard opening his duffel bag had discovered a sweater covered with white powder. While the residue had been merely the remains of a doughnut from last week, the finding kept them at the gate for an hour, and Mrs. K had been concerned that they would miss their flight as well as their connection in New York.

Deciding this trip can't get any worse – at least for now – Fallon leans back and falls asleep.

* * *

The transfer at JFK goes uneventfully, and the five don't even have to leave the plane. During the bustling transition of passengers, with bags moved in and out of the cramped overhead compartments, Mrs. K remains asleep – Angelica wonders if their instructor has been getting enough rest lately. Since the movies don't start until the trans-Atlantic phase of their flight, the four students busy themselves reading. While Maxine, Lang, and Angelica work on material due in class next week, Fallon pursues the in-flight magazine, moving to the editorials once the crossword proves too difficult. One of these articles is entitled *The Spore Scare*:

> "Anthrax is something to be concerned about . . . vats of the deadly bacteria can be maintained with minimal technology, and turned into weapons of vast destructive capability."

Fallon sets the magazine down for a second, pondering the issue: if it's so easy to make these weapons, why haven't attacks already occurred? Thinking to ask Mrs. K, he looks over the back of his seat, and sees that she is still fast asleep. He looks over at Maxine, who has traded her coursepack for a math textbook of some sort.

"What is that?" he asks.

"Linear algebra," she answers, hastily erasing a line on the paper interspersed between the opened pages. Looking up, and seeing the lack of comprehension on his face, she adds: "Matrices. It's math on matrices."

Removing his cap, Fallon absently scratches his head. "Never heard of it." Maxine shrugs as if his ignorance were entirely expected, and returns to punching numbers into her calculator. Trying desperately to break the silence setting between them, Fallon tries a different topic.

"So it looks like they're showing some good stuff on this trip," he says, flipping through the movies section of the magazine.

"You know," she replies, turning towards him again and barely concealing her irritation, "you really don't have to talk the whole trip. In fact, I actually have a lot of work to do. I think there's like ten radio channels," she adds, pointing to the headset in the pocket before him, "maybe you could listen to that."

Demoralized, Fallon takes the headset and tunes out the rest of the passengers, whose soft murmurings barely disturb him as he drifts off once more. Across the aisle, Angelica leans over Lang to get a look at the view outside.

"I think that might be the coastline," she says, turning back to the monitor in the seat back which displaying a digital image of their flight path. "We should be over Newfoundland by now."

Lang, clearly less than thrilled by this revelation, looks up only a moment from his book. "Do you see any cloud-rabbits, too? Actually," he waves his hand, "don't bother – I only want to know about kitty cat-shaped clouds. Please keep me apprised of the situation as it develops."

Angelica glares at him. "You know, you could at least try and be polite sometimes. Then maybe you wouldn't be going to prom alone."

She settles back into her chair, while he continues to glare at his book. Though he doesn't show it, her words sting him – it's not that he's been disliked at Fairview, just ignored by everyone but the teachers. Quiet and contemplative, he feels like any social life in high school has long passed him by.

A sudden noise jolts him from this reverie, and he looks over to see Fallon snoring loudly across the aisle, while Maxine sighs in frustration. Clearly, this is going to be a long flight.

* * *

Fallon is shaken awake hours later by Maxine, who hisses in his ear.

"Wake up, we're landing. They said we'll have to get off quickly – something about airspace regulations."

After getting over the initial confusion of waking, along with the realization that this might be the first time – and last – that Maxine actually touches him, Fallon notices that the lights are all off in the cabin. A trail of yellow dots lights the central aisle while, somewhere near the cabin, a faint red glow illuminates the gloom. Checking the ceiling above, he notices that the oxygen masks remain behind their pressure-loaded panels. Apparently, something else is wrong, and the stewardess voice carries a hint of distress over the speaker.

"Ladies and gentleman, we're afraid we've had to divert our flight path. We received a communication from JFK that unidentified spores were discovered on a US Airways flight to Hamburg, and for safety reasons, all flight within the last twenty four hours are being rerouted from major international hubs."

"What's going on?" Fallon whispers to Maxine, "if we can't land in Paris, where are we going?" As if in response, the flight attendant continues.

"Air traffic control in Paris has asked us to refrain from landing until the situation is resolved. We've been rerouted in the meantime to the air force base in Malkinagrad. The government of Malkinagrad has given us permission for all passengers to disembark before the flight crew continues to Hauser Air Base; we recommend all boarded passengers exit the aircraft during this period, as civilian access inside the Air Base is restricted, and we cannot guarantee that you will be reboarded in a timely fashion. Thank you for your patience, and please prepare for landing. Due to air space restrictions, we will only be able to remain at the Malkinagrad airport for two hours, so please disembark as quickly as possible. A service agent will contact you to reschedule your flight."

The landing gear whines below them as it emerges from the hull, though the night sky outside the window is too dark to tell how close they are to the landing strip.

"Where the heck is Malkinagrad?" says Fallon.

"It's between Kaliningrad and Lithuania," says Mrs. K, who is now seated behind Fallon and Maxine, leafing busily through their travel documents. "It's part of the former Soviet Union. Fortunately, I think we can get a direct flight from here back to Paris once this situation resolves – there's been a lot of biotechnology buildup around the capital in the last year, so the number of flights landing here is a lot more than it used to be. I think Pierpont actually may have a facility here."

Angelica leans in to hear the conversation. "Do you really think there was some kind of bioterrorism attack on that plane going to Germany? Seems pretty drastic to reroute everything out of New York like this. What do they think the spores are? Anthrax?"

"I don't know," says Mrs. K, "I just hope we can get out of here soon. I'm going to call the Pierpont representative as soon as we land – please get all your things together so we can get off the plane quickly."

Fallon leans over to retrieve his backpack, thinking that this flight has gotten a lot longer than even he expected.

Questions:

1. **If you've traveled recently, do you think it's likely that someone could get spores through airport security? Why or why not?**
2. **Based on what you know about the biology of anthrax, what safety precautions should passengers aboard the flight mentioned above take to avoid infection?**
3. **What quarantine measures might an airport use? How would they test to see if a passenger was infected with anthrax?**

Introduction:

Bacteria, viruses, parasites – over the last few units, we have seen a world that appears filled with disease-causing pathogens. However, now there are increasing fears that pathogens are in line to replace traditional bombs as an agent of warfare and terrorism. Although the government has responded with much research spending on bioterrorism countermeasures, how does one defend against a bioterrorism attack or even prepare troops to confront agents of biological warfare on the battlefield? While vaccination programs might seem a simple answer, the reality is more complicated. Such immunological defenses, as well as pharmacological measures, are an important topic of consideration for today's policy makers. We'll consider such policies in this unit, along with two disease agents that highlight the fears of biological weaponry: anthrax and smallpox.

Color-enhanced scanning electron micrograph shows splenic tissue from a monkey with inhalational anthrax; featured are rod-shaped bacilli (yellow) and an erythrocyte (red).

Goals:

By the end of this unit students should:

1. Define bioterrorism.
2. Describe the basic biology of both anthrax and smallpox: mode of infection, symptoms, and treatments.
3. Describe anti-bioterrorism strategies based on knowledge of the biology of a given disease.
4. Assess the potential for a given pathogen to be used as a bioterrorism agent.

Foundations of Biological Weaponry and Terror

The most direct definition of **biological warfare** is given in a NATO handbook:

". . . employment of biological agents to produce casualties in man or animals or damage to plants"[2, 3].

Of course, this does not explain *why* anyone would want to make a weapon out of a virus or bacterium. What advantages would such a biological agent have over a traditional bomb?

First, biological weapons are inexpensive. According to the same NATO report, a biological weapon can kill half the population of a square kilometer area for about $1.00. [Though this figure dates to 1969, even inflation would not raise it appreciably in relation to other forms of weaponry] [2]. Also, unlike traditional bombs, biological weapons specifically attack living organisms, avoiding damage to a city's infrastructure (and the consequent economics costs of reconstruction)[2]. Additionally, a biological weapon can act like a slow-ticking bomb. For example, a terrorist group could deposit a biological weapon in a water supply and exit harm's way as the ensuing epidemic might take months to develop[2].

Making a "biobomb"

In most cases, the steps involved in making a biological weapon are frighteningly straightforward. Essentially, all that is required is:

1. *A biological agent.* Typically acquired from the environment or a laboratory, such agents can be made through the use of molecular biology. For example, a toxin-producing gene can be inserted into a normally benign bacterium, making it deadly[4]. Additionally, such agents can sometimes be made from historical samples as seen with the resurrection of the 1918 flu virus and the recent synthesis of the polio virus[5, 6].

2. *Large amounts of the biological agent.* Once the agent has been acquired and/or sufficiently purified from contaminants, it has to be grown into large supplies. Bacteria and fungi are usually cultured in media on plates or in flasks, while viruses can be grown in chick embryos (similarly to how vaccine stock is generated)[4]. In addition, these large quantities of the agent must be packaged to avoid leaks that would harm the messenger. Bacteria, viruses, and other pathogens usually have optimal conditions for causing disease, including temperature, wind (if air dispersal is necessary), light, or person-to-person contact[4]. Freeze-drying is a common treatment as many pathogens are inactive at low temperature[4].

Many of the techniques involved in the creation of a biological weapon have been developed and refined in biomedical laboratories for decades. A bacterium is a bacterium, after all, and the same tools that are used to devise new cures can also help create terrible weapons. This very issue has led to much recent debate over "dual-use" research, where a technology can be both beneficial and harmful depending upon its use (see Case Study).

What Counts as a Biological Weapon?

There are a wide range of pathogens than can be "weaponized". In the wake of 9/11, researchers have attempted to more carefully define "high-risk" pathogens deserving particular attention[7-9]. Under their criteria, a "maximally credible threat" would be highly lethal, overwhelm healthcare and treatment resources, and generally instill panic[7]. Rising to the top of this list were anthrax and smallpox - the two pathogens we will take a closer look in this module.

Modern Biological Warfare

A Brief History of Biodefense Research in the 20th Century

It is difficult to say definitively when "biological warfare" began. From the sale of smallpox-laced blankets to Native Americans by European settlers to the Roman practice of "poisoning the well" by introducing pathogens to enemy water supplies, the fact stands that biological agents have been used aggressively for centuries. However, with the advent of modern science, the concept has taken on a new significance in an era when technology dominates warfare strategy. The possibility of mass-producing infectious pathogens has become a terrifying reality in the past century with the potential for man-made pandemics looming ever darker.

Table 1 | Examples of biological warfare during the past millennium

Year	Event
1155	Emperor Barbarossa poisons water wells with human bodies, Tortona, Italy
1346	Mongols catapult bodies of plague victims over the city walls of Caffa, Crimean Peninsula
1495	Spanish mix wine with blood of leprosy patients to sell to their French foes, Naples, Italy
1650	Polish fire saliva from rabid dogs towards their enemies
1675	First deal between German and French forces not to use 'poison bullets'
1763	British distribute blankets from smallpox patients to native Americans
1797	Napoleon floods the plains around Mantua, Italy, to enhance the spread of malaria
1863	Confederates sell clothing from yellow fever and smallpox patients to Union troops, USA

It is not clear whether any of these attacks caused the spread of disease. In Caffa, the plague might have spread naturally because of the unhygienic conditions in the beleaguered city. Similarly, the smallpox epidemic among Indians could have been caused by contact with settlers. In addition, yellow fever is spread only by infected mosquitoes. During their conquest of South America, the Spanish might also have used smallpox as a weapon. Nevertheless, the unintentional spread of diseases among native Americans killed about 90% of the pre-columbian population (McNeill, 1976).

Table source: *EMBO reports* 4, S1, S47–S52 (2003).

Unit 731

During WWII, the Japanese army maintained a covert biological warfare facility under the alias of "Water Purification Unit 731" in northeastern China[10, 11]. In this camp, around 1,000 prisoners of war were exposed to aerosolized *B. anthracis* spores and then examined post-mortem to study the progression of the resulting anthrax disease[10]. Besides conducting bioweapons research on human subjects, Unit 731 was also responsible for stockpiling supplies of

A series of horrific human experiments were conducted at Unit 731 during WWII.

Outside Reading: Medical Ethics
The use of data from biological research conducted on unwilling subjects by the German and Japanese scientific communities during WWII presents a moral quandary. Though the results of these studies were used by American researchers in their own work to benefit patients, is it ethical to take advantage of information extracted in so reprehensible a manner? *Strangers at the Bedside*, a 1992 book by historian David J. Rothman, provides some details of this and other abuses by medical researchers on both sides of the Atlantic. For those interested in expanding upon these issues, excerpts from the early segments of the book might generate some interesting classroom discussion on these dilemmas.

infectious agents. For example, many of the plague outbreaks in China during WWII are thought to originate from Japanese bombings with this arsenal[10]. Following the war, Unit 731 was dismantled, but the research data generated from work on human subjects was sold to the US in exchange for more lenient war-crime punishments[10].

Early US Bioweapons Research

During WWII, spurred by fears that Germany might develop biological agents to use with its bombs, the US developed a bioweapons research program at Fort Dietrick, Maryland in 1942 under the direction of the War Research Service (WRS)[12]. This program was expanded during the Korean War, including a countermeasures program in the event that US troops were infected with enemy biological agents. From the 1940s-60s, many classified experiments were conducted in which nonpathogenic (harmless) bacteria were spread over cities or through transportation thoroughfares to test the rate of epidemic expansion[12]. However, in 1969-70, President Nixon issued executive orders to terminate all of America's biological warfare research, and pledged that the US would never use such weapons[12]. Even though the offensive program was terminated, the US Army Medical Research Institute of Infectious Diseases (USAMRIID) remained to develop countermeasures to protect US troops[12].

Gruinard Island

Fearing that Germany might begin using biological weapons in its attacks, Britain initiated a series of biodefense experiments on Gruinard Island, a small rocky islet off the coast of Scotland[13]. In a series of tests from 1942-43, sheep were exposed to *B. anthracis* spores and autopsied to determine the virulence of the disease[13]. In 1942, an anthrax-infected sheep carcass washed up on the mainland where a dog subsequently consumed it and passed the bacterial infection to 24 other animals before the outbreak was contained[13, 14]. British officials claimed the body had not originated on the island but paid

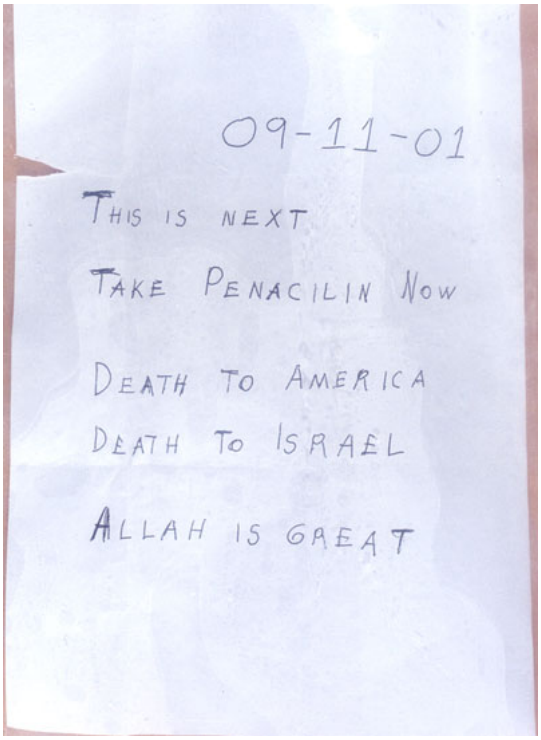
compensation nonetheless[14]. After the war, the island was deserted, but full decontamination procedures did not begin until the 70s and 80s (at least in part because more was known then about how to carry out sterilization procedures that would effectively kill the resilient spores)[15]. Only in 1987, after extensive chemical treatment reaching into the bedrock of the island, was Gruinard officially declared clear of anthrax spores[14].

The Sverdlovsk Incident

In 1979, a *B. anthracis* strain from a Russian bioweapons research facility at Sverdlovsk (now Ekaterinberg) led to one of the most deadly outbreaks ever, killing 68 people[16, 17]. Because the Russian program had been secret (in violation of the 1972 Biological Warfare Convention), the USSR tried to conceal the outbreak and quarantined the area extensively, claiming that the disease had spread from infected cattle meat and not from a mishap in bioweapons research[16]. It was not until 1991, with the fall of the USSR, that the full details of the incident became clear from an investigation launched by Boris Yeltsin[16]. These findings were further buoyed by information from a Russian scientific defector who confirmed that the Sverdlovsk facility was involved in industrial production of *B. anthracis*[16].

Today

The latest fears of bioterrorism emerged after 9/11 when several *B. anthracis*-laced envelopes turned up in the US. Abroad, Iraq has been suspected of carrying out bioweapons research, growing *B. anthracis* spores in large quantities for military use[18, 19]. In response to such concerns, the National Institutes of Health (NIH) has shifted its budget to fund more biodefense research[20]. This has led to much controversy over dual-use research, those projects that have both civilian and military applications; one example is the artificial synthesis of a Polio virus by researchers at State University of New York (SUNY) Stony Brook, reported in 2002 in the journal *Science*[5].



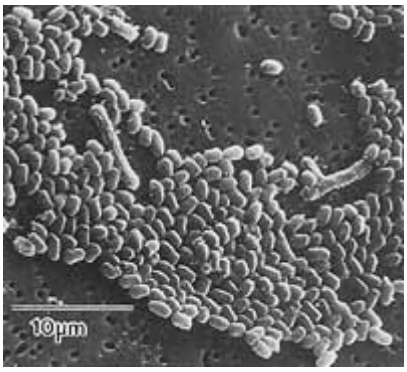
One of the *B. anthracis*-laced letters sent to Congress members during Fall, 2001.

Anthrax: The Cell Infiltrator

One might argue that the Book of Exodus records the impact of anthrax disease in 1490 BC. It reports: "And the Lord did that thing on the morrow, and all the cattle of Egypt died: but of the cattle of the children of Israel died not one." (Exodus 9:6) [21]*. An animal epidemic of anthrax perhaps?*

An immediate sign of such an epidemic might well be rotting meat. The smell is not just unpleasant: it may indicate that deadly anthrax spores have taken up residence within a decaying carcass. However, eating putrid flesh is not the only way to acquire the anthrax bacteria, as its spores can be dispersed by nothing but a gentle breeze. This is one of the factors that gives anthrax such deadly potential as a bioweapon and has led researchers racing to find countermeasures against this looming threat.

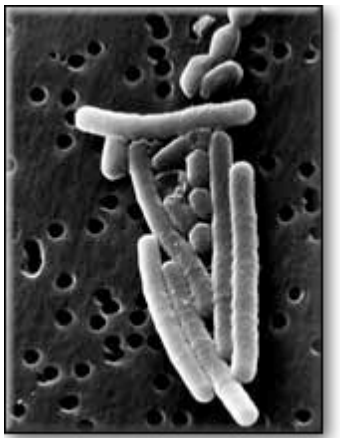
The Basics: What is Anthrax?



B. anthracis spores.

As is so often the case with scientific terminology in the mainstream, anthrax can be something of a misnomer. News reports tend to confuse the disease and the bacteria that cause it. To be precise, anthrax is the disease caused by the bacterium *Bacillus anthracis* (*B. anthracis*). This microorganism exists in two distinct forms: a non-virulent spore form and a virulent, infectious form.

The spore form allows the bacterium to survive under harsh conditions, lingering in soil for decades while waiting for favorable environmental conditions, or for the nutrients they need to divide[22]. As a spore, the bacterium is metabolically inactive – it does not carry out most of the energy-requiring activities of the virulent form, such as protein synthesis and reproduction[23]. To maintain this inactive state, the bacterium generates a hard, two-layer coating of protein that dissolves when it becomes active again[24]. The importance of the spore phase in the bacterium's life cycle is suggested by recent genetics research indicating that as many as 1/3rd of the organism's genes are devoted to making and maintaining the spore form[25]. Under a microscope, the spores look like seeds[26].


B. anthracis, virulent form.

On the other hand, the virulent form of the bacterium forms long strands under the microscope, and has a thinner coating of amino acids rather than the thick armor produced during the spore phase (more on this below)[26].


Anthrax-inflicted zebra.

Anthrax in nature: a disease of the animal kingdom

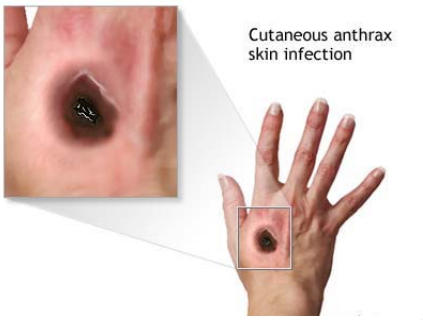
The spores of the anthrax bacterium can be found in most areas of the world, but particularly those with alkaline soil conditions favorable to *B. anthracis* growth[27]. The number of animals affected by the disease is as numerous as the climates that

can harbor it, ranging from cows, pigs, and sheep to wild creatures such as elephants and gazelles[27]. Birds, however, appear to be unharmed by the bacteria[27]. Animals are principally infected with anthrax spores following severe weather patterns (e.g. flooding or drought) which disturb the top layer of soil, bringing the spores to the surface[27]. Animals ingesting the spores can develop the disease. When these herbivores die, flies feeding on the meat can infect nearby vegetation through their excrement[27]. Further, carnivores can contract the disease by eating the carcasses of dead herbivores[27]. Sometimes even domesticated animals can acquire the infection through their feed (due to the addition of diseased carcasses in their food supply)[27]. Most animals die quickly from anthrax, though the particular symptoms often vary between species. Herbivores typically exhibit heavy breathing and fever along with bloody discharges; in pigs and wild carnivores, the disease often causes a swelling of the throat[27].

It is not hard to imagine how a human anthrax epidemic might start from animal infections, just as bubonic plague spread through Europe through diseased rats. In fact, the Black Death (aka The Plague) that ravaged Europe in the 14th Century may have resulted from a number of animal-transmitted diseases - including anthrax. Among the evidence for this latter theory is the discovery of *B. anthracis* spores in a "plague pit" in Scotland where victims of the disease were buried in a large group[28]. Given the manner in which the Black Death decimated the population of Medieval Europe, modern anxieties about such an outbreak are warranted.

Anthrax Can Occur in Different Forms

How deadly is anthrax? One indication is provided by the National Institute of Allergy and Infectious Disease (NIAID) which has currently classified the anthrax bacteria as a Class A bioterror threat –the same as smallpox and Ebola[20, 29]. However, the severity of anthrax disease depends upon how the bacteria are contracted:



Cutaneous anthrax.

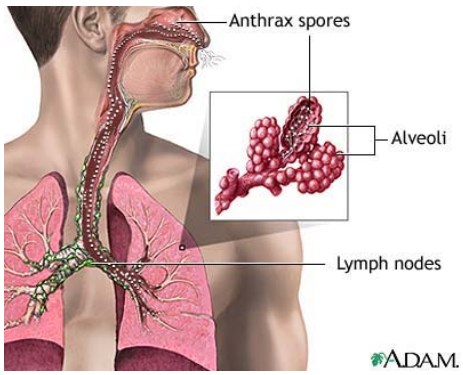
Cutaneous Anthrax:

In this form, the bacterium lodges in an open cut or sore, causing swelling and ultimately hardened black wounds[29]. In fact, the bacterial species name anthracis, the ancient Greek word for "coal", is derived from this phenotype[29]. Cutaneous anthrax is rare with only a handful of cases in the US occurring each year[29]. It is also the least virulent form of the bacteria with antibiotics usually proving sufficient to cure the infection[29].

Gastrointestinal Anthrax:

In its spore form, anthrax bacteria can linger in the soil for years. If consumed by an animal, the spores can become virulent by growing a protective capsule to evade the body's immune defenses[29]. In this encapsulated form, the bacteria can kill the host, and any meat taken from the body will be infected with the bacteria[29]. Eating B. anthracis-

laced meat can cause indigestion, diarrhea, fever, and ultimately death in 50% of victims if untreated[29]. However, no official cases have been recorded in the US[29].



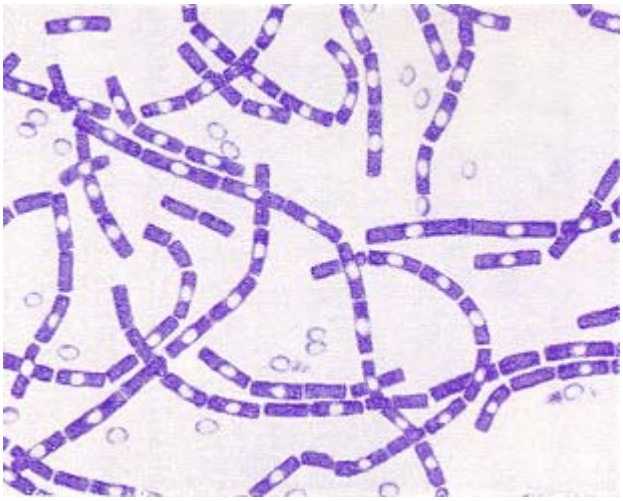
Inhalational anthrax.

Inhalational Anthrax:

The most deadly form of anthrax is transmitted through the inhalational route. If the spores are inhaled, they can revert to their bacterial form and infect the lungs and chest, causing mortality in over 75% of victims[30]. The disease progresses much like a flu, with symptoms including fever, vomiting, aches, and headaches which progresses to respiratory difficulties leading to shock and death[29]. Inhalational anthrax is even rarer than the cutaneous form with the last reported case (before the 2001 attacks) dating to 1976[29].

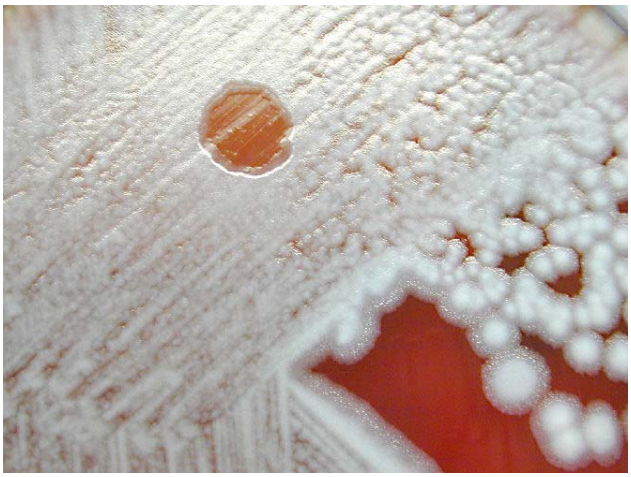
Detecting the Anthrax Bacteria

One general way to tell anthrax bacterium from other bacteria types is its rod-like shape[26]. In fact, the name *Bacillus* is used for any bacterium with this appearance[31]. Another way is using the Gram test discussed in the tuberculosis unit. Since *B. anthracis* is gram positive, it will appear purple-brown when colored with the Gram stain[26].



Gram-stained anthrax bacteria[26].

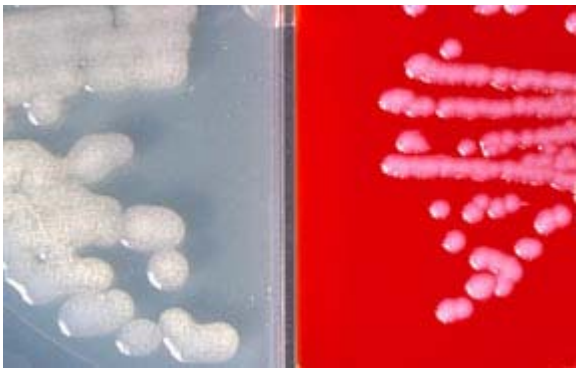
However, these two tests are not enough to identify the anthrax bacteria by themselves. To determine that a rod-like, Gram positive bacterium is indeed *B. anthracis*, researchers usually turn to the gamma-phage lysis test, in which a small virus (known as a bacteriophage) that specifically infects *B. anthracis* is applied to the unidentified bacterial colony[26]. If the phage produces holes in the culture, the sample is *B. anthracis*.



A positive gamma phage lysis test[26]

Hijacking the Human Cell

How exactly does the anthrax bacterium cause harm to the human body? The first sign of trouble occurs when the spores sprout a new protein "coat" made of a single kind of amino acid, replacing the two-layered shell surrounding the spore[32]. The new coat can be seen under the microscope as the bacterium's rough outer layer is replaced by a very smooth exterior[26]. T-cells (the white blood cells that patrol the body for microorganisms as a part of the immune system) rely on specific parts of the bacteria's outer coat for recognition as a harmful microorganism. When the anthrax bacteria produce a sleek protein coating, the T-cells are no longer able to recognize the bacteria as foreign invaders[32].

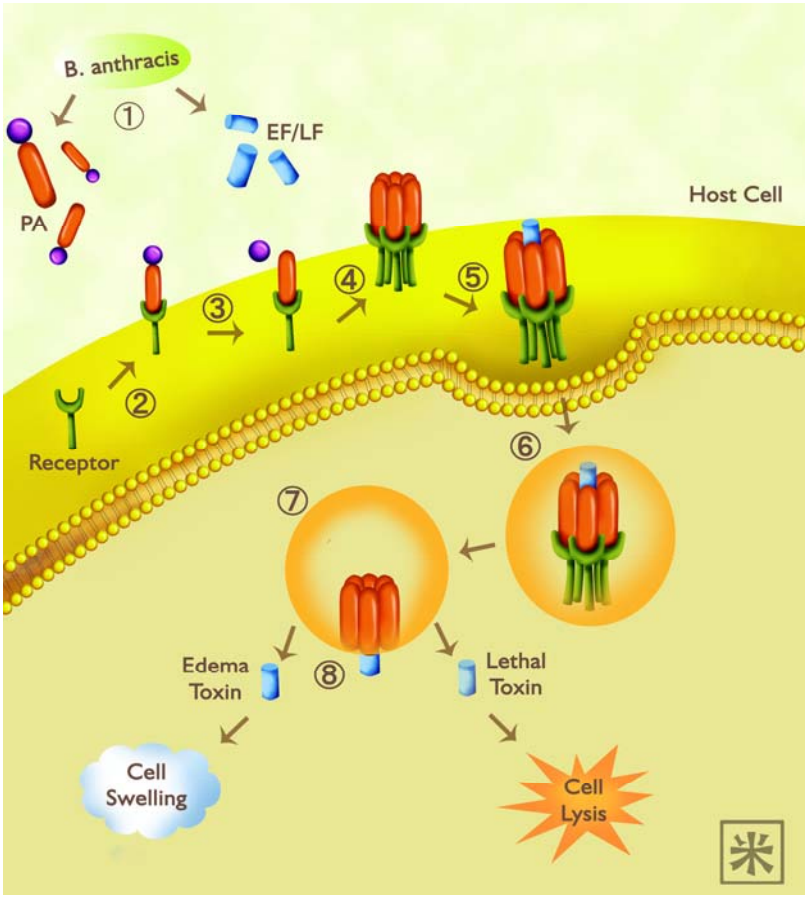


Anthrax bacterium goes from rough to smooth[32].

Now hidden from the immune system, the anthrax bacteria begin to secrete three different proteins into the blood: (1) Protective Factor (PA), (2) Edema Factor (EF), and (3) Lethal Factor (LF)[32]. Individually, each of these proteins is harmless. However, in combination, they initiate a cascade of events involved in anthrax disease progression. First, the released PA binds to a receptor on the surface of a human cell and in particular macrophages (those white blood cells that normally engulf bacteria)[32]. As a result of receptor binding, a portion of the PA is cleaved, allowing seven PAs to assemble together at the cell surface[32]. This group of seven PAs subsequently bind EF or LF, facilitating their uptake into the cell through endocytosis - a process in which a "bubble" or endosome containing the *B. anthracis* proteins buds off the plasma membrane into the

interior of the cell[32]. Within the endosome, a hole forms, allowing EF and LF to escape into the cell cytoplasm. There, the bacterial proteins elicit the most damage[32].

By altering ATP levels, EF alters the passage of water into and out of the cell, eventually causing the cell to swell[32]. Also, with insufficient supplies of ATP, cells that have a high energy requirement (like the macrophages mentioned above) quickly die, leaving the bacteria free to proliferate and cause more damage[32]. LF, on the other hand, disrupts a complex series of biochemical reactions within the cell (which are still not fully understood by scientists), ultimately causing the cell membrane to rupture[32]. A good animation of the above sequence can be viewed at: **http://www.wiley.com/legacy/college/boyer/0470003790/cutting_edge/anthrax/anthrax.htm.**



The Anthrax Infection Cycle

1. **The bacterium releases three proteins – protective antigen (PA), edema factor (EF), and lethal factor (LF).**
2. **A receptor (the anthrax toxin receptor, ATR) on the surface of the mammalian host cell binds to inactive PA.**
3. **PA is cleaved by a protease and is converted to its active form.**
4. **The active PA binds to other activated PA to form a heptamer.**
5. **EF or LF binds to the PA heptamer.**
6. **The PA-EF/LF complex is drawn into the host cell through endocytosis.**
7. **The acidic environment of the endosome induces a conformational change in the PA heptamer, which then pierces a hole in the endosome.**
8. **EF or LF is released into the cytoplasm where the toxins may disrupt normal cell function or even cause cell death.**

Original artwork by Senmaio Zhan

Fighting Anthrax: Then and Now

Anthrax has had an important place in antibacterial research since the earliest days of microbiology when a young scientist named Robert Koch was assigned to an isolated military post during the Franco-Prussian War[33].
Though cut off from an advanced laboratory, Koch decided to bide his time by testing whether the microbes he had identified earlier under a microscope were responsible for the anthrax outbreak killing the animals in the region[33]. In a series of experiments with limited resources, Koch demonstrated that: 1) bacteria from dead animal tissue could kill his laboratory mice and, 2) anthrax bacteria never exposed to animals were also lethal[33]. This was the first demonstration that disease could be transmitted by microorganisms, a landmark in medical science.

Robert Koch.

The venerable Louis Pasteur also played a major role in the fight against anthrax (and infectious disease in general). To demonstrate his immunology research, Dr. Pasteur arranged a public demonstration to show that sheep immunized with dead bacteria survived upon later injection with live *B. anthracis*[34]. Though the crowd gathering to watch might not have understood the biological mechanisms involved, vaccination through this same procedure would ultimately protect their descendents from all manner of lesser illnesses.

Today, the most well-known anthrax treatment is the antibiotic ciprofloxacin, known as "cipro" for short. Cipro works by blocking the bacteria's ability to divide. Normally, the bacteria compacts the long loop of DNA containing its genome with a protein called DNA gyrase[32]. The gyrase cuts a single strand of the loop, allowing it to be twisted favorably for storage within the bacteria. Cipro blocks the gyrase from rejoining the two ends of a DNA strand once they are cut, preventing the bacteria from dividing and multiplying[32].

However, it is important to realize that this is only effective if the bacteria *have not* already secreted the toxin proteins into the blood. Cipro does not actually kill the anthrax bacteria - it just prevents it from spreading further. The important clinical implication of this fact is that cipro cannot be used preventatively (or pre-exposure) because it is only effective once the bacteria have already infected the host. (This again relates back to antibiotic resistance due to overuse.

Current Research

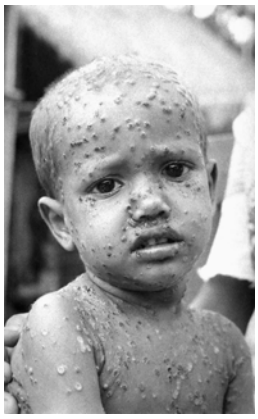
The PA, LF, and EF toxins were originally purified to use as a vaccine in 1970[26]. Using these toxins, and not an inactivated form of the bacterium, removed the possibility that the vaccine could cause anthrax. Still, the current anthrax vaccine remains imperfect, as demonstrated by the documented side-effects from mass vaccination programs instituted by the US military among servicemen and women[35].

In order to create more effective treatments to counteract the anthrax bacteria, scientists have now focused on specifically targeting PA, EF, and LF. Some have taken a

genetic approach to solving this problem by identifying genes that alter macrophage susceptibility to *B. anthracis* toxins. A pharmacological approach has focused on the synthesis of drugs to prevent PA from binding to the cell (and thereby preventing subsequent damage)[32]. Another synthesized therapy is artificial antibody that binds PA more tightly than its receptor, thereby preventing its binding to the cell[32]. In short, by targeting the disease on the molecular level, researchers hope to create better strategies for combating anthrax.

Smallpox: Dead or Alive?

It begins with symptoms that could be caused by many diseases – pounding headaches, exhaustion, and searing fevers. Only days later, the cold has become something far worse as a telltale rash begins to spread along the patient's body, soon swelling with pus and scabbing over to leave raised bumps over the infected skin. Even if the patient survives, the disfiguring scars can last a lifetime. This is the mark of smallpox – or it was, almost five decades ago, when the disease was still common in many parts of the world. In terms of mortality, smallpox tops the list: the smallpox virus has killed more members of the human population over the span of recorded history than all other infectious diseases combined[36].



A young smallpox victim.

In most of the diseases we have studied, mankind is in a pitched evolutionary battle with the viral co-inhabitants of this planet. Advances in biomedical technology may result in victorious skirmishes, but the war is seldom won as quickly-replicating viruses race to overcome vaccines and other drugs. Smallpox is one of the few true success stories, as the 1980 announcement by the World Health Organization of the worldwide elimination of smallpox marked a true victory. While smallpox vaccination used to be commonplace and widespread, its eradication made such treatment unnecessary. Except for a few isolated laboratory strains – preserved for posterity – smallpox is just a memory.

Or is it? Those same laboratory strains have many worried. Because smallpox has not been a public health threat for decades, stockpiles of vaccine are at an all-time low. If the virus were weaponized, there would be very little that could be done in response – and that is reason to be concerned.

Poxes Small and Large:

Much of what we know about the human smallpox virus comes from studying similar viruses that infect animals. This comparison works because the smallpox virus belongs to a family of closely related pathogens known as Orthopoxviruses. The members of this family are distinguished by their host (i.e. what kind of animal they can infect) and geographical distribution. Some, like the smallpox virus, are species-specific. For example (as seen in the picture below), poxviruses like variola major (smallpox) of

Acknowledgements: Part a, WHO; part b, Fenner © (1982) Academic Press; part b, part c, U. Wernery (United Arab Emirates) and H. Meyer (Germany).

humans (a), mousepox virus of mice (b) or camelpox virus of camels (c) remain largely restricted to one host species and rarely, if ever, cause infections outside of that species. Other poxviruses can infect multiple host species. It is the shared characteristics of this family that allow information about smallpox to be inferred from studies of its animal-infecting relatives in the viral evolutionary tree. A few of the more important members of the *Orthopoxvirus* family include:

Variola virus: This is the virus responsible for smallpox in humans. The name, first used to described the disease rather than the virus, is derived from the latin words *varius* (meaning "spotted") and *varus* (meaning "pimple"), and was coined in the 6th Century in Switzerland[37]. Later, English physicians would use the term *pockes*, based on the word *poc* (meaning "pouch") to describe diseases causing the same raised sores as smallpox[38]. The designation *small* came in the 15th Century, when it was necessary to distinguish the disease from the "great pox" – syphillis. The virus has two forms, called *major* and *minor*, based on the mortality rate for each strain. The classic form of smallpox is caused by *V. major*, while *V. minor* was recognized through epidemiological studies in the 20th Century[39]. Geographical variants of both *V. major and V. minor* also exist.

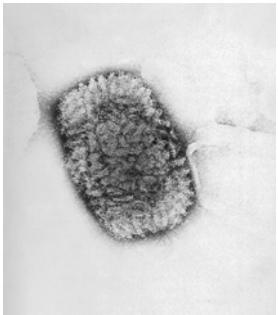
Vaccinia virus: Because *variola* is so dangerous to handle (requiring strict biohazard safety conditions), much of what we know about the smallpox virus actually comes from work on *vaccina*, a pox virus that infects several different animals including cattle[39]. This includes the cowpox virus that Jenner used in his landmark vaccination experiments (see historical section).

What About Chicken Pox?

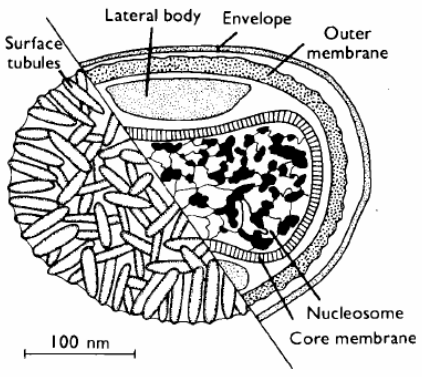
Despite the similarity of the names of the diseases they cause, the chicken pox virus, *Varicella zoster*, actually belongs to the Herpesvirus family. Like HIV, *V. zoster* can enter a latency period[40]. However, unlike HIV or influenza, it has a relatively stable genome[40].

The Deadly Brick

Researchers first observed pox viruses under the microscope at the end of the 19th century. Much larger than many viruses, poxes possess a brick-like appearance[39]. The genomes of the orthopox viruses are composed of DNA, not RNA[39]. Because DNA polymerase is much less error-prone than RNA polymerase, this feature has important clinical outcomes for mutation rate (discussed below). Zoom in and one can see that the brick structure is composed of three distinct components: (1) an inner "core" containing the DNA and associated proteins, (2) a protein shell that forms the outer



The smallpox virion.



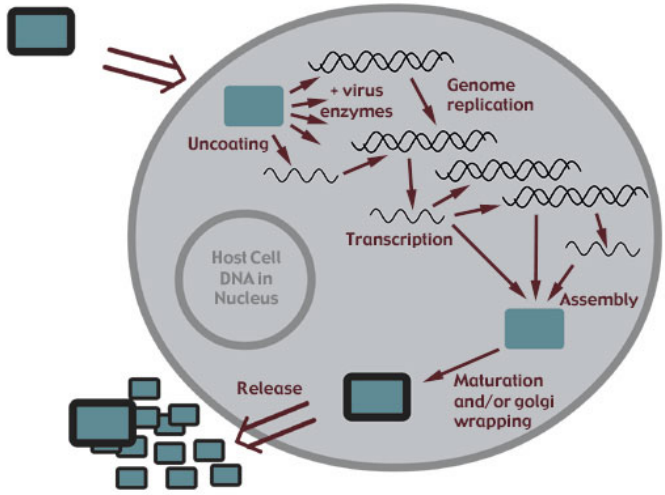
Schematic of smallpox virion.

membrane, and (3) a plasma membrane derived from the cell from which the virus budded. The virus does not always contain the outer membrane component. Sometimes, instead of budding, the host cell is broken apart and the virus exits wearing only its outer protein layer. While these "un-enveloped" viruses are still problematic, the lack of a cell-derived membrane has important implications in pathogenesis and vaccination[39].

The Pox Virus Life Cycle

The cycle by which the smallpox virus replicates is similar to other viruses. First, a free-floating virion penetrates the cell membrane of its target cell. How efficiently the virus gets through the host's membrane depends on whether or not it is coated by a plasma membrane[39]. Those pox virions with a membrane outer coating can enter a host cell more easily and, as a result, are more infectious.

Once inside, the pox virus, like HIV, sheds its outer coating and begins replicating. However, because its genome is made of DNA, the pox virus can copy itself in the cytoplasm unlike RNA viruses that must enter the nucleus of their host. The pox virus generate protein and DNA copies, recombines into a functional virion, and then buds off from the host cell, destroying it as a result. The cycle begins anew as the newly released virions go on to infect other cells.



Smallpox life cycle.

Clinical Symptoms

Even after the smallpox virus has infected a host, no symptoms are seen in the first two weeks[41]. This is a particularly dangerous time as every silently infected person can infect 10 to 15 more, who unless quarantined, go to infect 10 to 15 more themselves[41]. An infected individual initially looks and feels fine because the virus has not started actively replicating and shedding copied versions of itself throughout the body[41]. Once this proliferation has begun in earnest, the infected individual begins to experience flu-like symptoms. Just as the patient seems to be getting better, a terrible rash develops, particularly on the face[41]. The rash worsens, becoming pus-filled bumps that eventually scab over, leaving the pitted scars that are a hallmark of smallpox[41].

Less obvious – and even more dangerous – are the ulcers that develop inside the throat and nose of the infected individual. When the skin cells containing the virus die, they release the virus into neighboring saliva and digestive passageways. At this point, the individual's saliva becomes contagious, able to spread the virus by coughing if precautions are not taken[39]. The virus is also freed from the confines of the ulcer, and moves throughout the body through the conduit of the digestive system to infect any

organ it comes in contact with[39]. The more organs it infiltrates, the worse for the patient. Simply put, the virus overwhelms the body, killing cells of multiple organ systems.

Transmission

As noted above, transmission of the smallpox variola virus occurs exclusively from human to human with no insect intermediate or animal reservoir also containing the virus. It is mainly spread through aerosol when infected individuals cough up smallpox-laced mucous particles[42]. Once infected, patients are only contagious once the rash has developed and remain contagious even as the rash scabs[42]. Fallen scabs containing active virus particles can collect on bed sheets or clothing and must be subjected to proper disinfection procedures[42]. In the more severe *V. major*, the infected are often incapacitated, and so transmission can be minimized as long as exposure by healthy individuals is kept to a minimum[42]. However, in the case of *V. minor*, the symptoms are so mild that infected individuals may spread the virus easily during its infectious stage[42].

Drug Treatment?

One of the main reasons a smallpox outbreak would be so deadly is because there is no known drug treatment. The symptoms such as fever and headache can be subsided with traditional medications such as aspirin, but the virus itself cannot be killed by any drug in our current medicinal arsenal. More effective treatments may be on the horizon, though. Cidofovir, a viral DNA polymerase inhibitor used to treat cytomegalovirus (CMV) in AIDS patients, has been shown to kill smallpox virus in laboratory studies[43, 44]. However, the compound must be injected intravenously and causes kidney damage in the large concentrations required to penetrate smallpox-infected cells[44]. Currently, scientists are working to re-formulate the drug in a less toxic version. Until then, vaccination remains the primary protection against smallpox. To fully appreciate the impact of vaccination on the eradication of smallpox, first we must delve into some history….

The Origins of Smallpox

The earliest records of smallpox emerged from museum collections including mummies dating from 1570 to 1085 B.C.[45] From these specimens, scientists have concluded that Ramses V (a young Pharaoh monarch who died in his early thirties) likely died of smallpox[45]. With this disease and a civil war during his reign, it seems no great wonder that he died young! Other ancient civilizations proved more fortunate. Indeed, even though the global eradication of smallpox had to await modern technologies allowing large-scale vaccine production, mankind has known for millennia that it is possible to shield oneself against the virus. Striking evidence of this comes from the subcontinent of India, where medical texts from 400 C.E. contain what may be a description of an early vaccination procedure:

Pharaoh Ramses.

Take the fluid of the pock on the udder of the cow or on the arm between the shoulder and elbow of a human subject on the point of a lancer, and lance with it the arms between the shoulders and elbows until the blood appears. Then, mixing this fluid with the blood, the fever of the smallpox will be produced[46].

If this account is true, then it appears that Indian physicians discovered the protective power of cowpox a millennia or more before it was known in the West[47]. Certainly, the clinical symptoms of the disease were recognized by these early doctors, as indicated by passages such as this:

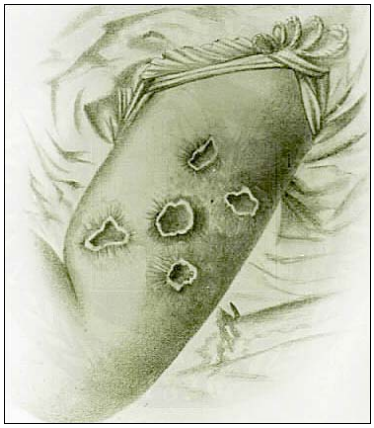
Before [smallpox] *appears, fever occurs, with pain over the body, but particularly in the back . . . . When bile is deranged, in this disease, severe pain is felt in the large and small joints, with cough, shaking, listlessness and langour; the palate, lips and tongue are dry with thirst and no appetite. The pustules are red, yellow, and white and they are accompanied with burning pain. This form soon ripens . . . . When air, bile and phlegm are deranged, in this disease the body has a blue colour, and the skin seems studded with rice. The pustules become black and flat, are depressed in the centre, with much pain. They ripen slowly . . . this form is cured with much difficulty, and it is called Charmo or fatal form*[48].

Indeed, it appears that the disease was so well-known in India that a Hindu Goddess of Smallpox named Sitala was venerated in many regions[49]. The afflicted would pray to Sitala to rid them of the disease, using drops from the water of immortality she was said to carry with her[50]. One of the ways in which Brahmin priests venerated Sitala was to journey the countryside each spring, inoculating villagers against smallpox as they went[51].

Edward Jenner.

Jenner and Pasteur: The Birth of Vaccination

Development of medical practices in the West comparable to India's actions would not come until the close of the 18th century when English physician Edward Jenner made a remarkable observation in his country home[52]. The dairy maids in the surrounding farms bore the lesions of cowpox, but never acquired smallpox: they were seemingly protected from the human virus[52]. Wondering whether this immunity could be replicated, Jenner injected a young helper with pus from a cowpox lesion from one of the milkmaids, and observed that the boy developed resistance to smallpox afterwards[52]. Jenner named his discovery *vaccine* (after the latin *vacca*, meaning "cow") though he did not understand fully why it worked[52]. The full explanation would await modern theories of

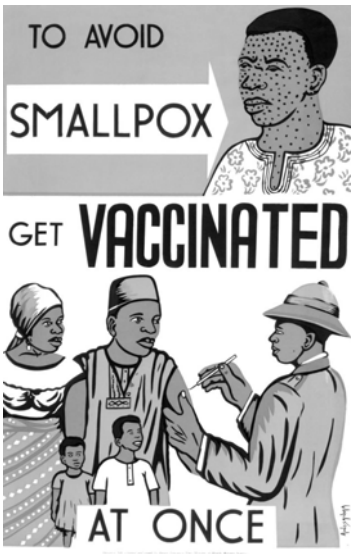


Cowpox-infected maid.

immunology, though Jenner's technique, refined by Louis Pasteur to combat rabies, would become immensely influential nonetheless[34].

Vaccination Takes Off

Vaccination would become increasingly widespread through the first half of the 20th century though it was never done extensively enough to eradicate smallpox. Even in countries such as England, where the disease had become uncommon, infected individuals from Africa spread a rash of cases in 1962, and only a quick surge of vaccinations prevented a full-blown epidemic[53]. However, the vaccinations could cause harm as well, resulting in severe adverse reactions in some individuals. To avoid having to negotiate this delicate balance between the benefits and shortcomings of vaccination, the disease would have to be completely eliminated[53].

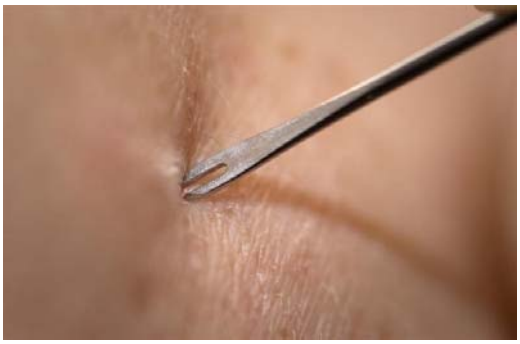


Vaccination campaign poster.

Global Eradication

Doubtless this need for a lasting solution drove the 1950-1958 vaccination campaigns in South America, Central America, and the Caribbean, complementing earlier efforts that had pushed cases in the United States into the single digits[53]. Based on this success a global campaign was launched by the World Health Organization in 1959, though it was not until 1967 that the effort truly intensified to meet the challenge of the task[53]. At this point, consistent supply networks were established, as well as extensive monitoring systems in many countries so that the effectiveness of the vaccination campaign could be confirmed over time[54].

Besides such organization, technological developments also aided this campaign. One was the bifurcated needle (shown to the right). Instead of a standard shot that punctured a vein, vaccinators employed a simple device developed in the 1960s by pharmaceutical firm Wyeth Laboratories. This horseshoe-shaped needle was dipped in vaccine then lightly tapped against a patient's skin to make a series of small punctures. In selfless move, Wyeth allowed the needle to be used without charging patent royalties. Besides their ease of use, the bifurcated needle also had the advantage of reusability: provided they were sterilized, each instruments could vaccinate hundreds of individuals[55]. In addition to providing needles, Wyeth was also manufacturing the Dryvax smallpox vaccine used during the eradication campaign[56].



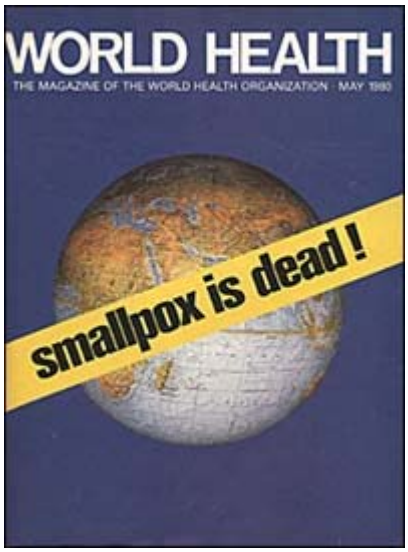
Bifurcated needle.

During the campaign, field workers would scour the globe, distributing vaccine and controlling outbreaks. Their efforts eventually met with success, with the last

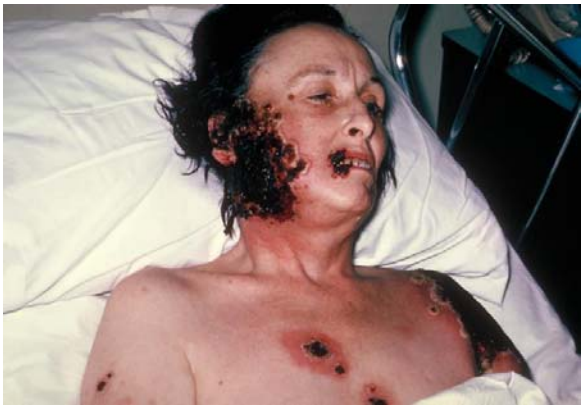
instance of smallpox infection recorded in Somalia in 1977. Another aberrant case cropped up in 1978 when a strain escaped a laboratory in England, but besides this, no other infections were reported. In 1980 the disease was declared extinct[57-59].

And Now?

Prior to its eradication, vaccination for smallpox was done using inactivated strains of vaccinia virus. However, only small stocks of the vaccine now remain and are not available for widespread distribution. Typically, only researchers in high-security facilities are now vaccinated for smallpox[60, 61]. Despite concerns about future biological warfare, the knowledge that the vaccine has side effects is a major argument against re-instituting more widespread smallpox vaccination in preparation for a bioterrorism attack. These complications usually result in skin rashes or, in more severe cases, potentially fatal tissue death. While health officials predict that only 1-2 out of every million vaccinated would actually die of such complications, the risks are still too much to reinstate vaccination on a large scale[41, 42].



A public health victory: smallpox is eliminated!



A victim suffers from the adverse effects of the smallpox vaccine.

Resurrecting the Pox?

The existence of laboratory strains of the virus means there is still a possibility of it being resurrected as a bioterror weapon. While the strains locked away at the CDC can probably be assumed safe from such abuse, the collapse of financial backing for Russian scientific research has raised concerns that private (and unfriendly) hands could now be funding work in former Soviet bioweapons facilities[62].

Is there a benefit to maintaining smallpox stocks in the laboratory? Some researchers claim that these supplies are necessary for further research. However, this seems suspicious given that most of what we know about the virus actually comes from animal forms such as *vaccinia*. Nevertheless, policy makers have supported retaining samples of the human virus. Even though the World Health Organization had promised the destruction of all remaining smallpox stocks in the US and Russia by 1999, the Clinton administration decided to reverse this plan in the US, claiming the samples were needed for future anti-viral research, or, in the event that the virus remerged, to develop

new vaccines[53]. However, the rescued stocks have actually generated little scientific interest, with no new pharmaceutical or vaccines developed in the years since they were salvaged from their planned destruction[53].

So how deadly would an outbreak be? Certainly, the lack of current vaccination makes smallpox a particularly dangerous threat, and since the vaccine is not used regularly in the US anymore, immunity levels among the American population are effectively nonexistent. Besides this, the virus is a tempting weapon because it can be easily grown and aerosolized[53]. Thus, because of this threat, the pros and cons of widespread vaccination are still being weighed, with the true threat of a smallpox outbreak remaining unknown.

Activity 1: Gulf War Syndrome

Are we protecting our military sufficiently from the dangers of chemical and biological weapons? Following the Gulf War, there has been increasing debate over whether servicemen and women were unnecessarily endangered by such exposure. Many have reported health conditions that have still not fully been explained, and the children of these veterans have shown an unusually high rate of birth defects. Is the media misrepresenting a series of coincidences as evidence of bioweapon exposure, or are the insidious and long-term effects of such weapons really to blame? In this activity, you'll consider both sides of this argument.

Your assignment is to browse the following website:

http://www.pbs.org/wgbh/pages/frontline/shows/syndrome/

Divide into two groups to address the debate from the following positions:

1. Gulf War syndrome does exist, and better protection is needed for military personnel. Find at least two interviews with veterans that justify this position.

2. Gulf War syndrome does not exist and has been created by media hype. Find at least two articles by experts that support this assessment.

Each group prepares a 10 minute statement outlining their opinion. After this, each is required to come up with a 5 minute rebuttal to the other presentation.

Hint: Even though you are only required to use two sources, it is to your benefit to have as many points of evidence as possible for the refutation phase!

Activity 2: How to Build a Poliovirus (The Risks of Research)

To defeat a disease, it's necessary to understand it on the most fundamental levels, dissecting its molecular building blocks to find out the best way to take it apart. In an effort to achieve victory over bacteria and viruses, scientists must grow the objects of their study long-term and acquire an intimate understanding of these pathogens. However, this kind of knowledge also serves as a blueprint for creating and maintaining these pathogens, a technique that could be put to adverse use in the hands of terrorists. Because such research has both potential benefits to human health, and the capacity to enable destructive weaponry, it is called "dual-use." Given the risks, should research on potential bioweapons be made public because it has relevance to human health, or should it be kept private, to make sure the next terrorist attack is not inspired by the contents of a scientific journal? In this case study, you will examine the most famous instance of this kind of dual-use research in recent memory: the synthesis of the poliovirus.

1. First read this article, which summarizes the findings of Wimmer and his colleagues, who reported artificial creation of the poliovirus in 2002:

http://www.sciencenews.org/articles/20020713/fob8.asp

2. Next, consider the response from Wimmer to accusations about the potential dangers of his research:

http://www.nature.com/embor/journal/v7/n1s/full/7400728.html

Based on these, address the following question:

Given the risk of bioterrorism, should this article have been published? Could it have been edited to make it appropriate? What guidelines do you think should exist?

Write a 2 page letter to the editors of a scientific magazine laying out your opinion. You should include citations, where relevant, from other examples of dual-use research, and conclude with a recommendation for future actions and policies.

Activity 3: Aum Shinrikyo and the Anthrax Attacks on Tokyo

Bioterrorism is not just a fear – it is already happening. In 1993, Japanese doomsday cult Aum Shinrikyo released anthrax spores from an office building in Tokyo. The group would become infamous in 1995 for a Sarin gas attack in the Tokyo subway, yet this earlier incident apparently caused no human outbreak. Why was the chemical attack so much more effective than the biological one? This activity examines why the group's anthrax attack may have been unsuccessful.

1. First brainstorm: what conditions are required for anthrax to be weaponized? How do these compare for the conditions for a chemical gas to be effective?

2. Have students read the article ***Bacillus anthracis*** **Incident, Kameido, Tokyo, 1993**. Consider the epidemiological findings in particular.

3. Would smallpox be less or more difficult for cult such as Aum Shinriyko to release? Why?

Activity 4: Going to Pasture

The Cow Pock-or-the-Wonderful Effects of the New Inoculation!
James Gillray (1757-1815)
Photographic reproduction of an etching appearing in
Vide--The Publications of ye Anti-Vaccine Society, June 12, 1802
National Library of Medicine Collection

The smallpox vaccine, originally prepared from the lesions of people infected with cowpox (a much milder disease contracted from cows), made many people fearful--of cow-borne disease, of usurping God's will, of the unknown. This 1802 cartoon shows Edward Jenner, the vaccine's discoverer, administering it, as previous vaccine recipients erupt with cow-like features.

1. Students will examine this painting and determine what is happening.
2. Why might people at Jenner's time in history have been afraid of vaccination? How has the artist taken advantage of science (and non-science) to portray his message?

Activity 5: Congressional Committee Hearing Simulation on Bioterrorism

Students will research a very timely topic and discover how many agencies are involved in the defense of our country against a bioterrorism attack and how much money they have been budgeted. Courtesy of Kenan Fellow Susan Hirsch's curriculum activities: www.ncsu.edu/kenan/fellows/2003/shirsch/bioterrorism/content.html.

Activity 6: Effects of Bioterrorism Seminar

This seminar activity consists of readings including the prologue from *The Anthrax Letters* (by Leonard Cole) and an article "What Leaders and Citizens Can Do," with quotes from former Soviet Union President Mikhail Gorbachev and former U.S. President Jimmy Carter. Discussion will follow. Courtesy of Kenan Fellow Susan Hirsch's curriculum activities. (www.ncsu.edu/kenan/fellows/2003/shirsch/bioterrorism/content.html

Activity 7: The Speckled Monster

This book tells the dramatic story of two parents who dared to fight back against smallpox. Included is the chapter "Rosebuds in Lily Skin", which tells how Lady Mary Wortley Montagu saved her daughter and helped save the city of London from the deadliest disease mankind had known. Discussion will follow. Courtesy of: Kenan Fellow Susan Hirsch's curriculum activities.
(www.ncsu.edu/kenan/fellows/2003/shirsch/bioterrorism/content.html

Activity 8: What Else is There?

Students will work as advisors to the White House. Their job is to identify and rate potential sources of biochemical warfare.

Divide the class into groups of 3-4 students each. Allow each group to choose one agent to study. Lists can be found at the CDC website (www.bt.cdc.gov/Agent/Agentlist.asp). Allow students to research their topic and prepare for their "debriefing with the President." Their presentations should include:

- Clear identity of the agent
- How the agent is obtained
- How the agent is detected
- How the agent could be used
- Possible scope of damage/destruction
- Was to defend or prepare for such an agent
- Security measures already in place
- Likelihood of an attack with their agent being implemented
- Visual aid (Powerpoint presentation, storyboard, or poster)

Each group then presents to the teacher who acts the role of the President. The rest of the class should pose questions to the presenters during and/or after their show. When all of the presentations have been completed, rank as a class the threats from the most dangerous to least likely. Courtesy of: Viki Babcock, pbs.org.

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Avian Flu: Pandemic of Tomorrow

"It is not if [avian flu] is going to happen . . . it is when, where, and how bad."[1]

- Michael Osterholm, head of the University of Minnesota Center for Infectious Disease Research and Policy, 2005

“Year of the Rooster”

“You have to be kidding,” says Maxine, looking about the spartan terminal of Malkinagrad International Airport. In the far corner, next to the tinted window overlooking their downed aircraft, a set of dangling wires marks the place where a fluorescent bulb once shone. The fixture’s glass cover is propped against the wall, but no workman’s ladder indicates that the light will be replaced anytime soon. Somewhere down the long hall towards baggage claim, she thinks she can hear a siren. “How long are we here for?”

“That depends on how fast this quarantine is resolved,” says Mrs. K, looking up from her cell phone, before the voice on the other end evidently returns. “Yes . . .,” she replies, nodding pensively, “. . . yes . . . no, we have all our luggage . . . I’m sorry, can you wait a moment?” Holding the phone awkwardly in one hand, she rifles through her purse for a pen and checkbook, roughly opening a page while talking into the receiver. She quickly takes down a number as a voice prattles away on the phone’s speaker. “Yes,” she replies, “I understand . . . tomorrow, alright. Thank you.” Putting the phone down, she turns to address her students. “That was the Pierpont representative – there’s no way we can get out of here before tomorrow, so they’ve arranged for us to stay at the embassy hotel across the street from the pharmaceutical district where the company has its installation here. I’m supposed to call them in the morning for an update on getting to Paris before Thursday.”

A rumbling sounds from outside and, turning to the waiting area’s numerous southward-facing windows, they see their plane soar back into the deepening gloom over the horizon. Though the quick approach of twilight makes it difficult to see clearly across the tarmac, it appears that there are no other aircraft currently parked at the landing strip. For better or worse, they’re stuck in Malkinagrad for the night.

“There’s one other thing,” says Mrs. K, as her students turn anxiously towards her. “I don’t want to alarm you, but the Pierpont representative informed me that there was an outbreak of avian influenza in a poultry market a few blocks from the pharmaceutical district last week. There weren’t any human cases reported, but, just to be safe, she suggested that we try and stay inside the hotel as much as possible.”

Angelica’s eyes bulge. “We’re trapped in nowhere *and* there’s an outbreak of an incurable virus?”

“Just stop,” grumbles Lang, stuffing a newspaper from the plane ride into his knapsack. “There’s nothing we can do, and complaining won’t make the situation any better.”

“Thanks,” she snaps, and the two students avoid eye contact for the next few minutes while following Mrs. K to the tiny ground transport desk. Fallon, looking distractedly about the run-down facility, wonders how many flights actually land here in a week. Fifty? Ten? None? Everyone else in the building appears to be an airline employee or one of the other passengers on their own flight. By all indications, Malkinagrad International Airport is a lonely place. At the ground transportation desk, Mrs. K calls the shuttle service for the hotel while her students slump among their luggage near the exit.

“I wonder how long we’ll be here,” wonders Maxine, checking that her wallet is still safely stowed inside her purse. “Maybe the hotel will have internet access . . . I bet this whole situation is all over CNN.”

"I brought my laptop," says Fallon, hoping that this is his opportunity to redeem himself in Maxine's eyes, "maybe there's wireless internet access."

"Yeah, as I recall, you almost got us kicked out of RDU for not taking it out of your bag *after* you caused that scare with your dirty shirt." She sighs, and he concedes defeat, at least for now.

Angelica, on the other side of the counter, turns to Lang. "Alright, I'm sorry I snapped at you, but I don't think you realize how rude you are sometimes. I don't understand how someone can be so artistic but so disinterested in other people."

Lang, slightly surprised, looks up from his book, fumbling for a reply. "I . . . I hate traveling, it's making me stressed." He looks down. "Um . . . I'm sorry too." As if to end the awkward exchange, the Pierpont shuttle pulls up outside the baggage claim, spraying brown ice over the sidewalk.

"Here we go," says Mrs. K, "the hotel isn't far from here." She grabs her suitcase, waving her hand for the students to hurry up and follow her to the waiting vehicle.

* * *

Compared to the airport, the hotel is surprisingly luxurious – probably an indication of where Pierpont's vast pharmaceutical wealth has been distributed in this beleaguered country. The quiet chamber music playing in the lobby almost drowns out the icy wind outside, and the trickling of water from the fountain beside the reception desk adds to the tranquility of the scene. Fortunately, the hotel has internet access on a computer console in the lobby, and Fallon busily clicks through news sites while Maxine reads anxiously over this shoulder.

"It looks like they're still resolving things," he says, scanning a BBC article posted only hours ago, "they don't even know what this stuff was on the flight." He continues scrolling down the page. "Apparently Paris won't allow any flights from New York to land until the lab tests come back. Looks like we're stuck."

Maxine rolls her eyes. "Great. Just great."

Fallon clicks on another news link. "Well there's some good news at least. It looks like all the poultry that Mrs. K was talking about earlier are being incinerated as a safety precaution – so at least we're safe there."

"You think so?" says Maxine. "I wonder where the virus came from. I mean, it had to get into the population somehow – my guess is that there are probably still infected ducks or something out there. Besides, I wonder how they got all the poultry farmers to agree to this . . . probably they didn't have much choice, even if it meant their year's stock is gone."

"Yeah . . ." replies Fallon, trying to think of something intelligent to say in response, and coming up with nothing. Instead, he continues to search the news sites, until Mrs. K approaches them, Angelica and Lang following closely behind.

"Alright, I just got off the phone with the Pierpont officials here," she says. "Since we're going to be stuck here for at least another day, they offered to take us on a tour of the facility across the street. A representative is going to meet us at nine tomorrow morning. I suggest you all go get some sleep, or you're going to have horrible jetlag tomorrow."

"I guess going outside is out of the question?" asks Angelica. "With this flu incident and all?"

"We were just reading about it online," says Fallon, "it looks like they're destroying all the animals in the market."

"I think they should destroy all the wild birds, too," says Maxine. "That's probably how it spread after all."

"You think so?" says Lang. "I thought avian flu came from Asia – you really think a sick bird flew all the way from Hong Kong to here? Wouldn't having avian flu make it harder for the bird to fly?"

"Oh . . . maybe," thinks Maxine. "But then how did the virus get here?"

"Well, whatever the answer is, I want you all to stay in the hotel," says Mrs. K. "I don't want to get into trouble with your parents for leading you around a quarantined city." She yawns, quickly covering her mouth. "Alright, I'm going to bed. Everyone needs to be in the lobby at 8:45. Goodnight." The four students watch her go, climbing the wide stairway in the lobby to the mezzanine where the elevators lead to the guest rooms.

"Did you notice that she slept through the entire flight?" asks Angelica. "And she's still exhausted. Strange."

"Yeah," says Maxine. "Maybe she's coming down with something." Remembering their recent conversation, she stops, and the others quickly pick up on the implication. "I'm sure it's nothing," she finally adds. "Anyway, I'm going too. Goodnight."

Maxine quickly climbs the stairs, almost tripping twice, while the others wait quietly beside the fountain, the music continuing placidly in the background.

Questions:

1. How likely is it that avian flu would be spread through migratory wild birds? What is evidence for and against this hypothesis?
2. Is culling the best solution for eliminating the risk of human infection? What are some obstacles to this kind of solution?
3. What kinds of conditions are required for humans to be infected with avian flu? In how much danger are our protagonists if they go outside?
4. If there were another report of bird flu, what steps should be taken to respond to the threat?

Introduction:

A malady has gripped the nation, leaving millions dead. The disease is killing almost as fast as the undertakers can bury the dead and seems to spread like the wind. No one is entirely sure where it came from, but its lethality is undeniable: most of its victims survive less than twenty four hours after contracting the virus.

This could be 1918. It could also be tomorrow. The versatile influenza virus, continually changing as it moves between species and modifies its behavior on a molecular level, was responsible for one of the worst pandemics ever recorded during WWI. It is also a threat looming on the horizon as scientists fear that a particularly virulent strain of influenza (H5N1) is preparing to jump from birds to humans in the very near future. In this module, we will both excavate the often forgotten history of the 1918 influenza pandemic and examine the biology behind what may well be the next great threat to humanity.

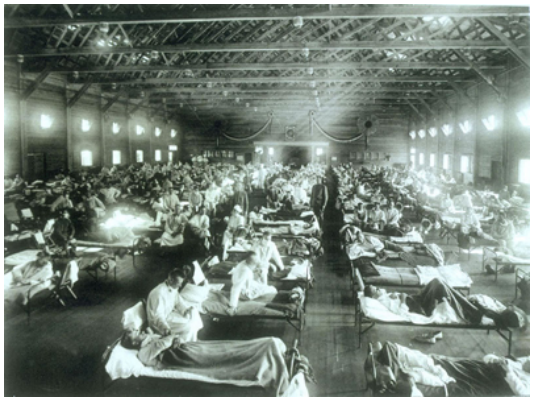
Goals:

By the end of this module, students should be able to:

1. Predict whether influenza is likely to spread within a given environment, and what public health measures might be effective in particular situations.
2. Analyze the probability of a virus being able to cross a species-species barrier given information about its pathogenesis and molecular makeup. Propose changes that would allow this cross-over to occur.
3. Predict whether an older influenza outbreak (like the 1918 "Spanish Flu") would have the same impact today.
4. Suggest possible vaccine targets based on the molecular basis of influenza replication.

Influenza Epidemics: From Past to Present

Although commonly misconceived as a "bad cold," influenza (or "the flu") is one illness that public health officials fear most[3]. In fact, the term "influenza" derives from Italian *influentia* – or to influence, and this disease has profoundly influenced human history. Since the sixteenth century, the world has endured 13 global outbreaks or pandemics of which humans possessed no partial immunity[3]. By conservative estimates, the 1918 flu pandemic alone resulted in the deaths of 20-40 million people worldwide in just four months – nearly twice as many people as those who died from battle wounds over the four years of WWI[3].



An emergency hospital at Camp Funston, Kansas, cared for large numbers of soldiers sickened by the 1918 flu. Courtesy of the National Museum of Health and Medicine, Armed Forces Institute of Pathology, Washington, D.C.

During the 1918 epidemic, the world may very well have understood the hysteria now building about avian influenza. In contrast to the massive media coverage predicting the potential rise of the avian influenza virus, the 1918 flu pandemic began without warning. At the time, countries were engaged in the combat of WWI. In March of that year, a single soldier at Fort Riley, Kansas was admitted to the base infirmary complaining of a sore throat, headache, and backache[5]. Within a month, the virus had reached most American cities including ports that would eventually ferry infected American soldiers to Europe for the war[6].

Epidemic versus Pandemic
Given how often these two terms are used (sometimes interchangeably), it seems prudent to provide a clear definition:

An **e**pidemic is a disease that spreads within the borders of a city or state[4]. A pandemic is an epidemic that has spread beyond national borders and is usually used in global terms[4].

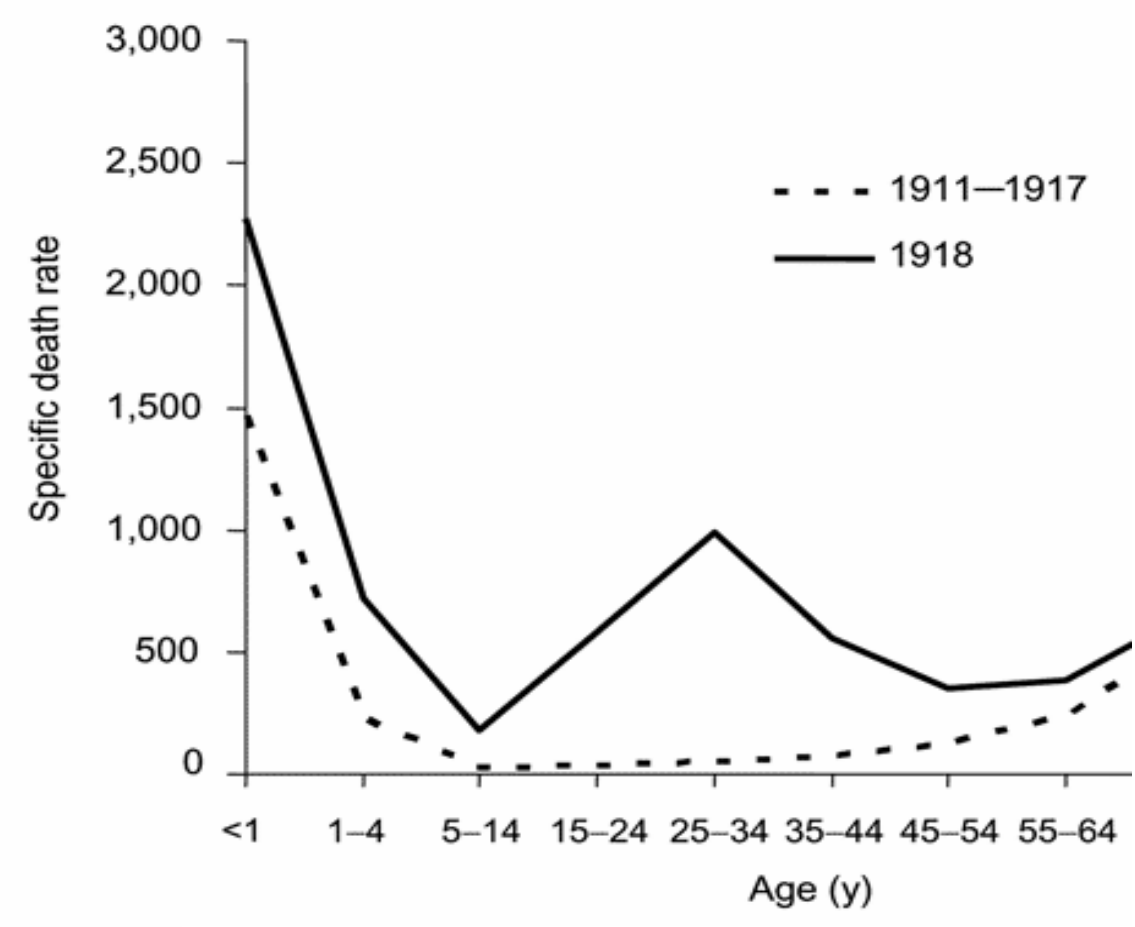
By June, media reports were beginning to report on the illness[3]. Because Spain was not participating in WWI, they did not need to worry about displaying weakness to the enemy in the form of a health catastrophe[7]. In a Spanish wire service to London, the headlines read: "A STRANGE FORM OF DISEASE OF EPIDEMIC CHARACTER HAS APPEARED IN MADRID…."[8] Eventually, this inadvertent headline would prompt public health officials to officially inaugurate 1918 as the year of the "Spanish Flu." Though the pandemic had gripped many countries, the flu in Spain was most heavily publicized. Interestingly, the Spanish referred to the disease as the "French flu"[9].

The arrival of fall did little to slow the virus. At a military facility near Boston, the flu took effect on stout young soldiers, killing 63 in one day[10]. The health disaster in the barracks paled in comparison however to the 851 New York City mortalities recorded in 24 hours on October 23[11]. Even when WWI ended in November, there was little celebration among the citizens of San Francisco who wore face masks to ward off infection[10]. However, after that fatal autumn, the disease vanished almost as mysteriously as it had appeared. What was to account for the swift arrival and departure of the disease?

Street car conductor in Seattle not allowing passengers aboard without a mask. 1918. Mass transit systems, with crowds of people in close quarters, were fertile venues for the spread of disease. In many large cities, public health officials required that passengers and employees wear masks as a precautionary measure.

Why the young?

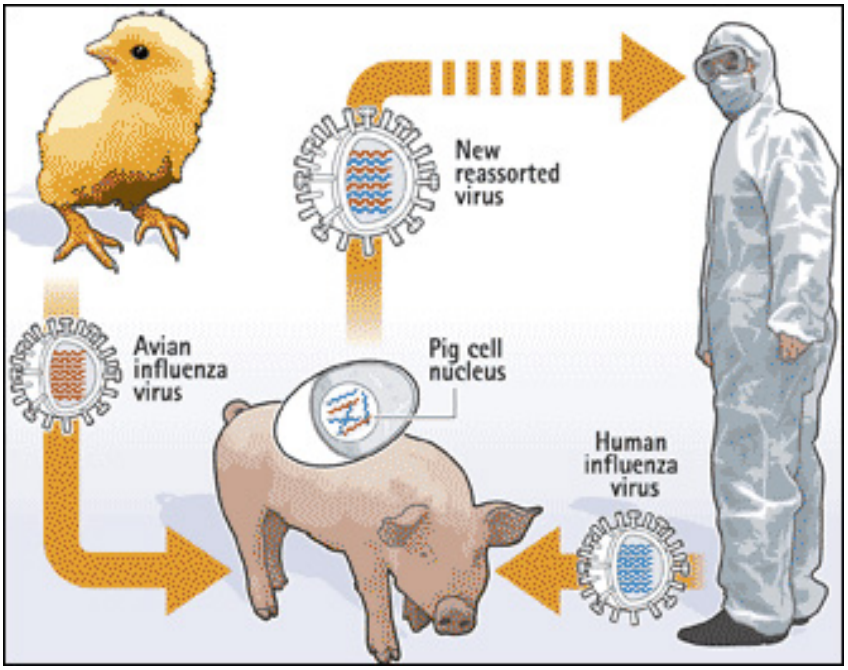
One continuing mystery of the 1918 flu is its lethality among adults of prime health. Conventionally, flu epidemics produce "U-shaped" mortality curves because death rates are highest among the very old and very young[12]. Often, healthy adults in between these age groups are largely unharmed. However, the Spanish flu showed a unique "W-curve" (see below) showing that, in addition to the high infant and elderly death rates, there was a sharp spike of fatalities among middle-aged patients[12]. What could explain this strange finding? One theory is that individuals born before 1889 might have been exposed to a similar (but less deadly) influenza that gave them partial immunity to the 1918 pandemic[13]. Thus, the peak in adult fatalities represents those born *after* this older virus infected the population and so did not acquire any degree of immunity prior to the Spanish flu.



"U-" and "W-" shaped combined influenza and pneumonia mortality, by age at death, per 100,000 persons in each age group, United States, 1911–1918. Influenza- and pneumonia-specific death rates are plotted for the interpandemic years 1911–1917 (dashed line) and for the pandemic year 1918 (solid line)[2]

Where Did the Spanish Flu Originate?

With WWI firmly set in the popular imagination, many at the time believed that Kaiser Wilhelm, the last German emperor whose policies helped to instigate the war, orchestrated the devastating flu pandemic[14]. Others have suggested that Germans landing in U-Boats poisoned Boston Harbor with the infection[14]. Additional theories included side effects from poison gas or even environmental factors unrelated to the conflict such as insects or stagnant air[14]. Though these explanations are unlikely, the answer to this question remains unresolved. Tantalizingly, a previously unknown illness with flu-like symptoms was observed in swine in the midwestern US mere months before the larger outbreak[15]. Furthermore, a similar disease was recorded by Chinese scientists half a world away[16,17]. As the Spanish flu wore on, the swine near sites of human infection were also plagued with symptoms similar to the human disease[16]. What could be the relationship between swine and human influenza infection? Statistical analysis has shown that the pigs could not have been the source of the human infection. On the contrary, the human virus appears to have been transmitted in the opposite direction: from humans to pigs[16]. The relationship remains pertinent today as pigs have also featured in the emerging threat of avian influenza. Since they are able to be infected by both human and avian strains of flu virus, they may serve as a "mixing vessel" where a more deadly human strain could evolve by mingling with avian virus also present in the pig's system[18].



FROM BIRD VIRUS TO HUMAN VIRUS Currently, H5N1 cannot be transmitted efficiently from human to human. In order for that to happen, it would have to join its genetic components to those of a human flu strain, in a process known as "reassortment." Historically, pigs have been good "mixing vessels" for this process, since pig cells--unlike bird or human cells--can be infected by both bird and human viruses. Inside the pig-cell nucleus, genetic segments of the two types of viruses replicate and mix, producing offspring with genes from both parent viruses. What was once a bird flu now has genes that enable it to spread more easily among humans.

Virus Hunters: Tracking the Flu

The desire to understand how the 1918 virus had been so deadly motivated a number of extraordinary expeditions that attempted to uncover the molecular remains of the Spanish flu. The story begins in 1949 when University of Iowa microbiologist Johan Hultin first learned about the fate of Brevig, Alaska[19]. In 1918, this small village of Inuit Eskimos had been decimated by the flu, with 72 of 80 villagers dying in the epidemic[19]. It is entirely possible that the villagers contracted the influenza virus in the nearby town of Teller where both outsiders and the Brevig natives brought supplies for trade[19]. Suspecting that he might be able to isolate the virus responsible for the pandemic from permafrost-preserved remains, Hultin journeyed to the Brevig site in 1951 to collect tissue samples from a mass grave[19]. Unfortunately, the exhuming yielded no viral particles[19].

Almost four decades later, the next person to take up the search for the 1918 influenza virus was not a scientist by training but a geographer named Kirsty Duncan who managed to track down a site in Norway where seven Spanish influenza victims had been buried in permafrost[20]. Traveling to the tiny town of Longyearbyen with a group of recruited virologists, Ducan located the graves, but after six years of work was ultimately unsuccessful in obtaining viral samples[20].

This difficult task would eventually fall to scientist Jeffrey Taubenberger who, in the 1990s, was trying to sequence the genome of viral particles from wax-encased specimens preserved by the military during the 1918 pandemic. As it so happened, Hultin would enter the picture again, contacting Taubenberger to propose another attempt at sequencing material from the Alaskan permafrost[19]. Returning to Brevig nearly 46 years after his first visit, Hultin found a sample of well-preserved lung tissue in the mass grave and returned it to Taubenberger's lab[19]. From 1997-2002, the lab team successfully determined the sequence of 8 viral RNA gene fragments, allowing the 1918 strain to then be further characterized. Perhaps more disturbingly, a team of researchers from Mount Sinai Medical School and the Armed Forces Institute of Pathology actually managed to create intact forms of the 1918 virus[21]. The researchers also determined that the virus probably jumped from birds to humans, a finding with parallel implications in the current avian flu scare[21,22].

Site of a mass grave in Brevig Mission, Alaska, where 72 people were buried following their deaths during the Spanish flu breakout of 1918.

Not surprisingly, some researchers have questioned whether the resurrection of the 1918 flu virus was wise, pointing to the possibility of the virus escaping the lab, or, more dangerously, its potential use as a bioterrorism weapon[23]. This became an even greater concern after a group at the Centers for Disease Control recreated the virus from

Taubengerger's sequence data and showed that infected mice had 39,000 times more influenza particles in their lungs after 4 days than is usual for modern day influenza strains[24]. This kind of reconstruction is made disturbingly convenient by the fact that, as a condition for publication, the genomic sequence for the 1918 flu was submitted to a public-access website[23]. Thus, anyone could order DNA with this sequence, and few companies currently screen their customer's purchases[23]. Further, it is not clear at this point that there is a direct public health benefit to studying the virus[23]. While this basic level of understanding about the virus' behavior may ultimately be used in combating a current avian flu epidemic, the risk of another outbreak may outweigh the benefits[23]. However, researchers working on the virus have pointed out that the current human population has at least some resistance to the 1918 influenza since the modern day seasonal variants are partially derived from the Spanish flu[23]. Fortunately, this means that an outbreak of the 1918 flu might not cause the same impact now that it did nearly a century ago.

Animal Studies in Infectious Disease Research

Among the many laboratory animals used in scientific studies, ferrets have had a key role in the history of influenza virology. During the 1930s at the National Institute for Medical Research in the United Kingdom, scientists were attempting to develop a vaccine for influenza but were having no luck with the various organisms with which they tried to test this treatment[25]. Every animal tested was unable to be infected by human influenza virus, making it impossible to test the effectiveness of the vaccine. Finally, they turned to a group of lab ferrets who were being employed in another research project[25]. This time they succeeded, not only by switching animals, but by injecting the virus nasally[25]. Over time, changes in influenza virus in blood samples collected from laboratory ferrets illuminated how the pathogen changed each year, and even today such samples are used to gauge the current state of influenza virus during the development of flu vaccines each autumn[26].

But *How* Deadly Was the Spanish Flu?

Despite the death toll of the 1918 epidemic, the fact remains that, of those infected, most recovered from their symptoms in 3-5 days[16]. Additionally, scientists at the time studied the bodies of the deceased, and even though viruses could not yet be isolated, bacterial infection was discovered in the lungs of many Spanish flu victims[27]. This raises the important question: were secondary bacterial infections at least partially responsible for the lethality of 1918 influenza? If true, we may now be better prepared for another influenza epidemic because our ability to treat secondary bacterial infections (with antibiotics) is stronger now than in 1918.

Flash Forward: Avian Flu (1997-2006)

Scientists and public health officials now have more pressing concerns than viruses from the turn of the century as a new killer flu virus appears to be on the rise. Among the thousands of viral species that currently exist, few are as widely feared as the Highly Pathogenic Avian Influenza Virus (HPAI), also known as H5N1. This threat story began in the poultry farms outside Hong Kong.

Out of the Marsh in Hong Kong

In March 1997, the chickens near the Mai Po, Hong Kong marshes were dying in droves, apparently struck by a nightmarish disease causing "body-wide hemorrhage," until "the birds literally melt[ed]"[28] The infection – recognized quickly as HPAI – spread to nearby farms until the outbreak was finally contained by the destruction of the diseased stocks[29]. The government workers culling the chickens did not initially fear infection since the virus was believed incapable of spreading from birds to humans[30].

However, this assumption was shattered scant months later when a young boy exhibiting flu-like symptoms was checked into nearby Queen Elizabeth Hospital[29]. The patient succumbed within weeks to the illness, and, fearing the worst, the Hong Kong doctors sent off tissue samples bearing an unidentified influenza strain to researchers around the globe for testing[29]. Scientists in the Netherlands were the first to identify the strain as HPAI[28]. Though there was initial disbelief that the avian virus had jumped to humans, it was later confirmed that the young boy had been killed by a virus that varied by a mere three amino-acids from the avian strain[31]. Molecularly speaking, the bridge between species had been perilously short.

Physical contact with infected poultry appears to be required for human H5N1 infection.

Why was this so surprising? Initially, scientists had believed that avian flu strains could crossover to humans only if the viral genome was intermingled with human strains (e.g. if both pathogens simultaneously infected a pig). However, the discovery in Hong Kong proved that only a slight rearrangement was necessary and, further, that an intermediate organism was not required for the virus to leap from bird to humans[29]. Additionally, this first case and most that would follow would be linked to the handling of diseased poultry[32].

Though it appeared at first that the toddler was an isolated case, several other infected patients were admitted to Hong Kong hospitals in November and December[29]. CDC officials rushed to the scene, fearing the possibility of individuals being co-infected with both an avian strain and a typical human strain available during the regular flu season[29]. If this happened, the normal human strain and the avian interloper might well swap genetic material, leading to a deadlier hybrid with improved capacity to infect humans. So far, the spread of the disease had been limited by its low transmission rate, but such genetic mixing might destroy this barrier to infection.

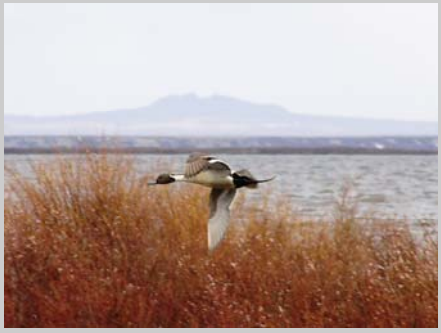
Destroying H5N1-infected poultry.

When poultry began dying *en masse* at the same time as the new human cases, Hong Kong's public health officials decided to take no risks by ordering both the destruction of 1.6 million chickens and a ban on live poultry imports from neighboring Guandong province[29]. As the weeks went by, no more human cases were reported, and it seemed that the situation was contained – at least for the time being.

The Virus Returns

In following years, HPAI crossed health surveillance radars intermittently, cropping up in Asian poultry markets in 2001 and 2002. The virus appeared to be growing stronger though, as tests on laboratory mice demonstrated astonishing lethality[33]. Attempting to contain an outbreak, Hong Kong health officials again ordered that vast poultry populations be slaughtered[34]. In December 2002, outbreaks among fowl in Hong Kong's parks raised the disturbing possibility that the virus had quickly evolved from poultry to infect the previously unaffected wild birds of the region[35]. Subsequently in early 2003, when several members of a family visiting mainland China died of the avian virus, health experts found their earlier fears of a human epidemic renewed[36].

Make Way for Ducklings?

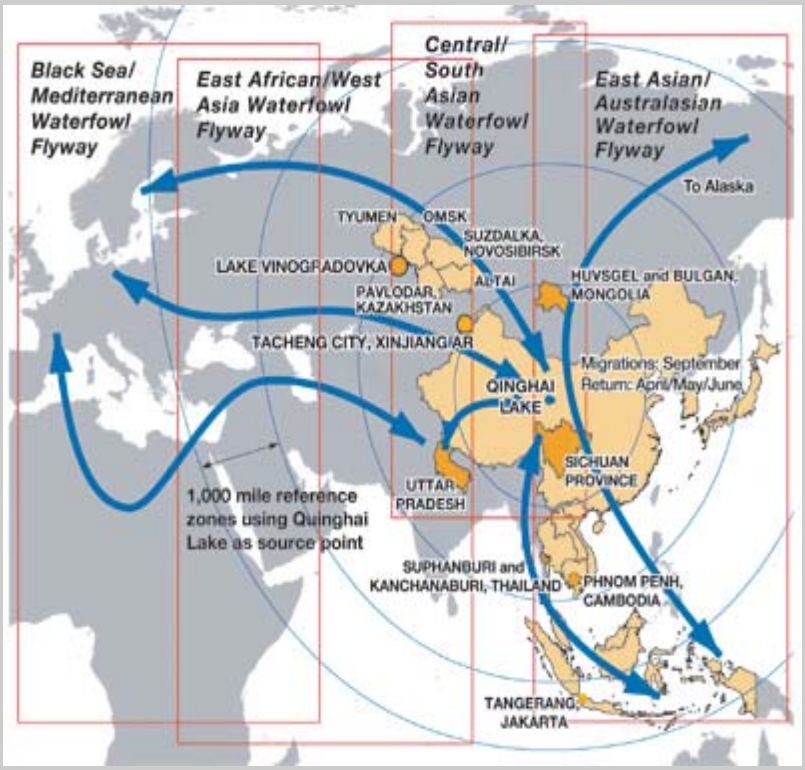


Given that avian flu has originated many times in waterfowl, it is perhaps natural to wonder whether seasonal migration might spread the virus among nations. In 2005, a suggestive clue that the virus might spread on a large scale through water fowl populations was documented at Qinghai Lake in China, where 6000 migratory birds were found dead from HPAI[37]. Before then, cases of avian influenza among such birds were found as small outbreaks in nearby poultry farms[37]. The idea that the virus could spread through migratory routes was bolstered by the fact that viral samples acquired along migratory paths in multiple countries, including human cases in Turkey, were extremely similar to those recovered at Qinghai Lake[37].

Because the virus could be spread through bird droppings, it could be dispersed throughout a migration route without contact ever occurring between the birds and humans along the way. Skeptics of this model point out that previously documented cases of avian flu in humans have all involved sustained contact between the two species (such as the environment in a poultry factory). They contend then that brief exposure to a passing flock might not be enough to generate an outbreak[38].

Additionally, the fact remains that not many birds fly between continents, and certainly those infected with the virus are less likely to be capable of completing such a journey. Further, once they land, infected birds would probably die before significantly spreading the disease. On the other hand, there have been reports of increasing avian resistance to the virus including asymptomatic ducks that carry avian influenza without any outward signs of infection[38]. If this trend continues, migratory flocks might well become incubators for international flu pandemics. However, it's worth keeping in mind that if the flu did jump to humans from a migratory bird population, the birds would become irrelevant. The virus can only maintain

specificity for one species at a time, so by adapting to humans, it would lose its ability to spread through birds[39]. But, of course, at that point, it would be spread by human-human contact.



Waterfowl flyways: Many waterbirds (ducks, geese, swans) migrate between wetlands in the northern breeding areas and southern non-breeding areas and, in doing so, regularly cross the borders of two or more countries. The birds can cover up to 1,000 miles per day. WFP Emergency Preparedness and Response Branch (OSAP), USAID.

Smoke and Mirrors: Covering up the Outbreak

In China and Thailand, early bird flu outbreaks were concealed by public health officials or, in the case of Thailand, the reigning poultry industry magnates. In Thai poultry factories, workers reported that diseased animals were being pushed through the processing lines ahead of veterinary health inspections[40]. It took outbreaks in nearby South Korea and Vietnam to reveal the extent of avian flu in the region[29]. However, like in Thailand, coverups had taken place in Vietnam, and it was only when doctors began investigating the mysterious deaths of several children that they discovered an avian flu outbreak had occurred only months earlier[41]. Even as another epidemic was announced in Japan, Thailand continued to conceal evidence of avian flu, paying farmers with infected flocks to stay quiet[29]. The Prime Minister even televised a broadcast of himself eating chicken to try to calm the nervous public's fears[42]. It was only in January 2004, after two human cases, that a government faction opposed to the Prime Minister forced the avian flu outbreaks to be announced[29]. Indonesia and China's coverups soon became public knowledge as well, making it clearer how widespread the virus had actually become[29].

Gauging the Pandemic

In September 2004, the first human-to-human transmission of the avian flu virus between a Thai citizen and her dying daughter was documented[29]. Amidst this and earlier human cases reported in Asia, WHO officials met in November to assess the risk of a

pandemic and came up with frightening figures. Though estimates between researchers vary, numbers from 50 million to 1 billion fatalities for a pandemic flu have been given[43]. Clearly, the impact is almost unfathomable.

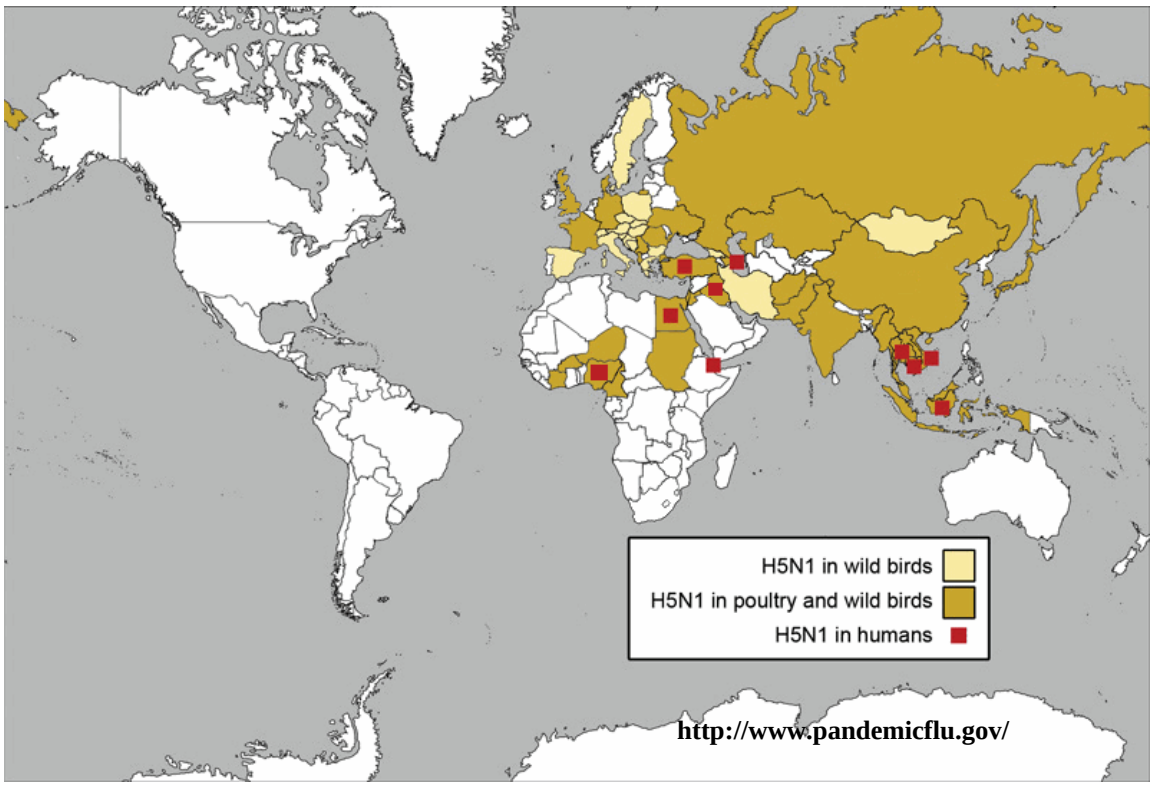
No Vaccines in Sight

Leading influenza virologists warned that the world was unprepared for a flu pandemic and that nations should start stockpiling antivirals (Tamiflu)[44,45]. However, the US was investing more money at this point in stockpiling anthrax and smallpox defenses in the event of a bioterrorism attack[29]. Pharmaceutical companies also shied away from influenza vaccine because it was difficult to produce and generally only worked for one season[29]. The potential disastrousness of ignoring flu vaccine stocks was suggested in 2004 when Liverpool pharmaceutical company Chiron discovered deadly bacteria in several influenza vaccine stocks, resulting in half the US supply of vaccine being lost for that autumn[29].

The Year of the Rooster and Onward

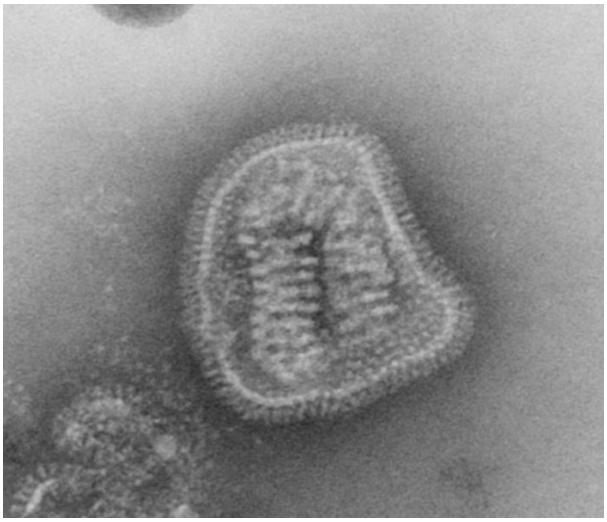
In 2005, additional H5N1 outbreaks in Asia occurred, including another case of human-to-human transmission in Vietnam in March[46]. As the summer and autumn wore on, cases would crop up in Russia, the Phillippines, and even as far away as Greece, Romania, and Turkey[47]. The virus was increasingly global, and, by 2006, a case of transmission between 8 people in Indonesia was recorded – indicating that it was also growing more infectious[48]. The threat of a global pandemic still looms large: the virus remains prevalent among bird populations, and it seems only a matter of time before it becomes widely infectious in humans.

Nations With Confirmed Cases H5N1 Avian Influenza (Feb. 2007)



Biology of the Influenza Virus

If cases of the illness worldwide and news reports are any indication, the H5N1 avian flu virus could be mankind's next greatest threat. But what is this killer, and how does it work?



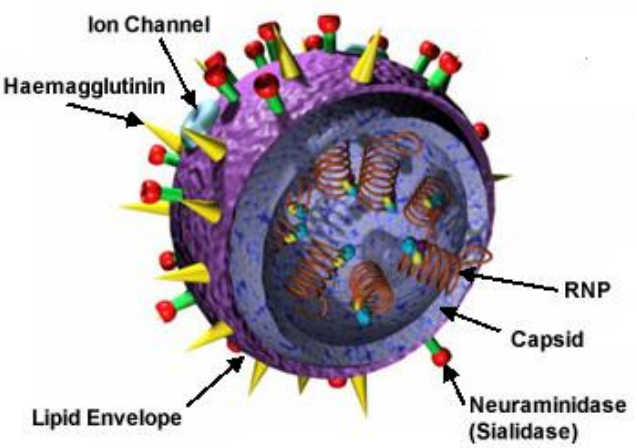
The influenza virus.

Introducing the Influenza Virus

The influenza virus, like all of its viral cousins, is a shell of protein and lipid protecting a nucleic acid core. In, addition to these general features, it has characteristics typical to the *orthomyoxoviridae* family to which it belongs, including a complicated structure of plasma membrane derived from the host cell enveloping sequential protein shells and, finally the virus' RNA genome (remember, as we discussed in connection with HIV, that viruses are classified principally by whether they have DNA or RNA genomes, how they replicate, and whether they have membranes around them derived from a host cell)[49].

Inside the innermost protein shell are eight segments of single-strand RNA containing the genetic instructions for making new copies of the virus. Its shape is

roughly round but could also be elongated or irregularly shaped. Additionally, the virion surface is composed of an outer layer of protein "spikes"[50]. There are two different types of these "spikes": one is the protein hemagglutinin (HA), which allows the virus to stick to a cell and initiate infection while the other is the protein neuraminidase (NA), which cuts up membrane lipids, allowing the virus to enter and exit the cell[51]. More specifically, NA cuts off an acidic molecule from these lipids called salicylic acid, a molecule to which HA might bind instead of the receptor. Further, this cutting is useful both in allowing the virus to enter the cell (e.g., HA is able to attach to the right place to get in), it also helps the virus escape the cell by allowing newly formed virions to disengage from any salicylic acid molecules that are holding them to the cell[52]. In other words, the NA cuts the molecular string that keeps a new viral particle tied to the host cell.



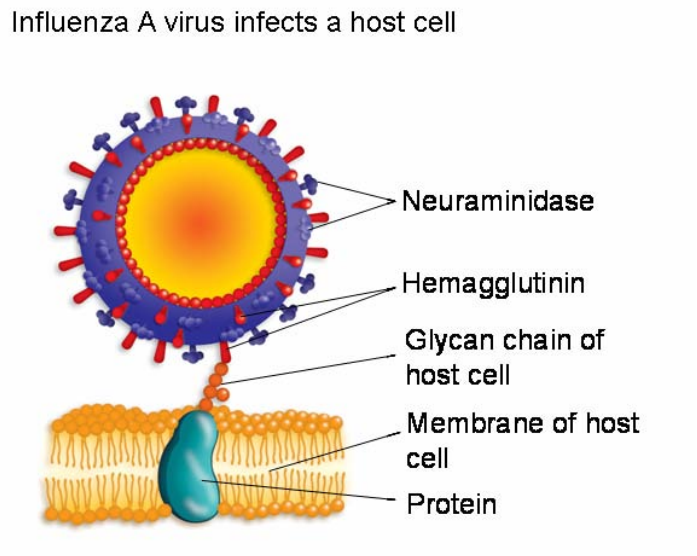
Among all influenza viral strains, there exist many isoforms of HA and NA, each varying in amino acid sequence[50]. However, each individual virus strain possesses only one version of these proteins, and consequently, each influenza strain can be named based on which isoforms are present. For example, the avian flu virus, H5N1, indicates that the virus has HA isoform #5 and NA isoform #1 within its shell. The ability for the virus to evolve different variants of HA and NA on the virion surface helps to elude detection by immune cells.

On a larger scale, influenza viruses can also be classified into three other general classes (A, B, C) based on the presence of different internal proteins. The B and C forms mostly infect children, causing mild respiratory illness[3].The A form, on the other hand, infects a wide variety of higher organisms including birds and mammals, causing more serious illnesses and the potential for epidemics.

Sticky Tabs for Infiltration

Like HIV and the smallpox virus, the influenza virus recognizes particular receptor molecules on the outside of a human cell. In this case, the appropriate receptors are usually found in the cells of the respiratory tract such as the epithelial cell lining of the throat, bronchial tubes, and trachea. The availability and identity of the receptors accounts for why some viruses infect particular species better than others. For example, a chicken has different receptor molecules on the surface of its cells than a human, and the viral HA proteins usually stick more strongly to the receptor of one species than another. The cases of avian flu in which the virus jumped from birds to humans can be explained by viral mutations in the composition of the virus' outer coat, resulting in "stickier" virions that now bind to human cells[53]. There has also been evidence that the avian flu virus is more difficult to transmit between humans because only human cells deep within the respiratory tract have the necessary receptors to the stick to the virus[54].

How does this interaction between receptor and viral HA work? To explain this, it's helpful to remember what receptors normally do, which is recognize molecules outside the cell. The amino acid sequence of a given receptor determines its three dimensional structure, which subsequently only pairs with a specific target molecule. When it recognizes its target molecule, the receptor sets off a cascade of secondary chemical events inside the cell with widely varying results.



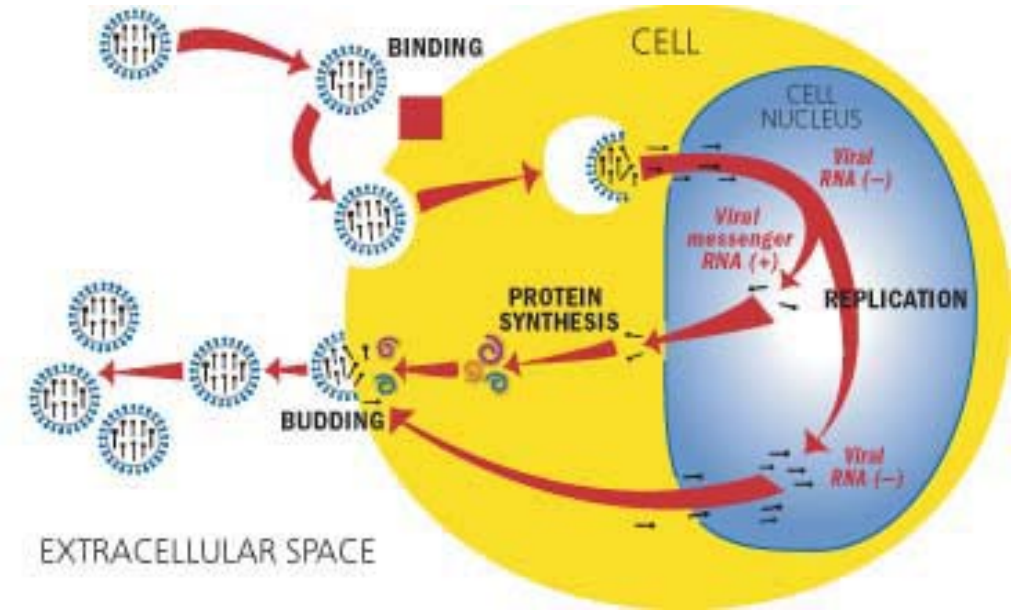
One possible result is receptor endocytosis, a process that allows the bound virus to enter a cell. Once the outer shell of the flu virus latches on to one of these anchor receptors, it is engulfed by the cell[50,55]. Inside this membrane pocket, the virus fuses with the surrounding lipid and slips through this thin barrier into the cell's cytoplasm with the help of the protein M2 found in the protein shell of the virus, which forms a proton/ion permeable channel through the plasma membrane around the virus and consequently lowering the pH inside the endosome by admitting protons[51,56,57]. Here – in an environment that does not immediately destroy fragile RNA – the outer shell cracks open, allowing the viral genome to initiate propagation within the host cell[57].

Reverse Messaging:

Once inside the cell, the viral RNA moves into the nucleus[58]. Here, the viral RNA is replicated by the notoriously error-prone RNA polymerase, making each successive generation slightly different than its predecessor (discussed further below)[59]. Unlike HIV, the viral RNA does not integrate within the host's genome, so infections are acute rather than chronic. When messenger RNAs generated from the viral genome in the nucleus pass into the cytoplasm, the viral proteins can then be synthesized using the cell's own ribosomes or protein-making machinery[60]. Ultimately, the cell will be overwhelmed by the number of viruses inside it and die.

Comprehension Questions:

1. What part of the influenza virus is responsible for sticking to its host cell membrane?
2. How does the virus alter the pH inside the endosome containing it?
3. What nucleic acid is the influenza genome made from?



After binding to a receptor on the cell membrane, the influenza virus moves into the cytoplasm where the virus shell opens, releasing RNA. In the nucleus, viral RNA is copied to messenger RNA which moves back to the cytoplasm as a template to make more viral proteins. Copies of the viral RNA join with the viral proteins to make more viruses, which bud on the outer surface of the cell and seek new cells to infect.

Transmission

Between birds in the wild, the most common route for avian influenza transmission is through fecal matter, since the virus replicates efficiently in the cells lining the intestinal tracts of these animals[61,62]. When this fecal matter enters a pond or other body of water, it can spread the virus to other birds via this tainted water supply[39]. Interestingly, though, carnivores that eat infected birds are not susceptible to infection[62].

The influenza virus is often spread by aerosol transmission.

Interestingly, among domesticated chickens contact transmission seems to be an important route of infection, with studies suggesting that it is more efficient than aerosol transmission[63]. Not surprisingly, the density of chickens in a hutch also affects the rate of viral transmission[63].

The main route of regular, seasonal influenza transmission in humans is aerosol, in which particles of respiratory fluid (saliva, mucus) containing the virus are spread by coughing, sneezing, or other actions that expel the aerosol[64]. If these virus-laden droplets are inhaled by another individual, they can cause infection[64]. However, the influenza

virus can also be spread through physical contact with an infected individual, since the infected person may have touched their eyes, nose, or mouth and transferred aerosol particles to their skin[64]. Finally, indirect transmission is also possible if an infected individual transfers virulent aerosol particles to a surface or object that is then touched by uninfected people[64].

In the case of avian influenza, transmission between birds and humans has usually occurred as a result of close contact between the patient and an infected poultry population. Examples of such contact include regular plucking or handling of such birds, or consumption of infected animals[65]. Unlike the more common seasonal flu, avian influenza in humans has **only** been transmitted between individuals through physical contact, not aerosol. This explains why outbreaks have usually been observed in family groups[65].

Cover up! An early public health poster.

Scientists worry that this may change over time. The virus could accumulate mutations (from normal replication errors) that could confer aerosol transmission of the virus[66]. Alternatively, transmission from the environment to human populations is also possible[65]. If droppings from infected birds enter a water supply, the virus could potentially infect humans[65].

Clinical Symptoms of Infection

Though the molecular means by which all influenza viruses enter a cell are similar, the outward effects of this infiltration can be quite different. Because of this, it is important to distinguish between the three forms of influenza discussed in this unit, because each has different clinical symptoms: they are (1) seasonal flu, (2) avian influenza in humans, and (3) avian influenza in birds.

In the seasonal form, it only takes 48 hours for symptoms to appear after infection. These signs can include headache, fever, weariness, chills, and dry throat. However, the severity of these symptoms depends upon the specific class of virus. C-class viruses, for example, cause mild reactions, while A- and B-class viruses result in more serious infections. Pneumonia, in which the lungs fill with inflammatory white blood cells, is a common secondary symptom of influenza[67]. Both young children and the elderly are at increased risk for serious complications arising from influenza infection[68]. Because its symptoms, such as pneumonia, can be caused by other pathogens, diagnosing

influenza is not always easy (see below). This can be further complicated by the fact that these symptoms can be the result of either (1) the immune response to the virus itself, or (2) the weakening of the immune system by the virus, allowing other pathogens, like bacteria, to infect the body. Thus, while the virus may be the underlying cause of a patient's symptoms, it may be tricky to discern this among the secondary effects caused by simultaneous infections that the virus makes possible.

Human infection with avian influenza includes both the symptoms of the regular seasonal flu mentioned above but also more serious conditions such as Acute Respiratory Distress Syndrome (ARDS)[69]. Unlike the seasonal flu, the period of incubation can be longer, ranging from 2 to 17 days[65]. Lethality has been recorded at 89%, with many victims dying 9 to 10 days after infection[70].

The clinical course of the influenza A virus found in water fowl is different. Since there are two types of the virus – highly pathogenic and lowly pathogenic – the symptoms can either be mild or strikingly deadly. In its low pathogenic form, the virus causes ruffled feathers, decreased egg production, and swelling of the head and neck due to fluid buildup in the animal's tissue[71]. The highly pathogenic form spreads quickly through the respiratory and cardiovascular systems of infected animals, causing extensive bleeding and swelling. Mortality rates can reach 100% in the first 48 hours[71].

What exactly causes such differences in the clinical symptoms of the virus? Recent genomics research has indicated that the human and avian viruses differ by 52 distinct sites in their genomes including genes coding for proteins that induce cell death in the infected host[53]. Other sites encode the proteins responsible for viral replication, and thus may control how fast and in what volumes the virus propagates[53]. Interestingly, similar analysis of the 1918 virus has shown that it is more similar to the avian form than the human form, and these specific points of similarity may suggest what mutations are necessary for an avian strain to become highly infectious in humans[72]. Such comparisons may prove invaluable in eventually combating an avian flu pandemic.

The Ever-Evolving Influenza

While many influenza outbreaks follow the typical yearly cycle familiar in most countries, the pandemic outbreaks of influenza A that happen roughly every decade are caused by a phenomenon known as antigenic shift, in which a new viral strain develops to which the human population has no immunity[67]. There are several proposed mechanisms for how this shift might occur. One is that the RNA components of two different strains of influenza A simultaneously infect a single cell in an intermediate species (for example, with avian flu the intermediate would be domestic poultry or a pig serving as a genetic go-between for humanity and wild water fowl) and, upon replicating, merge into a mix of the two original viruses (see Diagram on following page)[67]. By swapping genetic material in the form of nucleic acids, two species-specific viruses generate an intermediate strain that is better able to cross the species barrier that separates mankind and wild bird. Another theory holds that only a certain number of influenza A strains exist, and that pandemics result when the immunity of the human population to a particular strain has fallen over time[67]. Finally, viruses from animals may genetically adapt so that they are able to infect humans[67]. An example of this is recent research demonstrating that a minor switch in the virus' affinity for a particular cell surface

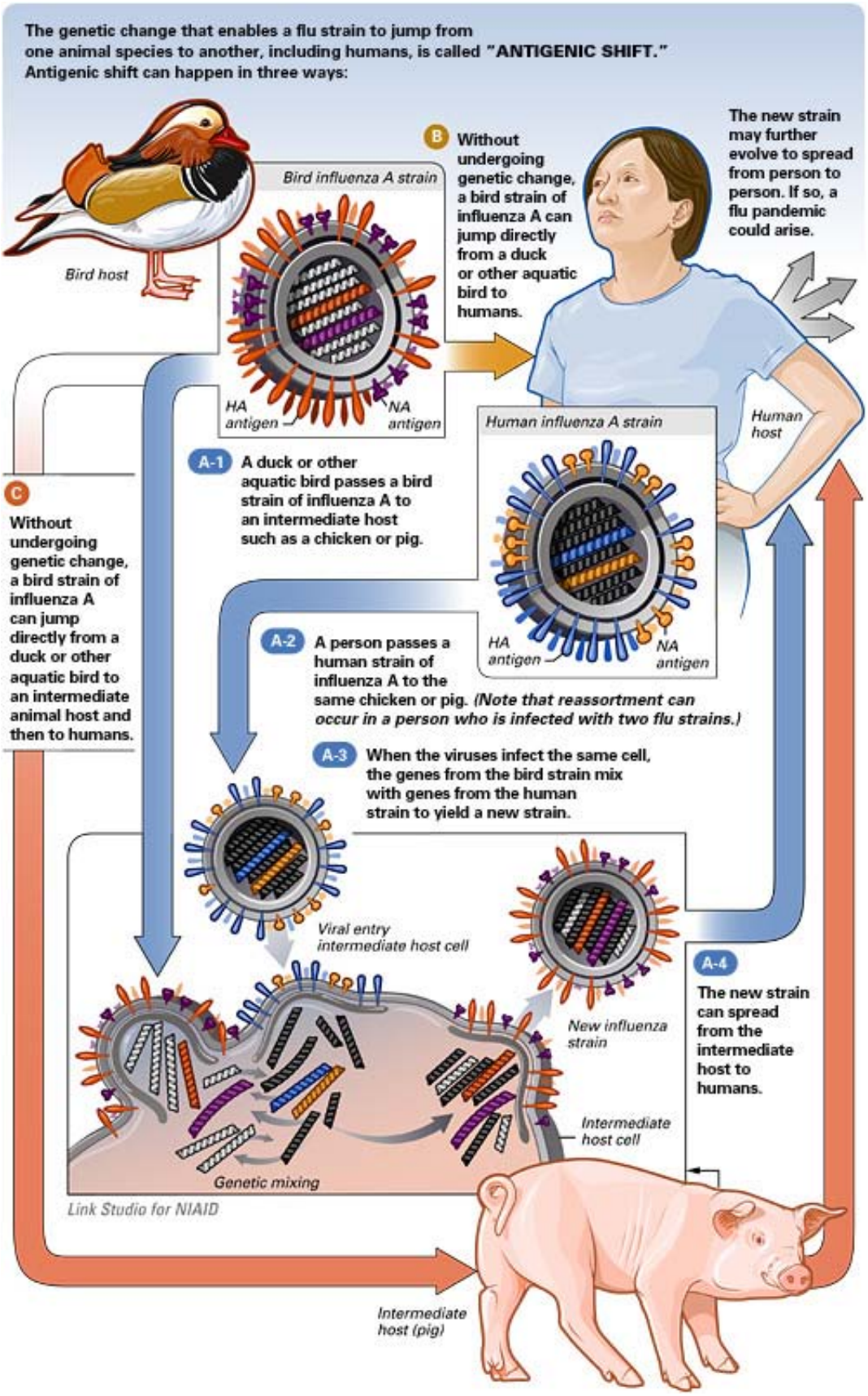
receptor would make it much more likely to infect the cells of humans instead of birds[73]. A mutation generated by the relatively error-prone RNA polymerase when it copies the viral RNA genome might be enough to allow such a change in affinity.

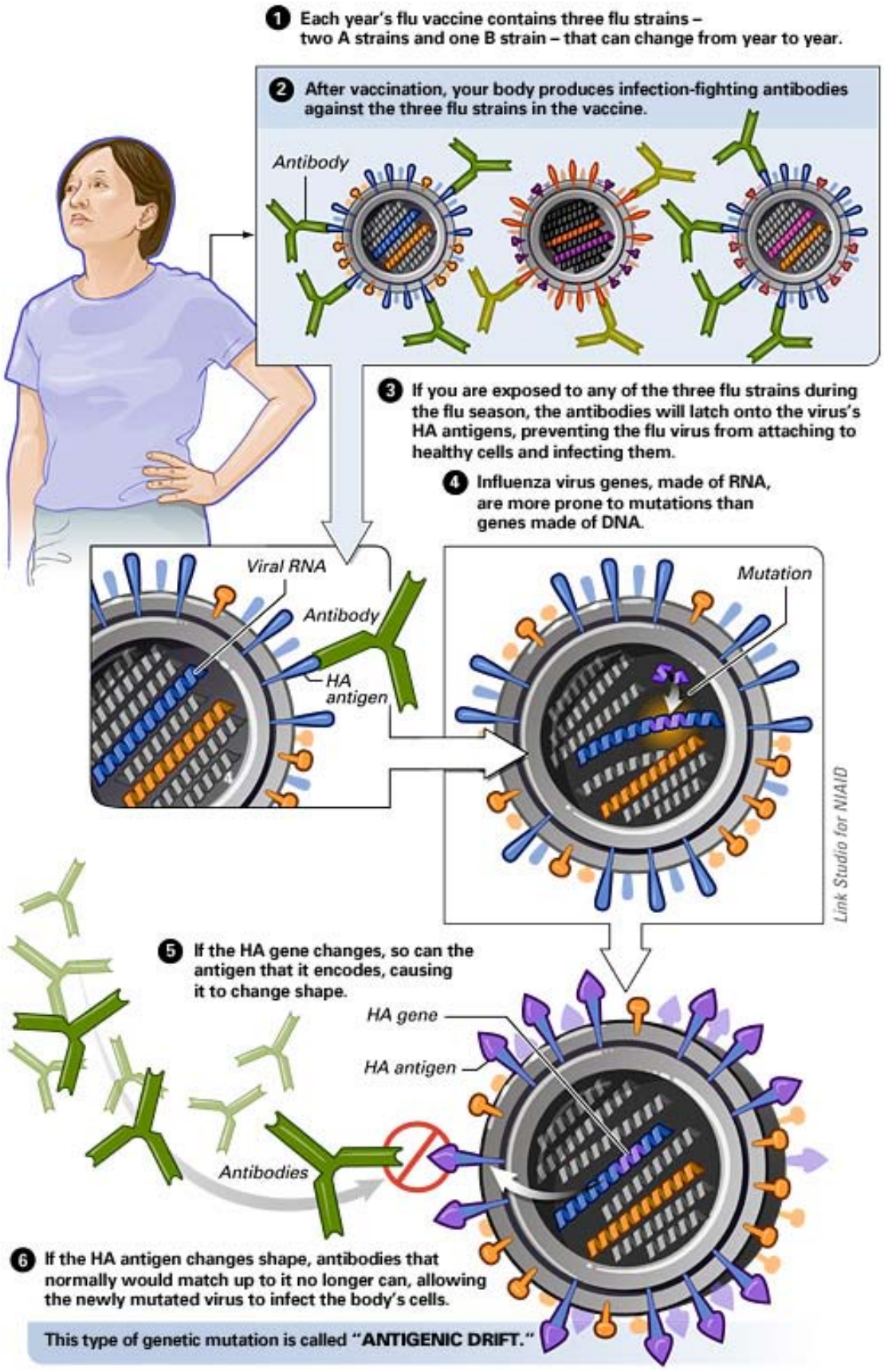
Aside from pandemics, yearly variations in influenza strains can be caused through antigenic drift, in which natural mutations and selective pressure from the host immune system lead to the development of tougher viral strains[67]. In other words, the error-prone RNA polymerase replication mentioned above leads to viruses with slightly different molecular composition, such as HA molecules that cannot be recognized by the antibodies in a host. The host's immune system will destroy all the non-mutated viruses, leaving only this small population of deviant types intact; these are the viruses that then spawn the next "generation" of influenza.

However, even if the flu does become more virulent to humans, a pandemic will be impeded if the virus cannot easily pass between human hosts. As mentioned above, recent studies have suggested that the avian influenza virus prefers binding to cells deep within the lungs, as opposed to the upper respiratory tract[54,74]. Though this usually causes infection to be more deadly, it also means that coughing and sneezing (the means through which the virus is usually spread in humans) are less likely to pass avian influenza between hosts since the mucus droplets from the upper tract that are usually expelled would not contain the virus[54,74].

Comprehension Questions:

1. Give an example of a mutation that would allow an avian influenza virus to infect humans? What enzyme is usually responsible for these mutations?
2. Why is an intermediate animal sometimes required for the influenza virus to travel between species?
3. How is the influenza virus usually transmitted in humans? In wild ducks?


The genetic change that enables a flu strain to jump from one animal species to another, including humans, is called "ANTIGENIC SHIFT." Antigenic shift can happen in three ways:
Bird host
Bird influenza A strain
HA antigen
NA antigen
B Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to humans.
The new strain may further evolve to spread from person to person. If so, a flu pandemic could arise.
Human host
Human influenza A strain
HA antigen
NA antigen
A-1 A duck or other aquatic bird passes a bird strain of influenza A to an intermediate host such as a chicken or pig.
C Without undergoing genetic change, a bird strain of influenza A can jump directly from a duck or other aquatic bird to an intermediate animal host and then to humans.
A-2 A person passes a human strain of influenza A to the same chicken or pig. (Note that reassortment can occur in a person who is infected with two flu strains.)
A-3 When the viruses infect the same cell, the genes from the bird strain mix with genes from the human strain to yield a new strain.
Viral entry intermediate host cell
New influenza strain
A-4 The new strain can spread from the intermediate host to humans.
Intermediate host cell
Genetic mixing
Link Studio for NIAID
Intermediate host (pig)


1 Each year's flu vaccine contains three flu strains – two A strains and one B strain – that can change from year to year.
2 After vaccination, your body produces infection-fighting antibodies against the three flu strains in the vaccine.
Antibody
3 If you are exposed to any of the three flu strains during the flu season, the antibodies will latch onto the virus's HA antigens, preventing the flu virus from attaching to healthy cells and infecting them.
4 Influenza virus genes, made of RNA, are more prone to mutations than genes made of DNA.
Viral RNA
Antibody
HA antigen
Mutation
Link Studio for NIAID
5 If the HA gene changes, so can the antigen that it encodes, causing it to change shape.
HA gene
HA antigen
Antibodies
6 If the HA antigen changes shape, antibodies that normally would match up to it no longer can, allowing the newly mutated virus to infect the body's cells.
This type of genetic mutation is called "ANTIGENIC DRIFT."

Diagnosing Influenza
There are three main ways that influenza virus is diagnosed in the human patient:

1. By obtaining viral samples from throat swabs or similar "mucus" tissue, the influenza virus can be amplified in a system such as a chick embryo, after which a number of chemical tests can be performed to identify the particular strain and class of virus[67].

2. Cells from an infected patient or animal can be tested using fluorescent markers that bind to cell-surface proteins generated in response to infections with specific viral strains[67].

3. The presence of the virus can be indirectly assayed by studying the levels of anti-influenza antibodies present in a patients' blood[67].

Antiviral Treatments

1. One class of therapies called M2 inhibitors (including *amantadine* and *rimantidine)* inhibit replication of the influenza virus by preventing the outer shell of the influenza from releasing its contents into the host cell because the M2 protein is not able to function and lower the pH inside the endosome enough for the shell to open[76]. As the RNA that allows the virus to replicate remains trapped within its shell, the virus is functionally inoperable[76]. Importantly, the influenza A virus has demonstrated an alarming ability to develop resistance to these two compounds during treatment[76]. Many strains, including the H5N1 avian flu strain, now exist that cannot be effectively treated by amantadine and rimantidine[76]. Thus, in its latest recommendation, the CDC advises that these compounds not be used in the US until the resistant strains of influenza have declined[76].

Antibiotics and Influenza: Risk of Overprescription
In the rush to protect ourselves from health scares such as avian influenza, it is important not to let the treatments become more problematic than the threat they are meant to combat. An example of this is the prescription of antibiotics to treat flu symptoms, largely because bacterial pneumonia can result from influenza infection. However, it is crucial to remember that antibiotics treat *bacteria*, not *viruses*; furthermore, since there are also viral forms of pneumonia, it is possible to misprescribe antibiotics for an infection that antibiotic therapy cannot treat. Ineffectiveness is only one part of the problem, however. Overprescribing antibiotics provides bacteria more exposure to antibiotics, and the selective pressure to develop antibiotic resistance (a phenomenon described in the TB unit). While avian flu seems the most threatening concern, drug-resistant bacteria have actually also caused health scares in Hong Kong[75]. In short, it is necessary to remember that the wrong cure can be as harmful as many diseases.

2. Another strategy is demonstrated by antineuraminidase compounds (also known as NA inhibitors including *zanamavir* and *oseltamavir)*[76]. Tamiflu falls in this class of antivirals, which target the NA proteins in the outer shell of the virus[76]. Besides helping the virus to get inside a host cell (as was discussed earlier), it turns out that the NA proteins also help the HA proteins to target the host cell receptors[50]. Without NA proteins, the HA proteins might accidentally bind to complex sugar molecules outside the cell instead of the membrane itself. Normally, the NA proteins degrade these sugars, thus keeping the HA free and ready to bind to the host cell receptors[50].

The Tamiflu Controversy:
In an ideal world, pharmaceutical companies would be motivated solely by the desire to improve the health of their customers. However, when medical and economic incentives mingle, as they do in the real world, the results are usually less than pure. In a case such as avian flu, the potential financial windfall from possessing the only cure to the pandemic is certainly considerable.

Consider Tamiflu for example…

The active medicinal compound in Tamiflu is oseltamavir. It is derived from *shikimate*, a molecule that is also an essential component of the amino acid synthesis pathway in bacteria. Currently, the standard means of isolating shikimate is from the star anise plant, which grows in only four regions of China. However, researchers are working on genetically modifying *E. coli* so that the chemical can be artificially created[77]. Tamiflu is a neuraminidase inhibitor, so it blocks the entry of viruses into a host cell, and prevents newly synthesized viruses from exiting.

Originally developed by the firm Gilead, Tamiflu was licensed to the Swiss company Roche to allow increased production[78]. However, in late 2005, Gilead accused Roche of not sufficiently promoting the drug, and sought to revoke its license[78]. Roche, however, claims it is doing its job, and that Gilead's objections are unfounded[78].

Licensing has also been an issue among Roche and its international governmental clients. Because the avian flu pandemic is looming, some argued that compulsory licensing (i.e. the drug must be licensed to governments that want to generate their own stocks of vaccine) should be allowed[79]. An international conference in October 2005 debated this issue, and shortly thereafter Taiwan began development of Tamiflu in violation of Roche's patent[80]. Though Roche has since licensed production rights to several firms, the matter of intellectual property versus humanitarian precedent is far from settled[81].

Further, some have suggested that the avian flu panic and production of Tamiflu are linked: by generating hysteria, naysayers claim, corporate leaders are creating a situation in which they can generate millions from Tamiflu sales[82]. Whether such arguments are perceptive, or whether the flu does become a real pandemic, remains to be seen.

Vaccinations

Many are familiar with flu vaccines (i.e. "the flu shot"). There are several different strategies for preventing infection with the influenza virus (many of which are similar to those discussed in the HIV/AIDS module)[67]:

1. Vaccines with live virus (this is the most common form used today)
2. Vaccines using chemically "killed" viruses.
3. Vaccines using only a select portion of the viral coat, such as the NA or HA proteins.
4. Vaccines with live, but inactive forms of the virus.

While this last strategy has proven effective in clinical research, the live virus vaccine is still the standard treatment except for children[83]. The main problem is that the inactive virus must be prepared in a very pure form before it is approved as a vaccine, a procedure that can take up to 2 years to develop[67].

However, influenza vaccine production is a seasonal game. Unlike a DNA virus (like the smallpox virus) whose careful genetic proofreading minimizes mistakes upon replication, an RNA virus' use of the error-prone RNA polymerase allows mutations to propagate from generation to generation. The surface proteins (HA and NA) are almost always changed, allowing viral strains to elude detection by the immune system[67]. Antibodies generated for last year's flu strain may offer no protection against this year's variation. Similarly, a drug or vaccine that takes 2 years to develop will already be a year too late - by the time it is available, the influenza virus will have already mutated from the original strain on which the vaccine was based[67].

Nonpharmaceutical Interventions

While drugs and vaccines are one way to combat the influenza virus, the spread of a pandemic strain could also be limited through effective public health measures. The first principle of such efforts is to remove infected individuals from contact with the rest of the population and preventing neighbors or other contact from interacting with the patient while antiviral treatment is used. In severe cases, the World Health Organization has recommended closing schools and limiting air travel[84]. In the specific case of avian influenza, government officials tried to force poultry to be regularly removed from open markets during the 2001 Hong Kong outbreak[45]. This was meant to minimize the chance that the virus would replicate and spread, though eliminating the sale of live poultry would have solved the problem more efficiently[45]. However, cultural tradition and the economic importance of the live poultry markets in Hong Kong make such a change unfeasible[45].

Comprehension Questions:

1. What viral component(s) would you put in a vaccine? How does this allow the body to fight influenza? Why do you need a new vaccine each season?
2. How is influenza diagnosed? Why do you think you might need to grow the virus in a chick embryo for certain tests?
3. What are two mechanisms by which an antiviral compound can halt the activity of the influenza virus?

Fallout shelters for bird flu?

Activity 1: The Tamiflu Controversy

Students will read the USA Today article "***Avian flu scare has Tamiflu maker navigating minefield"*** and consider the following questions:

1. How is this situation the same as supplying HIV drugs to African nations? How is it different?
2. Is stockpiling Tamiflu a wise strategy? Based on what you know about the biology of the influenza virus, could you propose an experiment to determine if Tamiflu is effective?

Activity 2: In the News

Because avian flu is a developing story, for each week of this unit students will present "clips from the headlines" about bird flu. For each article, students should be able to:

1. State the main argument of the article.
2. Analyze whether the scientific facts in an article agree with their knowledge of influenza biology. If applicable, state how the article might influence policy makers' plans to combat a flu pandemic.

Activity 3: Flu Response

What does the US plan to do in the event of an influenza pandemic? Using the national strategic plan (available online; www.whitehouse.gov/homeland/pandemic-influenza.html), students should each choose one chapter and summarize the key points for their peers. Important question to consider include:

1. What are the main ways to prevent the spread of bird flu?
2. What can be done at a national level? At a local level? Which do you think is more important and why?
3. What are important international considerations?

Activity 4: Modeling an Avian-Human Hybrid Flu Virus

For a global outbreak of influenza to occur, three conditions must be met: a new virus subtype must arise; this subtype must be able to cause serious illness in humans; and it must spread easily from person to person – and continue to do so. The first two conditions have been met with H5N1 human infections. Scientists are working to better understand different influenza A strains in hopes of preventing the spread of this virus.

Here, students will learn about the structure of influenza A viruses, how they replicate in a cell, and how, when a person is simultaneously infected with an avian and a human virus, their RNAs can mix. Using the provided worksheet, students make avian and human influenza virus models, infect a lung cell, and make a hybrid virus that has some avian and some human RNA segments. They will see that the hybrids have surface proteins from both the avian and human influenza A strains, and that unfortunately, the

human immune system does not quickly recognize this combination of surface proteins. Courtesy of NOVA Science NOW.

Activity 5: Bird migration

Students will research and draw bird migration patterns around the world to determine feasibility of interhemisphere transfer to the US. Resource for teachers: Birds and Influenza H5N1 Virus Movement to and within North America" (provided).

Activity 6: Videos: "Bird Flu: How Safe Are We?"; "Secrets of the Dead: Killer Flu"

Activity 7: Journal of the Pandemic

Students will create fictional journals, role-playing as inhabitants of the United States during the 1918 flu. They might even look up their own family history and see if any of their relatives died of influenza during that period.

Activity 8: Movie: "Contagion"

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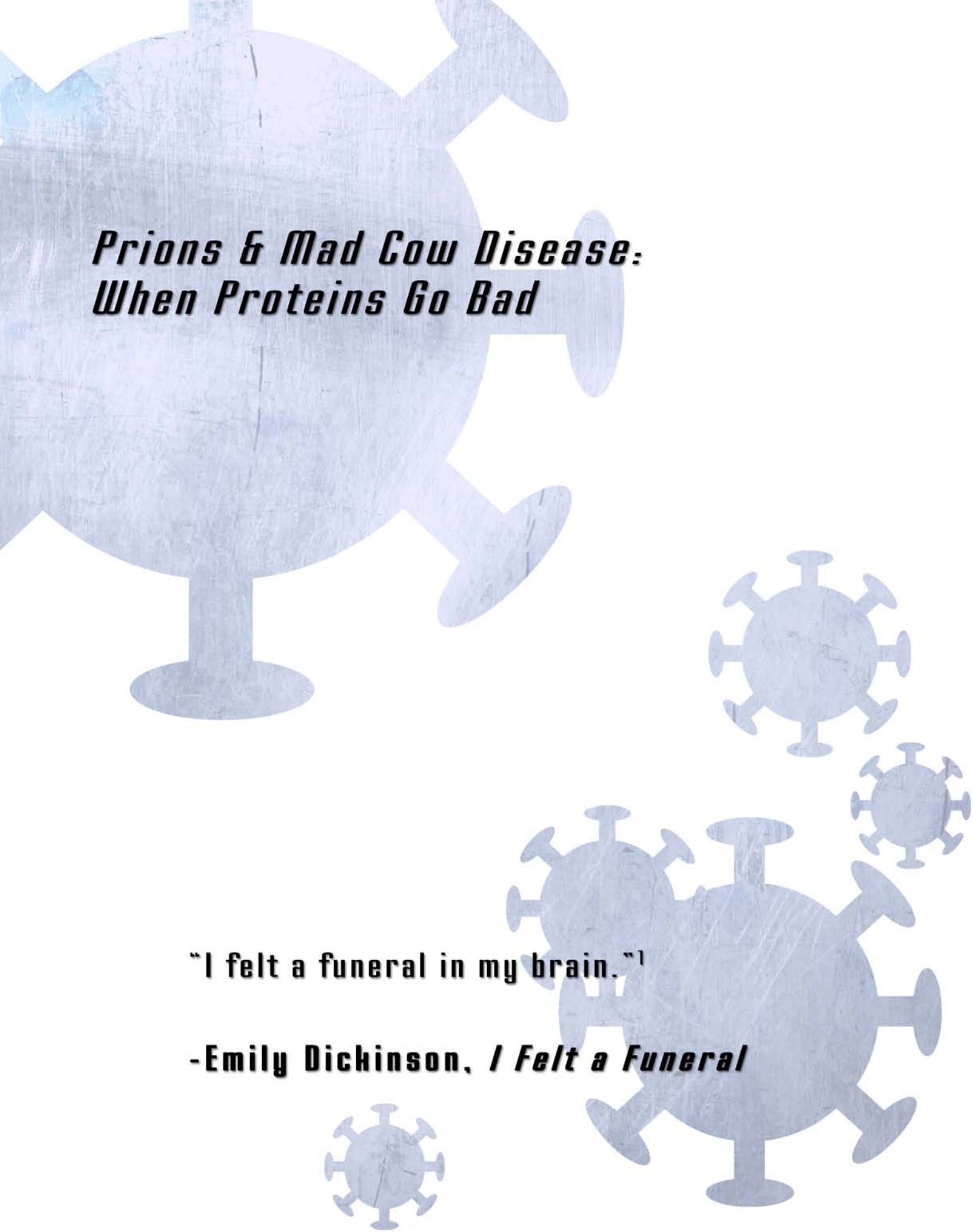
84 2006-2007.

Prions & Mad Cow Disease: When Proteins Go Bad



"I felt a funeral in my brain."[1]

-Emily Dickinson, *I Felt a Funeral*

“Tangled Threads”

Mrs. K’s four students stand shivering as they follow their instructor and a Pierpont representative across the street from the white facade of the hotel to the grey and glass mountain of the pharmaceuticals plant. Despite the icy blasts that continue whipping through the narrow gap between the two buildings, their guide continues his speech.

“None of the facilities over here are actually over five years old,” he says. “Western development was impossible in Malkinagrad before the Soviet Union collapsed, and it took a decade after that for biotech industry to decide it was even a *good* idea to set up shop here.”

“So why *did* Pierpont move here?” asks Angelica.

“Well,” says the representative, “besides the declining property values – which makes a place like this a lot cheaper to build than it would be in, say, the US – we have an unprecedented chance to make a difference here. Any antibiotics we develop can go directly towards alleviating TB in the gulags, and a whole population in desperate need of the medical resources a firm like ours can provide.”

Waiting for the wind to pick up enough to obscure his comment, Lang leans over to the other students, whispering: “I bet they don’t mind the lack of FDA regulation either. I wonder what actually goes on in this place.” Fallon shudders (and not merely from the cold).

If Mrs. K is similarly skeptical about the charitable activities of their sponsor, she keeps them to herself. Instead, she examines the immense building before them, whose hundreds of windows must equate to an enormous heating bill in this weather.

“We heard about a bird flu outbreak last night,” she says, “has it been resolved?”

“Oh, that.” The representative pauses. “I think that the government had all the birds destroyed. We haven’t heard of any cases of human infection yet, but, then again, we have extremely strict quarantines. There’s not much that gets in or out of here without us knowing, microbial or otherwise. Which reminds me, you’ll have to leave most of your things in the waiting area when we get inside – can’t risk contamination.”

Up ahead, they can see the yawning profile of the park garage underneath the building along with the slender ramp leading up to the main entrance. Somewhere below, the sound of several engines reverberates loudly enough to be heard about the wind. The icy gusts pick up again, howling down the street as the group walks more quickly towards the door of the Pierpont facility.

* * *

After a lengthy check-in process, the group stands in the gigantic lobby of the Pierpont building, lit far overhead by a series of oblong fluorescent bulbs. Curiously, the music is the same as the hotel across the street. On the far side, near a bank of long windows, is a line of palm trees. Leaving the company representative and their teacher behind, the four students wander over to this odd garden. Fallon removes his baseball cap to examine the bark up close before noting the waxy texture of the leaf – it’s fake, though convincing from a distance.

"Class," calls Mrs. K, her voice echoing against the ceiling, "don't wander off. They have a tour planned for us." Shuffling back towards the center of the lobby, the students approach just in time to hear the representative begin.

"This entire chamber is climate-controlled," he starts. "There's a set of enormous heaters on the roof that circulate air through the building – you can probably take your coats off, in fact."

"And yet, they have fake plants," mutters Maxine.

"Yeah . . . and for all the money they're supposedly spending on health care, the surrounding area is a dump compared to this place," adds Lang, making sure to stay out of earshot of their tour guide, who continues rambling about the facility."

"If you follow me this way, we'll proceed to the mezzanine, where most of the low-biohazard laboratories are located. The elevator here is actually powered by the same engines as a Boeing 747 – it can get to the top of the building in 20 seconds."

"I wonder if it will take off," snickers Angelica – unfortunately, Mrs. K hears and gives her a disapproving glare. The elevator door closes on the group, and, with a jolt, begins its climb.

* * *

The group walks through seemingly endless halls of sterile laboratories, studying topics they've never heard, and which their guide makes little effort to explain. None of the technicians seem to notice them, adding to the mechanical – and unnerving – atmosphere of the facility.

"The labs in this corridor are all devoted to Marfan's Syndrome . . ." the representative continues. Though Mrs. K continues to nod politely, her students can tell that she, too, is becoming bored – it's the same expression she wears when one of their presentations in class runs too long. Finally, though they recognize a phrase in the representative's speech.

"This over here is our prion laboratory . . ."

"Wait! Did you see prions?" asks Maxine, blushing a bit when she realizes how loud her exclamation was.

"Yes . . . you know something about prions?" asks their guide, seemingly amazed that any high school student knows anything about the research here.

"We studied Mad Cow disease and all the human ones in class," she replies, rattling off a list of illnesses. "Kuru, Creutzfeld-Jakob . . ."

"It's *Y*akob," corrects Lang. "Like *Y*usef."

"Thanks," says Maxine, shooting him an irritated glare before turning back to the attendant. "What exactly are you working on?"

"A lot of things," he says. "Actually, this is one of Pierpont's big areas of research right now. On one hand, we're simply interested in determining what makes a disease-causing prion different from the kind that is normally in a cell. More generally though, we want to know how information might be transmitted through proteins, just like it is through DNA." He pauses for a moment, then adds, "If you're looking for something to study, I guarantee you there'll be a Nobel Prize for the lucky person who discovers protein-based inheritance."

"Can we see inside?" asks Angelica.

The attendant looks at his watch. “I suppose . . . we have about an hour before I have to get back to work. Let me see . . . we can’t really go in the tissue prep room – as your probably know, it’s really hard to inactivate prions, so there are all sorts of safety precautions. But here, we can duck in and look at the modeling facility for a second.”

He leads them into a room very different from most of the laboratories they’ve passed. On the far wall is a small bench with the standard collection of bottles and tubes, but most of the room is taken up by computers.

“What is all this stuff?” asks Fallon, looking at one of the screens where what appears to be a piece of colored string periodically twists into odd shapes before unfolding again into a straight line.

“Well, as it so happens, a lot of the work we do here is theoretical,” says their guide. “That loop there is a rather simple representation of a prion protein – the program you’re watching is trying to predict what it will look like folded.

“Folded? You mean the three-dimensional structure that a protein possesses?” asks Maxine. On the screen, the thread unrolls again and repeats the folding process, undergoing a slightly different pattern of creases to reach the final product.

“Yeah….hard to believe such a small thing – such a small difference – can cause such horrible diseases,” says Angelica.

“Indeed,” agrees the representative. “But that’s what we have to figure out. How does a slight misfolding in a protein cause a brain to self-destruct? And how do we cure it?”

Questions:

1. **What are prions? Why are they different than any other pathogen described in this course?**

2. **There is actually new evidence that <u>chaperone proteins</u> might be involved with prion transmission[2]. Find out why.**

3. **How might you suggest treating prion disease? What about halting its transmission?**

Introduction:

Would you believe that infectious disease can also be transmitted in the absence of the typical players like viruses, bacteria, or parasites? Scientists have been shocked to discover that proteins, comprising the fundamental structure and function of every living thing, could also be involved in transmissible disease. Malformed proteins, including prions, are the topic of this unit. The term "prion" was coined by Nobel Laureate Stanley Pruisner in 1982, as an acronym for "proteinaceous infectious particle"[3]. Though the last two decades have revealed more about the peculiar behavior of these misshapen proteins, much remains unknown. Distinct from bacteria and viruses, prions continue to challenge our deep-seated biochemical assumptions about infectious disease.

Goals:

By the end of this unit, students should be able to:

1. Describe the prion hypothesis, including arguments for and against its validity.
2. Distinguish between prion diseases in animals and humans.
3. Describe possible therapeutic strategies based on biochemical knowledge they have acquired in previous units.
4. Make judgments about the safety of eating beef relative to other everyday risks.

Why Prions? The Itchy Sheep Hypothesis

To begin, it may seem puzzling that scientists would even suspect the existence of misshapen proteins as the cause of infectious disease. What led to this theory in the first place? The answer, it turns out, is sheep. For centuries, shepherds have sometimes noticed peculiar behavior in their flocks – a formerly healthy sheep will begin to lose weight and have difficulty walking about its pen. The sick animal also begins to rub against fence posts or other rough surfaces as if to alleviate an unbearable itch. The disease – *scrapie* - actually gets its name from this scraping movement[4, 5]. Eventually, the animal becomes paralyzed and succumbs to starvation[6].

The scraggly profile of a scrapie-infected sheep. Photo credit:

Traditionally, scientists thought the disease only occurs in sheep with a inherent genetic weakness that leads them to develop scrapie when exposed to the prions we will discuss later[7]. However, recent research suggests that, in addition to this genetic predisposition, scrapie can be caused by truly infectious prions that effect even flocks thought to be genetically resistant to scrapie[8]. Generally, sheep acquire the disease by ingesting prion particles in their environment[9]. Also, these particles can be transmitted between members of a flock, or prenatally from mothers to their young[10].

Scrapie In Action

To view a movie of a scrapie-affected sheep, please visit:
http://www.neurocenter-bern.ch/scrapie_e.shtml

After the sheep's death, brain dissection would show that the animal's brain and other neural tissue were decayed[5]. Because the sheep's nervous system malfunctions as the individual neurons die, sensory signals are unable to successfully make their way from the skin to the brain. As a result of its deteriorating nervous system, the scrapie-infected sheep does not respond normally to stimuli such as pressure and begins to uncontrollably itch[5].

But what had caused the sheep's brain to deteriorate in the first place? When scientists first began to study scrapie, they had immense trouble tracking down an origin. Hoping to isolate a pathogen from tissue in scrapie-infected sheep, they employed a common test called filtration. As we have learned throughout this course, viruses are much smaller in size than bacteria, parasites, and other infectious pathogens. Consequently, a tissue sample (like blood) from a bacterial infection would be non-infectious if it was first filtered through a membrane with pores small enough to allow only viruses to pass. In other words, the filtrate (or solution collected after filtration) would no longer contain the bacteria or parasites because the pores would have obstructed their passage. Using this technique, scientists found that the filtrates remained infectious even after being passed through the membrane, suggesting that bacteria or larger parasites were not to blame. Perhaps viruses were the cause?

However, viruses need genetic material such as DNA or RNA to replicate. Using this knowledge, scientists next treated scrapie-infected tissues with chemicals that destroy nucleic acids and then examined the tissue's infective nature[3]. Surprisingly, the tissue remained infectious, suggesting that a viral cause was unlikely as well. If the cause was not bacterial nor parasitic nor viral, what could it be?

Despite a lack of an identifiable pathogen, scrapie can still be transmitted by blood transfusion between sheep, suggesting that *something* is there. One clue to a cause, however, may be the misshapen protein clusters that are always present in the neural tissue of scrapie-infected sheep. In fact, these clusters must be present in a tissue sample in order to be infectious[5]. So, scientists next applied chemicals that destroy proteins to the scrapie-infected tissue. The result: the tissue could no longer transmit scrapie. With this treatment, the infectious nature of the unidentified "pathogen" had been lost[5]. But could this mean that a protein could be to blame?

The Prion Particle

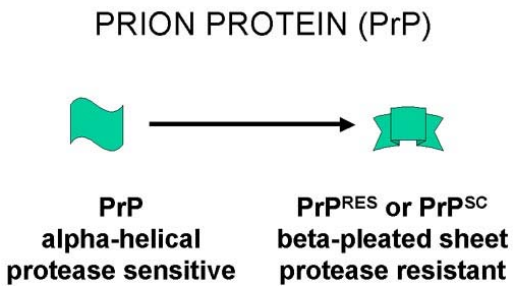
Originally, the idea that a protein could cause disease was thought impossible. The infectious agent responsible for scrapie was simply known as the "scrapie agent." Scientists did not know what it was, but assumed that a bacteria or virus was involved[11]. The most that researchers were willing to admit was that experiments showed a protein

was *associated* with this "scrapie agent," without suggesting that the protein could cause disease by itself[11]. It took a bold leap by future Nobel Laureate Stanley Prusiner to suggest that, indeed, a protein could cause disease, and he created the term "prion" to describe this unprecedented pathogen[3].

The Mysterious PrP

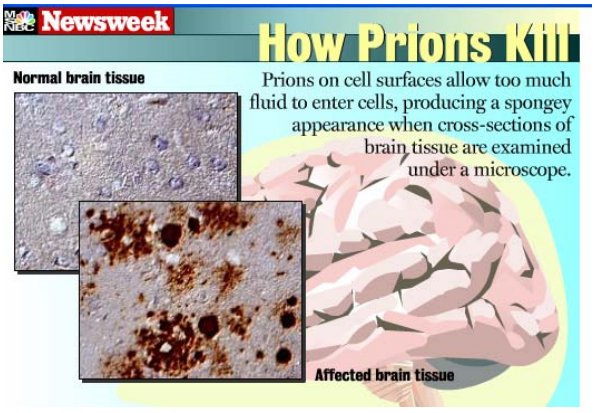
Normally, the prion protein (or PrP) adheres to the plasma membrane of neurons by a sugar anchor[12, 13]. In this position, it is thought to help transmit chemical signals between adjacent nervous cells as a part of the normal process by which a sensory experience (be it touch, taste, smell, or otherwise) is turned into the electrochemical language read by the brain[14]. When unneeded, PrP is degraded by proteases, the protein-cleaving enzymes discussed in previous units[15].

However, normal PrP can undergo a structural change to generate a disease-causing form (called PrP^{sc} for its ability to now cause scrapie). Though the amino acid sequences of PrP and PrP^{sc} are identical, the two proteins differ significantly in their three-dimensional structure. Experimental evidence has confirmed this: PrP consists mostly of alpha-helices while PrP^{sc} is composed mainly of beta-sheets[15]. Once formed, a PrP^{sc} can propagate the formation of additional PrP^{sc}s[16]. Just like a single unstable domino can knock over an entire domino line, one PrP^{sc} can convert normal PrPs into aberrant forms[16]. At each step, the new PrP^{sc} can convert more PrP just as each fallen domino can knock over other dominoes.



From normal to infective: the prion protein's deadly structure transistion.

Importantly, because of this structural change, PrP^{sc} can no longer be harmlessly cleaved by proteases and consequently begins to accumulate[15]. Because of their abnormal shape, PrP^{sc} proteins tend to stick to one another, and over time, the PrP^{sc} molecules cluster to form long chains called amyloid fibers[7]. These protein clusters are toxic to neurons, causing neuronal death and ultimately the neurodegeneration seen in scrapie-infected sheep[7]. A nice animation of this process can be viewed at: http://mt.gov/liv/animalhealth/diseases/BSE/pathology.asp.



The pathology of a prion-infected brain.

The idea that a change in protein structure causes disease is not new. As we saw in the malaria unit, sickle cell anemia is the result of misfolded hemoglobin. What is unexpected about PrP, however, is that different forms of the disease may be caused by different "misfoldings" – in other words, the 3-D structure of PrP "encodes" its disease-causing properties[15]. In a world where information of this kind is traditionally maintained in DNA or RNA, this is a radical notion.

Beyond Nucleic Acid: A Protein-Only Disease?

Going back to our investigation, how did scientists determine that proteins could be infectious? Remember the evidence thus far[15]:

1. The infectious agent is smaller than a bacterium.
2. The infectious agent is not destroyed by chemicals that disrupt nucleic acids.
3. Chemicals that destroy proteins are able to render infectious scrapie tissue non-infectious.

The idea of a protein disease was actually first proposed in 1967, predating the discovery of prions by many years[19]. At this time it was also declared absurd, but further evidence suggests that proteins may in fact be able to copy themselves in a limited sense. One theory for the formation of PrP^{sc}, for example, is that a PrP^{sc} molecule acts as a template for a catalyzed conversion of PrP to PrP^{sc} – a similar idea in some ways to the replication of DNA[16, 20,21,22].

L-Forms: Slippery Bacteria

An important point to keep in mind is that prion theory (in terms of infectious disease) is still just a theory. Many experts still doubt the concept that proteins alone can cause scrapie, mad cow, and related human disease. Every experiment is open to interpretation.

One such case is the filtration of bacteria. Many bacteria cannot pass through a membrane because of their rigid cell wall. However, some bacteria (including one class called *L-forms)* lack this wall or have an unusually flexible wall[17]. This consequently allow L-forms to pass through membranes that would usually exclude them[17]. Intriguingly, such L-forms of tuberculosis-causing bacteria are immune to chemicals that destroy nucleic acid and are invisible to the body's immune system – just like prions[18]. Thus, at this stage, a bacterial theory of mad cow is still conceivable.

Given that PrP^{sc} is always found in scrapie-infected sheep, the disease could be caused by an infectious protein[11]. While this is currently the leading theory for the cause of scrapie and other prion diseases, many scientists have a hard time accepting that a protein can be infectious, and believe that a small and peculiar virus is responsible for these diseases. Proponents of this latter theory claim that the nucleic acid is simply tightly bound to PrP – thus immune to nucleic-acid destroying processes - but lost during the purification process[20]. Another argument against the "protein-only" theory is that there exist different "strains" of scrapie in laboratory mice, distinguished by incubation time and the tissues where most symptoms occur[20]. These types of differences are usually encoded in viruses and bacteria through genetic differences. On the other hand, prion-theory supporters claim that differences in PrP folding account for these different "strains"[16].

The main argument against the "protein-only" hypothesis is that it is so unprecedented. No life form, including small infectious agents such as bacteria and viruses, has ever been discovered that does not use nucleic acids to store information. Thus, it seems impossible that an infectious agent could exist without DNA or RNA. However, there exists much evidence to support the "protein-only" hypothesis[15]:

1. By changing the amino acid sequence or three-dimensional conformation of PrP, scientists can generate different version of prion diseases in laboratory animals.
2. PrP^{sc} converts PrP to PrP^{sc} in a test tube.
3. Mice lacking the PrP gene cannot become infected with scrapie, but transplanting brain cells with normal PrP into these PrP-deficient mice allows them to develop the disease. Similarly, experiments with mice and hamsters have shown that mice genetically modified to produce the hamster form of PrP can develop scrapie if infected with hamster PrP^{sc}. Otherwise, hamster PrP^{sc} has no effect on the mice.

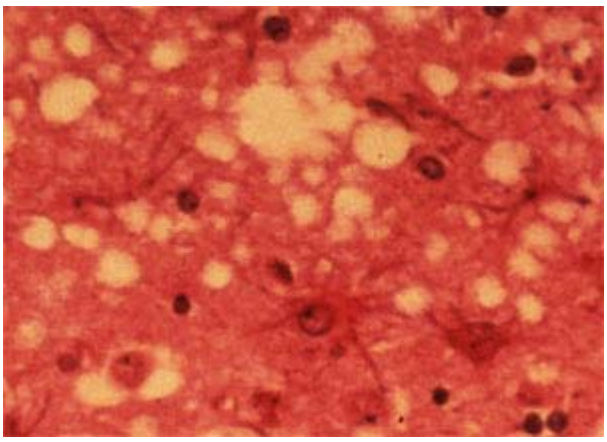
The experiments in (3) raise the question of whether prion diseases could be genetic as well as infectious. If possessing a certain gene allows a mouse to acquire a prion disease (and lacking that gene makes it immune), it appears that the disease might have some genetic basis. However, it has been shown that sheep that are genetically capable of acquiring scrapie do not do so in a sterile environment[15]. Therefore, it would appear that external factors cause the disease while genetics can only predispose a sheep to develop scrapie.

Prion Diseases

We have just examined what we know about prion diseases through studies on scrapie. However, prion diseases occur in many other animals including humans.

What is TSE?

One important term to define is TSE, an acronym for *Transmissable Spongiform Encephalopathy* and the medical term for prion diseases. Encephalopathy means "disease of the brain," and spongiform refers to the fact that, in prion diseases, the brain deteriorates in a pattern that resembles swiss cheese or a sponge. Thus, TSE is simply a fancy term describing a brain-deteriorating illness that can be passed between animals or humans.



Damage from prion protein gives the brains of affected cows a sponge-like appearance.

Animal Forms of TSE

While the classic example of a prion disease is scrapie, such diseases exist in other species as well[23]. Perhaps the most famous instance in the current media is Bovine Spongiform Encephalopathy (BSE), the disease more commonly known as "Mad Cow

Disease"[23]. Another notable animal TSE is Chronic Wasting Disease (CWD), which afflicts hoofed mammals in North America including deer[24]. However, unlike BSE, it is not known to be transmissible to humans. Prion infections have also been observed in mink, goats, and other captive animals[24].

Human Forms of TSE

In general, human prion diseases cause progressive loss of motor control, dementia (severe loss of memory or mental function), paralysis, and wasting (a great loss of body and muscle mass, in this case). TSE can also be accompanied by secondary infections including pneumonia.

There are many human diseases caused by prions. The most common is Creutzfeldt-Jacob Disease (CJD), a neurodegenerative disorder that occurs in both spontaneous (non-genetic) and genetically linked forms[25]. Even in the more common spontaneous form, CJD is relatively rare, afflicting only about 1 person in a million each year worldwide[25]. As with other neurodegenerative conditions like Alzheimer's disease, older patients are more likely to develop CJD[25]. For example, beyond age 50, the incidence of spontaneous CJD is approximately 3.4 per million individuals a year[25]. The spontaneous disease accounts for 85% of all reports of CJD[25].

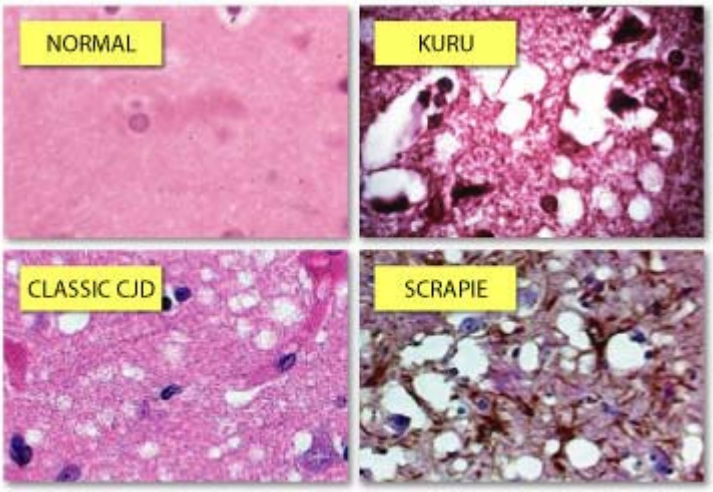
The rarer genetically-linked form of CJD accounts for only about 15% of cases. It is classified as autosomal dominant condition (i.e. it is due to a mutation in body cells, not germline cells, and only one copy is required to have a negative effect)[25]. Like familial Alzheimer's disease, genetically linked CJD affects younger individuals than the spontaneous disease. Genetically-linked CJD is further subdivided based on clinical symptoms, including forms such as Gerstmann-Straussler-Scheinker (GSS) and fatal familial insomnia. GSS is extremely rare, with an incidence of between 1 and 10 per 100 million per year, and is believed to be caused by a point mutation in the amino acid sequence of PrP[26, 27]. Notably, there are some populations demonstrating higher than average incidence of CJD, like Libyan-born Israeli Jews, for whom the incidence of the disease is 30 per million per year[5]. A genetic defect can also cause the rare familial fatal insomnia, a prion disease that usually afflicts related individuals. What begins as an inability to sleep progresses to loss of mobility reminiscent of Parkinson's disease, followed by a loss of mental function and, within 1-3 years, death. Like other prion diseases, there is no known cure[28].

Kuru victim.

There is also a variant form of CJD, denoted vCJD, which was first described in 1996 in the United Kingdom in connection with mad cow disease[5]. There is now strong scientific evidence that the same agent that causes mad cow also is responsible for vCJD in humans, especially since the only

common trait that the vCJD victims in Britain appear to share is that they ate beef[5].

Another notable human prion disease is Kuru, a neurodegenerative disorder propagated among cannibals in Papua New Guinea through the practice of eating the brain tissue of deceased family members[5]. Kuru is responsible for first bringing media attention to prion infections in the 1950s[5].



Thin slices of kuru, classic CJD and scrapie brain tissue under the microscope reveal holes that were formed after misfolded prion proteins kill neurons in the brain.

Modes of Infection

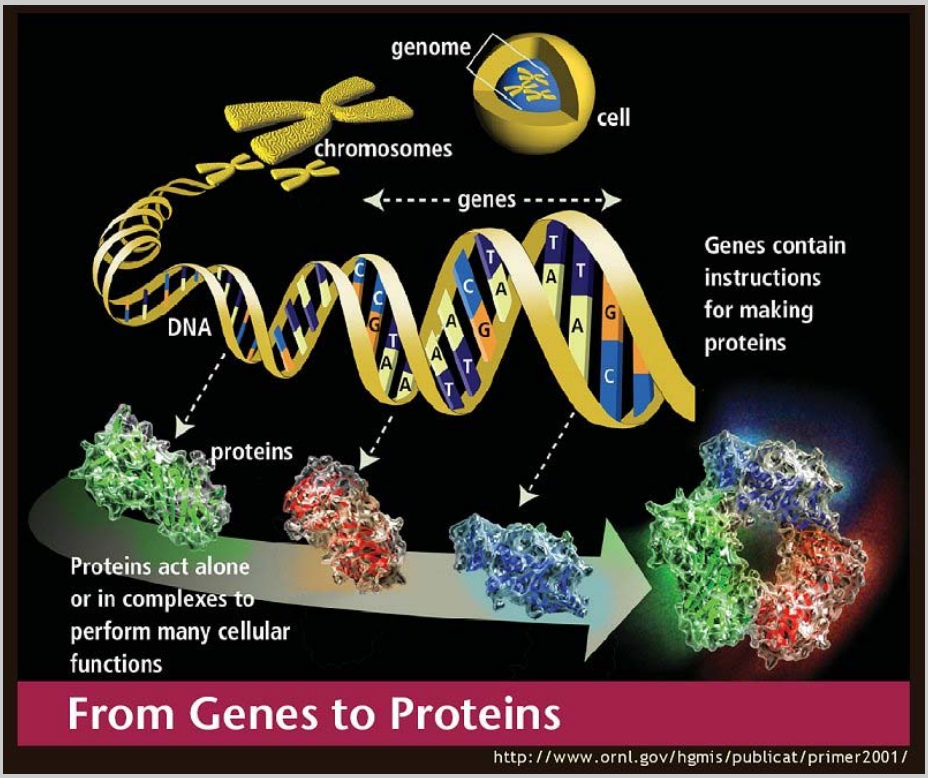
It is thought that there are at least two ways that human prion diseases are transmitted - through infectious agents from animals (such as ingested infected tissue) or through genetic heritage[5]. However, there have been recent fears of a third route: through infected tissue transplants (e.g. corneal transplants) or surgical instruments[5]. In fact, prions cannot be destroyed by boiling, alcohol, acid, standard sterilization methods, or radiation[29]. Prion-infected brains that have been sitting in formaldehyde for decades can still transmit spongiform disease![29] Standard surgical procedures may have to be modified in the future to accommodate protection from prions.

The ingestion of infected meat can result in prion transmission. But what is the relative risk?

The Central Dogma Upended?

In the last century, one of the great mysteries of the cell remained: how do cells pass on their inherent traits to progeny? In that vein, what type of molecule was responsible for storing our "genetic" information? Given the wide variation between individuals of a species, it seemed logical that an "inheritance factor" would be structurally complex. At the time, proteins – with their twenty amino acids and complex shape – seemed the leading candidate. Conversely, nucleic acids (with only 4 nucleotides) seemed much too simple to contain the code of life – until the landmark discovery of the genetic code by Watson and Crick in 1953. Further, Watson and Crick declared a "central dogma" of molecular biology: DNA is a template for RNA (by transcription), while RNA is a template for protein (by translation). Information could **only** flow in this order (DNA-RNA-protein). However, Crick did note at the time that "Our knowledge of molecular biology . . . is still far too incomplete to allow us to assert dogmatically that [this restriction] is correct," and further that "just one type of present-day cell could carry out any of the three unknown transfers [of protein to RNA or DNA, and RNA to DNA] would shake the whole intellectual basis of molecular biology." Interestingly enough, he noted: "There is, for example, the problem of the chemical nature of the agent of the disease scrapie . . ."[30]

Thus, even at this early juncture, it seems scientists recognized the peculiarity of prion diseases – and that they might upset the order of the central dogma.



Koch's Postulates Revisited

A main reason dissenters remain in the prion debate is that Koch's postulates – the principles that govern whether an agent is infectious – have not been fulfilled for prion diseases. The main difficulty is that prions cannot be grown in pure culture like bacteria or viruses[31]. Thus, scientists cannot infect laboratory animals with pure cultures of prions and demonstrate infectiousness in the manner demanded by Koch's postulates[31].

The Final Word?

> **"***Protein, so far as we know, does not replicate itself all by itself, not on this planet anyway. Looked at this way, the [prion] seems the strangest thing in all biology and, until someone in some laboratory figures out what it is, a candidate for Modern Wonder"*[32].
>
> *-Lewis Thomas*

How Prions Came to Be: A Brief History

The Origins of Scrapie

The origins of scrapie are unknown. Perhaps sheep have always been afflicted by the illness in low numbers, and the disease simply went unnoticed by human herders. What is understood with greater certainty is that incidences of scrapie were recorded during the 18th and 19th centuries when the exportation of sheep from Spain coincided with an increased occurrence of scrapie[33]. Furthermore, inbreeding sheep to improve wool quality, a practice encouraged by economic incentives, also increased the prevalence of scrapie[33]. When inbreeding ceased, scrapie levels simultaneously fell. The following 1759 document from Germany describes the disease[33]:

> *Some sheep also suffer from scrapie, which can be identified by the fact that affected animals lie down, bite at their feet and legs, rub their backs against posts, fail to thrive, stop feeding and finally become lame. They drag themselves along, gradually become emaciated and die. Scrapie is incurable. The best solution, therefore, is for a shepherd who notices that one of his animals is suffering from scrapie, to dispose of it quickly and slaughter it away from the manorial lands for consumption by the servants of the nobleman. A shepherd must isolate such an animal from healthy stock immediately because it is infectious and can cause serious harm to the flock[34].*

Based on this passage, one can conclude that scrapie is infectious in sheep, but not in the human servants eating the diseased animals[33]. So far, these conclusions have proven accurate (remember that all cases of vCJD in Britain were linked to *beef* consumption).

Discovering Kuru

Drawn to epidemiological mysteries across the world, the American physician Carleton Gajdusek came to Papua New Guinea in 1957 to investigate Kuru, a peculiar disease that caused the brain to progressively deteriorate over a 6-12 month period[35]. The effects of the disease had been devastating, decimating whole villages in the remote mountainous terrain where the Fore people lived[35]. The name Kuru means “trembling in fear” in the Fore language, describing the loss of motor function from the disease, but also perhaps the dread with which it was associated[29]. Peculiarly, the disease did not progress like other infections. The members of the tribe who developed the illness showed no fever or other signs of inflammation such as swelling, pain, and redness of the skin[29].

Given that the disease seemed to afflict family members, scientists originally thought it was genetic[35]. However, closer analysis revealed that the disease was too prevalent and too deadly to be genetic because such a serious condition would be quickly eliminated through natural selection in such a small population as the Fore villagers[35]. Seeking another explanation in brain tissue from deceased Kuru victims, Gajdusek tried to infect small laboratory mammals with the disease but was unsuccessful[36, 37]. It was

only when he repeated his experiments in chimpanzees that he was able to observe the disease, as the primates developed Kuru roughly three years after inoculation with tissue from dead humans[38].

It eventually became clear that the disease was transmitted through the Fore practice of mortuary cannibalism[35]. Following the death of a community member, the individual's family would dismember them, preparing food from their flesh and internal organs[35]. This practice was reflected in the epidemiology of the disease. Kuru had the highest prevalence among women, children, and the elderly because these individuals were the primary participants in cannibalism and therefore most likely to eat prion-infected "morsels" such as brain tissue[35].

In order to curb the epidemic, cannibalism was banned in New Guinea after 1959[39]. However, due to the long incubation period of Kuru, there continued to be cases among the elderly into the 1960s probably because they contracted the disease much earlier in life[29]. For his discoveries, Gajdusek was awarded the Nobel Prize in Medicine in 1976[36]. At the time, he hypothesized that the disease was due to a "slow virus" – a virus characterized by an abnormally long incubation period[38]. While this theory now seems unlikely (at least according to the prion hypothesis), Gajdusek's work on Kuru did stimulate work on other Kuru-like diseases, including scrapie and CJD.

Stanley Prusiner Describes Prions

From his first encounter with a patient afflicted with CJD in 1972, Stanley Prusiner was hooked on the problem of "slow virus" infections – the term used to describe CJD, Kuru, and scrapie at the time[40]. Working at the University of California at San Francisco, Prusiner voraciously pursued the available literature on CJD and its related illnesses and collaborated with other scientists to try to purify the agent responsible for these peculiar diseases[40]. Their efforts met with failure, and Prusiner lost his experimental funding from the Howard Hughes Medical Institute due to the seeming hopelessness of his work[40]. However, after finding support from other sources and continuing his research, it occurred to Prusiner that his inability to purify a virus responsible for scrapie might be because there was no virus to be found[40]. Perhaps he had uncovered an entirely new form of infection! He published a controversial paper in 1982 in which he coined the term "prion," and controversy followed[3]. Nevertheless, time and the continuing inability of other scientists to purify nucleic acids responsible for scrapie, along with further evidence in support of the "prion hypothesis," led to gradual acceptance of Pruisner's ideas and acknowledgement of their importance. For his contributions to the field, Pruisner was awarded the Nobel prize in Medicine and Physiology in 1997[36].

Stanley Prusiner, professor of neurology and biochemistry and biophysics at UCSF.

Mad Cow Outbreak

Beginning in 1986, Mad Cow Disease, formally known as BSE, has become a major health concern in the UK and the world at large. The outbreak of a neurodegenerative disorder among British cattle in 1986 prompted veterinary researchers to investigate the matter: what they discovered were similarities between the disease killing Britain's cow's and human prion illnesses such as CJD and Kuru[41].

Incidentally, the disease was being spread through the cow's feed which contained prion-infected brain tissue from other members of the herd. In response to this discovery, UK officials placed regulations on feed content. However, it appeared the regulations had been applied to laxly, and in 1996, inadequate precautions in British slaughterhouses were linked to a variant of CJD that had appeared in patients much younger than was typical for the disease[42]. Later, epidemiological studies suggested that the disease had originated from an antelope imported from South Africa to a British safari park in the 1970s[42]. Scientists believe that the infected antelope was ground into cattle feed, transmitting the prions responsible for vCJD to herds of cows and ultimately to humans[42]. The disease led to a ban by the European Union (EU) of beef import from Britain that lasted for 10 years[33]. That same year, the British government began destroying herds most at risk for BSE infection[42]. Though the public was terrified by the spread of vCJD (which had now killed 165 people in Britain), the danger of infection turned out to be lower than first thought[43]. Only individuals with a particular mutation in the PrP gene, present in about 40% of the population, can develop the disease[44].

Though it was originally hoped to be limited to British cattle supplies, BSE began cropping up in herds worldwide. It was first identified in France in 2000, followed soon by Germany and the remaining countries in the EU[45]. Infected cows were later discovered in the US and Canada, leading experts to criticize government officials for not looking hard enough for the illness[46]. In response, both the US and Canada have begun massive quarantines and testing programs[46]. On the scientific front, researchers have begun genetic studies to engineer BSE resistant cattle. A promising recent find shows that prion-resistant goat fetuses can be created by deleting the PrP gene[47].

Activity 1: A Cure for vCJD?

If you were a research scientist, how would you go about finding a cure for vCJD? Using the pharmacological principles that have been introduced in this course, propose a possible treatment to prion diseases. Your solution should address the following points:

1. What step in the molecular/cellular mechanism of the disease would you target? Why?
2. How would your solution interact with the body's immune system?
3. If you are proposing a vaccine, what would you vaccinate with and why? Why might vaccination be less effective than other strategies?
4. How would you deliver your treatment to the patient?
5. How would you know that your treatment was working?

Activity 2: What are the Odds?

What is the risk of contracting mad cow disease? Should you be worried? The students' assignment is to figure out these statistics, and do a comparative analysis. Are they surprised by the results? Why might mad cow seem more dangerous than many more common threats, even though the risk of infection with a prion disease may be extremely low? A helpful starting point may be the following website from Mayo Clinic:

http://www.mayoclinic.com/health/infectious-disease/ID00003

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Epiloque:
The Cure

Epilogue: The Cure

The lights have dim, followed with the clinking of glassware as the table settings around the room are cleared away. As if on cue the music begins, a throbbing bass that could belong to a hundred different songs. In response, girls in sparkling dresses begin dragging tuxedoed boys to the center of the room, and the dance commences.

"Don't even *think* about it."

Lang continues to glare at some point along the far wall, while Angelica, cut off before she had even asked him to dance, instead reaches for the water pitcher again.

"Some fun you are," she answers, watching the last of the liquid drip into the fluted glass. The four classmates sit at a table large enough for eight – for some reason, the prom committee had put their group in this corner, even though there appear to be smaller, unoccupied tables closer to the front of the room. Maxine had made a joke about being "quarantined" when they entered, but as the night wears on, it becomes less amusing somehow.

"You know," says Fallon, attempting to break the silence that falls between the four, "this really isn't that bad. I heard last year there that a pipe broke in the kitchen and ruined half the food. That's why they switched this year."

"Remind me why I'm here again?" asks Lang.

"Oh come on," answers Fallon, "you wouldn't want to miss your senior prom, would you?"

"Wanna bet?" returns his surly classmate. Fallon sighs, but realizes that Lang being here at all is something of a miracle. A lot had changed this year; the two had hardly spoken five words to each other for the past three years of high school, before their class with Mrs. K. Fallon would never have imagined that they would be sharing a limousine to prom – even if Lang seemed less than pleased to be here.

The fact that all four of them would be here would have been even more surprising to Fallon a year ago. Sitting on the other side of Lang, the two girls are an interesting contrast, Angelica in a black gown with silver thread, and Maxine in pink. Somehow, he suspects that in the past, Maxine would not have been caught dead with him here. Noticing him, she smiles back, brushing a stray blonde curl over her ear.

Yes, a lot had changed.

* * *

Their layover in nowhere had been mercifully brief. The spores on the Hamburg flight ended up being a previously unidentified but harmless strain of yeast. While their scientific guide at Pierpont had attempted to explain how exciting this discovery was as he accompanied them in the airport shuttle the day after their tour, but the evolutionary significance seemed far less important to them than the fact that they could depart for Paris.

While the conference had proven memorable – many of the speakers had written articles or books they had read in class – the city itself was the highlight of the trip. During their free time, the four had climbed the Eiffel Tower, and had several pictures looking down over the Parisian streets. Mrs. K, who was afraid of heights, had remained below. Though she was unaware of it, her absence was partly responsible for what had happened that day.

It was a weak tourist season, and only a few other parties milled about the upper decks of the tower. Finding themselves more or less isolated, and freed for a moment from the eyes and

ears of their instructor, the four classmates had begun reminiscing about their experiences together the year. Even Lang, usually so unfriendly, had laughed out loud several times at particular recollections and had gotten quite involved in the conversation.

Gradually, the conversation drifted away from the class itself, to other memories of high school. Fallon had surprised himself, sharing stories he had previously only told his soccer teammates – somehow over the course of the year, these three acquaintances had become friends.

He forgot who it was who suggested the idea – maybe it was Angelica, she seemed to enjoy these kinds of things. In any event, the four had finally decided, as a kind of game, to each write down something on a piece of paper that they had never told anyone else, and exchange it with another member of the group. Additionally, they agreed they would only read them after graduation. Fallon's note had almost blown away over Paris while he kneeled, trying to think what to write. Starting, erasing, and starting again, he tried to write something eloquent and failed. Finally, giving up, he wrote only one line:

Maxine, I've had a crush on you for three years.

Folding their respective notes, they had quickly exchanged them – he with Maxine, Angelica with Lang. Then, imposing upon a fellow tourist, they had taken one last group picture above the Parisian skyline, the wind sweeping their hair while the sun began to set. It remained a desktop image on Angelica's computer for years to come, and when they asked, she would tell her college dorm mates that it was the happiest day of her life thus far.

* * *

The class had concluded soon after their return home to North Carolina. Since this was an experimental curriculum, the Pierpont team wanted time to evaluate feedback before the end of the school year, meaning that they finished their last session weeks prior to finals in their other courses. Mrs. K had given them each a book as a keepsake on that last day, and told them it had been the most memorable class she had taught. As the bell rang, they found themselves all a bit sad, realizing they would never again sit around this room, learning about the next great threats to humanity.

Though none had been part of the same social circle beforehand, the four had become an unexpected social circle in the weeks following the end of Biology 301. Fallon and Lang had begun eating lunch together, with Maxine, then Angelica, joining them. They became so used to each other's company that it had seemed almost a foregone conclusion that they would all go to prom together – and here they were.

* * *

Angelica finally manages to pull Lang out of his seat, leading her reluctant date by the hand towards the dance floor, and leaving Maxine and Fallon alone for a moment. Once the other two are out of earshot, Maxine leans over, whispering:

"I have a confession to make."

Fallon, not expecting this, looks puzzled. "What? Is my tie crooked again?" He'd made the mistake of trying to tie the bow himself, and it had been coming loose all night.

"I read it."

It takes him a moment to understand her implication, after which his face turns the color of her dress. Before he can stammer out an explanation though, she continues, smirking:

"Don't worry – I already knew. You're not exactly subtle, you know." She pauses briefly. "Cute, but not subtle." With a knowing wink, she takes his hand, following Angelica and Lang. Fallon follows, relieved and excited at the same time.

"Funny, we'll all be at the same place next year, you know?" he says, as his eyes adjust to the dimming lights. Through some strange coincidence – and, at least on his part, standardized test scores that were far better than others would have guessed – the four were all headed to the same college in the fall.

"I know," she answers, spotting Angelica within the crowd. "And we'll have four more years to remind Lang about how ridiculous he looks right now," she adds, cringing at the sight of her classmates attempting to dance. "I feel embarrassed just watching him."

Fallon laughs, wondering how Lang will feel to be made fun of for a change. "He's going to hear about this all the way home tonight."

"Honestly though," she continues, "he's changed a lot lately. I thought I'd never see the boy smile, but now he spends so much time telling jokes – even if they're always at everyone else's expense."

Fallon remembers something Mrs. K had said often. "Laughter is the best medicine – I guess it cured his personality."

Maxine laughs. "Laughter and love: they're the cure for everything." He blushes again, and she rolls her eyes. "All right, let's see if you can dance any better then your friend over there."

The music plays on, and, at least tonight, life isn't a disease, just a condition.

"it will not be simple, it will not be long
it will take little time, it will take all your thought
it will take all your heart, it will take all your breath
it will be short, it will not be simple"
-Adrienne Rich, *"Final Notations"*

The End

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Tuberculosis

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C – 6

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C – 7

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Small-Pox/Anthrax

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D-22
National Library of Medicine/NIH
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National Library of Medicine/NIH
http://www.nlm.nih.gov/exhibition/smallpox/sp_vaccination.html

D-23
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ID#:2841

D-24
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CDC ID#:
4593

D-28

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E-5
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Alberto Cuadra, Harvard School of Public Health
http://www.hsph.harvard.edu/review/rvw_winter06/rvwwinter06_flucatchers.html

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http://www.gi.alaska.edu/ScienceForum/ASF17/1772.html

E-10
US Department of State
www.state.gov

US Department of State
http://www.state.gov/g/avianflu/

E-12
NBII Wildlife Disease Information Node
http://wildlifedisease.nbii.gov/aiworkshop/WorkshopWebDocs/NorthernPintail_AIWorkshop_FolderPicture.jpg

E-13
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E-16

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E-27
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http://www.archives.gov/education/lessons/fallout-docs/index.html?template=print

Prions

F-5
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F-7
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http://pathmicro.med.sc.edu/mhunt/prion1.jpg

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http://mt.gov/liv/animalhealth/diseases/BSE/pathology.asp

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http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001/1.shtml

F-15
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