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The Origin of Plagues: Old and New

Richard M. Krause

Viruses and bacteria emerge in new and old forms to cause disease epidemics. Some microorganisms recur when changing life-styles (including increased international travel) offer new opportunities; others arise from new genetic variations. These various epidemics connect the future with the past, offering lessons for guarding the health of generations to come—lessons learned from diseases such as tuberculosis, toxic shock syndrome, Lyme disease, streptococcal infection, influenza, and acquired immunodeficiency syndrome (AIDS). The public must be vigilant to the possibility of new epidemics, learn more about the biology and epidemiology of microbes, and strengthen systems of surveillance and detection.

Acquired immunodeficiency syndrome is the most contemporary example of human vulnerability to the microbial world, and there is genuine concern that another "new" microbe, or a genetic variation of an old one, can and will "go global" as AIDS has done. A new epidemic may be incubating even now in the crowded, unsanitary mega-cities of the developing world, or in remote jungles in Africa, South America, or Asia—once sparsely inhabited regions that have recently been altered by modern civilization (*1–4*).

AIDS is not the only case of a microbial threat to human beings in the late 20th century. Since the 1970s, a series of unanticipated outbreaks of microbial diseases startled inhabitants of the United States. Legionnaires' disease, toxic shock syndrome, and Lyme disease all happened before the recognition of AIDS in 1981.

In addition to these threats within the United States, strange epidemics have been occurring elsewhere in the world. In Africa, outbreaks of the deadly Ebola virus took the lives of 50% of the people who became infected. A majority of the doctors and nurses who treated these patients also died of the disease. After fatal but localized outbreaks of Ebola fever, the disease failed to become a worldwide epidemic. At the same time, however, the AIDS virus, human immunodeficiency virus (HIV), was spreading from the rural communities of Africa to the towns and cities, infecting thousands of people in Central Africa alone. After a few years, HIV, which has a long latency period, manifested itself when those who were infected developed the medical symptoms of full-blown disease. By that time, AIDS had spread from Africa to the United States, Europe, Asia, and elsewhere.

Many factors interact and contribute to the reemergence of old infectious diseases or the emergence of new ones. More often than not, epidemics occur because of changes in the patterns of human behavior, social organization, urbanization, and agriculture. However, the most important factor is the spread of microbial organisms from points of origin as a result of the migration and travel of their human hosts (*5*).

In ancient times, infectious diseases spread slowly but steadily along caravan routes throughout the Roman and Asian world (*6*, *7*). From A.D. 165 to 180, measles was spread along the caravan routes, and from A.D. 251 to 266 smallpox was carried. One-third of the population died. Such a catastrophe did not recur until the bubonic plague spread from Asia to Europe in the 13th and 14th centuries. This occurred when the horsemen of the Mongol armies raced across the steppes of Asia, transmitting the disease from the point of origin in northern Burma. They carried fleas infected with plague bacillus. From there the plague moved farther eastward via the caravan routes to Europe and elsewhere. After 1492, the oceans became highways that further extended the dispersal of disease agents. It became possible for plagues such as smallpox and measles to circle the globe within a year. The oceans remained the predominant route of transmission until the present era of mass air travel. Today, airborne travelers incubating infections can reach any point on the globe within 24 hours. As a consequence, worldwide exposure to a highly infectious virus, such as influenza, occurs in a matter of weeks.

However, microbes are not idle bystanders, waiting for new opportunities offered by human mobility, ignorance, or neglect. Microbes possess remarkable genetic versatility that enables them to develop new pathogenic vigor, to escape population immunity by acquiring new antigens, and to develop antibiotic resistance.

For these reasons, it is necessary to be prepared for new epidemics caused by old

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microbes as well as those caused by new microbes that appear as a consequence of evolutionary events. Examples of early and recent epidemics are described here to illustrate the principles that govern the occurrence of plagues.

Old Microbes, New Epidemics

Tuberculosis. Although tuberculosis (TB) is discussed elsewhere (*8*), it is useful to recall the epidemic of TB in the 18th and 19th centuries. In the tragic story of TB (*9*), it becomes clear that the slums of the big industrial cities were cauldrons for the incubation of TB; the disease then spread to the upper classes and rural communities until it threatened "the very survival of the European race" (*9*). As McNeil has noted, quoting from *Piers Plowman*, "A fair field full of folk makes a [fertile] feeding ground" for microbes (*7*). Tuberculosis is again advancing, into populations that have been rendered susceptible to antibiotic-resistant strains by poverty, malnourishment, and infection with HIV.

Toxic shock syndrome. Nosocomial infections and surgical and trauma wound infections are frequently caused by *Staphylococcus aureus*. However, an unexpected occurrence in 1980 was the epidemic of toxic shock syndrome (TSS) caused by this organism. Changes in human behavior also preceded this epidemic, which occurred among young, primarily white women, with an onset during menstruation (*10*). A strong correlation was found between TSS and the presence of *S. aureus* in vaginal or cervical cultures of affected patients. A series of studies identified TSST-1 toxin as the most frequent cause of this syndrome (*11*). The pathogenesis of TSS involves the establishment of a toxin-producing strain in an individual not immune to TSS under conditions that favor the formation of the toxin. Such conditions prevailed with the introduction of certain brands of hyperabsorbent tampons. Their prolonged intravaginal use enhanced the aerobic surface area in the vagina, which favored the growth of intravaginal *S. aureus* and the production of TSST-1.

Public education and removal of the hyperabsorbent tampons from the market resulted in a marked decrease in the number of TSS cases in menstruating females (Fig. 1). This disease, as a complication of tampon use, has now almost disappeared. Sporadic cases of TSS have occurred before and since this epidemic, when penetrating or surgical wounds in individuals lacking immunity to TSS become infected with toxin-producing strains of *S. aureus*. TSS also persists as a threat to patients receiving hemodialysis.

Over 100 years ago, the Scottish surgeon

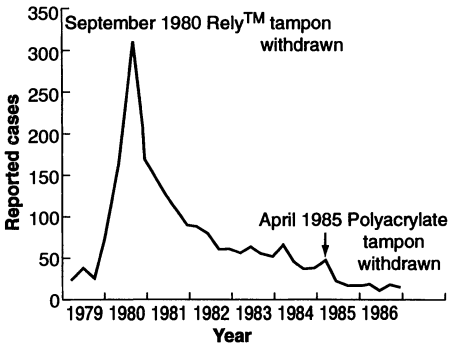

Fig. 1. Reported cases of toxic shock syndrome by year; passive surveillance, United States, 1979 to 1986 (*11*).

Sir Alexander Ogsden proved that *S. aureus* was a frequent cause of surgical wound sepsis. Mortality was very high because of unsanitary conditions but was greatly diminished with the introduction of sterile surgical techniques and, later, antibiotics (*12*). However, *S. aureus* is very adept in developing resistance to multiple antibiotics (*13*). For this reason, infections of surgical and trauma wounds are frequently difficult to treat. For reasons that are still unclear, a "new" strain of *S. aureus* arose in the 1950s that caused frequent outbreaks of severe infections in nurseries for the newborn and pediatric wards of hospitals in most countries of the world; however, these outbreaks subsided a decade later.

Future outbreaks of TSS might be caused by changes in medical practices or by possible alterations in the epidemiology and bacteriology of *S. aureus*. It is conceivable, for example, that *S. aureus* will acquire the genetic capacity to produce a "new" TSS toxin to which most humans are not immune. For all of these reasons, immunological approaches to the control of staphylococcal disease should be promoted that include the development of both active and passive immunization against the toxin or toxins and the surface carbohydrate antigens (*12*).

Lyme disease. Lyme disease is another bacterial infection whose outbreak startled the public. The disease emerged from obscurity as a result of changes in the habits of people and as a result of the ecological relation between the tick vector and its animal host. The illness is an old disease that recurred sporadically during the 19th century; it was then of unknown cause, but became known as Lyme disease in the mid-1970s as a result of an epidemic of arthritis that occurred in the village of Old Lyme, Connecticut (*14*). Since then, the number of cases has increased substantially. More than 10,000 cases were reported this past year.

Interest in Lyme disease was heightened with the discovery that chronic infection with the spirochete resulted in chronic inflammatory arthritis similar in many respects to rheumatoid arthritis. For this reason, the illness has become a model for the study of rheumatic diseases in humans. Lyme disease is a generalized infection; additional complications include cardiac inflammation and neurological abnormalities that resemble multiple sclerosis and neurosyphilis (*15*).

In 1982, the disease-causing spirochete, which was subsequently named *Borrellia burgdorferi*, was discovered (*16*). The reservoir of the spirochete in the northeastern United States is the white-footed mouse, on which the deer tick feeds and then becomes infected. This tick, *Ixodid dammini*, transmits the disease to humans. The adult-infected ticks mate in the fur of white-tailed deer (*15*) and then populate the landscape to which humans are exposed.

Lyme disease has become the most prevalent vector-borne disease in the United States (*17*). Although initially identified primarily in New England and Wisconsin, it has spread to other regions. An important factor in the spread of this epidemic has been the rapid increase in the deer population in the eastern United States, which has led to a large increase in the tick population. According to wildlife biologists, deer had almost disappeared in the northeastern United States by the end of the 19th century. Since then, the deer population has increased steadily, and by 1985 there were an estimated 13 million white-tailed deer in the United States. As the range of the deer spread, so did the range of the infected deer tick. During this time, the urban population moved to the suburbs; some people acquired second homes in rural areas, and outdoor recreation enthusiasts were exposed to and frequently bitten by infected ticks. Events were on a collision course.

This epidemic will continue until people follow the recommended precautions to prevent and avoid tick bites, until people seek prompt diagnosis and treatment with antibiotics after receiving a tick bite in an endemic region, and until the serological tests to detect infection are more reliable than current methods. An early first step in containing this epidemic is the formulation of an experimental vaccine that protects mice from illness produced by direct injection of spirochetes (*18*) or by infected tick bites (*19*).

Only recently has a satisfactory explanation for the occurrence of several hundred cases of Lyme disease in California and other western states been presented (*20*). The dusky-footed woodrat serves as the spirochete reservoir, but the transmission requires a two-cycle pattern involving two ticks distinct from the northeastern United States tick, *I. dammini*. The spirochete-

infected tick, *I. neotomae*, only bites the dusky-footed woodrat, and this rat maintains the reservoir. Another tick, *I. pacificus*, bites both the rat and the human and is responsible for transmission of the disease to humans. It is too early to know what events might enhance the "bi-cycle" transmission of Lyme disease in the western United States and whether the disease will become as prevalent as in the northeastern United States.

Old Viruses, New Epidemics

There are numerous examples of old viruses that have caused new epidemics as a consequence of changes in human practices and social behavior. Epidemic poliomyelitis emerged in the first half of this century when modern sanitation delayed exposure of the virus until adolescence or adulthood, at which time it produced infection in the central nervous system and severe paralysis. Before the introduction of modern sanitation, polio infection was acquired during infancy, at which time it seldom caused paralysis but provided lifelong immunity against subsequent polio infection and paralysis in later life. Thus, the sanitation and hygiene that helped prevent typhoid epidemics in an earlier era fostered the paralytic polio epidemic.

An outbreak of dengue hemorrhagic fever became an epidemic in southeast Asia in the 1950s, and more recently in Central and South America. This has occurred as a consequence of ecological changes that have resulted in the proliferation of the mosquito vector *Aedes aegypti*. The inadvertent introduction and wide dissemination of a second mosquito vector, *Aedes albopictus*, from Asia into the United States, has set the stage for a possible epidemic of dengue in this country. These and numerous other examples of new epidemics caused by old viruses are discussed in recent reviews (*2, 3*).

The Genetic Origin of New Microbes

Although people have influenced the distribution of microbes and the occurrence of epidemics, microbes have been more than simple opportunists. They have also been great innovators and have developed, through genetic versatility, new vigor for survival and species dominance. Examples of such genetic versatility for both bacteria and viruses are described below.

New Bacteria, New Epidemics

It is likely that many human bacterial infections, such as leprosy and TB, have survived successfully for hundreds of years, if not millennia. Nevertheless, genetic events can alter the antigenic characteristics of bacteria so that they can elude herd immunity, enhance pathogenicity by the acquisition of virulence factors, and result in the acquisition of antibiotic resistance.

Mutations comprise one source of such new properties. Of greater interest, however, has been the discovery that these new characteristics are acquired within a bacterial species or transferred between species by genes that are carried on extrachromosomal elements such as plasmids or on the DNA of bacterial phages. The genes associated with such elements are transferable from organism to organism by conjugation and transduction or by transformation. The genetic events involved in the appearance of antibiotic resistance among microbes are discussed elsewhere (*13, 21*).

A sudden epidemic outbreak of an infectious disease can be the consequence of microbial genetic events. For example, an outbreak of diphtheria occurred in Manchester, England, because the resident nontoxin-producing diphtheria microbe (which did not produce disease) was converted to a toxin-producing, virulent form (*22*). The origin of this conversion was traced to a child who returned by airplane to Manchester from Africa and who had become infected with a pathogenic, toxin-producing diphtheria bacillus. This organism contained the phage that carried the diphtheria toxin gene. Because multiple infections allowed toxigenic diphtheria bacilli to comingle in the community with nontoxic diphtheria bacilli, the toxin gene was spread to the latter by phages that possessed the toxin gene.

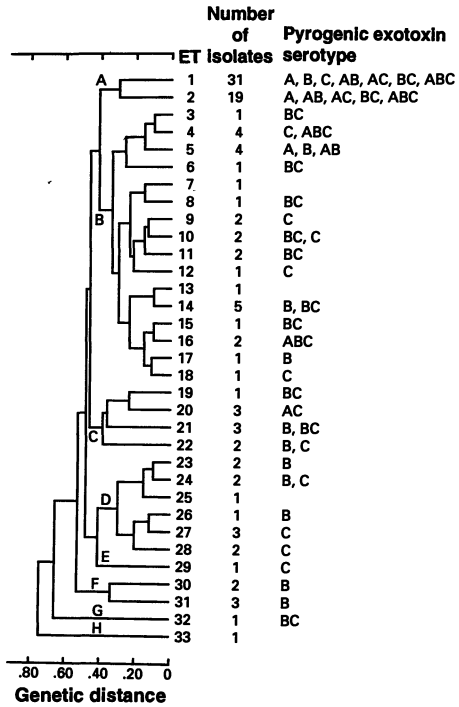

Fig. 2. Dendrogram showing estimates of genetic relations among 33 electrophoretic types (ETs) of 108 isolates of *Streptococcus pyogenes* group A, based on allele profiles at 12 enzyme loci. Eight major lineages are identified by letters A to H. Pyrogenic exotoxin serotypes of the isolates of each ET are indicated (*24*).

"New" streptococcal infections. There was a sense of disbelief among the general public when the media reported the unexpected death of the popular TV puppeteer Jim Henson. The disbelief arose because he died from a presumably ordinary streptococcal infection that is readily treatable with penicillin and normally resolves without complications. However, the infection progressed rapidly to a fulminating fatal pneumonia and irreversible shock, despite treatment with penicillin.

Although there is not an entirely satisfactory explanation for the occurrence of this virulent form of streptococcal disease [termed toxic shock-like syndrome (TSLS) because of its resemblance to *S. aureus* TSS], the evidence suggests that it is caused by certain strains of group A streptococci that produce potent "erythrogenic toxins." This severe form of streptococcal infection from which Jim Henson died made its appearance in the mid-1980s and was reported in Europe and throughout the United States. In one study of 30 patients, six died (*23*). Death from common streptococcal infections, including streptococcal sore throat, is very rare.

Streptococci are known to produce three distinct erythrogenic toxins: types A, B, and C. These toxins produce the rash of scarlet fever and possess powerful inflammatory and cytotoxic properties. In the early part of this century, streptococcal infections frequently resulted in a severe form of scarlet fever and toxic shock. Death was common. The streptococci cultivated from these patients produced the particularly potent toxin A. In contrast, modern strains that produce toxin types B and C are less likely to cause these lethal complications.

It now appears that the toxic streptococcal strains that more commonly produce the potent type A toxin have reemerged and are associated with TSLS. A relatively high frequency of exotoxin A among streptococcal isolates recovered from TSLS patients has been reported (*24*). Toxin A was produced by 28 of 31 strains isolated from patients with TSLS. In contrast, toxin A was produced by only 14 of 77 strains isolated from patients with streptococcal infections of the usual severity.

Multilocus enzyme electrophoresis was performed to further elucidate the genetic diversity and relations among the 31 TSLS and 77 non-TSLS streptococcal isolates (*24*). Thirty-three electrophoretic types

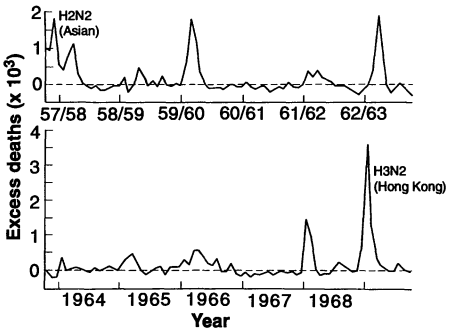

Fig. 3. Excess pneumonia and influenza mortality (than would have been predicted for a nonepidemic year), by 4-week periods, from 122 U.S. cities during periods of prevalence of A influenzas [modified from (*27*)]. The 1957 to 1958 Asian (H2N2 virus) and the 1968 to 1969 Hong Kong (H3N2 virus) influenza pandemics were due to "new" viruses that arose by genetic reassortment. The epidemics between the pandemics were due to mutants of the Asian influenza virus that had undergone point mutations in the antigenic genes. H2 and H3, hemagglutinin antigens 1 and 2, respectively. N2, neuraminidase 2.

(ETs) representing distinctive multilocus clonal genotypes were identified. More than two-thirds of the cases of TSLS were caused by strains of two related clones, ET-1 and ET-2 (Fig. 2). Recent pathogenic European isolates were also classified as ET-1 or ET-2. Nearly identical nucleotide sequences were found for the exotoxin A structural gene of six isolates from five ETs of diverse phylogenetic lineages. This finding suggests that the gene has been transferred from clones ET-1 and ET-2 and distributed horizontally among other clones, presumably by bacteriophage-mediated transfer. This supports the hypothesis that toxin A is a major factor in the pathogenesis of TSLS, although it is also conceivable that a product of a gene tightly linked to exotoxin A is involved in the pathogenesis of this severe disease.

The streptococcal strains from patients with serious disease may consist of a unique recent clone (*25*). Whether the streptococcal strains that cause TSLS are "new" or recurring strains is a complex question. In addition to phage-mediated genetic change, as noted above, other variables such as fluctuations in human immunity, host susceptibility, and the selective pressures of antibiotic therapy may have caused the recent arrival of the streptococci that produce TSLS. Whatever the final explanation, students of streptococcal disease recognize that they are dealing with something new in their clinical experience, and they must be alert to the prompt treatment of any streptococcal infection. There is also the danger that ET-1 and ET-2 strains will spread more broadly in the community and that the toxin gene may continue to spread horizontally to unrelated clonal types of group A streptococci.

New Viruses, New Epidemics

The emergence of novel viral agents is restrained in part by the requirement to preserve the structural integrity of the virus and its relation to a specific ecological niche. New viruses may occur, in spite of evolutionary constraints, as a consequence of genetic mechanisms such as point mutations and genetic reassortment (*2*). This phenomenon is dramatically represented by influenza viruses, which frequently generate "new" viruses that cause epidemics and pandemics.

Influenza. Point mutations that alter critical sites on the major hemagglutinin glycoprotein antigen of influenza virus allow the virus to elude the immunity of the population that occurred after exposure to prior strains of influenza virus. Such changes in the virus account for the antigenic drift in the hemagglutinin of wild-type viruses that occurs from year to year. Because the new hemagglutinin bears some resemblance to the old, antibodies to the old hemagglutinin may, but do not always, neutralize the "new" virus. Therefore, the "new" virus may cause infection even in those who previously had influenza.

The second origin of "new" and novel influenza viruses is genetic reassortment (*26*). This results in the occurrence of an entirely new hemagglutinin antigen against which there is little or no immunity, and may give rise to a pandemic of influenza. This occurred in 1957 (Asian flu) and in 1968 to 1969 (Hong Kong flu) (*27*) (Fig. 3). Both viruses possessed the same neuraminidase. The viruses causing these epidemics are thought to have arisen as a result of the capture by human viruses of one or more external genes from avian influenza viruses at the time of co-infection of the two viruses in the same animal or human. Such events most likely occurred in China or elsewhere in Asia and resulted in new viruses.

The generation of these new viruses could occur during co-infection because the influenza viruses carry their genetic material in eight separate gene segments. During the course of co-infection with different strains, these genes can be reassorted or recombined, producing novel viruses that possess gene combinations unlike those of either parent. Thus, Kilbourne notes, "The process is analogous to sexual reproduction. Influenza viruses can make evolutionary leaps without the need to gradually accumulate numerous favorable mutations" (*2*). For these reasons influenza is a moving target, and worldwide surveillance is needed to determine the emergence of new strains and their use in formulating an up-to-date influenza vaccine.

AIDS—A New Disease?

The AIDS epidemic is clearly new. The first sporadic cases occurred in Africa, the United States, and elsewhere. The epidemic gained momentum throughout the 1980s. Currently, more than a million people are infected in the United States, and many millions are infected worldwide, particularly in Africa and Central and South America. If a cure is not found, most or all of these individuals will die of overwhelming secondary infections that occur as a result of the severe immune deficiency. The World Health Organization has predicted that the epidemic will spread widely in India and elsewhere in Asia unless a means is found to stem the advance.

Although the AIDS epidemic is certainly new (AIDS does not mimic any previously known pestilence), the data so far leave unanswered whether HIV-1 and HIV-2 are new (*28*). Some believe that these viruses were transmitted from African monkeys or other primates to humans, but the evidence for this is not conclusive. Africa is a known reservoir of the nonhuman primate lentiviruses termed simian immunodeficiency virus (SIV), but only one of a number of the SIV strains characterized so far is closely related to HIV-1 (*29, 30*), the virus currently causing the largest number of AIDS cases. AIDS caused by HIV-2 is rare in the United States but quite common in West Africa, and is now occurring in western India. HIV-2 is closely related to SIV isolated from captive rhesus macaques (*31*). Computer programs have been used to construct evolutionary trees to determine the relatedness of viruses by comparing genetic sequences for homologies (*32*). It has been suggested (*33*) that known SIV strains appear to be more closely related to HIV-2 than to HIV-1. Nevertheless, these evolutionary relations depend on the proper alignment of homologous sequences, and there are disputes concerning the interpretation of these alignments and the conclusions.

Whatever the origin of the AIDS viruses, it is likely that they have been around for at least a century (and perhaps longer in

Table 1. The budget of the National Institute of Allergy and Infectious Diseases as a percentage of the total budget of the National Institutes of Health (*39*).

Year	Percentage
1965	9.0
1970	9.2
1975	5.7
1980	6.3
1985	7.2
1990	11.0
1992	10.7

Central Africa), causing sporadic human infection. Mathematical models have been used (*34, 35*) to examine the time course of the AIDS epidemic and the spread of the disease from a hypothetical initial focus or single village in Africa. The models have sought to determine the length of time it would take for the virus, emerging in one village, to spread to surrounding villages and rise to a detectable level (a seroprevalence of 1%). These models assume sporadic, limited personal interactions between one local village and a set of surrounding villages in the isolated outback of Africa. Such restricted interactions between villages were the most likely pattern for the spread of AIDS in the earlier years of the epidemic. According to the model, the number of cases of HIV infection would increase faster with time (*34*). These considerations indicate that there may have been a passage of 100 years between the emergence of AIDS at its putative source and the outbreak of the epidemic during the 1980s.

It is also likely that the AIDS epidemic gained momentum and emerged from this local dormancy as a result of changing cultural patterns, urbanization, and the migration of peoples, as well as long-distance land travel along new highways and commercial routes. Because it was no longer restricted to isolated locales, the AIDS virus spread rapidly to urban areas. Similar historical circumstances have been noted (*7*) for the occurrence of bubonic plague. Old social habits allowed people to coexist with the plague bacillus in the Burma-Unnan region long before the Mongol invasion. These people adopted a life-style which, although it did not eliminate the microbe, curtailed its spread. However, social disruption subsequently favored the spread of microbes.

Just as social and economic changes of the Industrial Revolution promoted the outbreak of TB (*9*), social forces in the United States arising out of changing patterns of sexual behavior and the use of addictive drugs during the past 30 years have enhanced the spread of AIDS (*36*). There is ample evidence that the epidemic of sexually transmitted diseases (STDs) has also contributed to the spread of AIDS. Only research and a combination of social education and health programs will prevent the further spread of STDs and AIDS (*37*).

Strategies for the Future

In the 1960s and 1970s, there were frequent pronouncements that infectious diseases had been conquered and were no longer major threats to health (*38*). The medical and scientific community and the public generally accepted this verdict, and the major research effort in the 1970s was concentrated on great "killers and cripplers," heart disease and cancer.

The reasons for Burnet's optimistic view can be traced to the advent of the sulfonamide drugs in the 1930s and penicillin in the 1940s, when hundreds of new antibiotics were discovered to treat infectious diseases such as gonorrhea, syphilis, pneumonia, TB, and typhoid fever. New vaccines prevented epidemics of measles, meningitis, rubella, polio, and hepatitis.

In view of this declining interest in infectious diseases, it is not surprising that research support for microbiology and virology declined for many years at the National Institutes of Health (NIH). The budget of the National Institute of Allergy and Infectious Diseases (NIAID) as a percentage of the NIH budget from 1965 to 1992 is shown in Table 1 (*39*). In 1965, NIAID received 9% of the NIH budget, but this declined to 5.7% in 1975 (*39*). The decline was slowly reversed when the public and the scientific community were confronted with the series of unexpected plagues discussed here and elsewhere (*40*). Throughout the 1980s the NIAID budget continued to increase as the AIDS epidemic progressed and as other research initiatives were undertaken to develop new vaccines and new methods for the prevention and treatment of epidemic diseases.

It is likely that epidemics will continue to occur in the future. Changes in human social behavior and customs will continue to provide opportunities for microbes to produce unexpected epidemics. We must be aware of the possible consequences of altering our behavior individually and as a society. Furthermore, science cannot halt the future occurrence of "new" microbes. These emerge from the evolutionary stream as a consequence of genetic events and selective pressures that favor the "new" over the "old."

Therefore, public health requires the renewal and expansion of research on the epidemiology and biology of microbes and awareness to the possibility of new epidemics. Although clinicians in New York and California detected the first cases of AIDS, it was the nationwide surveillance system of the Centers for Disease Control that identified the epidemic nature of the disease and its mode of dissemination. Such cooperation was also true for TSS, Legionnaires' disease, and Lyme disease. It is necessary to expand surveillance efforts both in the United States as well as in other regions of the world.

Surveillance alone, however, cannot detect the unexpected emergence of future plagues or prepare the defense against them. The accomplishments thus far in the fight against AIDS are based on 40 years of biomedical research. Research into the survival of microbes and their adaptation to new habitats is required. In addition, it is necessary to understand their genetic makeup and ability to cause disease, as well as the immunological processes that are mobilized by the body against microbial invasion and infection.

In conclusion, the migration of people and changes in individual and social behavior foster the occurrence of epidemics caused either by old microbes of by new ones that that emerge as a consequence of genetic events that enhance virulence and pathogenicity. These various factors must be considered in the development of strategies to cope with future epidemics.

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Structure-Based Strategies for Drug Design and Discovery

Irwin D. Kuntz

Most drugs have been discovered in random screens or by exploiting information about macromolecular receptors. One source of this information is in the structures of critical proteins and nucleic acids. The structure-based approach to design couples this information with specialized computer programs to propose novel enzyme inhibitors and other therapeutic agents. Iterated design cycles have produced compounds now in clinical trials. The combination of molecular structure determination and computation is emerging as an important tool for drug development. These ideas will be applied to acquired immunodeficiency syndrome (AIDS) and bacterial drug resistance.

Will the next generation of pharmaceuticals arise from a combination of crystallography and computational methods (*1–5*)? While I share the enthusiasm for structure-based drug design (*6–8*), it is the newest of several approaches to the lengthy process of finding and developing therapeutic agents (Table 1). One important discovery procedure is high-volume "random" screening of natural products, corporate databases of compounds, or peptides and oligonucleotides. Another method is the interception of specific biochemical mechanisms. Vaccine development is yet another route to anti-infectives. Finally, there are well-developed "active analog" approaches to improve upon initial discoveries. Any of these techniques, singly or in combination, can play a pivotal role in finding new drugs.

Can we design drugs from first principles, creating a molecule with a specific mode of action and acceptable biological properties? Today's answer is "no." What we can reliably expect is to design inhibitors, especially enzyme inhibitors, and to begin the long process of drug development from a sensible starting point.

Fifteen years ago, Seymour Cohen proposed a general paradigm for developing drugs for infectious diseases (*9*). Infectious agents, such as viruses, bacteria, fungi, or protozoa, encode or carry their own crucial enzymes and nucleic acids, which serve as obvious targets for intervention. In the succeeding decade and a half, the ability to identify, clone, express, and purify proteins and nucleic acids has increased enormously, making highly specific in vitro assay systems commonplace. These assays, in turn, lead to effective strategies for the discovery of a wide variety of inhibitors (*10*). Structural techniques have also advanced, and high-resolution molecular anatomies can be determined by crystallographic and magnetic resonance experiments. Thus the pieces are in place to extend Cohen's concept to structure-based design (*1, 3, 7, 11, 12*).

I will draw examples from AIDS and bacterial drug resistance. These two major health problems have three features in common: recent starting points as public health issues, known etiologies, and a large number of macromolecules as potential targets (Table 2).

Screening

The vast majority of drugs in the marketplace were derived from discoveries in large-scale screens or from analog development programs. Robotic systems can perform thousands of tests per day by means of radioactive labeling or spectroscopic detection, and further improvements can be expected (*13*). It is feasible to scan an entire corporate database (for example, 100,000 to 500,000 compounds) in less than a year's time. The coupling of cell metabolism to microsensors opens the door to rapid surveys of toxicity and function at the cellular level (*14*). The only current approved drugs against human immunodeficiency virus (HIV) were detected with screening techniques (*15*) and so were the original generation of antibiotics.

Understanding the biological or biochemical mechanism of a disease often suggests the types of molecules needed for new drugs (*16, 17*). Examples are substrate or cofactor analogs for thymidylate synthase as antitumor agents (*18, 19*) or the development of the captopril family of antihypertensives (*17*). In a similar manner, clavulinic acid acts as a β-lactamase inhibitor (*20*). Such efforts represent a proven route from test tube to pharmacy.

Substrate-Based Design of Protease Inhibitors

There are circumstances in which the "rational" design of inhibitors can be performed without a target structure. A good example is a two-step protocol for developing protease inhibitors: (i) characterize the substrate specificities of the protease; and (ii) synthesize peptides with similar features but with the hydrolyzable amide bond replaced by a nonreactive "isostere." The peptides can subsequently be optimized by modifications in the side chains or backbone. This approach has been used for renin inhibitors (*21*) and for inhibitors of the HIV-1 protease (*22*). One can proceed further by adding specific moieties such as chloromethyl ketones or phosphonates that are capable of forming transition-state analog complexes with the enzyme. Among the examples are inhibitors of the *Schistosoma mansoni* cercarial elastase (*23*) and carboxypeptidase A (*24*). It is reasonable to expect to obtain peptidelike inhibitors with nanomolar inhibitory constants in in vitro assays after 1 year of effort.

Table 1. Drug development steps (*71*).

Step	Years
Discovery and lead generation	1–2
Lead optimization	1–2
In vitro and in vivo assays	1–2
Toxicology trials	1–3
Human safety trials	1
Human efficacy trials	1–2
Total development time	6–12

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