Chapter 35 Epidemiology of Infectious Diseases

OUTLINE

Humankind has long been beset by diseases that spread rapidly among a population, with devastating effects. How such pestilences were spread was unknown until the nineteenth century and was attributed to various natural and supernatural forces. The use of the techniques of **epidemiology** (the study of disease occurrence and distribution) helped to solve these mysteries. Moreover, epidemiological studies have had practical benefits by leading to an understanding of how to control the spread of infectious diseases.

Some pathogenic microorganisms are transmitted by one major mode, whereas others may be transmitted by several modes. Pathogenic microorganisms may be disseminated by food, water, aerosols (microorganisms that are dispersed in air), or the bites of various infected arthropods or other animals. They may also be transmitted by direct contact with an infected person or animal or by contact with inanimate objects (**fomites**, singular **fomes** or **fomite**) contaminated by an infected host. Epidemiological studies have not only concentrated on the transmission of causative agents of disease but have also revealed that the host population itself can play an important role in determining whether an infectious disease will be **pandemic** (having a large number of cases that occur on a global scale within a short time period); **epidemic** (having many cases in a particular geographic region within a short time period); **endemic** (having a low

incidence but constantly present in a particular geographic region); or **sporadic** (having only an occasional occurrence).

How a pathogenic microorganism is transmitted does not necessarily depend on a knowledge of the nature of the microorganism, although this is very helpful. For instance, in 1855, nearly 30 years before Robert Koch discovered *Vibrio cholerae*, John Snow demonstated clearly that the causative agent of cholera was transmitted by drinking water contaminated with human feces; in a classic epidemiological study he traced the source of a London cholera epidemic to the Broad Street Pump in Golden Square. A more recent example is AIDS (acquired immunodeficiency syndrome): the modes of transmission of the causative agent of this disease were known since 1981, but identification of the probable causative agent of AIDS as being a retrovirus (HTLV-III) was not accomplished until 1984.

EPIDEMIOLOGICAL TECHNIQUES

The accumulation and organization of data about the occurrence and distribution of an infectious disease can provide clues as to the manner of transmission of the causative agent and the factors involved in acquiring a disease. The number of cases of a particular disease may be plotted versus time, geographic region, age, sex, race, occupation, or other parameters in order to determine what correlations may be present. Analyses of descriptive data are often helpful in determining the mode of spread of a disease or the factors contributing to outbreaks of the disease, as indicated by the following examples.

The finding of a *seasonal incidence* may provide important clues as to the mode of transmission of the causative agent. For example, an epidemic disease that occurs mainly during the colder months suggests an airborne mode of transmission, as in pneumonia, influenza, or chicken pox (Fig. 35-1). This is because during the colder months people are more likely to occupy crowded quarters and therefore are more likely to transmit microorganisms via aerosols generated through coughing and sneezing. On the other hand, the agent of a disease that occurs mainly in the warmer months would probably be transmitted by other means, e.g., by an arthropod that is prevalent during these months (as in tick-borne Rocky Mountain spotted fever) or by contaminated food (where warm temperatures may allow bacteria in improperly refrigerated food to multiply to high numbers; see salmonellosis, Fig. 35-2).

Geographic correlations may help to determine the mode of transmission. An epidemic occurring in a particular town or city suggests that a common factor

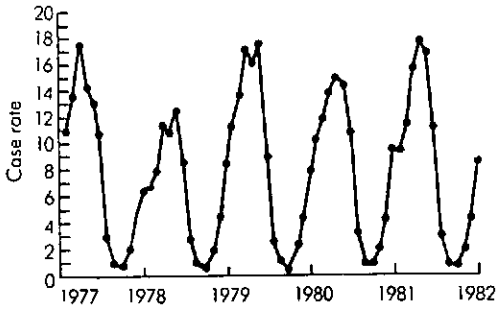


Figure 35-1. Reported case rates of varicella (chicken pox) per month per 100,000 population, United States, 1977–1981. The highest incidence occurs in the colder months, which is characteristic of an airborne infection. The peak incidence for 1981 was reached between March and May. (*Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.*)

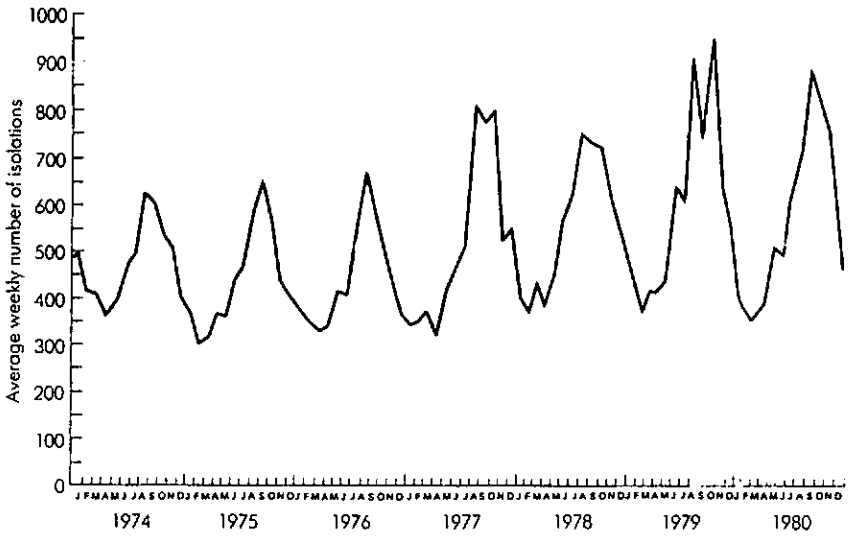


Figure 35-2. Reported isolations of salmonellas from humans, United States, 1974–1980. Each point represents the weekly average number of isolates for the month. The marked increase in the number of reported isolates during the warmer months suggests that conditions present during this time of year contribute to the spread of salmonellas. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1980, issued September 1981.)*

such as a particular water or food source may be involved, as in typhoid fever or bacillary dysentery. A sporadic disease restricted to a particular geographic area suggests that other factors may be involved; for example, human bubonic plague in the United States is limited to the western and southwestern regions and is acquired through occasional contact with the infected wild rodent population of those regions (Fig. 35-1).

Correlation of a disease with age groups affected by a disease may indicate factors of epidemiological importance. A disease that occurs mainly in the age group over 65 may be suggestive of a breakdown in immunity. For instance, most of the new active cases of tuberculosis in the United States occur in elderly persons (see Fig. 35-3), particularly males whose resistance has been lowered by factors such as malnutrition. Diseases that affect mainly children also suggest that a lack of active immunity may be a major factor. For example, whooping cough (pertussis) is most common in infants (Table 35-2). In contrast to many other infections, passive natural immunity acquired from the mother is relatively ineffective in protecting an infant against this particular disease, and thus vaccination of infants against whooping cough should be begun at about 2 months of age.

A correlation with *occupation* or *life style* can be made with some diseases. For instance, a disease that occurs mainly in veterinarians and slaughterhouse workers suggests that direct contact with the tissues of infected animals may be the mode of transmission, as in cases of human brucellosis in the United States. In another example, 91 percent of all cases of the disease called AIDS (see Chap. 33) have occurred in persons belonging to one or more of the following categories: homosexual or bisexual men (71 percent of cases); intravenous drug users such as heroin addicts (17 percent of cases); persons with hemophilia who receive injections of clotting factor prepared from the blood of many donors (1 percent of cases); heterosexual partners of members of these groups (1 percent of cases); and recipients of blood transfusions (1 percent of cases). This strongly

Table 35-1. Reported Cases of Plague in Humans by State, United States, 1960–1981

Area	Total
United States	165
Arizona	22
California	17
Colorado	12
Idaho	1
Nevada	3
New Mexico	99
Oregon	7
Texas	1
Utah	2
Wyoming	1
Other states	0

SOURCE: Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.

Table 35-2. Reported Cases of Pertussis (Whooping Cough) by Age, United States, 1982

Age Group	Cases
Under 1	960
1–4	474
5–9	181
10–14	88
15–19	35
20–24	24
25–29	22
30–39	29
40–49	9
50–59	2
60+	2
Age unknown	60
Total for all age groups	1,886

SOURCE: Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1982, issued December, 1983.

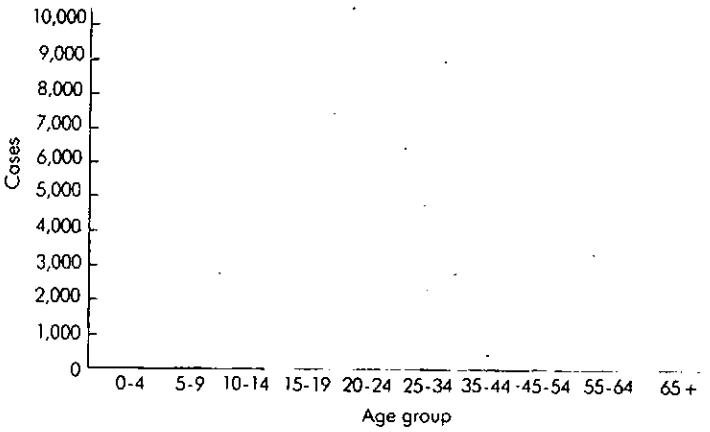


Figure 35-3. Reported cases of tuberculosis by age group, United States, 1980. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1980, issued September 1981.)*

indicates that the causative agent (probably a retrovirus called HTLV-III—a member of the human T-cell leukemia virus group) can be transmitted from person to person by sexual contact or, less commonly, via blood or contaminated hypodermic needles.

Epidemiological Markers

To trace the origin and manner of spread of an outbreak of disease, it is often useful to determine whether the *same strain* of a microbial species is responsible for all the cases. For example, let us suppose that an epidemic of streptococcal sore throat occurs in one part of a city, and a few days later another outbreak occurs in a different area of the city. Are the two outbreaks caused by the same strain of *Streptococcus pyogenes* which has been transported across the city, or are the outbreaks completely unrelated to one another, each having a completely different origin?

Such questions could be answered easily if each microbial strain were to carry some sort of identification, like the numbers emblazoned on football uniforms that allow an observer to keep track of the players. The cells of microbial strains obviously do not have identifying numbers etched on their cell walls; however, they often possess other properties which can serve the same purpose. That is, they have properties that can be used to specifically characterize or type the strains within a species.

Subdivision by Antigenic Composition. The M proteins that occur in the cell walls of *S. pyogenes* can be used to classify the strains of this species into serovars (different serological types), since 63 types of M proteins occur and each strain possesses only one type. In the two outbreaks of streptococcal sore throat mentioned previously, if both were found to be caused by, say, type 45, this would strongly indicate that the same strain was involved. Many other pathogenic species can be subdivided into serovars, such as *Streptococcus pneumoniae* (80 serovars, on the basis of capsular antigens); *Haemophilus influenzae* (serovars a to f, on the basis of capsular antigens); and *Leptospira interrogans*

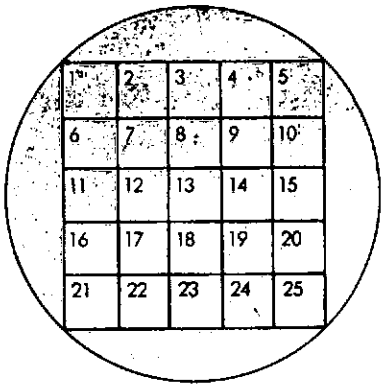


Figure 35-4. Phage-typing. The back of a Petri dish of agar medium was marked off into 16 squares. The surface of the medium was then swabbed with a strain of *Escherichia coli*. Each of 16 *E. coli* bacteriophages was inoculated onto the agar surface (phage 1 onto area 1, phage 2 onto area 2, etc.) After incubation for 6 h, zones of lysis developed where phages 12, 13, 16, and 18 had been inoculated. This phage-lysis pattern identified the *E. coli* strain as belonging to phage type A4B4, thus differentiating it from other strains of the species.

(180 serovars, on the basis of cell wall antigens). Influenza viruses are divided into three main types, A, B, and C, on the basis of their ribonucleoprotein (RNP) antigen (the S antigen). Thus all strains of type A share common RNP antigens; strains of types B and C have distinctly different ones. In addition, each type can be further divided into subtypes based on hemagglutinin (H) and neuraminidase (N) antigens.

Phage-typing. By applying a battery of different bacteriophages to a plate seeded with a particular strain, we can determine which phages will cause lysis of the bacteria and which will not. The pattern of susceptibility and resistance to phage lysis differs from one strain to another and can be used to characterize a strain as belonging to one or another phage type, or **phagovar** (see Fig. 35-4). For example, although the strains of *Salmonella typhi* cannot be distinguished from each other antigenically (i.e., they all belong to the same serovar), they can be subdivided into 33 distinct phagovars.

Resistance or susceptibility to various bacteriocins can also be used to type the strains of a species (see Chap. 12).

Biotyping. Differences in physiological properties can be used to classify strains into types (**biovars**) for epidemiological purposes. For instance, *Brucella abortus* can be subdivided into 8 biovars based on properties such as a growth requirement for CO_2, production of H_2S, and ability to grow in the presence of certain dilute dyes added to the culture medium.

Antibiotic Susceptibilty. Strains of *Staphylococcus aureus* are often differentiated on the basis of their susceptibility or resistance to a spectrum of antibiotics. The pattern of responses obtained constitutes an **antibiogram.** *S. aureus* can also be subdivided on the basis of either antigenic composition or phage lysis patterns.

ROLE OF THE HOST IN INFECTIOUS DISEASES

Carriers

Pathogenic microorganisms can be transmitted to healthy persons by **carriers**—persons who harbor the organisms. Such carriers can be of three types: (1) persons who have a clinical case of infectious disease; (2) persons who have had a disease and recovered from it, but still harbor the pathogenic organisms

for some period of time **(convalescent carriers)**; and (3) persons who harbor pathogens in their bodies, yet are not ill **(healthy carriers)**, i.e., they have an **asymptomatic** infection.

The occurrence of healthy carriers indicates that merely acquiring a pathogenic microorganism may not automatically ensure that disease will result. Whether disease develops depends to a great extent on the natural resistance and immune state of the person. For example, 20 to 40 percent of the population harbors virulent *S. pneumoniae* in the upper respiratory tract, yet it is only when resistance is lowered that pneumococcal pneumonia develops. Factors that can decrease resistance toward pneumococcal pneumonia include alcoholic intoxication, anesthesia, or the occurrence of a mild primary respiratory infection such as the common cold.

Convalescent and healthy carriers may harbor pathogenic microorganisms for only a few days or weeks **(casual carriers)**; but in some instances they may harbor them for months, years, or even for life **(chronic carriers)**. For example, about 3 percent of persons recovering from typhoid fever become chronic carriers. In these carriers the typhoid bacilli have established a persistent, harmless infection of the gall bladder or bile ducts, from which they pass to the intestine and are excreted in the feces. Some typhoid carriers harbor the organisms in the urinary bladder. The stories of several persistent carriers have been recorded, but the most notorious is that of Mary Mallon, better known as Typhoid Mary, who was responsible for at least 10 outbreaks of typhoid fever, involving 51 cases and 3 deaths.

Chronic or casual healthy carriers can be treated with drugs to remove the focus of infection, or, in some cases, the organ harboring the microorganisms can be removed by surgery. For instance, the carrier state of a chronic typhoid carrier can usually be eliminated by the use of antibiotics (ampicillin) or by surgical removal of the gall bladder.

Herd Immunity

In order for an infectious disease to occur in epidemic form, the causative microrganism must be transmitted easily from one susceptible host to another within the population. Unless a sufficient proportion of the population is susceptible, the disease can occur only in an endemic or sporadic form. For instance, if 70 percent of schoolchildren in a population are immunized against poliomyelitis, epidemics of this disease are unlikely to occur even among the remaining 30 percent who were not immunized. These latter children enjoy what has been termed **herd immunity**. This is not true immunity but merely an expression of the unlikelihood that a susceptible individual will encounter the causative agent of the disease.

AIRBORNE TRANSMISSION

Many microbial pathogens have an airborne mode of transmission and cause infections of the respiratory tract. The infections caused by such airborne organisms tend to occur in epidemic form, appearing explosively and attacking large numbers of people within a short time. Their incidence usually increases during the fall and winter when people are more likely to occupy crowded quarters. The causative microorganisms occur in secretions from the nose and throat of infected individuals and can be transmitted directly to healthy indi-

Figure 35-5. High-speed photograph of an aerosol generated by sneezing. *(Courtesy of Marshall W. Jennison and the American Society for Microbiology.)*

viduals by aerosols (fine sprays producing droplets that remain suspended in air for a time) generated by coughs, sneezes (see Fig. 35-5), or even talking. Microorganisms that cause respiratory infections can also be transmitted indirectly via fomites such as drinking glasses, eating utensils, and handkerchiefs that have recently been used by an infected person.

Droplets and Droplet Nuclei

The size of bacteria-containing droplets expelled into the air by coughing and sneezing determines the time period during which they can remain suspended and also determines their ability to be trapped on the moist surfaces of the respiratory tract during inhalation. In general, larger droplets (10 μm or more in diameter) tend to settle out after traveling only a few feet. If inhaled, most become trapped in the nasal baffle and nasopharyx, from where they can reach the oropharynx by the downward flow of mucus. Smaller droplets (1 to 4 μm tend to evaporate rapidly, leaving **droplet nuclei** (the residue of solid material left after drying, e.g., bacterial cells). Such nuclei can remain suspended in air for hours or days, travel long distances, and serve as a continuing source of infection if the bacteria remain viable when dry. Viability is governed by a complex set of circumstances including (1) the atmospheric conditions, e.g., humidity, sunlight, and temperature; (2) the size of the particles bearing the organisms; and (3) the degree of susceptibility or resistance of the particular microbial species to the new physical environment. If inhaled, small droplets or droplet nuclei tend to escape being trapped in the nasopharynx; instead, they can reach the lungs and be retained there.

Infectious Dust

As indicated above, large aerosol droplets settle out rapidly from the air on various surfaces, such as textiles used by a patient, where they dry. Nasal and throat discharges from a patient also can contaminate surfaces and become dry. Disturbance of this dried material by bedmaking, handling a handkerchief hav-

ing dried secretions on it, or sweeping a floor in the patient's room can generate dust particles which add microorganisms to the circulating air. The survival of microorganisms for relatively long periods in dust creates a significant hazard, particularly in hospital areas, and can contribute to nosocomial diseases (diseases acquired in a hospital). Tubercle bacilli have been isolated from the dust of sanitoria; diphtheria bacilli and hemolytic streptococci have been found in floor dust near patients or carriers harboring these organisms. The bacterial content of room air under various conditions is shown in Fig. 35-6. An illustration of the effect that occupants of a room have upon the numbers of airborne microorganisms is shown in Fig. 35-7.

Epidemiology of Influenza

One of the most familiar examples of airborne infection is epidemic influenza, or "flu" as it is commonly known. Epidemics of influenza occur in cycles; those caused by type A strains of the virus commonly follow a 2- to 3-year cycle and those caused by type B strains have a 4- to 6-year cycle. Type C strains rarely, if ever, give rise to epidemics; they cause subclinical infections or small outbreaks of the disease among children.

In addition to epidemics, influenza can occur in the form of pandemics having rapid global spread. Such pandemics illustrate the enormous geographic range and the rapid spread that airborne diseases can achieve. Great pandemics of influenza have occurred at intervals of 10 to 30 years or even longer and are caused by type A influenza virus strains. These strains have new subtype antigens, i.e., new varieties of the H (hemagglutinin) and N (neuraminidase) antigens that are different from those of the strains that preceded them. Antibodies against

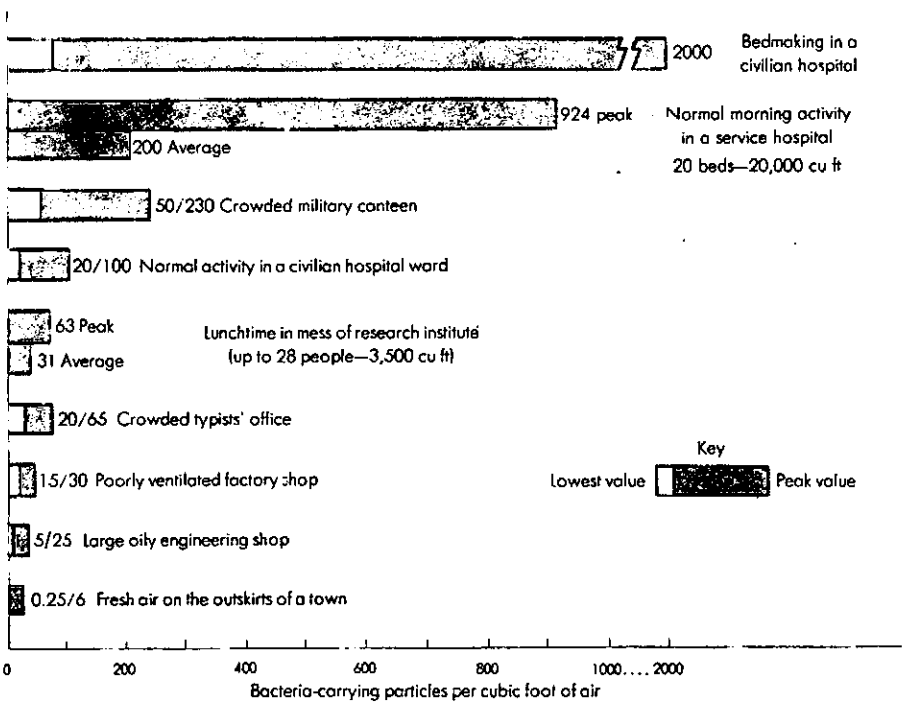


Figure 35-6. Bacterial content of air in civilian and military establishments, as measured with a slit sampler. [*From F. P. Ellis and E. F. Raymond, "Studies in Air Hygiene," Med Res Council (GB), Spec Rep Ser 262, 1948. By permission of the Controller of H. M. Stationery Office, London.*]

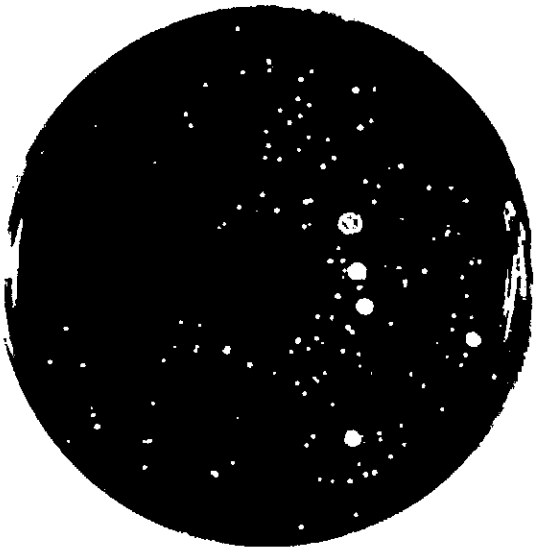
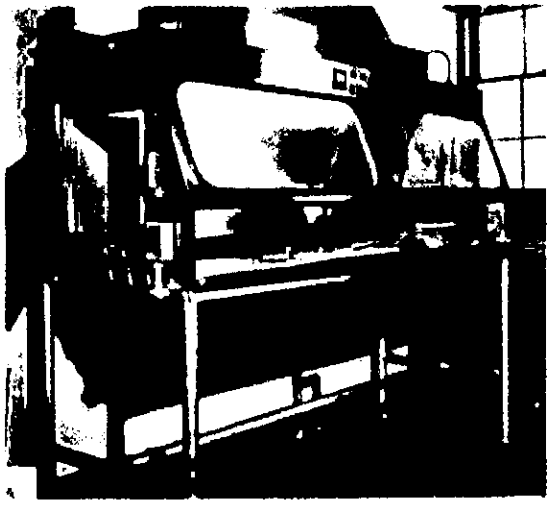


Figure 35-7. Flora of room air with and without occupants. The right side of the plate represents the sample taken while two people were working in a laboratory; the left side of the plate represents the sample taken when the room was unoccupied. The sampling was performed with a slit sampling device. *(Environmental Services Branch, National Institute of Health, Public Health Service.)*



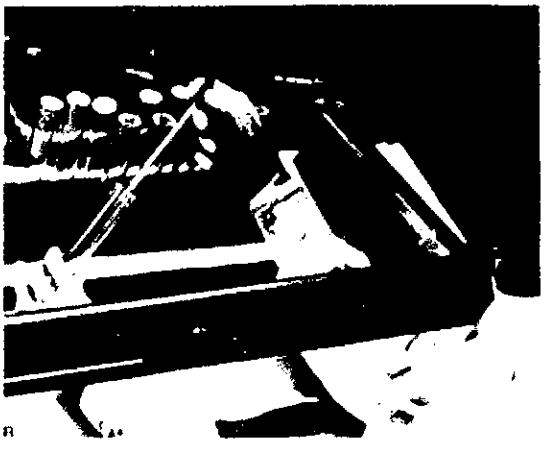


Figure 35-8. Example of one type of safety cabinet used to protect personnel from infectious agents. (A) Open-front bacteriologic safety cabinet. (B) Pipetting operation in open-front safety cabinet. The technician is protected by the glass shield, rubber gloves, and gown. Room air is drawn into the cabinet at the front and ultimately passes through a HEPA (high-efficiency particulate air) filter before leaving the cabinet, thereby preventing the escape of infectious aerosols generated within the cabinet. [*Courtesy of the U.S. Naval Biological Laboratory and W. W. Umbreit (ed.), Advances in Applied Microbiology, vol. 3, Academic, New York, 1961.*]

the H and N antigens of the virus are protective, and recovery from influenza confers a degree of active natural immunity on a population; however, antibodies formed by a host population against previous subtypes of influenza virus do not protect against new H and N subtypes. Therefore, when alteration of their antigenic makeup occurs, influenza viruses can cause new pandemics in populations that had previously been immune to influenza.

Hazards of Aerosols in the Clinical Microbiology Laboratory

Microbiologists are very much concerned by the fact that many routine laboratory procedures can generate aerosols of infectious microorganisms. Laboratory workers have acquired infections during microbiological investigations; more-

Influenza Pandemics—A Recurring Problem

The most disastrous influenza outbreak was the 1918–1919 pandemic, which was worldwide in its distribution and took more than 20 million lives. Although techniques for isolating and characterizing influenza virus were not available at the time, the strain is believed to be similar to one isolated from swine in 1930 which possessed the following variant H and N antigens: HswlN1. This was deduced from the fact that the blood serum of persons born between 1918 and 1929 contained antibodies to the swine virus H and N antigens, whereas the serum of persons born after that period did not. In other words, these antibodies provided an **immunological record** of the influenza virus strains that had been prevalent during the 1918–1929 period.

Marked changes in the H and N antigens occurred in 1947, 1957, and 1968, giving rise to strains having the antigenic variations H1N1, H2N2, and H3N2, respectively. Each change resulted in a pandemic. For example, after the emergence of the H2N2 variant, a pandemic began in the central part of mainland China in February 1957. From there it spread widely in China, then to Hong Kong, and then to other parts of the world. Shortly after September 1957, epidemics swept the United States. Although not as severe as the pandemic of 1918, it is estimated that nearly half the population of the United States became ill and more than 8,000 deaths were caused directly or indirectly by this new antigenic variant. Before this pandemic occurred, it had been found that the blood serum of people 70 to 90 years old already contained antibodies against the new strain. This was of interest because it implied that a similar strain may have caused an epidemic reported to have occurred in 1890. In 1968 the H3N2 variant appeared in Hong Kong and again a pandemic occurred. Still another variant was isolated in 1976 at Fort Dix, New Jersey; its antigenic type was HSw1N1, resembling that of the highly virulent strain thought to be responsible for the 1918 pandemic. This subtype was predicted to give rise to a new and serious pandemic; however, it failed to gain predominance over the H3N2 subtypes that were still prevalent. In 1977 still another subtype emerged, H1N1, similar to the subtype that had been prevalent in 1947, and spread throughout the world. The sequence in which these variants have emerged suggests that antigenic variation in type A virus may occur in a *repeating* or *cyclic fashion* rather than as an endless progression of new subtypes. Future comparisons of new variants with past variants will help to confirm or negate this hypothesis.

over, many infections that are not normally transmitted by aerosols in the general population, such as typhoid fever, can be acquired via aerosols in the laboratory. Certain technical manipulations create larger amounts of aerosols than others: among these are inserting a hot loop into a culture, streaking an inoculum on a rough agar surface, and blowing out the last drop of culture from a pipette. Aerosol formation from these sources can be avoided or greatly reduced by using good laboratory techniques. With highly contagious organisms such as *Francisella tularensis*, even a slight amount of aerosol can be dangerous since only one or a few cells can cause infection. Consequently, many safety devices have been designed to protect laboratory workers from this hazard. Bacteriological safety cabinets like that in Fig. 35-8 are available for working with particularly dangerous organisms.

WATERBORNE TRANSMISSION

Waterborne pathogens usually cause intestinal infections, such as typhoid fever, shigellosis, or cholera. Such infections are usually acquired by the consumption of polluted water containing human fecal matter from patients or healthy carriers. When human feces pollute a municipal water supply or other common source of drinking water, the outbreaks of intestinal disease tend to be of the epidemic type, and the source of drinking water is the common factor linking the various cases. The following example illustrates this principle.

Case History

A wedding reception was held in August at a country club in Pennsylvania. Over the next three days 90 of 119 guests developed gastrointestinal illness. Surveys revealed that only those who drank water at the reception developed the illness. A survey of club members who had played golf at the country club but who had not attended the wedding reception indicated that 60 of 113 golfers had also experienced gastrointestinal illness. Moreover, in the previous three months, 73 percent of the golfers surveyed had become ill. The drinking of water from fountains on the golf course was significantly associated with illness ($p = <0.01$). *Shigella sonnei* was isolated from stool samples from some of the wedding guests and golfers. Water for the country club and the golf course fountains came from an old private drilled well. The water was routinely chlorinated, but early in July the automatic chlorinator broke down and it was not functioning at the time of the outbreak. Coliforms, indicative of fecal contamination, could be cultured from the water supply of the club. Based on sample survey results, over 1,000 persons may have acquired shigellosis from the contaminated well.

The drinking of water is not always required for transmission of some waterborne infections. For instance, leptospirosis—a nonintestinal disease characterized by bacteremia and kidney damage—can be acquired merely by coming into contact with water contaminated with the urine from infected domestic or wild animals (e.g., by swimming in a farm pond frequented by infected cattle, or by working in a rat-infested sewer). The leptospires can penetrate the conjunctiva of the eye, abrasions in the skin, or mucous membranes of the nose and mouth.

FOODBORNE TRANSMISSION

Foodborne diseases may be intestinal diseases but can be other types as well. With regard to their epidemiology, they can be divided into two major categories. (1) In **foodborne intoxications**, such as botulism or staphylococcal food poisoning, the causative microorganisms produce an exotoxin in food; when a person consumes the food, the toxin is ingested and gives rise to the disease. (2) In **foodborne infections**, the causative organisms are ingested; these subsequently grow within the body and cause damage.

Epidemiology of Foodborne Intoxications

In staphylococcal food poisoning, human carriers are responsible for contaminating a food with enterotoxigenic strains of *Staphylococcus aureus*. These carriers usually harbor the organisms in the nose (**nasal carriers**). The sequence of events is usually as follows:

1 The hands of a carrier become contaminated with nasal secretions.
2 The carrier's hands inoculate food during its preparation.
3 The food is stored for several hours without being properly refrigerated, and during this period the staphylococci multiply and produce enterotoxin.
4 The food is consumed, raw or cooked. Cooking does not destroy the toxin: the crude form of the toxin is heat-stable and resists boiling for 30 min or more.

The foods involved are usually such items as milk products, custards, processed meat spreads, cream puff fillings, sandwich spreads, turkey stuffing, and potato salad. Prevention of staphylococcal food poisoning depends mainly on refrigeration of these food items so that multiplication of the staphylococci will not occur. The following example illustrates many of the principles involved.

Case History

Two scheduled airplane flights originating in Rome, Italy, and one charter flight originating in Lisbon, Portugal, landed in United States airports carrying a total of 246 passengers acutely ill with severe nausea and vomiting. Passengers on the first two flights were served identical lunches which consisted of salad, chicken, vegetables, rolls, and custard dessert. A survey revealed a significant association between illness and eating the custard dessert ($p = <0.005$). The lunch served to passengers aboard the third flight had a different entrée and vegetables but included the same custard dessert. On all three flights, first-class passengers and crew members were served different lunches without the custard dessert and none experienced illness. Results from several laboratories indicated the presence of high numbers of *S. aureus* in the custard. Epidemiologic investigation revealed that a catering facility located in Lisbon had provided the lunches for the three flights. The custard was produced from egg yolk, sugar, milk, gelatin, chocolate, gooseberry juice, and strawberry jelly; its preparation each morning required several pouring and chilling steps during a 4-hour period. It was then packed into individual passenger trays and stored in a holding area for 2 hours until placed aboard the plane. The holding area temperature was 62°F (rather than the usual refrigerator temperature) and apparently had been so for several weeks; therefore, the total time the custard was held at a temperature greater than 60°F was over 4 hours; sufficient to allow multiplication of enterotoxigenic staphylococci.

In botulism, soil is usually the source of endospores of the causative organism, *Clostridium botulinum*. Food is easily contaminated by soil and thus by the spores. If the food is an uncooked, processed food such as smoked fish or a cured ham, subsequent storage may allow the spores to germinate, the bacteria to grow, and the neurotoxin to be produced, if appropriate anaerobic conditions exist within the food. Alternatively, if the spore-contaminated food is canned, improper canning procedures (insufficient temperature or time) may allow some of the spores to survive. During a subsequent storage period the spores may germinate, and toxin may then be produced within the anaerobic interior of the can or jar. Botulism occurs sporadically in home-canned foods and only rarely in commercially canned foods. Home-canned foods are less subject to quality control, as illustrated by the following example.

Case History

Four women in Nevada ate a lunch which included a freshly prepared beet salad made from home-canned beets. In the next three days, three of the women had onset of botulism, and the severity of their symptoms corresponded to the amount of salad consumed. A sample of blood serum from one of the women taken prior to her treatment with botulinal antitoxin was positive for type A botulinum toxin by the mouse toxin-neutralization test. Type A botulinum toxin was similarly demonstrated in samples of leftover salad and leftover beets. Pressure cooking had not been employed during the canning of the beets. The beet salad reportedly tasted normal when eaten.

Epidemiology of Food-borne Infections

Foods such as meat, milk, and eggs may come from an animal infected during its life by a pathogen. For example, infection of chickens, turkeys, swine, and cattle by certain *Salmonella* serovars is common. If such food is stored at a warm temperature, the salmonellas may multiply sufficiently to cause infection of persons who consume the food. This is illustrated by the following example.

Case History

During a one-week period, the Wisconsin Division of Health received reports that 7 individuals residing in three contiguous rural counties had become infected with *Salmonella* serovar *saintpaul*. All of the 7 persons had attended the same church dinner. A questionnaire returned by 188 of the 352 persons who had attended the dinner indicated that 19 persons had experienced recent gastrointestinal illness. Everyone attending the dinner ate chicken, but all 19 ill persons and only 53 of the 169 non-ill persons ate before 12:30 P.M. A food preparer, fearing a shortage of chicken, had hurriedly panfried a single batch of chicken for 10 minutes per side, and then placed the batch in a warming roaster at 175°F until served. These chickens were served until 12:30 P.M. but were removed when several persons complained of undercooked chicken.

A second way in which food may become contaminated is by means of human or animal carriers who have access to the food during its storage or preparation. The following example illustrates this kind of transmission.

Case History

During a two-month period, 18 cases of typhoid fever were diagnosed in Michigan among 310 people who had consumed a luncheon served at a community banquet hall. Of the 18 cases, 16 were confirmed by culturing *Salmonella typhi* from blood or stools. All isolates were phagovar E_1 and had the same antibiogram. No specific food could be incriminated. A chronic carrier of *S. typhi* was identified among the food handlers. This individual had participated in the preparation of all or most of the foods served, and *S. typhi* of the same phagovar and antibiogram as that obtained from the patients was isolated from a rectal swab.

The control of foodborne or waterborne infections depends primarily upon preventing the contamination of food and water supplies. This can be done effectively by means of such sanitary measures as proper disposal of human wastes, purification of water supplies, and use of sanitary methods in production and handling of food (including milk). Carriers of enteric organisms such as salmonellas and shigellas must not be permitted to participate in the handling and preparation of food. Detection of carriers, inspection of foods and food processing plants for occurrence of enteric pathogens, and identification of improperly treated water sources are important functions of local and state public health agencies.

TRANSMISSION BY DIRECT CONTACT

Transmission by Person-to-Person Contact

Of outstanding interest among the human direct-contact diseases are the venereal or sexually transmitted diseases (STDs) such as gonorrhea, syphilis, genital herpes, chancroid, nongonococcal urethritis, lymphogranuloma venereum, and granuloma inguinale.

Epidemiology of Gonorrhea. With the discovery during World War II that penicillin was effective in treating gonorrhea, it was predicted that this important STD would eventually be eliminated. Unfortunately, its incidence, which had hit an all-time low in the United States about 1958, took an upward turn, with a steady increase in the annual number of reported cases until 1978. Some of the factors involved were (1) the introduction of oral contraceptives and contraceptive intrauterine devices, which contributed to an increase in sexual freedom and to a decrease in use of spermicidal preparations and condoms (both of which afford some protection against gonorrhea); (2) the inability of public health departments and physicians to trace all the contacts of the many carriers and clinically infected persons; and (3) the emergence of certain strains of gonococci that produce penicillinase and, therefore, are resistant to penicillin (however, the number of isolates of this sort, although on the increase, is still relatively low). In the United States the annual number of reported cases of gonorrhea reached an all-time high of 1,013,436 in 1978, and the rate has decreased only slightly in subsequent years. A high incidence of gonorrhea has not been limited to the United States but has been reported in all parts of the world. The exposure and incidence is highest in the 15- to 29-year age group (Table 35-3).

Table 35-3. Reported Cases of Gonorrhea by Age in the United States, 1982

Age Group	Cases
0–14	10,453
15–19	235,086
20–24	363,135
25–29	195,037
30–39	121,208
40–49	25,972
50+	9,742
Total for all age groups	960,633

SOURCE: Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1982, issued December, 1983.

Gonorrhea can also be acquired by a newborn infant from an infected mother during passage through the birth canal, resulting in a blinding conjunctivitis (gonococcal ophthalmia neonatorum). It has been estimated that 10 percent of all cases of blindness may be due to this type of transmission of the gonococcus. For this reason it is a standard practice to place drops of silver nitrate solution or an antibiotic into the eyes of the newborn to prevent this conjunctivitis.

A rare third type of gonorrhea, vulvovaginitis, may occur in little girls, who may acquire the infection from fomites such as bedclothes, towels, and common bathtubs.

Control of sexually transmitted gonorrhea is accomplished by treatment of patients or carriers with penicillin (or spectinomycin in the instance of peni-

cillin-resistant strains) and also by identification and treatment of other persons who have had contact with these patients or carriers.

Epidemiology of Syphilis. The syphilis spirochete is transmitted mainly by sexual intercourse; however, the organism can also be acquired by an infant in utero from an infected mother (congenital syphilis). The spirochete can also be transmitted by blood transfusion with contaminated blood. The incidence of syphilis, whose long-term effects are serious, is fortunately about 30 times less frequent than gonorrhea, but its distribution by age groups follows the same pattern.

In both syphilis and gonorrhea, the causative microorganisms are easily killed by drying and other environmental influences. This may help to explain why these organisms are restricted mainly to transmission by intimate contact.

Epidemiology of Genital Herpes. In recent years a marked increase in the number of cases of genital herpes has occurred. The disease is caused by the herpes simplex virus type 2. In the United States it has been estimated that there are 400,000 new cases of genital herpes each year and that between 5 and 20 million individuals have the infection. The disease cannot be cured by antibiotics, although the drug acyclovir shows promise as an antiherpes agent. The only method presently available for prevention of this and other viral diseases of the genital tract is the identification of patients and their contacts and avoidance of sexual intercourse with infected persons.

Transmission by Blood or Blood Products

Some microbial pathogens can be transmitted from person to person by transfusion of blood or blood products or by contact with traces of blood or blood serum on hypodermic syringes, syringe needles, tattoo needles, contaminated razors, and similar items that have been used by more than one person without being properly sterilized after each use. A classic example is viral hepatitis type B, a disease that is common among drug addicts. It is also associated with blood bank workers, dentists, and surgeons, who, because of their occupations, can come into contact with infected blood or blood products. There is some evidence that the hepatitis type B virus may also be transmitted by saliva and by sexual contact.

Transmission by Direct Contact with Animals

Some pathogens can be transmitted via contact with the tissues of infected animals. Diseases caused by such pathogens often have an occupational incidence, being contracted mainly by hunters, veterinarians, butchers, slaughterhouse workers, and the like. Examples are brucellosis, tularemia, and anthrax.

Epidemiology of Tularemia. Tularemia is mainly a disease of wild animals. About 1 percent of wild rabbits are infected, and over 90 percent of human cases are contracted from contact with these animals. The causative agent, *F. tularensis;* is an exceptionally small bacterium and has the ability to penetrate small abrasions on human skin and perhaps even unbroken skin. In the initial stage of the disease, a papule often appears on the skin of the fingers or hands and eventually becomes an open sore; this lesion indicates the site of entry of the pathogen into the body.

Case History

In November a 19-year-old man went deer hunting with friends and relatives in central Washington state. Four days later festering sores appeared on his hands, legs, and knees. Spiking fevers followed and eventually the disease was diagnosed as tularemia on the basis of development of a high antibody titer against *F. tularensis*. Despite repeated attempts to elicit a history of exposure to wild rabbits, none was obtained until after Christmas, when a relative recalled that the hunter had found a partially dismembered dead rabbit. The hunter had amputated the front paws for good luck charms, which he gave to another hunter in the party. The rabbit had been handled with bare hands that were bruised and scratched from the hunter's occupation as an automobile mechanic. The recipient of the "good luck charms" remained well; however, he had discarded the paws and they were therefore unavailable for culture.

Wound Infections

Infections may occur whenever a laceration or other type of wound is contaminated with pathogenic microorganisms. Wounds can be contaminated by organisms from the skin; from the object that inflicted the wound; or from various other sources such as soil, clothing, feces, or aerosols. For instance, the major causative agent of gas gangrene, *Clostridium perfringens*, is a normal inhabitant of the intestinal tract of humans and animals. Similarly, the spores of *Clostridium tetani*, the causative agent of tetanus, are common in animal feces and soil. Contamination from these sources frequently occurs in traumatic injuries, e.g., automobile accidents, gunshot wounds, battle injuries, and the like. Wounds most conducive to infection by anaerobic bacteria such as *Clostridium* or *Bacteroides* species are generally deep and ragged, with devitalized tissue. Consequently, the most important step in preventing such infections is to surgically remove the dead tissue and blood clots to prevent development of anaerobic conditions.

It should be recognized that surgical incisions are also wounds and can become contaminated, giving rise to postoperative infections. For instance, staphylococci are commonly present on the skin and, unless the skin is disinfected, they may gain entrance to the body via an incision. Surgical wounds may also become infected from environmental sources such as aerosols and infectious dust in the operating room or in the hospital ward or from contact with articles such as bedpans and bedclothes.

ARTHROPOD-BORNE TRANSMISSION

Arthropod-borne infections have an extensive distribution over the face of the globe. Through the centuries, such diseases have produced much suffering, economic loss, and death in the human population. On innumerable occasions, these diseases have reached pandemic proportions.

Arthropods associated with human infections serve as vectors for pathogenic microorganisms. A **vector** is an organism, such as an insect, that transports a pathogen. Some arthropods serve merely as mechanical vectors of pathogens. The common housefly, *Musca domestica*, is the classic example. The diseases transmitted by it includes salmonellosis, poliomyelitis, infectious hepatitis, amoe-

Table 35-4. Representative Protozoan Diseases of Humans Transmitted by Arthropods as Biological Vectors

Disease	Etiologic Agent (Geographic Distribution)	Biological Vector	Interrelationship of Arthropod-Pathogen-Human
Chagas' disease	*Trypanosoma cruzi* (Continental Latin America)	Reduviid bugs (*Triatoma* spp., *Panstrongylus* spp.)	Pathogen multiplies in midgut of insect; inoculated in humans by rubbing onto skin or into conjunctiva
African trypanosomiasis (sleeping sickness)	*T. gambiense* (West and Central Africa) *T. rhodesiense* (East and Central Africa)	Tsetse flies (*Glossina* spp.)	Pathogen multiplies in midgut and salivary glands of fly; humans inoculated by bite
Malaria	*Plasmodium vivax* *P. malariae* *P. falciparum* *P. ovale* (Regions with warm climates)	Mosquitoes (*Anopheles* spp.)	Pathogen completes sexual cycle, then multiplies by sporogony in mosquito; humans inoculated by bite
Leishmaniasis	*Leishmania donovani* (China, India, Africa, Mediterranean area, continental Latin America) *L. tropica* (Mediterranean area to western India) *L. brasiliensis* (Mexico to northern Argentina)	Sandflies (*Phlebotomus* spp.)	Pathogen multiplies in midgut of fly; humans inoculated by bite

Table 35-5. Representative Bacterial Diseases of Humans Transmitted by Arthropods as Biological Vectors

Disease	Etiologic Agent (Geographic Distribution)	Biological Vector	Interrelationship of Arthropod-Pathogen-Human
Plague	*Yersinia pestis* (Africa, Asia, South America, western United States)	Rodent fleas (*Xenopsylla cheopis*); human fleas (*Pulex irritans*); body louse (*Pediculus humanus*)	Pathogen multiplies in gut of flea; humans inoculated by bite of flea
Tularemia	*Francisella tularensis* (North America, Asia, Europe)	Ticks (*Dermacentor* spp., *Amblyomma* spp., etc.); deerflies (*Chrysops discalis*)	Pathogen multiplies in gut and hemocoel (body cavity through which blood circulates); congenitally transmitted in some ticks; humans inoculated through bite or crushing of tick
Epidemic relapsing fever	*Borrelia recurrentis* (Asia, Africa, Latin America)	Body louse (*Pediculus humanus*)	Pathogen multiplies in tissues of louse outside gut; humans inoculated by crushing louse on skin
Rocky Mountain spotted fever	*Rickettsia rickettsii* (North America, Mexico, Colombia, Brazil)	Ticks (*Dermacentor* spp., *Amblyomma* spp., *Ornithodoros* spp., etc.)	Pathogen multiplies in wall of tick's midgut; congenitally transferred in tick; humans inoculated through bite

Table 35-5. (*continued*)

Disease	Etiologic Agent (Geographic Distribution)	Biological Vector	Interrelationship of Arthropod-Pathogen-Human
Scrub typhus	*Rickettsia tsutsugamushi* (Asia, Australia, Pacific Islands)	Red mites (*Trombicula* spp.)	Pathogen multiplies in gut of mite; congenitally transmitted in mite; humans infected through bite of larval mite
Rickettsialpox	*Rickettsia akari* (United States, Russia, Korea, Africa)	Mouse mite (*Allodermanyssus sanguineus*)	Pathogen multiplies in gut of mite; humans infected through bite
Classical typhus fever	*Rickettsia prowazekii* (Worldwide)	Body louse (*Pediculus humanus*)	Pathogen multiplies in epithelium of louse's midgut; humans inoculated through bite, feces, or crushing of louse on skin
Trench fever	*Rochalimaea quintana* (Europe, Africa, North America)	Body louse (*Pediculus humanus*)	Pathogen multiplies in midgut of louse; humans inoculated by feces or crushing of louse on skin
Murine typhus	*Rickettsia typhi* (Worldwide)	Fleas' (*Xenopsylla cheopis* and others)	Pathogen multiplies in epithelium of midgut of flea; humans infected through bite

Table 35-6. Representative Viral Diseases of Humans Transmitted by Arthropods as Biological Vectors

Disease	Etiologic Agent (Geographic Distribution)	Biological Vector	Interrelationship of Arthropod-Pathogen-Human
Yellow fever	Yellow fever virus (a togavirus) (Africa, South America)	Mosquitoes (*Aedes aegypti, Haemagogus* spp.)	Pathogen multiplies in tissues of mosquitoes; humans inoculated through bite
Dengue fever	Dengue fever virus (a togavirus) (Southern and Southeast Asia, Pacific Islands, Northern Australia, Greece, Caribbean Islands, Nigeria, Latin America)	Mosquitoes (*Aedes* spp., *Armigeres oturbans*)	Pathogen multiplies in tissues of mosquitoes; humans inoculated through bite
Eastern, western, and Venezuelan equine encephalitis	Encephalitis viruses (belong to the togaviruses) (Western hemisphere)	Mosquitoes (*Aedes* spp., *Culex* spp., *Mansonia* spp.)	Pathogen multiplies in tissues of mosquitoes; humans inoculated through bite
Colorado tick fever	Colorado tick fever virus (an orbivirus) (Western United States)	Wood ticks (*Dermacentor andersoni*)	Pathogen multiplies in tissues of tick; humans inoculated through bite

biasis, and other enteric diseases. However, in most arthropod-borne diseases, the arthropod serves as a **biological vector**, i.e., one in which the pathogen undergoes a period of incubation or development. Various diseases in which biological vectors are involved are shown in the accompanying tables. Table 35-4 shows representative diseases of humans caused by protozoa and transmitted by arthropods. Table 35-5 shows representative human diseases of bac-

terial origin transmitted by arthropods. The majority of these bacterial diseases, as shown in the table, are caused by rickettsias—obligate intracellular parasites that are transmitted by lice, ticks, mites, or fleas. Table 35-6 shows representative human infections caused by viruses and transmitted by arthropods. Note that there are many kinds of encephalitis diseases, with different viral agents and varied geographical distributions. Also notice that mosquitoes and ticks are the main vectors involved in the transmission of the arthropod-borne viruses.

Examples of Arthropod-Borne Infections

Epidemiology of Plague. Plague pandemics ravaged Asia and Europe for centuries. The Great Plague, which started in 542, was reputedly responsible for over 100,000,000 deaths in 50 years. The Black Death, the great plague pandemic of the fourteenth century, was considered the worst catastrophe to strike Europe, and perhaps even the world. It resulted in the death of an estimated one-third of the world's population. The last pandemic of the nineteenth century began in central Asia in 1871 and spread to other parts of the world. In India alone during the years 1898–1918 it was responsible for 10,000,000 deaths.

The epidemiology of plague is complex. Plague is mainly a disease of wild rodents and is spread from animal to animal by fleas. In the United States, plague made its first appearance in 1900 at the port of San Francisco, being imported by rats on ships. The domestic rats of the city were soon infected and from them the disease spread to various wild rodents such as ground squirrels, prairie dogs, and wood rats. Thus plague spread eastward from the Pacific coast, and today a rodent reservoir of infection exists in the southwestern United States. A few sporadic human infections occur every year as the result of contact with infected wild rodents; however, epidemics of human plague have not yet occurred in this country.

Human plague epidemics follow a sequence of events. Domestic rats, i.e., rats that share human habitations, contract plague via fleas from infected wild rodents. The blood of infected rats contains large numbers of plague bacilli. When a rat flea ingests this contaminated blood, the action of a bacterial coagulase may cause a clot to form in the insect's proventriculus. This blockage prevents the access of food to the midgut (stomach) of the insect, and a flea in this condition becomes very hungry. As the rat hosts die, the fleas leave the corpses and begin to attack human hosts. Because of the blockage of the proventriculus, the human blood that is ingested by a flea becomes mixed with plague bacilli and is regurgitated into the flea bite. Fleas whose proventriculus is not blocked may excrete feces containing plague bacilli onto the skin during feeding; when a human scratches the flea bites, the flea feces can be rubbed into the bites, thereby transmitting the organism.

Once a human contracts bubonic plague, the disease can be transmitted from person to person via human fleas or human lice. This type of transmission requires heavily louse- or flea-infested populations, which were common in temperate regions such as Europe before the advent of sanitation. The wearing of thick clothing inhabited by lice or fleas was highly conducive to contracting not only plague but other diseases as well, such as louse-borne epidemic typhus. In some bubonic plague patients, the bacilli may reach the lungs and establish a pneumonia (pneumonic plague). This form of plague is highly contagious because it can be transmitted from person to person via aerosols and is no longer dependent on arthropods.

Control of plague depends first upon control and elimination of domestic rats and rat fleas; it is impractical to eliminate the wild rodent reservoirs of infection. Other preventive measures include immunization programs and elimination of human fleas and lice.

Epidemiology of Rocky Mountain Spotted Fever. Despite its name, Rocky Mountain spotted fever (RMSF) occurs throughout the temperate zones of the western hemisphere, and in the United States it is far more prevalent in the east than the west (Fig. 35-9). The causative agent, *Rickettsia rickettsii*, is transmitted in the east mainly by dog ticks and in the west by wood ticks. These arthropods serve as a reservoir of infection as well as vectors. The rickettsias are not pathogenic for the ticks; indeed, they become hereditary and are transmitted to the tick offspring by transovarian passage. The rickettsias are widely distributed throughout the body of a tick, and transmission to humans or animals occurs via infected saliva during biting. Although ticks probably constitute the primary reservoir of infection, there is also an animal reservoir of infection (wild rabbits, dogs, sheep, and rodents) in which the disease is perpetuated by associated ticks.

RMSF occurs mainly in the summer months and in persons engaged in outdoor pursuits, since such persons are most likely to be bitten by ticks. A characteristic rash usually appears first on the ankles and wrists and later spreads to the rest of the body. In many cases an initial diagnosis of measles, meningococcemia, scarlet fever, or other diseases involving a rash is made before RMSF is suspected; however, a history of tick bites is suggestive of RMSF. Prompt treatment with a tetracycline is essential; penicillin is not effective in treatment.

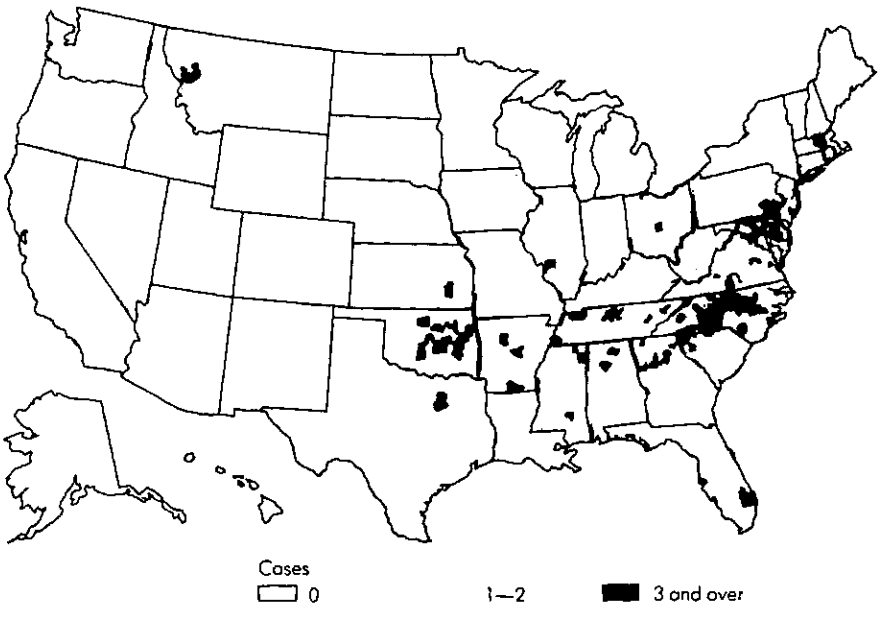


Figure 35-9. Reported cases of Rocky Mountain spotted fever by county, United States, 1981. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.)*

Case History

A 9-year-old boy from Nebraska became ill with a rash and a temperature of 102°F. Over the next three days these symptoms persisted and were accompanied by conjunctivitis, muscle aches, and joint pains. The rash became generalized and extended to the child's palms and soles, and lymph nodes were enlarged in the cervical area. The patient gave a history of having received multiple tick bites within two weeks prior to onset of the illness. A presumptive diagnosis of Rocky Mountain spotted fever was made and treatment was initiated with oxytetracycline. The child made a full recovery. The presumptive diagnosis was later confirmed by serological analysis of blood serum specimens collected at 4 and 34 days after onset of the illness: complement fixation tests indicated that the child had produced an active antibody response against RMSF antigen.

Epidemiology of Yellow Fever. Yellow fever is the most serious arthropod-borne viral disease of the tropics. For more than 200 years after the first identifiable outbreak in the Yucatan in 1648, it was one of the great scourges of the world. Although yellow fever now occurs in South and Central America and in Africa, during the nineteenth century it was also prevalent in the eastern United States, causing at least half a million cases. As late as 1905, New Orleans and other southern American ports had an epidemic that involved at least 5,000 cases and many deaths. The last indigenous case in the United States occurred in 1911; the last imported case occurred in 1923.

Yellow fever results from two basically different cycles of virus transmission, urban and sylvatic (jungle), as shown in Fig. 35-10. In the urban cycle, the virus is transmitted from person to person by bites of the *Aedes aegypti* mosquito. The blood meal taken from a patient contains the virus, which multiplies for 10 to 15 days in the mosquito's intestinal tissue. After the virus appears in the salivary glands, the mosquito can transmit the infection. Once infected, the mosquito remains infectious for the rest of its life.

Sylvatic yellow fever is caused by the same yellow fever virus but occurs in wild animals, mainly monkeys. The virus is transmitted among them, and sometimes to humans, by mosquitoes other than *A. aegypti*. In the rain forests of South and Central America, species of treetop *Haemagogus* or *Sabethes*

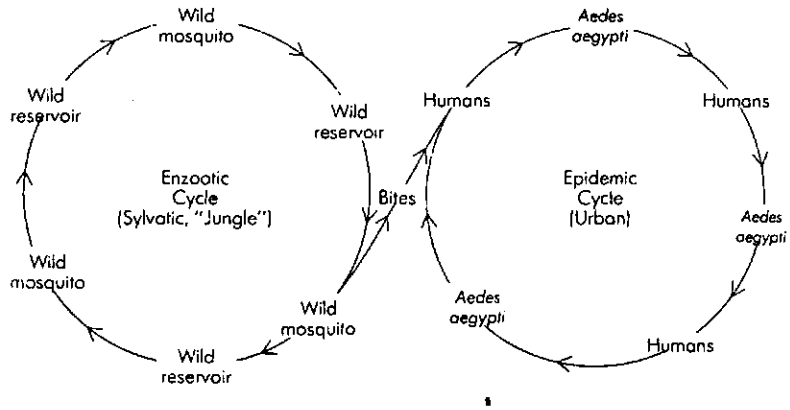


Figure 35-10. Relationship between enzootic (sylvatic) and epidemic (urban) transmission cycles of yellow fever.

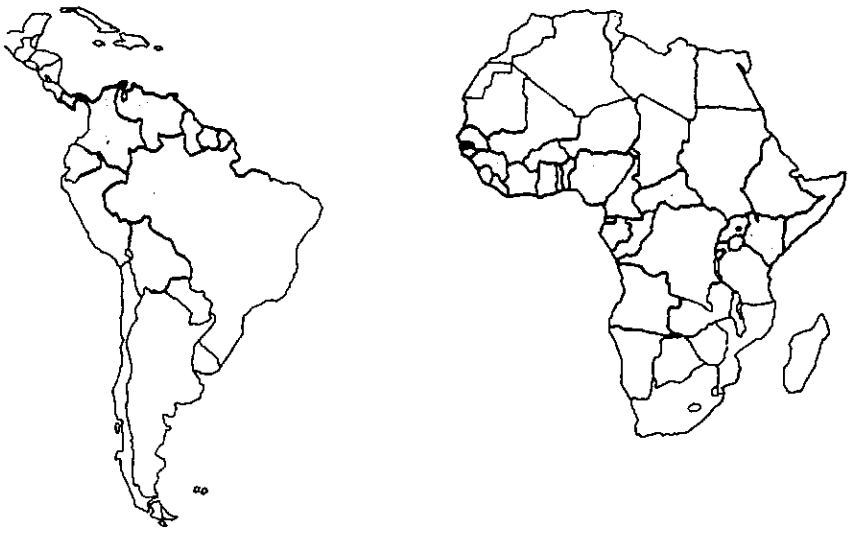


Figure 35-11. Regions where yellow fever is endemic (shaded areas) in South America and Africa.

mosquitoes maintain transmission in the wild animal reservoir. When humans enter the jungle, sporadic cases or local outbreaks may occur because of mosquito bites. In Africa, the mosquito-primate cycle is maintained by *Aedes africanus*, a species which seldom feeds on humans. However, the mosquito *Aedes simpsoni* feeds upon the primates encroaching on village gardens and it can then transmit the virus to humans. The threat of yellow fever always exists in urban areas of the tropical and semitropical regions because of the existence of the sylvatic cycles. Once yellow fever is reintroduced into an urban area, the urban human-mosquito-human cycle can be reinitiated, with the possibility of developing epidemics. The current endemic zones of yellow fever in South America and Africa are shown in Fig. 35-11. Why yellow fever has never invaded Asia despite widespread distribution of human-biting *A. aegypti* mosquitoes is an enigma of medical epidemiology.

Urban yellow fever can be prevented by eradicating *A. aegypti* mosquitoes or by suppressing their numbers to the extent that they no longer perpetuate infection. Control of the sylvatic form is impractical, however, because of the widespread animal reservoir of infection. However, jungle yellow fever can be effectively prevented in humans by immunization.

QUESTIONS

1 Distinguish between the terms *sporadic*, *endemic*, *epidemic*, and *pandemic*, and give examples of diseases to which these terms apply.

2 How does the seasonal incidence of influenza differ from that of (a) Rocky Mountain spotted fever and (b) salmonellosis? Indicate the basis for the differences.

3 Certain diseases, such as tuberculosis and pneumococcal pneumonia, have their highest incidence in elderly or aged persons. What might account for this?

4 Assume that two outbreaks of a bacterial disease occur in different areas of a city. How might you go about obtaining evidence that would confirm or negate the hypothesis that both outbreaks are caused by the same bacterial strain?

5 What evidence suggests that antigenic changes in type A influenza viruses may occur in a cyclic fashion?

6 Give an example of a pathogen that has a high rate of healthy carriers but causes a relatively low incidence of clinical disease. What other factors besides acquiring the organism may be important in occurrence of clinical disease?

7 *Streptococcus pyogenes* is the causative agent of streptococcal sore throat and is transmitted mainly by aerosols.
(a) How might it be transmitted by fomites?
(b) How might it be transmitted by infectious dust?
(c) How could it cause a wound infection?

8 To what hazards can aerosols contribute in the clinical microbiology laboratory, and how can these hazards be prevented?

9 Give an example of a waterborne disease in which ingestion of contaminated water is not required in order to contract the infection.

10 Give the sequence of events leading to (a) an outbreak of staphylococcal food poisoning and (b) an outbreak of botulism. How may such outbreaks be prevented?

11 Give the sequence of events leading to an outbreak of foodborne salmonellosis. How might such outbreaks be prevented?

12 Indicate the epidemiological differences between gonorrhea, vulvovaginitis, and gonococcal opthalmia neonatorum, all caused by *Neisseria gonorrhoeae*.

13 What is meant by the term *animal reservoir of infection*?
(a) Why might veterinarians and slaughterhouse workers be more likely to contract brucellosis than persons having other occupations?
(b) Why might hunters be more likely to contract tularemia than other persons?
(c) Why might a person in the southwestern United States be more likely to contract bubonic plague than a person in the eastern United States?

14 Describe the sequence of events leading to an epidemic of human bubonic plague. What are the differences between the person-to-person transmission of bubonic plague versus pneumonic plague?

15 Indicate the epidemiological differences between sylvatic yellow fever and urban yellow fever. Why would it be easier to eradicate the latter rather than the former?

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Chapter 36 Microbial Agents of Disease: Bacteria

OUTLINE

Spirochetes
Treponema

Microaerophilic Vibrioid Gram-negative Bacteria
Campylobacter

Aerobic Gram-negative Rods
Pseudomonas • *Legionella* • *Brucella* • *Bordetella*

Aerobic Gram-negative Cocci
Neisseria

Facultatively Anaerobic Gram-negative Rods
Escherichia • *Salmonella* • *Shigella* • *Yersinia* • *Vibrio* • *Haemophilus*

Anaerobic Gram-negative Nonsporeforming Rods

Rickettsias and Chlamydias
Rickettsia • *Chlamydia*

Mycoplasmas
Mycoplasma

Facultatively Anaerobic Gram-positive Cocci
Staphylococcus

Aerotolerant Fermentative Gram-positive Cocci
Streptococcus

Aerobic/Facultatively Anaerobic Gram-positive Sporeforming Rods
Bacillus

Anaerobic Sporeforming Rods
Clostridium

Nonsporeforming Gram-positive Rods of Regular Shape
Listeria

Nonsporeforming Gram-positive Rods of Irregular Shape
Corynebacterium

Mycobacteria
Mycobacterium

Bacterial Pathogens of Plants

Bacterial Pathogens of Insects

Although great attention has been paid to bacteria that cause disease in humans and animals, in fact relatively few of the thousands of bacterial species that occur in nature are pathogenic. Consequently, it is important for the clinical microbiologist to be able to distinguish a pathogenic species from the many harmless species that may also occur in clinical specimens. We usually cannot do this by merely observing a Gram stain from a lesion or streaking an agar plate: it is necessary to choose the proper culture media for isolation of the pathogen and to apply appropriate characterization tests in a systematic fashion. We must also determine the antimicrobial agents to which the pathogen is susceptible. All of these things need to be done *as quickly as possible*. Speed coupled with accuracy may make the difference between life and death, and the clinical laboratory is quite different in its aims and perspectives from the microbiology laboratory in an academic or research environment. Clinical microbiology is a demanding profession that requires a maximum of skill, responsibility, specialized knowledge of pathogenic microorganisms, and attention to a myriad of details attendant on the isolation and identification of those organisms.

In addition to bacteria which are pathogenic for humans and animals, some bacteria cause diseases of plants and insects and are consequently of great economic importance to agriculture.

This chapter serves only as an introduction to the subject of pathogenic bacteriology. It highlights the important features of some selected bacterial pathogens and the diseases which they cause. The major characteristics and taxonomic features of the genera discussed in this chapter have been described in Chaps. 13 and 14.

SPIROCHETES

The genera *Treponema*, *Borrelia*, and *Leptospira* contain species that are pathogenic for humans. Diseases caused by these species are listed in Table 36-1.

Treponema

The most important pathogen of this genus is *Treponema pallidum* subsp. *pallidum*, the causative agent of syphilis. Syphilis is a well-known and much dreaded sexually transmitted disease. Of untreated patients, about 25 percent exhibit a spontaneous cure, about 40 percent develop signs and symptoms of tertiary syphilis but do not die from the disease, and about 35 percent die from tertiary syphilis. Syphilis occurs only in humans and is transmitted by direct sexual contact (venereal syphilis) or by placental transfer from an infected mother to the fetus during the first 4 months of pregnancy (congenital syphilis). In 80 percent of the cases of congenital syphilis, the child is born asymptomatic but develops signs of syphilis several weeks or months later.

Venereal syphilis progresses in three stages. Only in the first two stages is the patient able to transmit the infection to others. The primary stage develops after an incubation period of 10 to 90 days during which the treponemes attach to host cells, multiply locally, invade the lymphatic system and blood, and become distributed throughout the body. The first sign of the disease is the chancre, a painless ulcer with a hard margin which develops on the genitals or on other areas of the body. Darkfield microscopy usually reveals treponemes in this lesion (Fig. 36-1). During the primary stage the patient does not feel ill, and the chancre heals within 25 to 40 days.

Table 36-1. Spirochetes Pathogenic for Humans

Genus and Species	Natural Host	Disease
Treponema		
T. pallidum		
subsp. *pallidum*	Humans	Syphilis, venereal (sexually transmitted) or congenital
subsp. *pertenue*	Humans	Yaws, a skin disease common in tropical countries in both hemispheres; transmitted by direct contact
subsp. *endemicum*	Humans	Nonvenereal endemic syphilis (bejel); occurs in the Middle East, Africa, Southeast Asia, and Yugoslavia; transmitted by direct contact
T. carateum	Humans	Pinta, a skin disease found in tropical countries in the Western hemisphere; transmitted by direct contact
Borrelia		
B. recurrentis	Humans	Epidemic relapsing fever; occurs in Asia, Africa, and Latin America; transmitted from person to person by body louse (*Pediculus humanus*)
B. hermsii *B. parkeri* *B. turicatae* *B. hispanica* and others	Wild rodents	Endemic relapsing fever; usually contracted by those engaged in outdoor activities; has a worldwide distribution; transmitted to humans by *Ornithodoros* ticks; a recently described disease called Lyme disease, characterized by a skin eruption, is caused by a *Borrelia*-like spirochete that is transmitted by *Ixodes* ticks
Leptospira		
L. interrogans	Various wild and domestic animals	Leptospirosis, characterized by a bacteremia, fever, muscular pain, and nephritis; transmitted by contact with or ingestion of water containing infected animal urine

The secondary stage develops after 2 to 6 months. A generalized eruption appears on the skin and mucous membranes of the body (Fig. 36-2), and the patient may have lymphadenopathy (swollen lymph nodes), malaise (a vague feeling of bodily discomfort), and a slight fever or headache. The treponemes

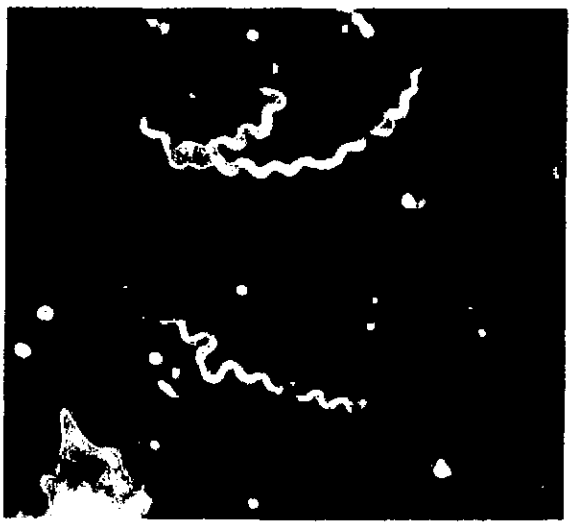

Figure 36-1. *Treponema pallidum* (X3000) in exudate, as seen by dark-field microscopy. *(Courtesy of General Biological Supply House, Inc.)*

Figure 36-2. Left: Secondary syphilis is ushered in by a widespread rash. Right: In secondary syphilis, lesions swarming with spirochetes may occur on the mucous membranes. Note the lesion on the lip. *(Courtesy of Armed Forces Institute of Pathology.)*

Figure 36-3. In tertiary, or late, syphilis, lesions called gummas rupture and result in ulcers. *(Courtesy of Armed Forces Institute of Pathology.)*

may be present in the lesions but are often hard to demonstrate. The skin and mucous membrane lesions disappear after 3 weeks to 3 months. A latent stage, which may last from 3 to 30 years, may intervene between the secondary and tertiary stages.

Approximately 75 percent of untreated patients will progress to the late or tertiary stage and may exhibit signs and symptoms ranging from mild to severe. Even when tertiary syphilis is mild, patients usually have 5 to 10 years taken off their life span and may also exhibit blindness, difficulty in hearing, and other symptoms of "early senility," i.e., being old before one's time. In more severe forms of tertiary syphilis, brain damage may occur, giving rise to paresis (general paralysis of the insane); the symptoms include failing memory, personality changes, insomnia, headaches, and delusions, and paresis may progress to the point where the patient must be institutionalized. Tabes dorsalis results from damage to the spinal cord: the patient becomes uncoordinated and has loss of reflexes, uncontrollable urinations, loss of perception and sensation, impotency, lancinating ("lightning") pains, blindness, and loss of hearing. In addition to these symptoms, damage to blood vessels such as the aorta or those nourishing the central nervous system can occur. Disfiguring granulomatous lesions called gummas may appear on or within various parts of the body (Fig. 36-3).

T. pallidum cannot be cultivated on laboratory media. It can only be grown, with difficulty, in the testes of rabbits or under special conditions in tissue

culture. Consequently, the laboratory diagnosis of syphilis does not depend on isolation of *T. pallidum* but rather on the demonstration of antibodies in the patient's serum which have formed in response to the infection. Simple serologic screening tests are used initially for this purpose, such as the VDRL (Venereal Disease Research Laboratory) test or the RPR (rapid plasma reagin) test. These tests are simple and inexpensive, but they are nonspecific; i.e., they use a nontreponemal antigen, cardiolipin (usually prepared from beef heart muscle), to detect the presence of Wassermann antibodies (anticardiolipin antibodies), which occur in a syphilitic patient's serum. However, certain other diseases, e.g., malaria, lupus erythematosus, rheumatoid arthritis, and infectious mononucleosis, may also stimulate the formation of Wassermann antibodies. Thus a positive reaction must be confirmed by a specific test which can detect antitreponemal antibodies, i.e., a test in which a preparation of *T. pallidum* is used as the antigen. Two widely used specific tests are the fluorescent treponemal antibody (FTA) test and the *T. pallidum* hemagglutination (TPH) test.

For treatment of syphilis, penicillin is the drug of choice; however, the longer the disease progresses, the more difficult it is to cure. Immunization against syphilis is not yet possible because of the inability to culture *T. pallidum* for use in a vaccine. Recently, genes from *T. pallidum* have been cloned in *Escherichia coli*; this suggests a way to produce *T. pallidum* antigens for use in immunization without actually having to culture *T. pallidum* itself. With regard to active natural immunity, persons who have recovered from the disease are susceptible to reinfection on subsequent exposure.

MICROAEROPHILIC VIBRIOID GRAM-NEGATIVE BACTERIA

Campylobacter

The small, motile, vibrio-shaped organisms belonging to the genus *Campylobacter* (Fig. 36-4) are mainly parasites and pathogens of cattle, sheep, and other domestic animals. Some can also cause gastroenteritis and blood infections in humans; of these, *C. jejuni* has received the greatest attention. This species causes as many cases of diarrhea as *Salmonella* and *Shigella*. Transmission to humans occurs mainly by ingestion of food or water containing fecal matter from infected animals, and the disease has its highest incidence during the summer months. Person-to-person transmission rarely occurs. *C. jejuni* infects people of all ages, although it is isolated most frequently from persons 10 to 29 years old. The organism produces acute exudative and hemorrhagic inflammation of the wall of the small and large intestine, although the exact mechanism

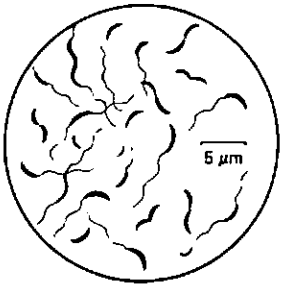

Figure 36-4. Drawing of cells of *Campylobacter*. The flagella are not seen by ordinary staining. *(Erwin F. Lessel; illustrator.)*

by which illness is caused is not known. Some strains have been reported to produce an enterotoxin that is similar to the cholera enterotoxin. *C. jejuni* infections can be treated with erythromycin or tetracycline.

Bacteriological diagnosis depends on isolation of *C. jejuni* from stool samples. A medium containing various antibiotics is used to suppress other intestinal bacteria while allowing growth of *C. jejuni*. Because campylobacters are microaerophilic, cultures must be incubated under a gas atmosphere containing approximately 6 percent O_2. Growth occurs best at 42°C.

AEROBIC GRAM-NEGATIVE RODS

Pseudomonas

Several species of *Pseudomonas* are pathogenic, but most are opportunistic pathogens. The most serious animal pathogens are *P. mallei* and *P. pseudomallei*. *P. mallei* is a true parasite, occurring only in animal hosts; it is the causative agent of glanders, a disease to which nearly all warm-blooded animals are susceptible except cattle, pigs, and pigeons. *P. pseudomallei* is a tropical and subtropical soil organism that can cause a disease known as melioidosis in a large variety of animals and occasionally in humans.

Of the many species of *Pseudomonas*, the species most frequently encountered in human clinical specimens is *P. aeruginosa*. In addition to an endotoxin, many strains produce a lethal exotoxin (**toxin A**), which has a mechanism of action identical to that of diphtheria toxin (see Chap. 31). Other virulence factors include a **hemolysin**, a **leukocidin**, **proteases** which cause tissue necrosis, and an **exocellular polysaccharide** that inhibits phagocytosis. *P. aeruginosa* is commonly found in soil and water but can occur in many other places as well, including hospital environments; e.g., it has been isolated from disinfectant solutions, bedside water containers, hand creams, flower-vase water, sinks, respiratory equipment, and many other hospital items. It is the causative agent of many **nosocomial infections** (infections acquired in a hospital), including infections following surgery, burn infections, and urinary tract infections. With regard to the latter, catheterization (i.e., the insertion of a rubber or plastic tube called a *catheter* into the urethra in order to obtain urine specimens) can introduce *P. aeruginosa* into the body. Other factors that may predispose toward *P. aeruginosa* infections in hospital patients are immunosuppressive agents, antibiotics, and irradiation. Cystic fibrosis patients are especially prone to develop fatal infections. *P. aeruginosa* is resistant to many common antibiotics, although gentamicin and carbenicillin often are effective.

Legionella

These small Gram-negative rods (Fig. 36-5) cause legionellosis—a type of bronchopneumonia—in humans, and all *Legionella* species are potentially pathogenic. Humans probably acquire the organisms from environmental sources (see Chap. 13), and person-to-person transmission does not seem to occur.

Legionellosis was first recognized as a new form of pneumonia in 1976 as the result of an outbreak of illness at an American Legion convention in Philadelphia in which 182 persons were affected and 29 died. At first the causative agent could not be isolated, but the Centers for Disease Control in Atlanta were eventually able to culture the organism in guinea pigs and embryonated chicken eggs and on special laboratory media. Once the organism was isolated, it became clear that legionellosis was not a new disease and that it had existed at least as

Figure 36-5. Scanning electron micrograh of the Legionnaires' disease bacterium, *Legionella pneumophila* (X16,200). *(Courtesy of D. D. Ourth, D. L. Smalley, and C. G. Hollis, Memphis State University.)*

far back as 1947. This was discovered by using the newly isolated bacterium as the antigen in immunofluorescence tests in order to detect the presence of serum antibodies against the organisms. The tests indicated that such antibodies were present in sera which had been preserved from various patients who, years earlier, had died of pneumonias of unknown origin.

Several species of *Legionella* have now been described, of which the best-known is *L. pneumophila*. This species may cause two kinds of disease syndromes. The first, known as Legionnaires' disease, is a severe bronchopneumonia characterized by fever, chills, and a nonproductive (dry) cough and sometimes by chest pain, abdominal pain, vomiting, diarrhea, and mental confusion. The fatality rate is 15 percent or higher. The disease tends to occur most frequently in men above the age of 50. Since 1976, more than 26 outbreaks and nearly 1,000 cases have been detected throughout the United States and in more than 15 other countries. The second type of legionellosis, termed Pontiac fever, is a less severe disease characterized by fever, chills, headache, dry cough, and muscle pain and is self-limiting and nonfatal.

Control of legionellosis is complicated by the wide environmental occurrence of the organisms, but it has been suggested that such measures as incorporation of germicides into the water of central air-conditioning cooling towers may be effective, especially during the warmer months when the incidence of legionellosis is highest. The drug of choice for treatment of *L. pneumophila* infections is erythromycin. No vaccine is presently available for prevention of legionellosis.

Brucella

Brucella species are mainly parasites and pathogens of domestic animals. However, three species—*B. melitensis* (occurring in sheep and goats), *B. abortus* (occurring in cattle), and *B. suis* (occurring in swine)—can cause brucellosis in humans, who acquire the disease from the animal hosts. Before the widespread use of pasteurization, unpasteurized milk from cows and goats was an important vehicle for many outbreaks of human brucellosis, and *B. abortus* and *B. melitensis* were the species most frequently encountered. At present, however, *B. suis* is the species most frequently isolated from human infection, being acquired through direct contact with infected swine or by inhalation of aerosols generated during processing of the meat from infected swine. Consequently,

brucellosis is an occupational disease for veterinarians, butchers, and slaughterhouse workers.

In humans the disease is characterized by generalized aches and pains of the muscles and joints, headaches, chills and night sweats, and a prolonged, irregular (undulating) fever which continues into a chronic stage. Brucellas initially multiply within lymph nodes and later pass to the bloodstream. Here, antibodies formed by the patient act in conjunction with complement to cause bacteriolysis and liberation of **endotoxin**, which causes the fever response and other generalized symptoms. Many of the bloodborne bacteria are removed by macrophages of the liver, spleen, lymph nodes, and bone marrow. The bacteria continue to survive and grow within the macrophages; thus brucellas are mainly intracellular pathogens. Antibodies are ineffective in curing the infection because they cannot easily penetrate the cells within which the bacteria are growing. The only effective immunity in brucellosis is cell-mediated immunity, and the lesions within the body are **granulomas**, i.e., nodules which are composed of macrophages and T lymphocytes and which are similar to those occurring in tuberculosis (see later in this chapter).

Although spontaneous abortion is not a formal feature of human brucellosis, it is a very important feature of brucellosis in cattle and other domestic animals. Brucellas localize in the reproductive organs of these animals because of the presence of the 4-carbon sugar alcohol erythritol, a compound that is highly stimulatory to the growth of the bacteria. Erythritol does not occur in the reproductive organs of humans.

In the laboratory diagnosis of brucellosis, the organisms are difficult to isolate and usually diagnosis is based on demonstration of a rising level of anti-*Brucella* antibodies in the patient's serum.

Tetracycline is the drug of choice for treatment of brucellosis in humans. No vaccine is available for human immunization, but live attenuated vaccines are available for immunization of cattle, sheep, and goats.

Bordetella

The main human pathogen of this genus is *Bordetella pertussis* (Fig. 36-6), the causative agent of pertussis (whooping cough). This species attaches preferentially to ciliated bronchial epithelial cells and produces four virulence factors: (1) an **endotoxin**; (2) **pertussigen**, which induces lymphocytosis (excessive numbers of lymphocytes in the blood), causes increased sensitivity to histamine, and induces hypoglycemia; (3) the **HLT** toxin (heat-labile toxin), which may cause damage to the epithelium of the respiratory tract; and (4) an antiphagocytic capsule.

Figure 36-6. *Bordetella pertussis* (X1,300). Cells of this species range from 0.2 to 0.3 by 1.0 μm. *(Courtesy of J. R. Porter.)*

Whooping cough is mainly a childhood disease. About 15 percent of the cases and 85 percent of the deaths occur in children under 2 years of age. The disease is characterized by a paroxysmal cough that ends with an inspiratory crowing sound, or "whoop." During the paroxysms, cyanosis, vomiting, and hemorrhages of the nose, eyes, and even brain may occur. Inability to eat or retain food may result in malnutrition, making the patient susceptible to secondary infections. Bronchopneumonia is a common complication, and deafness and other permanent damage may result.

Diagnosis can be confirmed by isolation of the organism from nasopharyngeal swabs. The organism will not grow on ordinary culture media because such media contain toxic substances such as unsaturated fatty acids. Starch must be

added to adsorb these toxic substances (detoxify the medium). A suitable starch-containing medium is **Bordet-Gengou agar**, to which penicillin is added to suppress the growth of other respiratory tract flora.

Erythromycin is the drug of choice for treating cases of whooping cough.

Vaccination of infants and children has reduced the incidence of whooping cough dramatically. Most pediatricians now give pertussis vaccine (a suspension of killed *B. pertussis*) along with tetanus and diphtheria toxoids to infants at 2, 4, 6, and 18 months of age. Vaccination is repeated at 4 or 5 years of age.

AEROBIC GRAM-NEGATIVE COCCI

Neisseria

The two most important pathogens of this genus are *Neisseria meningitidis* (Fig. 36-7), the causative agent of meningococcal meningitis, and *N. gonorrhoeae* (Fig. 36-8), which causes gonorrhea.

Neisseria meningitidis. Meningococci possess a **capsule** that helps to inhibit phagocytosis. Several serovars (antigenic types) occur and are differentiated by the polysaccharide composition of the capsule; serovars A, B, and C are the most common. Meningococci also possess a powerful **endotoxin**.

Humans are the only natural hosts for meningococci. The bacteria are transmitted by airborne means from patients with active infections and from convalescent or healthy carriers. Epidemics of meningococcal meningitis occur infrequently, but isolated cases may appear at any time. The disease is endemic in many large groups of "people in the herd," e.g., in the military and in college dormitories, where the majority of the population are young adults. Such groups experience rather frequent outbreaks due in part to introduction of new carriers or susceptibles. During an epidemic the carrier rate may be very high, even 80 to 90 percent, whereas the incidence of clinical disease is very low, 0.01 to 0.3 percent. Those who do develop the disease are very seriously ill and the mortality rate is high, 85 percent without treatment.

Figure 36-7. *Neisseria meningitidis* in cerebrospinal fluid, showing the characteristic diplococcus arrangement. The large objects are pus cells (neutrophils) (X1,400). *(U.S. Army photograph.)*

Meningococcal meningitis is characterized by excessive nasal secretions, sore throat, headache, fever, pain in the neck and back, loss of mental alertness, and sometimes a skin rash. Death can occur within 24 h after the symptoms begin, and therefore prompt diagnosis of meningococcal meningitis and treatment with penicillin is essential.

Presumptive diagnosis is made by demonstration of Gram-negative diplococci in stained smears of spinal fluid (Fig. 36-7). Confirmation is made by isolating the organism on prewarmed plates of a rich, blood-containing medium, with incubation under an atmosphere enriched in CO_2.

Penicillin is the drug of choice for treatment of meningococcal meningitis. A vaccine is available for epidemic control of meningococcal meningitis and consists of the purified capsular polysaccharides of serovars A and C; the serovar B polysaccharide appears not to be highly immunogenic.

Neisseria gonorrhoeae. Virulence factors of gonococci include **pili**, which allow adherence of gonococci to host cells; an antiphagocytic **capsule**; and a potent **endotoxin**.

Gonorrhea is transmitted mainly by sexual contact, except for the forms called vulvovaginitis and gonococcal opthalmia neonatorum (see Chap. 35). In the

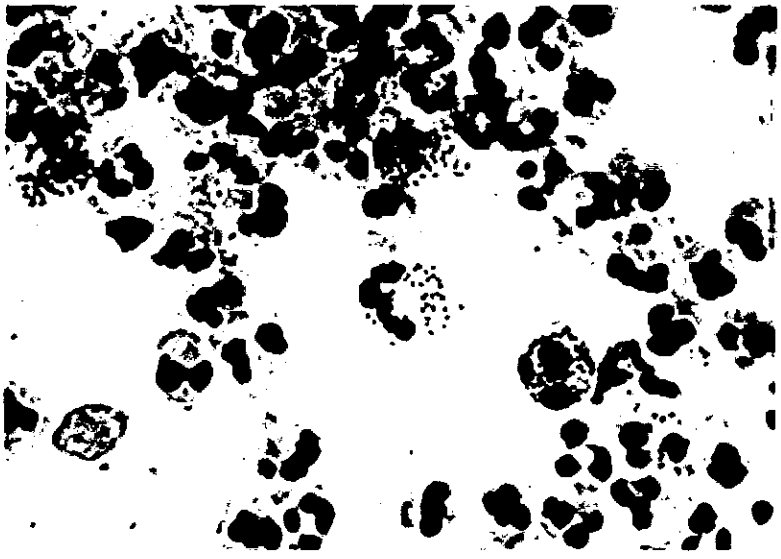

Figure 36-8. *Neisseria gonorrhoeae* in urethral exudate. Many of the bacteria occur within the cytoplasm of pus cells (neutrophils). These bacteria range from 0.6 to 1.0 μm in diameter. *(Courtesy of C. Phillip Miller.)*

male the primary site of infection is the urethra; in the female it is the cervix. The disease is usually more obvious in males because of pain during urination and a yellowish discharge from the urethra. The infection may extend to the prostate and epididymis. Between 20 and 80 percent of females are asymptomatic; those having symptoms may have painful urination, vaginal discharge, fever, and abdominal pain. In females the disease may ascend the genital tract and in 10 percent of cases the fallopian tubes become infected, leading to pelvic inflammatory disease (PID), a major cause of sterility. Other complications may include arthritis, endocarditis, and meningitis.

In the laboratory diagnosis of gonorrhea, smears from exudates often show the organisms inside neutrophils (Fig. 36-8). Isolation is done on Thayer-Martin medium, a rich blood-containing agar to which are added antibiotics to suppress the growth of other bacteria. *Neisseria* colonies are oxidase-positive, as indicated by a reddish-purple color that develops when the colonies are flooded with 1% tetramethyl-p-phenylenediamine reagent. Species identification is made on the basis of biochemical and serological tests.

Penicillin is usually used for treatment; spectinomycin is used for cases caused by penicillin-resistant strains. A vaccine is presently being developed, based on the ability of secretory IgA antibodies to prevent piliary attachment of the bacteria to host tissue.

FACULTATIVELY ANAEROBIC GRAM-NEGATIVE RODS

Escherichia

Although *E. coli* is part of the normal flora of the intestinal tract, certain strains can cause a moderate to severe gastroenteritis in humans and animals. Enteropathogenic strains colonize the jejunum and upper ileum of the small intestine and cause acute gastroenteritis in newborns and in infants up to 2 years of age. Enteroinvasive strains invade the epithelial cells of the large intestine and cause diarrhea in older children and adults. Enterotoxigenic (enterotoxin-producing)

strains produce one or both of two different toxins: a **heat-stable toxin (ST)** and a **heat-labile toxin (LT)**. Both toxins cause diarrhea in adults and infants. The LT stimulates adenylate cyclase activity in a manner similar to that of cholera toxin (see Chap. 31), whereas the ST stimulates guanylate cyclase activity. Enterotoxigenic strains of *E. coli* are often associated with traveler's diarrhea, a common disease contracted by tourists when visiting developing countries.

Other strains of *E. coli* which are usually harmless in their normal habitat (the intestine) can cause disease when they gain access to other sites or tissues. These diseases include urinary tract infections, septic infections, bacteremia, meningitis, pulmonary infections, abscesses, and skin and wound infections.

Salmonella

Over 2000 serovars of *Salmonella* exist, all of which are pathogenic for humans and often for animals. They are characterized by different combinations of **O antigens** (heat-stable outer membrane polysaccharides; see Fig. 5-21) and **H antigens** (heat-labile flagellar protein antigens). O antigens are designated by numbers 1 to 67. There are two major categories of H antigens, called phase 1 and phase 2. Each phase 1 antigen occurs in only a few serovars and is designated by a letter, a to z (z_1 to z_{59}). Fewer kinds of phase 2 antigens occur, but they are widely distributed among serovars and are usually designated by numbers. In addition to O and H antigens, a **Vi antigen** (capsular antigen, which can be destroyed at 100°C) occurs in *S. typhi* and a few other serovars. *Salmonella* serovars often have been assigned names as if they were species, but some are designated only by their antigenic formulas. Antigenic formulas are written as in this example: 6,7:r:1,7 (representing O antigens 6 and 7: phase 1 antigen r: phase 2 antigens 1 and 7). In the **Kauffmann-White scheme**, those serovars with particular O antigens in common are collected into O groups and arranged alphabetically by H antigens within the group. Table 36-2 gives the names of a few serovars commonly encountered in human infections, together with their antigenic formulas.

Salmonellas may cause three kinds of infections: enteric fever (typhoid or paratyphoid fever), gastroenteritis, and septicemia. Each of these has certain characteristic features.

Typhoid fever occurs only in humans and is caused by *S. typhi*, which is transmitted via food or water (see Chap. 35). The disease is characterized by a continued fever, inflammation of the intestine, formation of intestinal ulcers, and enlargement of the spleen. The disease begins in the small intestine, where the bacteria attach to the epithelium of the intestinal wall, penetrate this layer, multiply in the mesenteric lymph nodes, and eventually reach the bloodstream.

Table 36-2. Some *Salmonella* Serovars Commonly Encountered in Human Infections

	Kauffmann-White		
Serovar Name	O Group	Formula	Human Disease
S. paratyphi A	A	1,2,12:a:–	Enteric (paratyphoid) fever or gastroenteritis
S. typhimurium	B	1,4,5,12:i:1,2	Gastroenteritis, septicemia, or focal infection
S. choleraesuis	C	6,7:c:1,5	Enteric fever or gastroenteritis
S. typhi	D	9,12 [Vi]:d:–	Enteric (typhoid) fever
S. enteritidis	D	1,9,12:g,m:–	Gastroenteritis

Here, lysis of some of the bacteria by the combined action of antibodies and complement results in the liberation of endotoxin, which causes various generalized disease symptoms such as fever. Some of the bacteria are excreted in the urine; others are removed from the blood by macrophages of the liver, spleen, lymph nodes, and bone marrow, in which they survive and multiply. Some pass from the liver to the gall bladder and bile ducts and are secreted into the intestine, where they establish a secondary infection and may cause diarrhea. Bacterial excretion in the feces may occur for weeks, and some persons become chronic carriers (see Chap. 35). Laboratory diagnosis of typhoid fever is based on isolation of the organism from blood samples during the first and second weeks or from stool samples during the second and third weeks. The most effective antibiotics for treatment of typhoid fever are ampicillin and chloramphenicol.

Recovery from an attack of typhoid fever does confer a lasting immunity, probably of the cell-mediated type, and second attacks in the same individual are rare. Immunization can be achieved by vaccination with acetone-killed whole cells of *S. typhi* and can confer protection for 3 years. Live attenuated vaccines for oral administration are presently being tested; these may stimulate the formation of secretory antibodies in intestinal fluid and thereby prevent initial attachment of *S. typhi* to the intestinal wall.

Foodborne gastroenteritis is caused by many serovars of *Salmonella*, some of which are associated with animal as well as human infection. The epidemiology of the disease is described in Chap. 35. After an incubation period of 12 to 24 h, the illness commences with a severe headache followed by nausea, moderate vomiting, severe diarrhea, abdominal pain, and fever. The disease usually lasts for 2 to 5 days. Unlike typhoid fever, there is seldom a bacteremia, and the disease is mainly an intestinal infection. The mechanism by which the diarrhea is caused is not clear. It may be due merely to invasion of the intestinal wall, which occurs in both the small and large intestine and results in appearance of blood, mucus, and leukocytes in the stools, or it may be due to an enterotoxin. An enterotoxin has been demonstrated in certain *Salmonella* strains.

Laboratory diagnosis of *Salmonella* gastroenteritis depends on isolation of the causative *Salmonella* strain from stool specimens. Treatment of *Salmonella* gastroenteritis with antibiotics is not recommended except in severe cases (which usually occur in infants or elderly persons) because the disease is usually self-limiting and the drugs do not decrease the duration of the disease. To prevent dehydration of the body from the diarrhea, intravenous replacement of fluid and electrolytes may be required.

A vaccine against *Salmonella* gastroenteritis is impractical because of the large number of *Salmonella* serovars.

Septicemia can be caused by any *Salmonella* serovar, although *S. choleraesuis* is the most frequent. From the intestinal tract the bacteria presumably reach the bloodstream in a manner similar to that described above for *S. typhi*. In the bloodstream the bacteria multiply, causing a recurring high fever, chills, loss of appetite, and weight loss. Gastroenteritis seldom occurs, and although the organisms can be isolated from blood samples they cannot be isolated from stools. The disease may persist for a long time in chronic form. The bacteria in the blood are distributed to various parts of the body, where they may cause meningitis, pneumonia, abscesses, nephritis, osteomyelitis, or endocarditis. Ampi-

cillin or chloramphenicol are effective for treatment of septicemia caused by *Salmonella*.

Shigella

Shigellas are restricted to humans as hosts. Four species exist, which correspond to four antigenic groups: *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). These groups are based on O antigens only (shigellas have no flagellar H antigens and are nonmotile). In the United States, *S. sonnei* is common in the north and *S. flexneri* in the south; *S. dysenteriae* and *S. boydii* seldom occur.

The main virulence factor of shigellae is their endotoxin; however, some shigellas, such as *S. dysenteriae* type 1, can produce an enterotoxin (shiga toxin) as well, which causes fluid accumulation in the intestine. The mechanisms by which the shiga toxin causes this fluid accumulation is unknown. In addition to its action as an enterotoxin, the shiga toxin is also a cytotoxin that kills various kinds of tissue cells by inactivating the 60S subunit of eucaryotic ribosomes, thus inhibiting protein synthesis. Originally the shiga toxin was thought to be a neurotoxin because it caused paralysis when administered to rabbits. However, such paralysis could be rapidly reversed by treatment with antitoxin, which was not characteristic of the type of paralysis caused by a true neurotoxin (such as botulinum or tetanus toxins). Other evidence indicates that the shiga toxin probably causes paralysis by damaging the walls of blood vessels, resulting in fluid accumulation and pressure buildup in the central nervous system.

Shigellosis, also known as bacillary dysentery, is characterized by inflammation of the wall of the large intestine, with consequent diarrhea and stools containing blood, mucus, and pus. Fever is sometimes present, presumably due to absorption of endotoxin from the intestine. Unlike salmonellas, shigellas never penetrate beyond the intestinal wall, and blood cultures are invariably negative. Laboratory diagnosis depends on isolating shigellas from diarrheic stools or rectal swabs.

In severe cases of shigellosis, dehydration of the body may necessitate intravenous replacement of fluid and electrolytes. Ampicillin or a combination of trimethoprim and sulfamethoxazole can decrease the duration of the disease.

Yersinia

Three species of this genus have been studied extensively: *Yersinia pestis*, the causative agent of plague, and *Y. pseudotuberculosis* and *Y. enterocolitica*, which cause gastroenteritis.

Y. pestis is a plump, nonmotile Gram-negative rod which, when stained in smears of animal tissue by special methods, such as Wayson's method, exhibits **bipolar staining** (deeply stained area at both poles; Fig. 36-9). Unlike most pathogenic bacteria, *Y. pestis* grows best at 25°C rather than 37°C. Virulence factors produced by *Y. pestis* include (1) an antiphagocytic capsule termed **fraction 1**, composed of carbohydrate and protein; (2) the **VW complex**, a complex of a cell-wall protein (V) and a lipoprotein (W), and which is also antiphagocytic; (3) an **endotoxin**; and (4) the **murine toxin**, a protein toxin that is lethal for rats, mice, and other animals susceptible to plague. The role of the murine toxin in plague is not yet certain.

The epidemiology of bubonic and pneumonic plague is described in Chap.

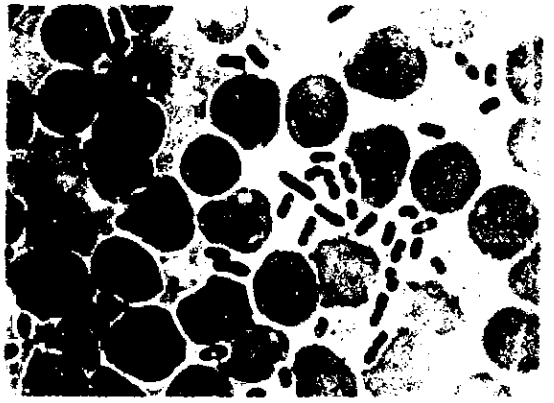

Figure 36-9. *Yersinia pestis* organisms show bipolar staining in this smear of mouse blood. The average size of this organism is 1.0 by 2.0 μm. The large round objects are erythrocytes. *(Courtesy of U.S. Naval Biological Laboratory.)*

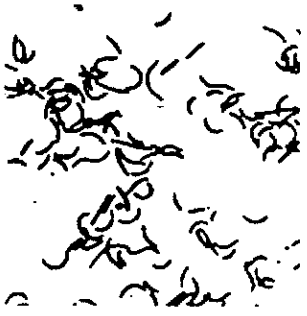

Figure 36-10. *Vibrio cholerae* (X1,500). *(Courtesy of J. Nowak, Documenta Microbiologica, part 1, "Bakterien," Gustav Fischer Verlag, Jena, Germany, 1927.)*

35. The bubonic form is characterized by chills, fever, nausea, vomiting, general weakness, and enlarged, inflamed lymph glands (bubos). The exudates from bubos are filled with plague bacilli. In untreated cases of bubonic plague the mortality rate is 50 percent. Pneumonic plague is a pneumonia characterized by a thin, watery sputum with bright red streaks of blood, and it has nearly a 100 percent mortality rate if untreated.

Streptomycin is effective for treatment of plague cases. Prevention of plague can be achieved by the control measures described in Chap. 35. Vaccines consisting of killed whole cells or live attenuated strains are available. The vaccines stimulate development of antibodies against fraction 1 and the V/W antigens, thereby enhancing phagocytosis.

Y. pseudotuberculosis is closely related genetically to *Y. pestis* and occurs in nearly all animal species, especially in rodents. It can cause acute mesenteric lymphadenitis in humans, a disease that is sometimes mistaken for appendicitis. In outbreaks, the initial cases are probably contracted by ingestion of food or water contaminated by feces from an infected animal, but secondary cases may result from the primary case via human fecal contamination. The virulence factors possessed by the organism are not known.

Y. enterocolitica also occurs in a wide range of animals and has much the same kind of transmission to humans as *Y. pseudotuberculosis*. It causes gastroenteritis, mainly in children, and in some parts of the world it is as common as *Shigella*. How widespread the infections are in the United States has not yet been evaluated. An enterotoxin similar to the ST toxin of *E. coli* has been demonstrated in laboratory cultures. Both *Y. enterocolitica* and *Y. pseudotuberculosis* grow best at 28 to 29°C, and isolation is often difficult on plates incubated at 37°C.

Vibrio

The most important species of this genus is *Vibrio cholerae* (Fig. 36-10), the causative agent of cholera. Other pathogenic species include two marine species: *V. parahaemolyticus*, which causes an acute gastroenteritis contracted by consumption of raw or inadequately cooked seafood, and *V. vulnificus*, which causes wound infections as well as fatal septicemias.

V. cholerae strains are assigned to various serovars based on their somatic O

antigens. The strains that cause epidemic and pandemic cholera belong to serovar O1. For epidemiological purposes, this serovar is further divided into two biovars: "el tor," which is hemolytic on sheep blood agar, and "classical," which is nonhemolytic. The major virulence factor produced by serovar O1 strains is the cholera enterotoxin. The mechanism by which this toxin causes severe diarrhea is discussed in detail in Chap. 31. Also important for virulence is the ability of the vibrios to adhere to the epithelium of the small intestine; however, the mechanism of this adherence is not yet known.

Some strains of *V. cholerae* do not belong to serovar O1 and are called nonagglutinable (NAG) strains (because O1 antiserum does not agglutinate them) or noncholera vibrios (NCV). Such strains are widespread in freshwater and estuarine environments. They produce an enterotoxin similar to cholera enterotoxin and can cause a mild choleralike disease.

Cholera is a disease of antiquity and has been the cause of untold suffering and death. It was endemic in parts of India for centuries but began to invade the rest of the world in the early part of the nineteenth century, causing a series of six great pandemics between 1817 to 1923. A seventh pandemic, caused by the el tor biovar, began in 1960 in Hong Kong and by 1971 had spread to the Philippines, Indonesia, the Middle East, Russia, Africa, and even to Spain and Portugal. The Pakistani-Indian War of 1971, with its massive population shifts, resulted in thousands of deaths due to cholera. Although presently rare in the United States, cholera was prevalent during the nineteenth century. In the twentieth century cholera has been imported many times but epidemics have not occurred; however, in 1978 about a dozen cases occurred in Louisiana and a few other cases occurred in Texas in 1981.

Cholera is transmitted in water and food contaminated with excreta from patients and convalescent carriers. In the small intestine the organisms adhere to the epithelium, multiply, and produce the enterotoxin. No penetration of the intestinal wall or invasion of the body occurs. The symptoms include vomiting and profuse diarrheal (rice-water) stools that result in severe dehydration, loss of minerals, increased blood acidity, and hemoconcentration. Replacement of fluids, salt, and bicarbonates is essential to treatment, and supplemental tetracycline therapy can help to eliminate the vibrios from the intestinal tract.

Bacteriological diagnosis can best be made by isolating *V. cholerae* from the diarrheic stools. Selective media have a pH of 8.5, which inhibits most other intestinal bacteria. Identification is based on biochemical tests and agglutination of the cells by O1 antiserum.

A vaccine is available, consisting of killed whole cells; however, it only confers about 50 percent protection for about 3 to 6 months. In general, control by improved sanitation is preferable to immunization for prevention of outbreaks.

Haemophilus

The members of this genus require the X factor (heme) and/or the V factor (nicotinamide adenine dinucleotide, NAD) for growth. A medium called chocolate agar provides these factors and is prepared by adding blood to melted agar at 45°C and then heating the medium to 80°C to rupture the blood cells.

Of the *Haemophilus* species pathogenic for humans, one of the most important is *H. influenzae*, the leading cause of meningitis in children between 6 weeks and 2 years of age. Despite its name, *H. influenzae* does not cause influenza

(which is caused by a virus); however, it can cause a severe secondary pneumonia in influenza patients. The major virulence factors produced by this species are (1) an antiphagocytic capsule, (2) an endotoxin, and (3) a heat-stable factor that causes loss of cilia from epithelial cells lining the respiratory tract. There are 6 capsular serovars, a to f; of these, serovar b is most frequently found in severe infections. Nonencapsulated strains commonly occur in the human respiratory tract, but these are much less virulent than encapsulated strains and act only as opportunistic pathogens.

H. influenzae has an airborne person-to-person mode of transmission. The most severe disease caused by this species is meningitis in children. The mortality rate in untreated cases is 90 to 100 percent, and even those who survive may be afflicted by deafness, speech impediments, behavioral problems, or other manifestations of damage to the central nervous system. The limits of the age group that is most susceptible to *Haemophilus* meningitis are determined by the fact that children 6 to 8 weeks to 2 to 3 years old no longer have the maternal bactericidal antibodies (acquired by passive natural immunization) that previously protected them, and they do not begin to make bactericidal antibodies of their own until about 3 years of age. No vaccine is presently available; however, an experimental vaccine consisting of purified b capsular polysaccharide may prove to be effective.

Another infection caused by *H. influenzae* is acute bacterial epiglottitis, which occurs mainly in older children. In this disease the epiglottis is inflamed and may become swollen to the extent that blockage of the trachea occurs and breathing is obstructed. Death can occur within 24 h of onset of the infection.

Immediate antibiotic therapy is necessary for cases of *Haemophilus* meningitis and epiglottitis because of the rapid course and high mortality rate of these infections. Initial treatment is accomplished by a combination of ampicillin and chloramphenicol. Ampicillin alone is often effective; however, nearly 10 percent of *H. influenzae* strains are now resistant to this antibiotic because of a β-lactamase that is coded for by a transmissible plasmid. Resistance to chloramphenicol occurs only rarely; however, this antibiotic may cause toxic effects in the body. Treatment with chloramphenicol may be discontinued if subsequent tests for β-lactamase production prove to be negative.

ANAEROBIC GRAM-NEGATIVE NONSPORE-FORMING RODS

Of the various genera of this group, *Bacteroides* and *Fusobacterium* have the greatest significance for clinical bacteriology, and *B. fragilis* is the most commonly encountered species. Members of *Bacteroides* and *Fusobacterium* occur as part of the normal flora of the intestine, oral cavity, nasopharynx, oropharynx, vagina, and urethra, and in these locations they are relatively harmless. However, if they gain access to other areas of the body, as by wounds, bowel surgery and other kinds of surgery, human or animal bites, dental extractions, uterine infection after abortion, or similar means, then they may establish severe infections that are usually characterized by abscess formation and tissue destruction. Abscesses may be formed in any part of the body, but most often near some mucosal surface that provided that portal of entry. Gas is frequently present in anaerobic infections, and if the lesions discharge fluid, this usually has a foul odor. Factors leading to such infections are those that cause depletion of oxygen in tissues and lower the oxidation-reduction potential, such as traumatic tissue

injury (wounds), lack of local blood circulation, presence of facultative bacteria that can use up oxygen, or substances that are toxic to tissue. Anaerobic infections are often refractory to some ordinary antibiotics such as aminoglycosides. Identification of the bacterial species involved is helpful because many species have predictable patterns of susceptibility to various antibiotics; however, anaerobic infections are often **polymicrobial** (more than one species present), which may complicate treatment.

RICKETTSIAS AND CHLAMYDIAS

Rickettsia

These arthropod-borne intracellular parasites cause a variety of infections in humans. The epidemiology of Rocky Mountain spotted fever has been discussed in Chap. 35, and Table 35-5 lists other rickettsial diseases, the species which cause them, and their arthropod vectors.

One species, *Rickettsia prowazekii*, the causative agent of classical typhus fever (epidemic typhus), has been responsible for much human suffering and death. It is transmitted from human to human by means of body lice, which themselves die of the infection. Typhus fever begins with chills, fever, headache, generalized aches and pains, and exhaustion. The rickettsias multiply within the cells that form the walls of blood capillaries: damage to capillaries of the skin leads to a characteristic red or purple skin rash, and damage to brain capillaries results in neurological symptoms such as hallucinations, delirium, stupor, deafness, hand tremors, and interference with speech. The mortality rate for untreated cases is usually 10 to 40 percent but has been as high as 70 percent in some epidemics. The disease can be effectively treated with tetracycline or chloramphenicol. Laboratory diagnosis of typhus (and other rickettsial infections) is usually done by detecting the development of antirickettsial antibodies in the patient's serum.

Some who recover from typhus fever continue to harbor the rickettsias in a latent form in their lymph nodes. These persons serve as a reservoir to maintain the rickettsias between epidemics, and years after the primary infection these carriers may themselves experience a mild recurrence of typhus fever known as Brill-Zinsser disease.

Until recently it was believed that the human and the louse were the only hosts for *R. prowazekii*; however, the rickettsias have been found in flying squirrels in the southern United States, and it seems likely that occasional human cases of typhus which have appeared in this region may be attributable to these squirrels and their arthropod parasites.

Epidemic typhus is a disease that accompanies disaster, war, and famine. After it was introduced into Spain, possibly by soldiers who fought the Turks in Cyprus, typhus caused the death of 17,000 Spanish soldiers during the siege of Granada in 1489, where fewer than 3,000 were killed in combat. From Spain the disease spread to Italy. In 1528, when the French army was on the verge of victory in the siege of Naples, typhus struck down 30,000 soldiers, and those who were spared were forced to withdraw. Typhus epidemics had decisive effects on the war in the Balkans in the sixteenth century, the Thirty Years' War, the Napoleonic campaigns, and the Serbian campaigns during World War I. Epidemics occurred in Italy and Yugoslavia in World War II, and typhus spread from the German concentration camps to the civilian populations of Germany,

France, and England. It did not reach epidemic proportions in those countries, but in Japan and Korea, 30,000 cases were reported in 1946 and 1947. At present the disease is confined to a few endemic foci in Africa, Central America, and South America, and possibly in the southern United States.

Prevention of typhus epidemics can be achieved by applying insecticides to louse-infested humans and their clothing to destroy the arthropod vector. In addition, a vaccine consisting of killed whole rickettsias is available, although the immunity lasts only about a year.

Chlamydia

The chlamydias are obligate intracellular parasites that have a complex life cycle (Chap. 13). Unlike rickettsias, they are not transmitted by arthropod vectors.

Chlamydia trachomatis causes several types of infections. Some serovars cause trachoma, a chronic keratoconjunctivitis that is the single greatest cause of blindness in the world and is highly endemic in developing countries. The chlamydias are acquired by direct contact with a patient, fomites, or via flies.

In industrialized countries, other serovars of *C. trachomatis* are among the most common sexually transmitted agents of disease. Some infections are relatively mild, such as nongonococcal urethritis (NGU). (NGU may also be caused by a mycoplasma, *Ureaplasma urealyticum*.) Another relatively mild infection is inclusion conjunctivitis, an acute, purulent disease of the eyes that is acquired by a newborn from the mother's genital tract. The disease usually disappears spontaneously within a few months, even without treatment. Other *C. trachomatis* infections, more serious and invasive, may occur, such as lymphogranuloma venereum, which is characterized by enlarged lymph nodes in the analgenito region, inflammation of the rectum, and constitutional symptoms such as chills, fever, and headache.

Chlamydia psittaci causes psittacosis, an infection of birds which can be transmitted to humans, usually by inhalation of infectious dust derived from infected avian feces. For example, poultry handlers or workers in poultry processing plants may acquire the infection. Psittacosis has economic importance for agriculture: the mortality rate in flocks of domestic fowl can be as high as 30 percent. In humans the disease may range from a relatively mild respiratory infection to a severe pneumonia that may eventually involve the central nervous system, with encephalitis, coma, convulsions, and death.

Isolation of chlamydias is done by inoculation of tissue cultures or the yolk sac of embryonated chicken eggs; when stained by Giemsa's stain, the infected cells exhibit characteristic intracellular inclusions.

Among the antimicrobial agents used for treatment of chlamydial infections are tetracyclines, sulfonamides, and erythromycin. Effective vaccines for prevention of chlamydial infections are not available.

MYCOPLASMAS

Mycoplasma

The cell wall–less bacteria belonging to the genus *Mycoplasma* are parasites of mucous membranes and joints and are mainly pathogens of animals, but some species are pathogenic for humans. The excretion of **hydrogen peroxide** by mycoplasmas adherent to the surface of tissue cells appears to be an important factor in causing tissue damage.

Mycoplasma pneumoniae occurs only in humans and is the only *Mycoplasma* species that is found in the human respiratory tract. It has an airborne mode of transmission and is the causative agent of primary atypical pneumonia (also known as walking pneumonia). The disease most frequently affects persons 5 to 25 years old and is characterized by a gradual onset with headache, sore throat, fever, and cough. The mortality rate is less than 1 percent. Tetracycline is effective for treatment. On the basis of suitable isolation techniques, it has been estimated that *M. pneumoniae* is the causative agent in 25 percent of all pneumonias in young adults and 9 percent of the pneumonias in children. For isolation, high levels of penicillin are used in the culture media to inhibit other bacteria; these levels do not inhibit mycoplasmas because of their lack of a cell wall.

FACULTATIVELY ANAEROBIC GRAM-POSITIVE COCCI

Staphylococcus

The major pathogen of this genus is *Staphylococcus aureus* (Fig. 36-11), the causative agent of many suppurative processes ranging from localized abscesses which can occur anywhere in the body to fatal septicemias and pneumonias. *S. aureus* occurs in the nasopharynx, on normal skin, and in the intestines. Infections occur when staphylococci enter the body through breaks, cuts, and abrasions in the skin or mucous membranes.

Some of the virulence factors produced by human strains of *S. aureus* are described in Chap. 31: the **α toxin**, the **Panton-Valentine factor**, **protein A**, and **coagulase**. Other factors include the **δ toxin**, which damages tissue cells by its action as a phospholipase; and **lipase**, which catalyzes the hydrolysis of fats and oils on skin, in sebaceous secretions, and in blood plasma and may aid penetration of staphylococci into the body.

S. aureus causes localized infections in which the characteristic lesion is a walled-off "fort," the **abscess**—a cavity filled with pus cells (neutrophils), dead tissue, and bacteria. Fibrin is deposited at the periphery of the abscess; this may be aided by the staphylococcal coagulase. Neutrophils can penetrate the abscess

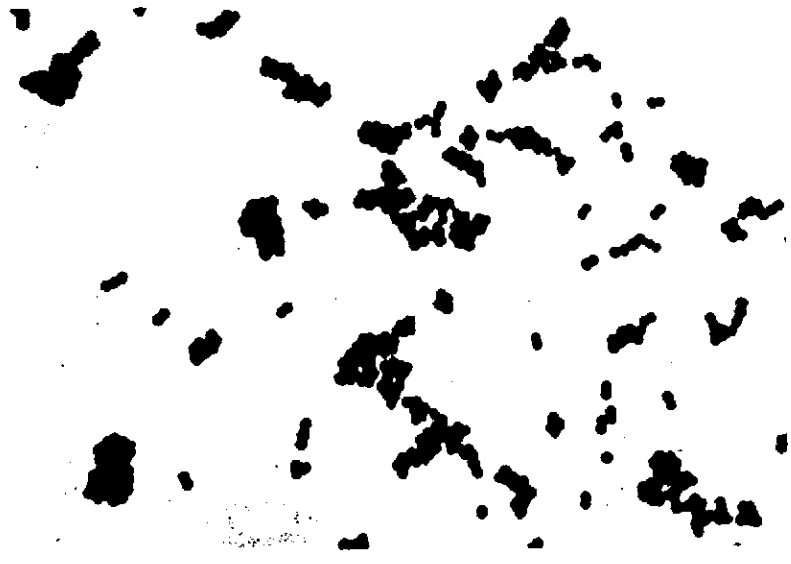

Figure 36-11. *Staphylococcus aureus* showing characteristic grapelike clusters of cocci (X1,300). *(Courtesy of U.S. Naval Biological Laboratory.)*

and, if not killed by the staphylococcal leukocidins, can phagocytoze the staphylococci. Phagocytosis can be greatly aided by antibodies; however, there is no circulation within an abscess and antibodies can penetrate the abscess only by a slow diffusion process. Antibiotics have a similar difficulty in reaching the staphylococci and may have to be administered to a patient for a long period in order to be effective.

Although *S. aureus* does not readily spread through tissues, the bacteria can sometimes be carried by the blood or within neutrophils to sites far removed from the original abscess. Indeed, nearly any organ or tissue may serve as a site for a secondary infection, leading to severe complications such as chronic osteomyelitis (bone infection that is difficult to treat because of poor penetration of the affected area by antibiotics), pneumonia, meningitis, endocarditis, and many more.

Another disease caused by *S. aureus* is toxic shock syndrome (TSS). Although TSS was first described in 1978, it may have occurred long before then as a complication arising from *S. aureus* infections. The disease occurs mainly in young women during a menstrual period, but males also may occasionally develop TSS from *S. aureus* infections. The symptoms, believed to be caused by **exotoxin C**, include fever, diarrhea, vomiting, shock, and a skin rash. Of 941 cases of TSS reported in 1980, 73 were fatal. TSS has been associated with the use of tampons, which may provide an environment for staphylococcal growth and toxin formation.

The treatment of staphylococcal infections is complicated not only by poor penetration by antibiotics into abscesses but also by the **multiple drug resistance** (resistance to several antibiotics, usually plasmid-mediated) that is exhibited by many clinical isolates of *S. aureus*. For instance, the use of penicillins for treating *S. aureus* infections would be desirable because of the bactericidal action of these antibiotics, but most clinical isolates of *S. aureus* are penicillin-resistant because they produce a plasmid-mediated β-lactamase. Certain semisynthetic penicillins, such as methicillin and oxacillin, are not destroyed by β-lactamase and thus can be effective in treating penicillin-resistant staphylococcal infections.

Certain strains of *S. aureus* that produce an **enterotoxin** can cause staphylococcal food poisoning. The epidemiology of this disease has been discussed in detail in Chap. 35. After consumption of an intoxicated food, symptoms begin within 1 to 6 hours and include severe nausea and vomiting and moderate diarrhea, but no fever. The disease lasts for about a day and, although extremely unpleasant, is not fatal.

AEROTOLERANT FERMENTATIVE GRAM-POSITIVE COCCI

Streptococcus

Certain *Streptococcus* species can cause infections in humans (Table 36-3). Identification of an isolate as belonging to a particular species depends on determination of the Lancefield group-specific antigens, on the type of hemolysis produced on blood agar, and often on a variety of additional biochemical tests. Of the pathogenic streptococci of human origin, *S. pyogenes* and *S. pneumoniae* are the most important.

Streptococcus pyogenes. This species comprises Lancefield group A. The cocci are arranged in long chains (Fig. 36-12), and colonies on blood agar are β-

Table 36-3. Some *Streptococcus* Species Pathogenic for Humans

Group and Species	Lancefield Group	Main Diseases Caused
Group A, B, and C streptococci		
S. pyogenes	A	Streptococcal sore throat (acute pharyngitis), scarlet fever, erysipelas, impetigo, acute glomerulonephritis, rheumatic fever, puerperal fever, septicemia
S. agalactiae	B	Meningitis and septicemia in newborn infants; occasionally other infections in adults; mastitis in cattle
S. equisimilis	C	Mild upper respiratory tract infections, erysipelas, puerperal fever; also various infections of animals
Group D enterococci		
S. faecalis	D	Urinary tract infections, endocarditis
S. faecium		
S. durans		
Group D nonenterococci		
S. bovis	D	Endocarditis
Viridans group		
S. sanguis	H	Endocarditis, dental caries
S. mutans	None	Endocarditis, dental caries
S. salivarius	K or none	Endocarditis
S. mitis	None	Endocarditis
Pneumococci		
S. pneumoniae	None	Lobar pneumonia

hemolytic (produce zones of clearing around the colonies). Major toxins or virulence factors produced by *S. pyogenes* are described in Chap. 31 and include the M protein, streptolysin O (SLO), streptolysin S (SLS), erythrogenic toxin, streptokinase, and deoxyribonuclease (DNase).

S. pyogenes is transmitted mainly by aerosols generated by carriers and clinical patients. Among the infections caused by *S. pyogenes* are streptococcal pharyngitis (streptococcal sore throat) and scarlet fever. These infections may give rise to complications, including inflammation of the middle ear (otitis media), mastoid bone (mastoiditis), sinuses (sinusitis), lungs (streptococcal pneumonia), heart valves (rheumatic fever), kidney (acute glomerulonephritis), and many others.

Streptococcal Pharyngitis. At least 300,000 cases of this disease occur each year in the United States. The disease is characterized by fever, enlargement of the lymph nodes of the neck, and a red, raw, and often bleeding throat surface. Scarlet fever is similar to the pharyngitis except for the skin rash that appears because of the erythrogenic toxin. The rash may extend to all parts of the body, appearing on the first or second day after onset of the disease. Clinical diagnosis of streptococcal pharyngitis and scarlet fever can be confirmed by isolating *S. pyogenes* from the pharynx. Specimens for cultural confirmation should be taken before antibiotic therapy is started. Penicillin and erythromycin are effective for treatment, which should be initiated as soon as possible because the

Figure 36-12. *Streptococcus pyogenes*, showing arrangement of the cells in chains (X1,600). *(Courtesy of J. Nowak, Documenta Microbiologica, part 1, "Bakterien," Gustav Fischer Verlag, Jena, Germany, 1927.)*

longer the steptococci continue to infect the body, the greater the chance that serious complications such as rheumatic fever may occur. If the organism cannot be isolated, circumstantial evidence for streptococcal infection can be obtained by detection of a rising level of antibodies in the patient's serum against certain streptococcal antigens, particularly SLO and DNase.

The only type of immunity that is protective against infection by S. *pyogenes* is humoral immunity: antibodies against M proteins act as opsonins, enhancing phagocytosis of the streptococci. Although it is theoretically possible to vaccinate against streptococcal infection by using M proteins as the immunizing antigens, as a matter of practicality it has not been possible to do so because of the great number of M proteins (over 60 types) that occur among the various streptococcal strains.

Rheumatic Fever. Inflammation and degeneration of the heart valves may follow approximately 3 percent of untreated cases of streptococcal pharyngitis or scarlet fever. Further damage to the heart increases with each subsequent streptococcal infection. The disease occurs most frequently in the preadolescent age group (3 to 10 years) and is a cause of death in the 5- to 20-year-old age group. Antibiotics are ineffective in curing the disease, and streptococci are usually not present in the heart or on the valves. The explanation of how the inflammation occurs in the absence of the bacteria is still not well understood, but it is likely to have an immunological basis. For instance, a similarity exists between certain S. *pyogenes* antigens and cardiac tissue antigens. In persons who have developed antibodies against the streptococcal antigens, the antibodies might cross-react with cardiac tissue and cause an inflammatory reaction.

Other Diseases. Acute glomerulonephritis (AGN) also appears to have an immunological basis. It is an inflammation, but not an infection, of the kidney that may occur following a S. *pyogenes* infection of the throat or skin. The disease can become chronic or even fatal; it can lead to kidney failure and to the necessity for the patient to be placed on a dialysis machine that carries out kidney functions.

Erysipelas is an acute skin infection caused by S. *pyogenes*. The lesions usually occur on the face and legs, and the skin becomes bright red, edematous, and covered with vesicles (fluid-filled blisters). The source of the streptococci may be the patient's respiratory tract, a carrier, or soiled linens, dressings, and other fomites. Control is best accomplished by good personal hygiene and by prophylaxis (preventive treatment) with antibiotics following known exposure.

Impetigo contagiosum is described by clinicians as a purulent dermatitis. Vesicular lesions appear most commonly on the face and hands but may cover the body and become crusted and ulcerated (form open sores). Impetigo is caused by S. *pyogenes* together with staphylococci and is transmitted by direct contact with a patient or contaminated objects. Because children are usually susceptible, epidemics are likely to spread rapidly through schools and camps unless controlled by good sanitary and hygienic practices.

Puerperal fever, sometimes called puerperal sepsis, is a streptococcal infection of the uterus of a mother and is acquired at childbirth. It is a serious disease but can be controlled by proper obstetrical practices. Sources of infection may

Figure 36-13. *Streptococcus pneumoniae*, showing diplococcus arrangement (X1,500). *(Courtesy of J. Nowak, Documenta Microbiologica, part 1, "Bakterien," Gustav Fischer Verlag, Jena, Germany, 1927.)*

be streptococci in the patient's genital, intestinal, or respiratory tract or on the skin of the patient or her attendants. Use of prophylactic antibiotics minimizes the danger of infection.

Streptococcus pneumoniae. Unlike *S. pyogenes*, this species is α-hemolytic and occurs mainly in the form of diplococci (Fig. 36-13). The major virulence factor is the **polysaccharide capsule**, which effectively inhibits phagocytosis; other virulence factors include an oxygen-labile hemolysin, **pneumolysin O**, which is similar in many respects to the streptolysin O of *S. pyogenes*.

Although pneumonia may be caused by many different organisms, at least 70 percent of bacterial pneumonias are caused by *S. pneumoniae*. Since 20 to 40 percent of the population harbors virulent strains of *S. pneumoniae*, contracting pneumococcal pneumonia is not merely a matter of acquiring the organism; the resistance of the host must also be lowered, as by a preliminary respiratory infection such as a common cold. In the lungs, pneumococci initiate an inflammatory response; fluid from nearby blood capillaries begins to fill the air sacs, and eventually the area of affected lung is no longer soft and spongy but rather is solid. Neutrophils also accumulate and attempt to phagocytoze the pneumococci; however, encapsulated pneumococci are highly resistant to phagocytosis unless the patient makes antibodies against the capsular polysaccharide. Even if the patient recovers, complications may subsequently occur, such as otitis media, mastoiditis, pneumococcal meningitis, septic arthritis, and endocarditis. Penicillin is the drug of choice for treatment of pneumococcal pneumonia; however, some strains of *S. pneumoniae* that are resistant to penicillin and other antibiotics have appeared in recent years.

Bacteriological diagnosis is based on isolating the organisms from the patient's sputum. A key test for identification is the ability of bile to induce lysis of pneumococci **(bile solubility test)**; other α-hemolytic streptococci are not affected. In another test, diagnostic antisera are used to cause the pneumococcal capsules to become easily visible under a microscope **(quellung reaction)**.

Only antibodies against the capsule of the pneumococcus are protective against pneumococcal pneumonia, and a vaccine has long been sought that would stimulate production of such antibodies. At first this seemed impractical because of the occurrence of over 80 kinds of capsular serovars. However, 14 of the serovars have been found to be responsible for at least 80 percent of the cases of pneumococcal pneumonia. Thus a vaccine consisting of the capsular polysaccharides from these 14 serovars was developed and is now licensed for use in the United States. Since pneumococcal pneumonia is a major killer of elderly persons, the vaccine is particularly recommended for this segment of the population.

AEROBIC/FACULTATIVELY ANAEROBIC GRAM-POSITIVE SPOREFORMING RODS

Bacillus

Although *Bacillus cereus* can cause a type of food poisoning in humans, only one species of the genus *Bacillus* is highly pathogenic for humans: *B. anthracis* (Fig. 36-14), the causative agent of anthrax. Two virulence factors are produced by this species: (1) the **anthrax toxin** (see Table 31-3) and (2) an antiphagocytic **polypeptide capsule.**

Anthrax is mainly a disease of herbivorous animals, particularly cattle and

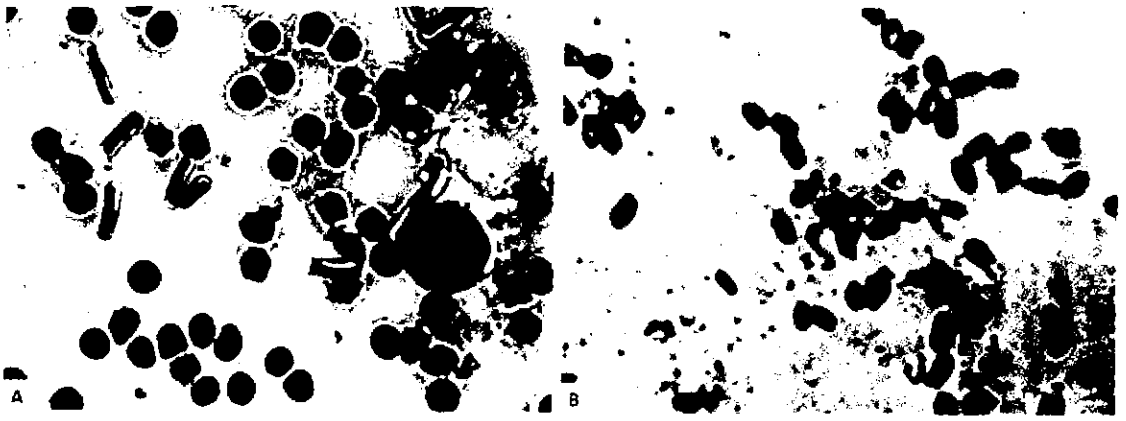

Figure 36-14. (A) Blood smear taken from a sheep that died of anthrax, showing vegetative capsulated *B. anthracis* (rod forms surrounded by a clear area). The large round objects are blood cells. (B) A group of *B. anthracis* spores. *(Courtesy of USDA, Bureau of Animal Industry.)*

sheep. Three forms of the disease occur, as defined on the basis of their mode of transmission. (1) **Intestinal** anthrax is frequently contracted by cattle and sheep that ingest anthrax spores by grazing on pastures, or on the hay produced from pastures, where anthrax-infected animals have died or been buried. Such pastures may remain infectious for many years. (2) **Cutaneous** anthrax is often contracted by cattle and sheep when anthrax spores get into a scratch or abrasion. Humans can also contract the disease by handling infected animals or products made from infected animals, such as hides, wool, and horn. (3) **Pulmonary** anthrax is contracted only by humans, who acquire the organism by inhaling dust from contaminated animal products. For instance, infectious dust may arise during the process of sorting or combing raw wool contaminated with anthrax spores. Regardless of the mode of transmission, anthrax begins as a localized infection of the skin, lungs, or intestine which, in fatal cases, develops into a septicemia.

Penicillin is the drug of choice for treatment of anthrax. A live attenuated vaccine is available for prevention of anthrax in cattle and sheep.

ANAEROBIC SPOREFORMING RODS

Several species of clostridia can cause disease in humans. Four of these species are discussed below.

Clostridium

Clostridium tetani. This species is the causative agent of tetanus. A characteristic feature of these bacteria is the formation of endospores at one end of the cell, giving the organisms a drumstick appearance (Fig. 36-15). The only virulence factor that is produced is a powerful **neurotoxin**. *C. tetani* occurs in the intestinal tracts of herbivorous animals and is widely distributed in soil. In wounds providing conditions favorable for its growth, *C. tetani* may grow and elaborate toxin. Such wounds are usually deep and ragged, with devitalized tissue in which aerobic or facultative organisms are also growing. The toxin becomes bound to nearby peripheral motor nerves and travels along these nerves to the central nervous system, where it exerts its effects (see Chap. 31). Symptoms include painful and violent contractions of the muscles, usually of the neck and jaw (restricting opening of the mouth, giving rise to the term *lockjaw*). This is

Figure 36-15. *Clostridium tetani.* Spores are terminal and swell the rods, producing a typical drumstick appearance. Cells range from 0.3 to 0.8 by 2.0 to 5.0 μm. *(Courtesy of General Biological Supply House, Inc.)*

Figure 36-16. *Clostridium perfringens.* The cells range from 1.0 to 1.5 μm wide by 4.0 to 8.0 μm long. Spores are not present and are not usually formed in ordinary laboratory media. Spores may develop in special media; if formed, they are located in the center of the cell and do not appreciably distort the parallel sides of the rods. *(U.S. Army photograph from the Armed Forces Institute of Pathology.)*

followed by paralysis of the thoracic muscles, frequently causing death from respiratory failure or cardiac failure. The mortality rate is 55 to 65 percent. Therapy involves the administration of muscle relaxants, and antitoxin is given to neutralize any toxin that has not yet become fixed to nerve tissue.

Immunization against tetanus is accomplished by a vaccine containing tetanus toxoid, which stimulates the body to produce antitoxin. Booster immunizations should be given every 10 years to maintain an adequate level of antitoxin. If a wound conducive to development of tetanus occurs in a nonimmunized person, tetanus antitoxin should be administered as soon as possible to neutralize any toxin that may be produced.

Clostridium perfringens. Several clostridia may cause gas gangrene in humans, but the majority of cases are caused by *C. perfringens* type A (Fig. 36-16), which occurs as part of the normal flora of the human intestine. The virulence factors produced by *C. perfringens* include the **α toxin** (a phospholipase that damages the membranes of erythrocytes and tissue cells), the **θ toxin** (an oxygen-labile hemolysin), the **κ toxin** (a collagenase that destroys connective tissue), the **μ toxin** (a hyaluronidase that may aid invasiveness), and the **ν toxin** (a deoxyribonuclease).

As in tetanus, gas gangrene results from contamination of wounds. The disease

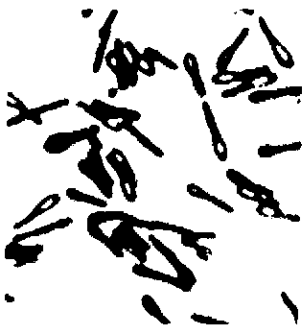

Figure 36-17. *Clostridium botulinum* (X1,500), showing many cells with oval, terminal, and subterminal spores. *(Courtesy of J. R. Porter.)*

is characterized by a toxemia, preceding and concurrent with the development of gas in the tissues. *C. perfringens* is highly invasive and spreads rapidly through tissues (see Chap. 31). Prevention of the disease rests on three measures: (1) surgical removal of dead tissue and blood clots that serve as breeding grounds for anaerobic bacteria, (2) administration of antitoxin, and (3) administration of antibiotics to stop growth of the bacteria. Once a case of gas gangrene has developed, the organisms are so rapidly invasive that it is difficult to halt their spread through tissues, and it may be necessary to amputate the affected limb to save the patient's life. An alternative treatment is the use of hyperbaric oxygen: the patient is placed in a chamber containing 3 atmospheres pressure of pure oxygen several times a day for half-hour periods. This treatment causes a high degree of tissue oxygenation that may halt the growth of the clostridia. However, none of these procedures may save the life of the patient if the clostridial toxins have become widely distributed throughout the body.

C. perfringens also causes a very common type of food poisoning. Various forms of raw meat or poultry may contain spores of *C. perfringens*. If the meat or poultry is cooked at a temperature which does not kill the spores and is subsequently kept at a temperature of 109 to 116°F for at least 2 hours, the clostridia may multiply. The food still appears and tastes normal, but after it is consumed the clostridia in the particles of ingested meat continue to grow and reach enormous numbers in the intestine. There they begin to sporulate, and during this process an enterotoxin is formed. The toxin causes abdominal cramps, diarrhea, and sometimes nausea and vomiting. The symptoms begin 8 to 24 hours after consumption of the food and last for 12 to 18 h.

Clostridium botulinum. This species (see Fig. 36-17) causes botulism, a paralytic disease of humans or animals. The disease is contracted by consuming food that contains the neurotoxin produced by these clostridia (see Chap. 35). The manner by which the toxin causes respiratory failure has been described in Chap. 31. The species is divided into 7 serovars, A to G, each of which produces an antigenically distinct kind of toxin. Botulism in humans is caused by *C. botulinum* serovars A, B, E, and (rarely) F. The spores of serovars A and B often occur in soil. Serovar E occurs mainly in the sediments of the Great Lakes and along the coasts of western North America and northern Japan, and the spores can contaminate fish or fish products. The epidemiology of foodborne botulism has been described in Chap. 35. Patients with botulism are treated with polyvalent antitoxin (i.e., antitoxin against toxin types A, B, and E) to neutralize any toxin that has not yet become fixed to nerve tissue. Patients experiencing respiratory difficulty may have to be placed on a mechanical respirator to prevent death from respiratory failure.

A type of botulism that is not acquired by ingestion of toxin can occur in infants 2 weeks to 6 months old. *C. botulinum* is not a normal part of the intestinal flora of humans and ordinarily cannot compete with normal flora organisms; however, in some infants whose normal intestinal flora is not yet well developed, ingested spores may be able to germinate and grow to some extent, producing enough toxin in the intestine to cause symptoms such as lethargy, excessive sleeping, poor head control, sluggish reflexes, and generalized weakness. In severe cases respiratory failure and death can occur.

Clostridium difficile. In the intestine of an adult person, *C. difficile* is either not present or is present in very low numbers. However, in patients treated with antibiotics such as ampicillin, clindamycin, chloramphenicol, tetracyclines, or cephalosporins, the normal flora may be inhibited to the point where *C. difficile* can compete effectively, producing a severe infection of the colon called pseudomembranous colitis. The disease is characterized by a watery diarrhea, inflammation of the colon wall, and a pseudomembrane (gray, white, or yellow patches on the intestinal wall). The mortality rate of untreated cases is 27 to 44 percent. Treatment of the disease by oral administration of vancomycin is usually effective. Two exotoxins appear to be involved in pseudomembranous colitis: an **enterotoxin**, toxin A, which causes fluid accumulation in the bowel, and a **cytotoxin**, toxin B, which kills tissue in the intestinal wall.

NONSPOREFORMING GRAM-POSITIVE RODS OF REGULAR SHAPE

Listeria

The cells of *Listeria monocytogenes* are short rods that may occur in chains. Four serovars can be distinguished based on surface antigens: 1, 2, 3, and 4; of these, 1 and 4 occur most often in human listeriosis. A **monocytosis-producing factor** can be extracted from the cells with chloroform and induces a hypernormal number of monocytes in the blood. An oxygen-labile **cytotoxic hemolysin** is also produced.

Listeriosis occurs in many species of mammals and birds. Symptoms are primarily neurological and are characterized by an acute encephalitis. Lesions (granulomas similar to those occurring in brucellosis, tularemia, or tuberculosis) are produced in many of the internal organs of infected animals. The organisms grow mainly within macrophages, and, as in brucellosis and tuberculosis, cell-mediated immunity is the only effective type of immunity. Humans can acquire *L. monocytogenes* from animals, although the mechanism of transmission is not known. In pregnant women, mild flulike symptoms may develop or the infection may be asymptomatic; however, the organisms may cross the placenta into the fetus and cause stillbirth or abortion. If the child is born apparently normal, meningitis or septicemia may develop 1 to 4 weeks later. Listeriosis mainly affects young children and aged persons; it also tends to occur in people whose immune mechanisms have been debilitated by some other type of illness such as alcoholism, diabetes, or cancer. Listeriosis can be treated effectively with penicillin G or ampicillin. No vaccine is presently available for prophylactic immunization.

NONSPOREFORMING GRAM-POSITIVE RODS OF IRREGULAR SHAPE

Corynebacterium

Three groups of corynebacteria occur: the animal or human parasites and pathogens, the plant pathogens, and the saprophytes found in soil and water. Human species include *C. diphtheriae*, the causative agent of diphtheria; *C. ulcerans*, which causes a diphtherialike disease; and *C. minutissimum*, which causes a skin disease called erythrasma.

C. diphtheriae (Fig. 36-18) produces several virulence factors. **Cord factor**, or **trehalose dimycolate** inactivates the mitochondrial membranes of phagocytes and other mammalian cells. **Diphthin** is a protease which inactivates IgA antibodies. **Neuraminidase** helps the bacteria to attach to mucous membranes of the throat by dissolving the mucus layer. **K antigens** are cell-wall proteins that

Figure 36-18. *Corynebacterium diphtheriae* (X1,300). *(Courtesy of J. Nowak, Documenta Microbiologica, part 1, "Bakterien," Gustav Fischer Verlag, Jena, Germany, 1927.)*

aid in attachment of the bacteria to host cells. The major virulence factor of *C. diphtheriae* is the **diphtheria exotoxin.** The gene that codes for this toxin (gene tox^+) is carried in the genome of a particular temperate bacteriophage called β, and a strain of *C. diphtheriae* produces toxin only when it harbors the β phage. Nontoxigenic strains of *C. diphtheriae* can be made toxigenic by infecting them with the β phage **(lysogenic or phage conversion).** The mechanism of action of the toxin as an inhibitor of protein synthesis is described in Chap. 31.

Diphtheria is mainly a disease of children. *C. diphtheriae* has an airborne mode of transmission and localizes in the tonsils, throat, and nose. The organisms are not invasive; however, the toxin produced by the organisms can circulate throughout the body, producing a general toxemia. Because of local necrosis of the throat cells, an inflammatory exudate may occur and develop into a tough **pseudomembrane** that can extend into the trachea and cause the patient to suffocate. Even when this does not occur, the systemic effects of the toxin are serious: the toxin is particularly damaging to heart muscle, nerve tissue, and kidneys. More than half the fatalities in diphtheria are due to cardiac damage. Treatment is by administration of diphtheria antitoxin; this should be done as early as possible because once the toxin has bound to mammalian cell membranes it can no longer be neutralized. Antibiotics are not effective in treatment because, unlike antitoxin, they cannot neutralize the toxin that has been produced. However, antibiotics can eliminate the carrier state in convalescent patients and thus help prevent further transmission of the disease. Laboratory confirmation of a clinical diagnosis is achieved by isolating the bacteria and demonstrating that they are toxigenic. Toxigenicity can be demonstrated by either an in vivo test using guinea pigs or an in vitro immunodiffusion test.

Once a very important disease and cause of death, diphtheria has become a clinical rarity in the United States because of two factors: (1) the detection of persons susceptible to diphtheria by means of the **Schick test** and (2) immunization of susceptible persons with diphtheria toxoid. The Schick test is performed by infection of a very small amount of diphtheria toxin into the skin. If the person is immune, the toxin is neutralized by antitoxin in the person's body and no skin reaction occurs; but if the person is susceptible (has no antitoxin), a local inflammatory reaction results.

MYCOBACTERIA

Mycobacterium

Both parasitic and saprophytic species of *Mycobacterium* occur; those that are pathogenic for humans are indicated in Table 36-4. Of particular importance is *M. tuberculosis*, which is responsible for over 90 percent of all cases of tuberculosis. It is almost exclusively a parasite of humans and has mainly an airborne mode of transmission.

M. tuberculosis can survive and multiply within phagocytic cells such as macrophages, and **cord factor** (trehalose dimycolate) in the bacterial walls can disrupt the respiration of mitochondria in phagocytes and tissue cells. Strains of *M. tuberculosis* possessing cord factor are virulent; strains lacking it are not. In cultures, the presence of cord factor is indicated when rough-looking colonies are formed due to the growth of the bacteria in cablelike arrangements (cords).

Pulmonary tuberculosis is a chronic, slowly advancing disease against which

Table 36-4. Some *Mycobacterium* Species Pathogenic for Humans

Group and Species	Clinical Significance
Tuberculosis group	
M. tuberculosis	Both species are pathogenic and cause tuberculosis; they regularly exhibit susceptibility to antituberculosis drugs
M. bovis	
Leprosy group	
M. leprae	Causes leprosy; has never been cultivated on laboratory media; can be grown in mouse footpads or in armadillos, where the temperature is favorable for growth (2 to 5°C below that of most mammals)
Runyon groups*	
I Photochromogens. Nonpigmented when grown in the dark; yellow pigment formed when grown in the light; slow-growing	
M. kansasii	Causes a tuberculosis-like disease.
M. marinum	Skin papules and ulcers; contracted from swimming in fresh or salt water; prefers temperature of 31°C for growth
II Scotochromogens. Red-orange pigment formed when grown in the dark or light; slow-growing	
M. scrofulaceum	Causes cervical adenitis in young children
M. szulgai	Pulmonary disease, adenitis, bursitis
III Nonchromogens. Nonpigmented in dark or light; slow-growing	
M. avium/M. intracellulare group	Tuberculosis-like disease in adults; lymphadenitis in children; usually resistant to ordinary antituberculosis drugs
M. xenopi	Tuberculosis-like disease
M. ulcerans	Ulceration and necrosis of skin
IV Rapid-growing; may be pigmented or nonpigmented	
M. fortuitum	Local abscess at the site of a trauma; occasionally causes a tuberculosis-like disease
M. chelonei	Has been isolated from patients with chronic respiratory disease

* The species in these four groups are environmental bacteria and do not appear to be directly transmissible from person to person.

cell-mediated immunity, rather than humoral immunity, is the major defense mechanism. The disease begins when the bacteria lodge within an air sac in the lungs. There they are rapidly ingested by macrophages, in which they multiply. The first evidence of infection is development of a hypersensitivity to the bacteria after about a month. This is determined by the **tuberculin test**, in which an extract of harmless proteins (**purified protein derivative**, or **PPD**) from *M. tuberculosis* is injected into the skin. A positive test is indicated by a red, swollen zone at the site of the injection within 48 h.

In untreated cases, as cell-mediated immunity develops, specific T lymphocytes migrate toward the bacilli in the lung and, upon contact with the bacilli, liberate lymphokines which attract macrophages to the area and cause them to accumulate. Eventually a small, pearl-gray nodule forms, consisting of bacilli, several concentric layers of macrophages, and an outer mantle of lymphocytes. This nodule, a type of granuloma, is called a **tubercle**.

In many instances, cell-mediated immunity develops sufficiently to be able to halt further advance of the infection. A lymphokine called macrophage activating factor (MAF) converts normal macrophages to angry or activated macrophages, which can arrest the growth of the tubercle bacilli. The tubercle becomes dormant and the infection remains subclinical. Tuberculin hypersensitivity will continue to exist as long as the dormant bacilli exist in the body—usually for a lifetime, unless the infection is eliminated by drug therapy.

In about 10 percent of untreated infections, cell-mediated immunity is not strong enough to halt the growth of the bacilli. The initial tubercle becomes larger and more of them develop. Within the enlarged tubercles, macrophages and tissue cells begin to die and fuse together to form an amorphous cheeselike mass (caseation necrosis). Even now the lesions may undergo a healing process called calcification, but if the infection progresses, eventually several tubercles coalesce to form an area of dead tissue that is large enough to be detected by a chest x-ray. As the area of dead tissue expands it may erode the wall of a bronchus, so that acid-fast bacilli begin to appear in the sputum coughed up by the patient (Fig. 36-19). The detection of acid-fast bacilli in sputum constitutes a presumptive laboratory test for tuberculosis, but isolation and identification of the mycobacteria are required for confirmation.

As the disease progresses the patient begins to exhibit loss of appetite, fatigue, weight loss, night sweats, and a persistent, worsening cough. If a blood vessel is eroded in the lungs, the sputum coughed up by the patient may become streaked with blood; also the tubercle bacilli may gain access to the blood and be transported to various parts of the body, establishing numerous secondary foci of infection. Death ultimately results when sufficient damage has occurred in the lungs or other vital organs.

The type of tuberculosis most prevalent in the United States today is *reactivation* tuberculosis, in which dormant bacilli from an old subclinical primary infection are no longer held in check and begin to proliferate rapidly. This form of the disease occurs most often in elderly persons whose resistance has been lowered by various factors such as malnutrition, alcoholism, and other stresses.

Chemotherapy is the most effective method for treatment of tuberculosis. If a person who has previously been tuberculin-negative becomes tuberculin-posi-

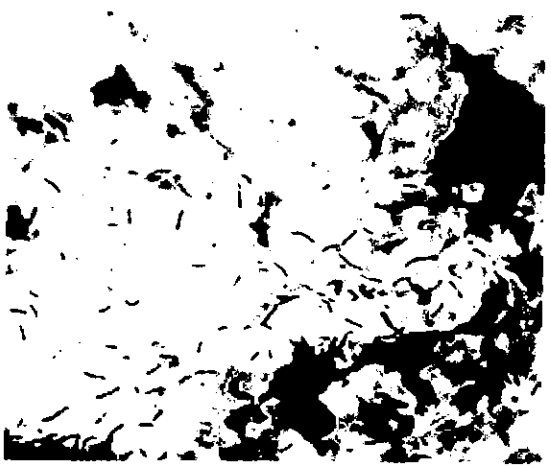

Figure 36-19. *Mycobacterium tuberculosis* in sputum, acid-fast stain (X800). *(U.S. Army photograph.)*

tive, antibacterial drugs are administered for several months to ensure that the infection will not progress to clinical tuberculosis. Treatment of a patient with clinical disease must be extended over a year or more because of the chronic nature of the disease and the walling off of the bacteria by tubercle formation. Among the effective drugs are isoniazid (INH), streptomycin, p-aminosalicylic acid (PAS), and rifampin.

A live attenuated vaccine (BCG vaccine) that induces cell-mediated immunity against tuberculosis is used in European countries and is about 80 percent effective. However, immunized individuals give a positive tuberculin test and thus this test is no longer of diagnostic value; for this reason the vaccine is not used in countries where the incidence of tuberculosis is relatively low, as in the United States.

BACTERIAL PATHOGENS OF PLANTS

Bacteria cause a wide variety of plant diseases characterized by such host reactions as galls (tumors composed of undifferentiated cells), wilts (loss of turgor), cankers (localized wounds or lesions resulting from necrosis of stems or bark), rots, deformed fruits, leaf spots, change in the color of plant parts, dwarfing, and retarded ripening of fruit. Bacterial plant pathogens may be disseminated in many ways—by wind, water, and soil movements; infected seeds and nursery stock; insect vectors; and infected farm tools, especially those used for pruning in orchards. Once inside the plant tissue, bacteria usually grow intercellularly. The following genera of bacteria contain plant pathogens; however, not all species in these genera are pathogenic.

Certain *Pseudomonas* species such as *P. syringae* cause leaf spot, leaf stripe, wilt, and similar diseases.

Xanthomonas spp., especially *X. campestris*, cause necrosis.

Erwinia spp. invade the tissues of living plants and produce dry necrosis, galls, wilts, and soft rots.

Agrobacterium spp. live in the soil or in the roots or stems of plants, where they produce galls (tumorlike growths).

The genus *Corynebacterium* contains plant pathogens that may be found in the soil as well as in diseased plants. They cause a vascular disease of alfalfa; ring rot of potatoes, grasses, and tomatoes; and diseases of many other plants.

Streptomyces spp. are responsible for potato scab, and a disease of sweet-potato roots and rootlets.

Spiroplasma spp. cause arthropod-borne disease in a wide range of crops and wild plants. The plants are often stunted and malformed.

Agrobacterium species are particularly noted for the ability to cause galls in plants. Galls are disorganized masses of plant cells, some large and swollen, others small and rapidly dividing (Fig. 36-20). As they develop, they may cut off the flow of water and nutrients in the plant, resulting in death. Crown gall, or plant cancer, may occur on fruit trees, sugar beets, and other broad-leafed plants where the stem comes out of the ground.

The causative agent of crown gall, *Agrobacterium tumefaciens*, has the unique ability of transforming normal plant cells into tumor cells. When the tumor

Figure 36-20. Crown gall, showing primary and secondary tumors and tumefaction in the petiole of one of the leaves of a sunflower plant (*Helianthus annus*). The tumor is the result of inoculation with *Agrobacterium tumefaciens*. *(Courtesy of Dr. Armin C. Braun, The Rockefeller Institute for Medical Research.)*

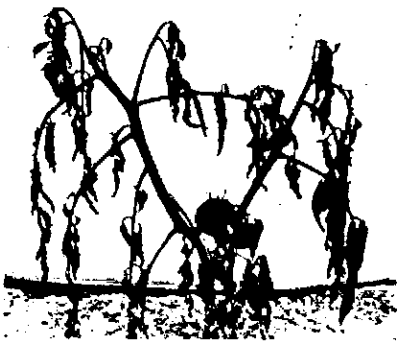

Figure 36-21. Bacterial wilt. Branches and stems of this tomato plant have wilted because of an infection with *Pseudomonas solanacearum*. *(Courtesy of USDA, Bureau of Plant Industry.)*

cells have become established, they continue to produce abnormal cells even in the absence of the bacterial pathogen; i.e., they multiply autonomously. The ability of *A. tumefaciens* to transform plant cells into cancer cells is due to a plasmid known as Ti; strains that do not possess this plasmid are not tumorigenic. During the course of infection of a wounded plant, the Ti plasmid is somehow transferred to the plant cells, where part of it is integrated into the nuclear material of the transformed plant cells. One unusual attribute of the transformed plant cells that is induced by the Ti plasmid is the ability to produce **opines** (unusual amino-acid derivatives such as octopine and nopaline); this creates a unique ecological niche for the cancer-inducing agrobacteria, since almost all strains of the bacteria can use the opines made by the cancer cells as a carbon and nitrogen source—an ability that is conferred on the bacteria by the Ti plasmid.

Agrobacteria are the only bacteria known to be involved in any form of cancerlike growth. As such they have served as a model system for mammalian cancer research.

Bacterial wilts are caused by the slime-producing bacteria that plug the passages in the plant through which water passes (Fig. 36-21). Wilt of sweet corn and some other varieties of corn is caused by *Erwinia stewartii*, bacterial wilt of cucumbers and muskmelons by *Erwinia tracheiphila*, that of tobacco by *Pseudomonas solanacearum*, and that of alfalfa by *Corynebacterium insidiosum*.

Cankers start in the water-conducting tissue of the plant, but they spread into the surrounding tissue. Bacterial canker of stone fruits decreases the yield of fruit or kills the trees. The most characteristic symptom of the disease is formation of canker accompanied by exudation of gummy substances, a symptom called **gummosis**. The bacterium that causes canker and gummosis is *Pseudomonas syringae*, which is not killed during the winter. Bacterial canker of

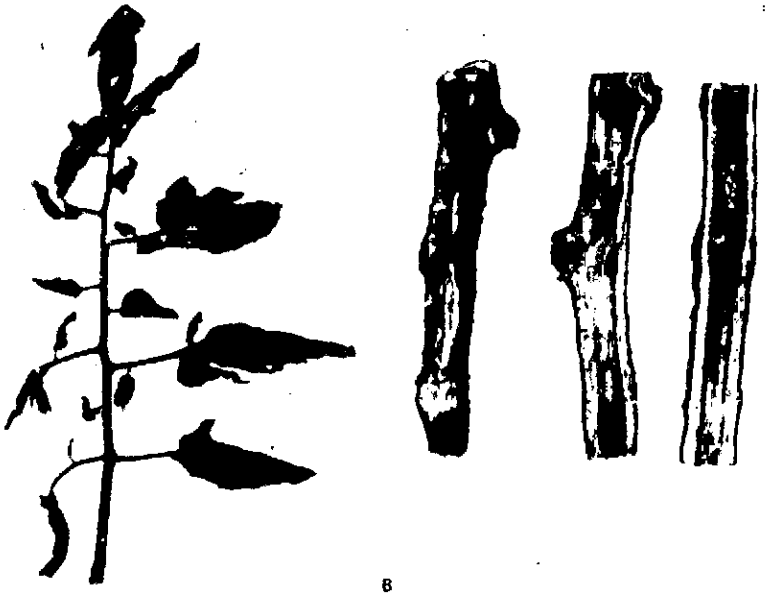

Figure 36-22. Bacterial canker. Leaves and stem of a tomato plant affected with bacterial canker, caused by *Corynebacterium michiganense*. (A) The curling and withering of leaflets that characterize this disease. (B) At the left is a portion of stem showing an open canker, and at the right are two stems cut lengthwise to show decay of the inner tissues. *(Courtesy of USDA, Bureau of Plant Industry.)*

Figure 36-23. Bacterial spot. These tomatoes show characteristic lesions of bacterial spot caused by *Xanthomonas campestris* pathovar *vesicatoria*. *(Courtesy of USDA, Bureau of Plant Industry.)*

tomatoes is due to *Corynebacterium michiganense* (Fig. 36-22). *Xanthomonas campestris* pathovar *citri* causes bacterial canker of citrus; in 1984 a variety of this organism was responsible for a serious outbreak of the disease in Florida.

Bacterial spot diseases usually occur on the leaves, but fruits and stems may be affected (Fig. 36-23). On peaches, bacterial spot is caused by *Xanthomonas campestris* pathovar *pruni*, and *P. syringae* pathovar *striafaciens* causes bacterial stripe of oats. Halo blight of oats is caused by *P. syringae* pathovar *coronafaciens*, which elaborates a toxic substance that produces yellowish areas surrounding the dead spots.

BACTERIAL PATHOGENS OF INSECTS

Some bacteria can cause insect diseases. Many of these diseases reach epidemic proportions, and if the insect species is beneficial, the diseases can cause great

Figure 36-24. Electron micrograph of *Bacillus thuringiensis*, showing parasporal bodies (PB) and oval spores. The parasporal bodies are composed of a crystallized protein that is toxic for *Lepidoptera* larvae. The bar represents 1 μm. *(Courtesy of David J. Vitale and George B. Chapman.)*

harm. On the other hand, if the insects are of a harmful species, limitation of their population by disease may be desirable. Indeed, diseases of insects appear to be nature's control mechanism for the prevention of mass destruction of plants and animals by arthropods. Diseases of honeybees, known as foulbroods, exemplify the type of insect disease that must be prevented. Foulbroods are caused by sporeforming bacilli such as *Bacillus larvae*. One of the first bacterial diseases of harmful insects to receive great attention is characterized by dysentery and septicemia in locusts and grasshoppers. It is caused by a variety of *Enterobacter aerogenes*.

The idea that microorganisms may be used to control arthropod pests is not new, having been successfully used on a laboratory scale by Metchnikoff in 1879. Practical difficulties have deterred its development and application; however, highly successful results have been achieved in the control of the Japanese beetle by inducing milky disease in their grubs. The disease causes the blood of the sick grubs to become filled with bacteria and spores, giving it a milky appearance. The causative organism of type A milky disease is a sporeforming bacterium, *Bacillus popilliae*, and the causative organism of type B milky disease is *Bacillus lentimorbus*. The spores of these bacteria are highly resistant to desiccation, heat, and cold, and they survive in the soil for years. When these bacteria are introduced into soil where the grubs of the beetles develop, some of the grubs become infected; as the grubs die, more bacterial spores are introduced into the soil. This method has resulted in the virtual elimination of Japanese beetles in areas formerly heavily infested.

Bacillus thuringiensis is a sporeforming bacillus which causes a disease in lepidopterous larvae, such as the Mediterranean flour moth and other undesirable insects. The bacilli produce a toxin, which occurs in the form of a protein crystal (parasporal body) (Fig. 36-24). When the bacteria are ingested by larvae, the crystals dissolve and cause erosion of the gut epithelium. The toxin is not harmful to higher animals.

Although the use of insect pathogens has not been widely practiced, further study will surely lead to greater application because of the following obvious advantages:

1 ***Permanency.*** Once applied, bacterial spores such as those causing milky disease of Japanese beetles persist in the soil for a long time.
2 ***Safety.*** Most insect pathogens are harmless to plants and animals, and the danger of poisonous chemical residues is eliminated.

QUESTIONS

1 Distinguish between nonspecific and specific serologic tests for syphilis.
2 What special conditions are required for isolation of *Campylobacter jejuni*? What other organisms described in this chapter require special physical or chemical cultural conditions?
3 What kind of evidence is there that legionellosis occurred prior to the first recognition of the disease in 1976?
4 Zoonoses are diseases of animals that can be transmitted to humans. List the various zoonoses discussed in this chapter.
5 Why is humoral immunity developed by a patient relatively ineffective in contributing to recovery from brucellosis, listeriosis, and tuberculosis?

6 What general type of vaccine is most effective in preventing (a) brucellosis or tuberculosis, (b) pneumococcal pneumonia or meningococcal meningitis, and (c) diphtheria or tetanus?
7 List the bacterial pathogens that produce an enterotoxin and those that produce an antiphagocytic capsule.
8 What is the basis for antigenic subdivision of (a) *Salmonella*, (b) *Shigella*, (c) *Clostridium botulinum*, (d) *Haemophilus influenzae*, (e) *Streptococcus pneumoniae*, and (f) *Neisseria meningitidis*?
9 What are the characteristic features of an anaerobic infection? What general factors help to initiate an anaerobic infection? What bacterial species is the one most frequently isolated from anaerobic infections?
10 How and where might *Rickettsia prowazekii* maintain itself between typhus epidemics?
11 Name three organisms that can cause a pneumonia and indicate characteristics that can differentiate them.
12 Describe the characteristic kind of lesion produced by *Staphylococcus aureus* in tissues. Why might it be difficult for antibiotics to inhibit the staphylococci located in such a lesion? Why might it also be difficult for antibodies produced by the patient to inhibit the staphylococci?
13 In what fundamental way does rheumatic fever differ from most other kinds of diseases caused by pathogenic bacteria?
14 *Clostridium perfringens* food poisoning is not a true food poisoning like that caused by *Staphylococcus aureus* or *Clostridium botulinum*. Why? Is infant botulism a type of food poisoning? Explain.
15 What are the measures used for prevention of gas gangrene? What is the basis for the use of hyperbaric oxygen for treatment of a patient with gas gangrene?
16 In what fundamental way does the Schick test differ from the tuberculin test?
17 What is the basis for crown-gall formation in plants and why is this plant disease of interest to those doing research on animal cancer?
18 What advantages does biological control of insect pests have over control by chemical insecticides?

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Chapter 37 Microbial Agents of Disease: Viruses

OUTLINE

Viruses Containing Single-Stranded (+) RNA
Picornaviridae (Picornaviruses) • *Togaviridae* (Togaviruses) • *Coronaviridae* (Coronaviruses)

Viruses Containing Single-Stranded (−) RNA
Orthomyxoviridae (Orthomyxoviruses) • *Paramyxoviridae* (Paramyxoviruses) • *Bunyaviridae* (Bunyaviruses) • *Arenaviridae* (Arenaviruses) • *Rhabdoviridae* (Rhabdoviruses)

Viruses Containing Double-Stranded RNA
Reoviridae

Viruses Containing Double-Stranded DNA
Poxviridae (Poxviruses) • *Adenoviridae* (Adenoviruses) • *Herpesviridae* (Herpesviruses) • *Papovaviridae* (Papovaviruses)

Viruses Containing Single-Stranded DNA
Parvoviridae (Parvoviruses)

RNA Tumor Viruses Requiring a DNA Intermediate for Replication
Retroviridae (Retroviruses)

Miscellaneous Viruses
Hepatitis Viruses • Slow Viruses

Viral Pathogens of Plants

Viral Pathogens of Insects
Occluded Viruses • Nonoccluded Viruses • Other Viruses

The fact that viruses are obligate parasites does not mean that they necessarily cause overt disease. Some viruses may cause latent or inapparent infections that never result in clinical signs or symptoms. Others can cause inapparent infections which may later give rise to clinical disease; here, the link between the virus and the disease has often been difficult to establish. Still other viruses are frank pathogens, causing disease in humans, animals, insects, or plants; these viruses have naturally received the most attention. Viruses that can cause human disease may be strictly limited to human hosts, or they may be mainly pathogens of animals, with humans serving only as accidental hosts. Viral pathogens that are restricted mainly or solely to animals or plants may cause great economic losses in agriculture. Laboratory identification of viral pathogens is more diffi-

cult than identification of bacterial pathogens, because we cannot ordinarily observe viruses directly with a light microscope or culture them on nonliving laboratory media. The use of tissue cultures and embryonated eggs does provide a means by which many viral pathogens can be cultivated in the laboratory; in addition, the antibody response of the patient can serve as circumstantial evidence for a particular infection. Viral diseases cannot be treated with the chemotherapeutic agents that are effective against bacteria (although a few antiviral drugs are being developed); consequently, great emphasis has been given to epidemiological and immunological methods by which viral infections can be prevented. For instance, such measures have resulted in total eradication of smallpox, and many other viral diseases have been greatly reduced in incidence. Moreover, recombinant DNA techniques are beginning to provide safer and less expensive vaccines than have been available in the past for prevention of viral infections, and they are also beginning to provide biochemical agents such as interferon, which have previously been difficult and expensive to obtain, for potential use in treatment of viral infections.

This chapter provides an introduction to the various viral pathogens and some selected diseases which they cause, as well as some of the ways in which these diseases can be diagnosed and prevented.

VIRUSES CONTAINING SINGLE-STRANDED (+) RNA

Picornaviridae (Picornaviruses)

Picornaviruses consist of small (22 to 30 nm in diameter) viruses composed of single-stranded RNA of the (+) type (which can function directly as mRNA in a host cell) contained within an icosahedral capsid (see Fig. 21-15). No lipid-containing envelope surrounds the nucleocapsid. The family includes **polioviruses, coxsackieviruses A and B,** and **echoviruses;** these picornaviruses are collectively referred to as **enteroviruses** because they are found in the intestines and excreted in the feces. They cause mainly intestinal infections and sometimes infections of the respiratory tract; neurological disease may also be produced. Other picornaviruses called **rhinoviruses** cause only the respiratory infections known collectively as the common cold. Still other picornaviruses, the **foot-and-mouth disease (FMD) viruses,** cause disease mainly in domestic animals.

Polioviruses. The morphology of polioviruses is depicted in Fig. 21-1A. Two outstanding characteristics of polioviruses are their affinity for nervous tissue and their narrow host range. Besides humans, most strains will infect only monkeys and chimpanzees. Three immunological types have been recognized. Type 1 is the common epidemic type, type 2 is associated with endemic infections, and type 3 is an occasional cause of epidemics.

Polioviruses are transmitted in nose and throat discharges and in the feces of infected individuals. Entry into the body, therefore, may be via the respiratory route or by the oral-intestinal route.

Polioviruses multiply in the oropharynx or intestinal mucosa, pass to the lymphatic system (tonsils or mesenteric lymph nodes), and eventually reach the bloodstream. In most instances the disease is *subclinical* (no symptoms). In 4 to 6 percent of cases, *nonparalytic* poliomyelitis occurs; this is characterized by fever and stiffness or pain in the neck and back muscles. In about 0.1 percent of cases, *paralytic* poliomyelitis occurs; the bloodborne virus infects the central

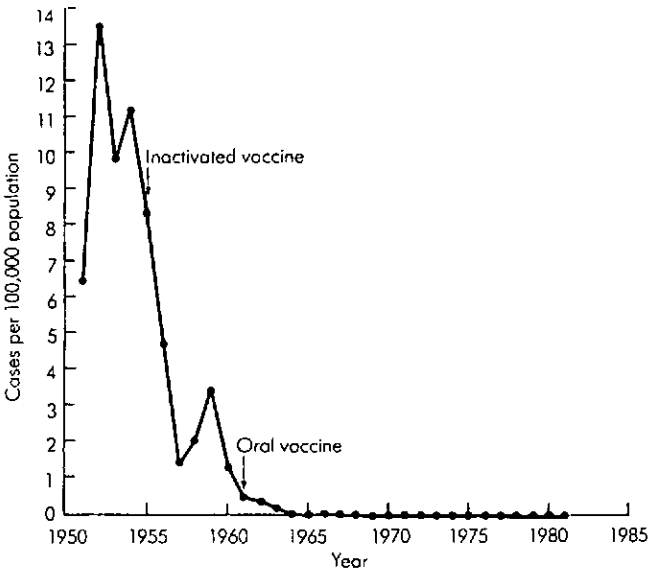

Figure 37-1. Reported case rates of paralytic poliomyelitis by year per 100,000 population, United States, 1951–1981. Arrows indicate when vaccination programs using the Salk (inactivated) vaccine and Sabin (oral) vaccine were begun. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.)*

nervous system and causes an inflammation of the gray matter of the spinal cord, especially the anterior horn, in which the cell bodies of motor neurons are located. When these cells are damaged, motor responses to the affected parts are weakened or destroyed. Paralytic poliomyelitis can result in death, although fatality rates have never exceeded 10 percent of the cases. The seriousness of the disease is also emphasized by the crippling effects on the survivors: one well-known example is that of Franklin D. Roosevelt, who acquired paralytic poliomyelitis as an adult and could walk only with the greatest difficulty for the rest of his life.

Two types of vaccines are available for immunization against poliomyelitis. The **Sabin vaccine** is an easily administered oral vaccine that is nearly 100 percent effective. It consists of live attenuated strains of the three immunological types. These strains infect the intestinal tract but, unlike virulent strains, they do not cause paralytic disease. Rather, they produce a long-lasting immunity by stimulating the formation of secretory IgA antibodies in the intestine and serum antibodies in the bloodstream. The secretory antibodies can neutralize the infectivity of virulent strains that may be subsequently encountered; thus these antibodies can prevent primary intestinal infection. The **Salk vaccine** is administered in a series of three intramuscular injections and is 70 to 90 percent effective. It consists of formalin-inactivated strains. This vaccine stimulates production of serum antibodies but not intestinal secretory IgA antibodies. Although the serum antibodies cannot prevent intestinal infection by a virulent poliovirus, they can prevent poliovirus in the bloodstream from reaching the spinal cord and causing paralysis.

In nations that have undertaken wide-scale immunization programs, paralytic poliomyelitis has decreased dramatically (Fig. 37-1). The vaccine presently used in the United States is the Sabin vaccine. Its use is not entirely without risk:

occasionally the type 3 mutant strain reverts to virulence, resulting in vaccine-associated paralytic poliomyelitis. The incidence is low (0.03 persons per 100,000 vaccinated persons) in comparison to disease rates of 7 to 13 per 100,000 population prior to vaccination programs (Fig. 37-1).

Rhinoviruses. Many kinds of viruses have been isolated from secretions from the respiratory tracts of persons with respiratory illness. Of these, rhinoviruses (Fig. 37-2) have been found to be major causes of the common cold, the most frequent of all human infections. Rhinoviruses differ from enteroviruses by being destroyed at pH values of less than 5 (such as would occur in the stomach) and by having an optimal growth temperature of 33°C rather than 37°C. These characteristics may help to explain why rhinoviruses are restricted to the respiratory tract.

Rhinoviruses are spread by droplets, by discharges from the nose and throat of an infected person, or by freshly contaminated fomites. After an incubation period of 12 to 72 h, the cold develops as an acute catarrhal infection of the nose, throat, sinuses, trachea, and bronchi, lasting approximately 2 to 7 days.

Antibodies appear in response to a rhinovirus infection, including secretory IgA antibodies in respiratory tract secretions. Such antibodies should protect against subsequent infections, yet a person may have several colds during a year. The reason is that there are at least 113 immunologically distinct types of rhinoviruses, and immunity against one type does not prevent infection by another type. No vaccine exists for prevention of the common cold because so many antigenic types would have to be included in the vaccine. Rhinoviruses are susceptible to human interferon, which may account for the fact that a person recovering from a cold often resists being reinfected for about 4 weeks.

Foot-and-Mouth Disease (FMD) Viruses. The FMD viruses are similar to the rhinoviruses and are the causative agents of foot-and-mouth disease, an acute communicable disease of cloven-hoofed animals, mainly cattle, sheep, goats, and swine. Humans are susceptible to infection and they may transmit the viruses to susceptible animals on their person or clothing. Animals which recover from the infection may act as asymptomatic carriers.

Entrance into a host is usually through abrasions of the skin and mucous membranes. In humans the disease is relatively mild and is characterized by

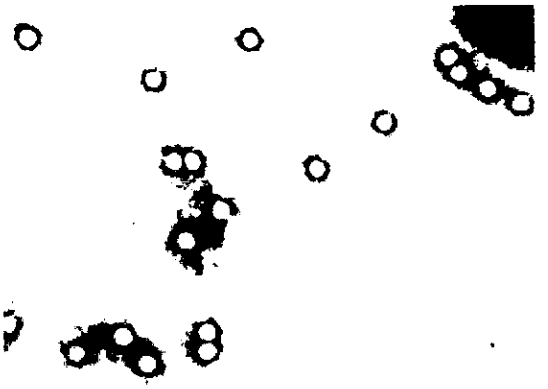

Figure 37-2. Electron micrograph of purified rhinovirus (X125,000). *(Courtesy of Frances Doane, University of Toronto.)*

Figure 37-3. Foot-and-mouth disease in animals causes lesions of (A) tongue, (B) teats, and (C) foot. *(Courtesy of USDA.)*

fever, salivation, and a vesicular (blisterlike) eruption on the mucous membranes of the oropharynx and on the skin of the palms, soles, fingers, and toes. In animals, vesicles occur on the mucosa of the mouth, between the claws of feet, and on other parts of the body (Fig. 37-3). The mortality rate in animals is low, but FMD causes great economic losses due to reduced milk and meat production and to abortion. Laboratory diagnosis of FMD is done by complement-fixation, virus-neutralization, or mouse-inoculation tests. FMD has been eliminated from the United States by enforcement of quarantine, inspection, and regulations prohibiting importation of susceptible animals and animal products from countries where the disease exists.

Recovery from infection confers immunity to the particular viral serotype involved. Vaccines are available and are prepared from inactivated virus grown in tissue cultures; however, outbreaks of disease are frequently linked to incompletely inactivated vaccines or escape of live virus from research and production facilities. Recently a novel vaccine was developed by recombinant DNA techniques. A gene for an FMD viral coat protein was spliced into an *Escherichia coli* plasmid, and bacteria containing this plasmid then produced the viral protein antigen. The antigen was extracted from the bacteria and used as a completely safe immunizing agent. Such an approach avoids the dangers and difficulties associated with whole virus vaccines.

Togaviridae (Togaviruses)

Togaviruses are spherical (40 to 90 nm in diameter), have an icosahedral nucleocapsid contained within a lipoprotein envelope (the word *toga* means "coat"), and possess single-stranded RNA of the (+) type (see Fig. 21-15). Four groups occur which are differentiated by their antigenic properties: the **alphaviruses** and **flaviviruses** cause arthropod-borne infections of humans, **rubellavirus** causes rubella (German measles), and **pestivirus** causes several animal diseases.

Alphaviruses and Flaviviruses. Alphaviruses infect mainly animals and are transmitted to humans by mosquitoes. Some of the diseases caused by alphaviruses are indicated in Table 37-1. The diseases are characterized by a viremia which gives rise to fever, joint pains, and sometimes a skin rash. In some instances the bloodborne virus may invade the central nervous system and cause an encephalitis characterized by high fever, delirium, convulsions, paralysis, and coma. Mortality rates vary with the kinds of virus; for example, eastern equine encephalitis has a high mortality rate (50 to 70 percent), whereas western equine encephalitis virus infections are seldom fatal (3 percent).

Flaviviruses also have an animal reservoir and are transmitted to humans by either mosquitoes or ticks, depending on the virus species (Table 37-1). Like alphaviruses, most of the flaviviruses cause fevers or encephalitis.

The yellow fever virus differs from other flaviviruses by causing a disease in which the liver, kidney, and other organs are affected. In addition to generalized symptoms (headache, fever, muscle pains, backache), yellow fever is characterized by **albuminuria** (albumin in the urine, indicative of kidney damage); **hematemesis** (vomiting of blood); **hepatomegaly** (enlargement of the liver); and **jaundice** (yellowing of skin and mucous membranes, indicative of liver damage). The fatality rate for yellow fever is 25 to 30 percent. The epidemiology of urban and sylvatic yellow fever and the measures for control of the disease have been described in Chap. 35.

***Rubivirus* (Rubellavirus).** Rubellavirus occurs only in humans and has an airborne transmission via respiratory secretions. The virus is the causative agent

Table 37-1. Some Examples of Alphaviruses and Flaviviruses

Virus	Reservoir	Vector	Occurrence	Disease in Humans
Alphaviruses				
Eastern equine encephalitis (EEE)	Birds	Mosquitoes	Western hemisphere	Encephalitis
Western equine encephalitis (WEE)	Birds	Mosquitoes	Western hemisphere	Encephalitis
Venezuelan equine encephalitis (VEE)	Rodents, horses	Mosquitoes	Western hemisphere	Acute febrile illness; sometimes encephalitis
Ross River	Mammals, humans	Mosquitoes	Australia, Pacific	Fever, rash, joint pains
Chikungunya	Monkeys, humans	Mosquitoes	Africa, India, Southeast Asia	Fever, rash, joint pains
Flaviviruses				
Yellow fever	Monkeys	Mosquitoes	Africa, South and Central America	Hemorrhagic fever, hepatitis, nephritis
Dengue	Humans	Mosquitoes	Tropics, worldwide	Fever, rash, joint pains
St. Louis encephalitis	Birds	Mosquitoes	Western hemisphere	Encephalitis
West Nile	Birds	Mosquitoes	Africa, Middle East, Europe	Fever, rash, joint pains
Japanese B encephalitis	Birds, swine	Mosquitoes	India, Japan, Far East	Encephalitis
Tickborne encephalitis	Rodents	Ticks	Europe, Asia	Encephalitis
Powassan	Rodents	Ticks	North America	Encephalitis

of rubella (German measles, or three-day measles)—a highly communicable disease that is unrelated to common measles (rubeola). Although rubella is communicable and usually occurs in epidemic form (every 7 to 10 years in the United States), the disease in general, as compared with rubeola, is of relatively short duration and of mild form.

After initial multiplication in the upper respiratory tract, the rubellavirus becomes distributed via the blood to the skin, lymph nodes, and joints. Respiratory symptoms do not usually occur. The disease is characterized by fever, malaise, lassitude, and other symptoms. A rash, which probably results from an immune response to viral antigens in infected skin cells, sometimes appears on the face and later on other parts of the body. A characteristic feature of the disease is swelling of lymph glands below the ear and at the nape of the neck.

The characteristic mild course of the disease can give a misleading impression of its importance. When rubella occurs in early pregnancy, it can cause serious congenital abnormalities in the fetus. During the 1964–1965 epidemic in the United States, it is estimated that there were 30,000 fetal deaths and that more than 20,000 children were born with defects of vision and hearing, heart disease, and mental retardation.

A vaccine prepared from live attenuated virus is available. Immunization with this vaccine in combination with the mumps and measles vaccine is recommended for all children at 15 months of age. Since the introduction of the rubella vaccine in 1969 there has been a 96 percent decrease in the incidence of rubella (Fig. 37-4).

Coronaviridae (Coronaviruses)

Coronaviruses have a helical nucleocapsid and contain single-stranded RNA of the (+) type. Their name is based on the occurrence of distinctive club-shaped particles of glycoprotein that project from the surface of the envelope and which give the effect of a crown or corona (see Fig. 21-15).

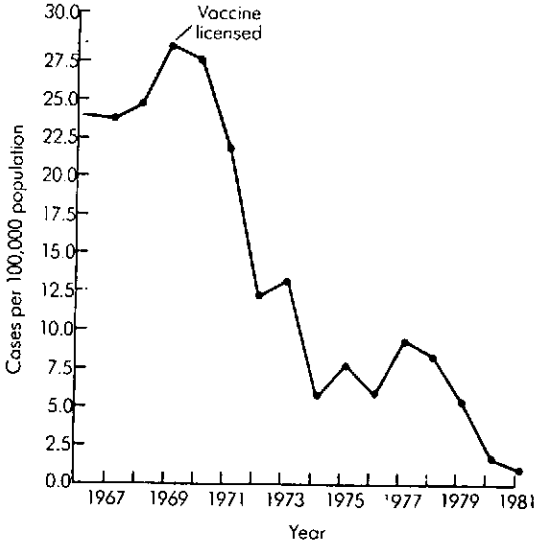

Figure 37-4. Reported cases of rubella (German measles) by year per 100,000 population, United States, 1966–1981. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.)*

Like rhinoviruses, human coronaviruses represent causative agents of the common cold. At least three immunological groups exist. Other coronaviruses are important animal pathogens, causing diseases such as avian infectious bronchitis of chickens, mouse hepatitis, and gastroenteritis of piglets.

VIRUSES CONTAINING SINGLE-STRANDED (−) RNA

Orthomyxoviridae (Orthomyxoviruses)

The only members of the family *Orthomyxoviridae* are the influenza viruses. They are usually spherical, 80 to 120 nm in diameter (see Fig. 21-15); or they may be filamentous, up to several μm in length (see Fig. 21-11). They have a helical nucleocapsid with a core of single-stranded RNA of the (−) type (which serves as a template for synthesis of a complementary strand that acts as the messenger RNA). The RNA occurs in eight separate pieces to which protein subunits are attached. This ribonucleoprotein is wound up to form a rounded mass and is covered by a lipid-containing envelope. Protein spikes project from the envelope; these are hemagglutinins. Between the spikes are mushroom-shaped protrusions composed of neuraminidase. The role of the hemagglutinins and neuraminidases in attachment to host cells is discussed in Chap. 31.

The epidemiology of influenza and the role of antigenic variation in the periodic occurrence of pandemics are discussed in Chap. 35. Influenza is characterized by nasal discharge, headache, muscle pains, sore throat, a marked weakness and exhaustion, and a tendency to develop secondary bacterial pneumonias. During the disease the virus remains localized in the respiratory tract, where it kills ciliated epithelial cells. This killing effect may actually be due to cytotoxic T lymphocytes that respond to the viral antigens on infected cells.

A synthetic drug, amantadine, can prevent and even help to cure influenza caused by type A strains; it acts by preventing the penetration and uncoating of the virus after it attaches to a host cell (see Chap. 21). Another drug, ribavirin, inhibits not only influenza viruses but also other RNA viruses and DNA viruses by preventing the synthesis of guanosine monophosphate used for nucleic acid synthesis. The drug also inhibits host cells; however, the inhibitory effect is greater on the viruses because of their greater demand for nucleic acid precursors.

Vaccination against influenza is accomplished by use of formalin-inactivated virus. Immunity is based on the development of antibodies against the hemagglutinin antigens, thereby preventing viral attachment to host cells. Since immunity is subtype-specific, a mixture of several of the hemagglutinin subtypes most likely to cause infection is used in the vaccine. The antibodies most effective in preventing viral attachment are secretory antibodies present in mucus; however, inactivated vaccines mainly stimulate development of serum antibodies rather than secretory antibodies. The latter would be more efficiently produced in response to live attenuated vaccines, and such vaccines are currently under development.

Paramyxoviridae (Paramyxoviruses)

The paramyxoviruses have a structure similar to that of the orthomyxoviruses; however, they are larger (125 to 250 nm; see Fig. 21-15), the RNA is nonsegmented, and the hemagglutinin and neuraminidase activities occur together in a single kind of surface glycoprotein (HN). A second kind of surface glycoprotein (F) has hemolytic activity. The family includes **parainfluenza viruses** (see Fig.

21-1D), which cause 30 to 40 percent of all acute respiratory infections in infants and children. The infections range from mild coldlike disease to severe croup, bronchiolitis, and pneumonia. Other examples of paramyxoviruses include **respiratory syncytial virus,** the major cause of bronchiolitis and pneumonia in infants less than 1 year old; **Newcastle disease virus,** which causes avian pneumoencephalitis, an important disease of chickens; **measles virus;** and **mumps virus.**

***Morbillivirus* (Measles Virus).** The measles virus occurs in respiratory tract secretions of patients who are in the early stages of measles and is transmitted by droplet infection. The portal of entry into the body is the upper respiratory tract or the conjunctiva.

Measles (rubeola) is one of the most common acute communicable human diseases, mainly affecting children but sometimes occurring in adults who have escaped previous infection. After initial multiplication, the virus becomes disseminated via the blood to the mucous membranes of the intestinal tract and urinary tract, to the skin, and to the central nervous system. Symptoms include fever; coldlike symptoms; cough; conjunctivitis; the occurrence of Koplik's spots (small bluish-white spots surrounded by a reddish area which occur on the mucous membranes of the cheeks and lips); and a characteristic red, blotchy skin rash. The rash probably results from an immune reaction with viral antigens on the surface of infected blood capillary cells, which in turn causes dilatation of the capillaries and leakage of blood into the tissues.

After exposure of a person to a known infection, prompt administration of gamma globulin (pooled antibodies from human blood) can prevent the disease or greatly modify its course. After measles develops, there is no effective treatment for the infection, although antibiotics may serve to check secondary bacterial invaders which may otherwise cause internal ear infection or pneumonia. Recovery from measles confers a high degree of immunity.

A rare, progressive, fatal disease of the central nervous system called subacute sclerosing panencephalitis (SSPE) may follow about 6 years after recovery from a case of measles. Although patients exhibit a high level of antibodies against the measles virus, it is not yet clear whether the SSPE virus is actually the measles virus or a variant of it.

The measles virus can be grown on tissue cultures of human or monkey cells and on chick embryos. Live vaccines prepared from such cultures by using the attenuated Edmonston strain are effective antibody stimulators, and immunization with the measles vaccine, in combination with the live rubella and mumps vaccines, is recommended for all healthy 15-month-old children. Vaccination programs begun in 1965 have resulted in a great decrease in the incidence of measles (Fig. 37-5).

Mumps Virus. Mumps virus is spread by droplets or fomites contaminated with infected saliva. Mumps (epidemic parotitis) is a common communicable disease that is endemic in most heavily populated areas. Epidemics are prevalent during the winter months, often occurring in schools and among military personnel. Most cases occur in children in the 5- to 15-year age group. Although mumps is a common childhood disease, it also attacks adults, in whom it may be a more serious infection.

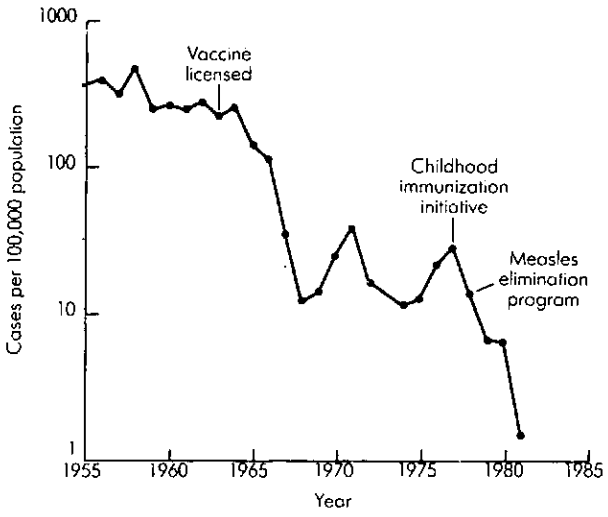

Figure 37-5. Reported cases of measles by year per 100,000 population, United States, 1955–1981. For 1981 the incidence of reported measles reached a record low of 1.4 cases per 100,000 population—a 99.5 percent reduction from the prevaccine period of 1955–1962, when the average annual incidence was 299.5 cases per 100,000 population. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, Annual Supplement Summary 1981, issued October 1982.)*

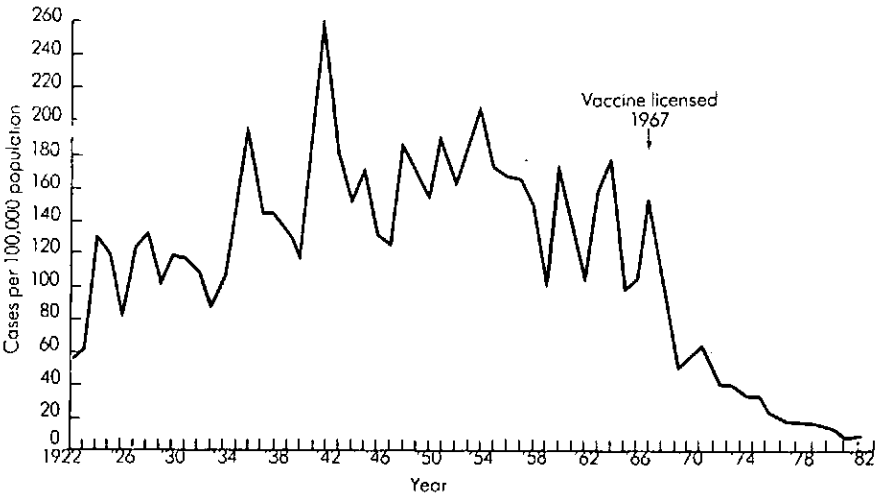

Figure 37-6. Reported cases of mumps per 100,000 population, United States, 1922–1982. *(Centers for Disease Control: Morbidity and Mortality Weekly Report, October 28, 1983.)*

In the body a viremia is produced that distributes the virus to various glands, in which viral multiplication occurs. Mumps is characterized by painful swelling, particularly of the parotid glands, although the salivary glands, the testes, the ovaries, the pancreas, and other glands may be involved. A common manifestation of mumps in adults is orchitis (inflammation of the testes) in males or inflammation of the ovaries in females.

Immunization is achieved by means of a live attenuated vaccine. Active immunization with the combined mumps-measles-rubella vaccine is recommended for all children at 15 months of age. Since the introduction of the vaccine in 1967, a 97 percent decrease in the number of reported cases of mumps has occurred (Fig. 37-6).

Bunyaviridae (Bunyaviruses)

Bunyaviruses are enveloped viruses having a helical nucleocapsid. They contain single-stranded RNA which is composed of three segments and is of the (−) type. The viruses range from 90 to 100 nm in diameter. Bunyaviruses cause fevers or encephalitis in humans and/or abortion and hepatitis in domestic animals. They are transmitted by mosquitoes, biting flies, or ticks. Some examples are La Crosse encephalitis (North America, mosquito-borne); Bunyamwera fever (Africa, mosquito-borne); and Crimean-Congo hemorrhagic fever (Africa and Asia, spread by ticks).

Arenaviridae (Arenaviruses)

Arenaviruses are similar in structure to bunyaviruses; however, they have a large average diameter (110 to 130 nm) and their RNA is composed of two segments. The prefix *arena-*, meaning "sand," is based on the occurrence of electron-dense granules (probably host ribosomes) that occur within the virions (see Fig. 21-15). Arenaviruses cause asymptomatic infections in rodents, which constitute the natural reservoir of infection. Transmission to humans probably occurs by contact with infected rodent feces and urine. Human arenavirus infections include (1) a *meningoencephalitis*, a debilitating but rarely fatal disease that is caused by lymphocytic choriomeningitis virus (LCV) and (2) *hemorrhagic fevers* such as Lassa fever, in which there is a high fatality rate. Lassa fever was first discovered in Nigeria in 1969 and at least four other outbreaks have since occurred in Africa. Death rates have varied from 20 to 66 percent. The Lassa virus has been isolated from a species of rodent that is widely distributed in the region, and it is likely that this species constitutes a reservoir for the virus. It is possible that contact with the rodent, its secretions, or articles contaminated by it may be responsible for the initial human infections in an outbreak. Unlike most arenavirus infections, Lassa fever is highly contagious and can be spread from person to person. The mode of transmission is uncertain but may be via aerosols or direct contact, and strict isolation techniques must be used for patients. Fatal infections have been contracted from laboratory procedures involving clinical specimens.

Rhabdoviridae (Rhabdoviruses)

These viruses are 70 to 80 by 130 to 240 nm in size and have a characteristic bullet shape (Fig. 37-7). They have a helical nucleocapsid and contain single-stranded RNA of the (−) type. Surrounding the nucleocapsid is a lipid envelope with surface glycoprotein antigens.

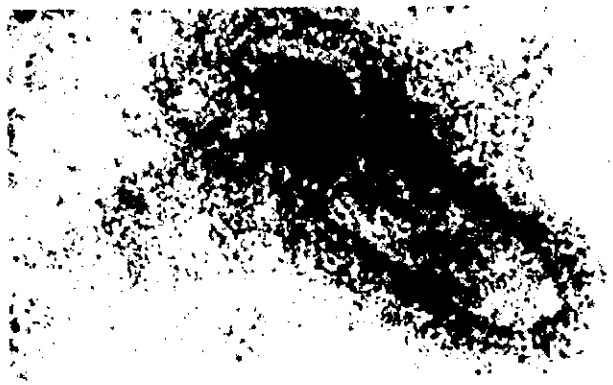

Figure 37-7. Vesicular stomatitis virus, showing the bullet shape that is characteristic of rhabdoviruses. Shown also is an irregular "handle" which may represent remains of a disrupted end (X200,000). *Courtesy of Margaret Gomersall, McGill University.)*

Discovery of a Killer Virus

The events surrounding the discovery of the Lassa fever virus were frightening. They began in 1969 at the Lassa mission hospital, located in a remote region of Nigeria, when a nurse contracted a strange and unidentifiable disease. The symptoms began with a severe sore throat, muscular aches, fever, and lassitude. During the next few days the patient showed an increase in temperature and development of ulcers in the back of the throat. Hemorrhaging of blood capillaries beneath the skin caused the skin to discolor. Despite intensive treatment, there was continued deterioration, with development of kidney failure, extreme difficulty in breathing, cyanosis (lack of oxygen in the blood), and cardiovascular collapse. The nurse died within a week after the illness began.

One of the nurses who had attended the patient also developed symptoms of the strange disease, became desperately ill, and died. About a week later, another nurse who had assisted at the autopsy began to develop symptoms of the illness. By the 12th day she was desperately ill. Arrangements were made for her to be flown to New York for treatment. There, in the isolation ward at Columbia Presbyterian Hospital, she slowly recovered from the illness. However, neither the causative agent of the disease nor an effective treatment for it were yet known.

Meanwhile, tissue and blood samples from all three victims had been sent to the Yale Arbovirus Research Unit in New Haven, Connecticut, where they had been inoculated into suckling mice and into tissue cultures. The tissue cultures showed cytopathic effects that were characteristic of a virus. Although the infant mice failed to become ill, the virus later proved to be highly fatal for adult mice. While information about the virus was accumulating, a Yale virologist who was studying the virus contracted Lassa fever. Although he was successfully treated with immune serum from the nurse who had recovered from the infection, another shock was to follow: a second Yale virologist, who had never worked with the Lassa virus and who had no known contact with it, inexplicably contracted the disease and died.

It was now clear that the usual safety precautions for dealing with infectious agents in a laboratory evidently were not sufficient for the Lassa virus. As a result, all research at Yale on the live virus was immediately halted; all live infectious material was transported to the new maximum security facility that had been constructed at the Centers for Disease Control (CDC) in Atlanta for containment and study of highly contagious pathogens, and where the Lassa virus was already under investigation.

At present, all studies of live Lassa virus, including the isolation and identification of the virus from clinical specimens, are restricted to laboratories such as those at the CDC which are specially equipped to deal with exceedingly hazardous microorganisms.

Rhabdoviruses cause disease in humans, animals, (vertebrates and invertebrates), and plants. One well known group of animal rhabdoviruses is the vesicular stomatitis virus (VSV) group, which causes vesicular (blisterlike) lesions in the mouth and other areas of the body of cattle, pigs, and horses. The mode of transmission is uncertain but may be by means of arthropods. Humans can contract a benign form of vesicular stomatitis characterized by malaise, fever, aches and pains, headache, nausea, and vomiting. Laboratory infections with VSV are common.

Rabies Virus. The most important member of the rhabdovirus family is the rabies virus, a parasite of domestic and wild mammals. Only one immunological type of this virus is known to exist. Transmission to humans occurs through the bite of an infected animal. Dogs, cats, bats, and skunks are most frequently the source of the virus infecting humans.

Rabies is essentially an overwhelming encephalomyelitis. In humans the incubation period from time of infection varies from 6 days to 1 year but is usually about 3 to 8 weeks. The development of symptoms and the length of the incubation period (in untreated cases) depend largely on the severity and location of the bite. It has been estimated that only 5 to 15 percent of all persons bitten by a rabid animal contract rabies. Symptoms in humans include severe headache and high fever, with alternating stages of excitement and depression. Patients have difficulty in swallowing, and slight stimuli incite muscular spasms in the throat and chest. Death usually follows paralysis or convulsive seizures. The mortality rate from rabies is nearly 100 percent.

If a person has been bitten by a rabid animal, the long incubation period for rabies allows time for measures to be taken to prevent the virus from reaching the central nervous system. These measures include a combination of passive immunization (by administration of immune human or horse globulin, which provides an immediate source of antibodies against the virus but which lasts for only about 14 days) and active immunization (administration of a rabies vaccine, to stimulate a longer-lasting production of antibodies by the patient).

Laboratory confirmation of rabies in the animal which has bitten the patient is done by any of several means: (1) detection of rabies virus antigen in clinical specimens by use of fluorescent antibodies; (2) isolation of the virus from saliva, urine, spinal fluid, or tissues by inoculation into the brains of mice; and (3) demonstration of inclusion bodies (**Negri bodies**, shown in Fig. 37-8) in the nerve cells of the brain. In all these tests, negative results do not exclude rabies, and for this reason the animal should not be destroyed prematurely but should be kept under observation for at least 2 weeks. If the animal develops symptoms of rabies, the human victim must be vaccinated.

In 1885, Pasteur produced an effective rabies vaccine by using brain tissue from rabbits in which rabies virus had been propagated. By drying the infected nerve tissue for increasing periods of time, the infectivity of the virus could be progressively diminished to the point where the preparation could be used to immunize persons who had been bitten by rabid animals. In 1919 Semple modified the Pasteur vaccine by adding phenol to completely inactivate the

Figure 37-8. Negri bodies, inclusion bodies (dark, round structures) found in Purkinje cells and cells of the hippocampus, are diagnostic of rabies. *(Courtesy of J. Nowak, Documenta Microbiologica, part 2, "Pilze und Protozoen," Gustav Fischer Verlag, Jena, Germany, 1930.)*

virus. One difficulty with the Pasteur and Semple vaccines is that they sometimes cause an allergic encephalitis in the recipient, because of the presence of nerve tissue in the vaccines. In 1949 a different type of vaccine was developed. It contained a live attenuated strain of rabies virus—the Flury strain—which could be grown in embryonated duck eggs. This vaccine has been widely used; however, it has relatively low immunogenicity, and although it lacks large amounts of nerve tissue antigens, it still can occasionally cause allergic encephalitis. In 1980 an inactivated vaccine prepared from virus propagated in cultures of diploid human cells devoid of nerve tissue antigens was licensed for use. This new vaccine appears to be both safe and highly immunogenic.

VIRUSES CONTAINING DOUBLE-STRANDED RNA

Reoviridae

This family consists of icosahedral viruses, 70 to 80 nm in diameter, having a double capsid, no envelope, and containing double-stranded RNA with 10 to 11 segments. Among the groups included in the family which affect humans and animals are the **orbiviruses, reoviruses,** and rotaviruses

Orbiviruses are arthropod-borne viruses, transmitted by mosquitoes, ticks, or sandflies. They cause serious diseases in humans and animals; for example, Colorado tick fever virus causes an acute, generalized infection of humans and occurs in the western regions of the United States and Canada (see Table 35-6).

Reoviruses are widely distributed among mammals and are inhabitants of the intestinal tract. Although they have been isolated from humans with mild respiratory tract and gastrointestinal disease, they commonly occur in healthy individuals as well, and their relationship to disease is not yet clear.

Figure 37-9. Electron micrograph of rotavirus (X80,000). *(Courtesy of M. Petric and Maria T. Szymanski, The Hospital for Sick Children, Toronto.)*

Rotaviruses (Fig. 37-9) are intestinal viruses that occur in humans and animals. Their name comes from their wheellike shape. In recent years rotaviruses have been increasingly recognized as frequent and important contagious agents of diarrhea in infants and children (6 months to 2 years old). Indeed, the World Health Organization (WHO) has estimated that, on a worldwide basis, rotaviruses are responsible for one-half of the cases of infantile diarrhea requiring hospitalization. Like polioviruses, rotaviruses have a fecal-oral mode of transmission, infect the intestine, and are shed in stools. Rotavirus diarrhea is characterized by fever, severe diarrhea, vomiting, and dehydration. Diagnosis is based either on demonstrating the virus in stools (usually by means of an ELISA test; see Chap. 34) or on finding an increasing level of serum antibodies against the virus.

VIRUSES CONTAINING DOUBLE-STRANDED DNA

Poxviridae (Poxviruses)

Poxviruses are the largest of all viruses and are brick-shaped (200 to 260 by 250 to 390 nm) or ovoid. They contain double-stranded DNA, protein, and lipid and have a dumbbell-shaped nucleoid (see Fig. 21-15) surrounded by two membrane layers. The outer surface is covered with threads or tubules. Six genera and 27 species of poxviruses exist, most of which are animal pathogens. Of the human pathogens, the variola (smallpox) virus unquestionably has been the most important.

Variola (Smallpox) Virus. This virus is transmitted by droplet infection, either directly from an infected person to another person or by handling of articles infected by the smallpox patient. The virus is believed to lodge in the naso-

pharynx and to invade the regional lymphatic system. This is followed by dissemination of the virus via the bloodstream to all tissues and especially the skin. An initial fever occurs, followed by a rash consisting of small papules that appear first along the hairline and later on the face and other parts of the body. These papules or pustules become larger and are filled with fluid. The fever recurs and the patient becomes severely ill with generalized symptoms. As the pustules regress, scabs are formed, which leave the craterlike scars characteristic of smallpox. There are two kinds of variola virus: (1) *variola major*, which causes severe symptoms and has a fatality rate of 10 to 30 percent; and (2) the less virulent *variola minor (alastrim)*, with a fatality rate of only 0.1 to 0.3 percent. Except for the difference in virulence, the two viruses cannot be distinguished.

Since mild cases may be difficult to diagnose and may be confused with chicken pox (varicella, caused by a herpesvirus), the laboratory uses several methods of diagnosis to aid the clinician: (1) electron microscopy to visualize the brick-shaped virions in skin exudates, or, alternatively, the use of light microscopy to demonstrate inclusion bodies (**Guarnieri bodies**, probably the virus itself) in stained cells (see Fig. 21-21); (2) cultivation of the virus on the chorioallantoic membrane of embryonated chicken eggs; or (3) serological detection of the viral antigen in smallpox lesions.

Although smallpox has been widespread for thousands of years, both endemically and epidemically, no cases of smallpox have appeared anywhere in the world since 1977. This total eradication of smallpox was achieved because of several factors: (1) the virus had no animal reservoir of infection; (2) no subclinical carrier state occurred; (3) no dormant (latent) infections occurred; (4) an effective vaccine was available; and (5) no antigenic variation in the virus occurred (in contrast, for example, to influenza viruses). These factors permitted development of a grand strategy for eradication which could be implemented on a worldwide basis and which ultimately proved successful (Table 37-2).

Protection against smallpox involves both humoral and cell-mediated immunity and is achieved by intradermal inoculation with a live vaccine. The vaccine consists of a virus known as **vaccinia** which is closely related antigenically to variola virus but which does not ordinarily produce viremia. It is important that a live vaccine be used; inactivated vaccines prepared from vaccinia virus extracted from infected cells are ineffective because they lack an important immunizing antigen. This antigen can be acquired only by a live virus during its natural release from host cells.

Although smallpox immunization will eventually cease because of the eradication of smallpox, another potential use for vaccinia virus is being developed. In 1983 the gene for the immunizing antigen of a herpesvirus was successfully incorporated into the vaccinia genome by recombinant DNA techniques. The altered vaccinia virus produced not only its own surface antigens but the herpes antigen as well. Similarly, vaccinia strains that can produce antigenic components of hepatitis or influenza viruses have been developed. It is possible that a single vaccinia strain could be developed that can make several different viral antigens simultaneously. Such genetically modified vaccinia strains are of great interest because of their potential use as safe, inexpensive vaccines for prevention of several diseases concomitantly.

Table 37-2. Grand Strategy for Eradicating Smallpox

	Phase 1: Attack	Phase 2: Consolidation	Phase 3: Maintenance
Areas	Areas where over 5 cases of smallpox per 100,000 people occur per year and where less than 80% of all segments of the population shows scars of primary vaccination	Areas with less than 5 cases per 100,000 people and where over 80% of all segments of the population show scars of primary vaccination	Areas free of endemic smallpox for more than 2 years but geographically situated in endemic continental areas.
Vaccination	Systematic mass vaccination	Continuing maintenance vaccination	Continuing maintenance vaccination
Intelligence	Establish prompt and regular reporting of smallpox by all existing health facilities	Extension of case-detection system to assure that all suspected smallpox cases are reported	Continuing of case-detection system to assure that all suspected smallpox cases are reported.
Field investigations	Epidemiological investigation of major outbreaks throughout the country and of all cases in areas where systematic mass vaccination has been done	Prompt epidemiological investigation of all cases to establish sources of infection and to exclude the possibility of unreported cases	Each case to be investigated as an emergency by an epidemiologist
Laboratory	Establish techniques and methods for the submission and examination of specimens for confirmation of diagnosis	Specimens studied from all isolated cases and representative samples from each outbreak	Specimens studied from every suspected case
Containment	Localized, intensive vaccination in communities where cases or outbreaks occur; isolation of cases if feasible and disinfection	Vaccination and observation of case contacts; isolation of cases and appropriate disinfection; localized, intensive vaccination in community	Vaccination and observation of case contacts; isolation of cases and appropriate disinfection; localized, intensive vaccination in community

SOURCE: *World Health*, The Magazine of the World Health Organization, January–February 1968.

Adenoviridae (Adenoviruses)

The adenoviruses are icosahedral viruses, 60 to 90 nm in diameter, which contain double-stranded DNA and do not possess a lipid envelope. At each of the 12 vertices of the icosahedron there is a fiberlike projection (see Fig. 21-15). These fibers have hemagglutinin activity and also probably are responsible for viral attachment to host cells. Two major kinds of adenoviruses occur: those which are isolated from humans and other mammals and those which are isolated from birds. The human adenoviruses are divided into four subgroups based on their ability to agglutinate monkey or rat red blood cells. Each subgroup in turn contains various antigenic types.

Adenoviruses cause acute, self-limiting respiratory and eye infections and have an airborne type of transmission. Diseases caused by adenoviruses include acute febrile pharyngitis, which occurs most often in infants and children; pharyngeal-conjunctival fever in children; acute respiratory disease (ARD), which occurs mainly in military recruits; adenovirus pneumonia, a complication of ARD; and acute follicular conjunctivitis in adults.

Adenoviruses may also cause inapparent or latent infections, particularly of lymphoid tissue. Indeed, adenoviruses were initially discovered by accident:

Table 37-3. Oncogenicity of Subgroups and Serotypes of Human Adenoviruses

Hemagglutination Subgroup	Serotypes	Oncogenicity in Newborn Hamsters
I	3, 7, 11, 14, 16, 21	Weak
II	8–10, 13, 15, 17, 19, 20, 22–30	None†
III	1, 2, 4–6	None†
IV	12, 18, 31	High

† But may cause in vitro transformation in tissue cultures.

tissue cultures had been prepared from apparently normal human tonsils and adenoids, yet these cultures exhibited degenerative changes (cytopathic effects) that were indicative of the presence of a virus.

Although adenoviruses are not known to cause cancer in humans, some strains, particularly those of subgroup IV, can cause cancer (are **oncogenic**) when inoculated into immunodeficient animals (newborn hamsters) (Table 37-3). These strains, as well as certain other strains that do not produce oncogenic effects in animals, can cause neoplastic transformation of cells grown in tissue culture (see Chap. 21). Such transformation occurs mainly in cells which, when infected, yield few or no viral progeny (**nonpermissive** cells). In the transformed cells, it can be shown that a portion of the viral DNA has become integrated into the genome of the host.

Herpesviridae (Herpesviruses)

Like adenoviruses, herpesviruses have an icosahedral nucleocapsid containing double-stranded DNA; however, the nucleocapsid is enclosed within a bilayered envelope from which extend numerous short projections (see Fig. 21-15). Moreover, herpesviruses are much larger than adenoviruses, having a diameter of 180 to 200 nm.

Herpesviruses cause disease in a wide variety of animals and in humans. One of the animal herpesviruses is Marek's disease virus (MDV), which affects the epithelium of feather follicles in chickens. The virus has special significance because it can also cause neoplastic transformation of chicken T lymphocytes, resulting in lymphoma—a type of cancer. Some human herpesviruses may also be related to cancer, although whether the relationship is a causal one is uncertain. A discussion of some human herpesviruses follows.

Herpes Simplex Virus Type 1 (HSV-1). Primary infections with HSV-1 are common and may be contracted by children over 6 months old. By adulthood the proportion of persons who have been infected is 50 percent or higher. On initial contact with HSV-1, more than 90 percent of persons develop a subclinical form of *primary* herpes. Clinical infections are usually self-limiting and include acute gingivostomatitis (vesicular eruption in the mouth), pharyngitis, cold sores, keratoconjunctivitis, or skin lesions (on nongenital areas). Occasionally, more severe and even fatal infections occur, such as encephalitis.

Following the primary infection (clinical or subclinical), people develop neutralizing antibodies and maintain them for the rest of their lives. Despite these antibodies, the virus continues to occur in a *latent* form. The latent virus may sometimes be reactivated by environmental factors such as heat and cold. by

hormonal or emotional disturbances, or by other stimuli. This is recurrent herpes and is usually characterized by superficial vesicles (cold sores, fever blisters).

HSV-1 has been related to head and neck cancers, but whether it actually causes these cancers is uncertain. Under certain conditions, HSV-1 (and also HSV-2, discussed below) can cause neoplastic transformation in tissue cultures of hamster embryo cells and human fibroblasts.

Herpes Simplex Virus Type 2 (HSV-2). HSV-2 shares some antigens with HSV-1 but it also has some unique antigens and other distinctive properties. In recent years HSV-2 has been recognized as the causative agent of primary and recurrent disease involving the genital tract.

Primary genital herpes is most commonly transmitted by sexual contact. Infection is associated with small, painful blisters on the cervix, vagina, urethra, and anus in women; in men, the lesions are on the penis, in the urethra, or around the anus. The lesions become crusted and heal without leaving scars. The virus is present in the lesions, and the disease is most contagious when lesions are present; however, the disease may sometimes be contagious even when lesions are absent. Other aspects of the clinical syndrome include fever, painful urination, inflammation of the inguinal lymph glands, and genital soreness. Infection in pregnant women may result in serious neonatal disease with dissemination of the virus to the skin, eyes, central nervous system, and visceral organs of the newborn.

After the lesions disappear, the virus remains latent for periods from a few weeks to a year or longer, after which the symptoms may recur. Recurrent genital herpes is frequent. In women, the cervix is usually involved, but the infection is often subclinical; in men, herpetic vesicles are commonly present on the penis as in primary genital herpes. Several studies have shown a definite link between venereal herpes and cancer of the cervix or prostate, although it has not been proved that HSV-2 actually causes the cancer.

Genital herpes, like all virus diseases, cannot be cured by antibiotics. Topical application of the drug acyclovir can shorten the healing time in primary genital herpes but has little effect in treatment of the recurrent form; however, recent evidence suggests that oral or intravenous administration of acyclovir may help to suppress the recurrent form. Vaccines to prevent genital herpes are under development: recombinant DNA techniques are being used to insert genes for individual herpesvirus surface antigens into harmless bacteria. The antigens can then be produced by the bacteria and used for immunization, thereby avoiding the difficulties involved in propagating and inactivating infectious virus in order to make a vaccine. (See also vaccinia virus as a carrier of herpes antigens, described earlier in this chapter.)

Other Human Herpesviruses. Varicella-zoster virus (VZV) causes two diseases. Varicella (chicken pox) is a mild infection of children characterized by a vesicular skin rash. Herpes zoster (shingles) is a disease caused by activation of latent virus from a previous varicella infection. It occurs mainly in adults and is characterized by a vesicular eruption and a very painful inflammation of sensory nerves. Epstein-Barr virus (EBV) is the causative agent of infectious mononucleosis, a self-limiting disease which occurs in up to 80 percent of the population

Table 37-4. Some Polyomaviruses and Papillomaviruses

Virus	Diseases Caused
Polyomaviruses	
Mouse polyoma	Causes cancer when injected into newborn mice and hamsters; in tissue culture, transforms nonpermissive cells of secondary cultures of rat and hamster embryos; occurs widely in wild and laboratory mice, being transmitted to offspring by excretions and secretions
SV 40	Occurs in monkeys; first discovered as a latent virus in cultures of rhesus monkey kidney cells; causes sarcomas in newborn hamsters; transforms secondary cultures of hamster, rat, or mouse embryo cells
Human SV 40–like: BK, JC	Widespread in human populations; acquired during childhood; produce tumors in hamsters and transform hamster cells in vitro; BK is not known to cause any human disease; JC probably causes a rare, fatal disease (not a cancer) of the nervous system in adults (progressive multifocal leukoencephalopathy)
Papillomaviruses	
Infectious wart viruses	Cause benign tumors (warts) in humans; nearly all individuals have been infected by some wart viruses by the age of 20; cell-mediated immunity is probably effective in suppression or regression of disease
Rabbit papilloma	Causes warts in wild cottontail rabbits—usually benign but may become malignant; domestic rabbits are highly susceptible and tumors eventually become malignant

(peak incidence in the 15- to 20-year-old group) but which is usually subclinical. Patients exhibit fatigue, malaise, sore throat, fever, swollen cervical lymph nodes, and an increased level of blood monocytes. EBV is also thought to be the causative agent of Burkitt's lymphoma, a cancer of B lymphocytes; however, the causal relationship is not yet proved.

Papovaviridae (Papovaviruses)

The name *papovavirus* is derived from the names of three of the viruses included in the family: rabbit *pa*pilloma virus, mouse *p*olyoma virus, and *v*acuolating agent SV 40. Papovaviruses are small, nonenveloped, icosahedral, double-stranded DNA viruses that induce tumors in animals. They are divided into two major groups, polyomaviruses (45 nm in diameter) and papillomaviruses (55 nm in diameter). The oncogenic potential of these viruses is indicated in Table 37-4. Except for the human wart viruses, none appear to be tumorigenic in humans.

VIRUSES CONTAINING SINGLE-STRANDED DNA

Parvoviridae (Parvoviruses)

Parvoviruses are small (about 20 nm in diameter), icosahedral, nonenveloped viruses that contain single-stranded DNA. Some parvoviruses are defective, i.e., they cannot replicate autonomously but only when the host cell is also infected by an adenovirus (helper virus); consequently, such parvoviruses are termed adenovirus-associated viruses (AAV). Humans are commonly affected with AAV, but the AAV apparently do not cause any overt disease and do not contribute to the symptoms caused by the adenovirus.

Other parvoviruses do not require a helper virus for replication; however, they do require that the host cell be in the S period of its growth cycle (i.e., the period during which DNA is being synthesized). Consequently, these viruses grow best in rapidly dividing cells. Parvoviruses can infect a variety of animals; for example, bovine parvovirus causes diarrhea and abortion in cattle. With

regard to human infections, a parvovirus known as B19 has been implicated in aplastic crisis, a disease occurring in persons with sickle cell anemia: when rapid cell division occurs to produce precursors of new blood cells, the virus can multiply in these precursor cells and kill them. B19 has also been implicated in erythema infectiosum, a highly contagious rash occurring in children.

RNA TUMOR VIRUSES REQUIRING A DNA INTERMEDIATE FOR REPLICATION

Retroviridae (Retroviruses)

This heterogeneous family consists of enveloped viruses that contain single-stranded (+) RNA and cause tumors in chickens and mammals. An outstanding characteristic of retroviruses is the occurrence of an RNA-dependent DNA polymerase known as reverse transcriptase; indeed, the prefix retro- is derived from the activity of this enzyme. The action of reverse transcriptase is to synthesize a DNA strand complementary to the viral RNA strand (see Chap. 21). The single-stranded DNA subsequently serves as a template for synthesis of a complementary DNA strand, thereby resulting in double-stranded DNA. This double-stranded DNA is required for the tumorigenic properties of the viruses and becomes integrated into the host-cell genome. In this integrated form (provirus) it is transcribed to produce new viral RNA.

The relation between retroviruses and oncogenes is discussed in Chap. 21.

Human T-cell leukemia viruses (HTLVs) comprise a group of retroviruses that

Identification of the Probable Causative Agent of AIDS

Although the modes of transmission of the causative agent of AIDS had been known since 1981 (see Chap. 35), identification of HTLV-III as the probable causative agent of the disease was not accomplished until 1984. That a virus was the cause of AIDS had been suspected; however, the virus could not be isolated from the T cells of AIDS patients in quantities sufficient for study. This was because the T cells, which might have allowed the virus to proliferate when grown in tissue culture, failed to grow. Robert Gallo of the National Institutes of Health and his colleagues believed that if the virus was present in these T cells, it might merely be killing the cells before much viral proliferation could occur. A killing action on T cells would be consistent with the severe decrease in the level of T cells that occurs in AIDS patients.

The breakthrough came when a suitable permissive T-cell tissue-culture system was developed by Gallo and his colleagues. They found a T-cell line which allowed propagation of the AIDS virus but was not killed by the virus. With the new system, the virus was obtained for the first time in amounts sufficient to allow its antigenic properties to be determined. The virus was related to HTLV-I and HTLV-II but belonged to a new subgroup. The new virus was isolated from 18 of 21 individuals with "pre-AIDS" (mild, early symptoms of AIDS) and from 26 of 72 patients with clinical cases of AIDS. Further evidence for the role of HTLV-III in AIDS was provided when antibodies against the virus were detected in 43 of 49 AIDS patients. The antibodies were not detected in 185 of 186 control individuals, even in individuals having various other disorders of the immune system. Subsequent improved detection techniques have indicated the presence of antibodies against HTLV-III in 100 percent of AIDS and pre-AIDS patients tested but none in control individuals. These data provide strong circumstantial evidence that HTLV-III is the causative agent of AIDS.

are **lymphotrophic**, i.e., that either inhibit T-cell (T-lymphocyte) function, transform T cells, or kill T cells. There are presently three subgroups: HTLV-I, HTLV-II, and HTLV-III. HTLV-I is the subgroup most commonly isolated and causes a type of T-cell leukemia in adults which is endemic in certain areas of Japan, the Caribbean, and Africa. HTLV-II has been isolated from a patient with a different type of leukemia—hairy-cell leukemia. HTLV-III is the probable causative agent of acquired immunodeficiency syndrome (AIDS), a disease characterized by severely decreased levels of helper T lymphocytes (T cells that recognize an antigen and then facilitate a B cell response to it; see Chap. 33).

Examples of animal retroviruses include mouse mammary tumor viruses; avian sarcoma viruses; avian leukemia viruses; and murine, feline, porcine, bovine, and monkey leukemia viruses. It should be noted that transmission of some retroviruses is **exogenous**, or **horizontal**; i.e., a healthy animal contracts the infectious virus from an animal harboring the virus. For other retroviruses the transmission is **endogenous**, or **vertical**; i.e., the virus is carried in the form of noninfectious provirus (the viral genome is integrated into the host cell genome) and thus is transmitted hereditarily to offspring. Infectious virus occurs only upon spontaneous induction of the provirus or by treatment with mutagens.

MISCELLANEOUS VIRUSES

Hepatitis Viruses

Hepatitis A virus (HAV) is a small icosahedral virus 27 nm in diameter and contains single-stranded RNA; it is probably related to the picornaviruses. In contrast, hepatitis B virus (HBV) is an enveloped virus 42 nm in diameter and contains double-stranded DNA. HBV is also distinguished by a specific surface antigen designated **HBsAg**, or **Australia antigen**. The diseases caused by these two viruses are acute systemic infections primarily affecting the liver, but they differ in their epidemiology and other characteristics. HAV can infect chimpanzees and marmosets and can be propagated, with difficulty, in tissue cultures. HBV can be grown only in chimpanzees.

Viral Hepatitis Type A. This disease has also been called acute epidemic hepatitis, infective hepatitis, and short-incubation hepatitis. The major mode of transmission of HAV is similar to that of poliovirus, that is, by the fecal-oral route. Infections with HAV are endemic in nursery schools, mental institutions, and all establishments or societies where there is a high risk of fecal contamination. The disease is generally limited to humans.

Following ingestion, the acid resistance of HAV allows it to pass through the stomach into the small intestine. The virus infects the mucosal epithelial cells, replicates, and spreads to adjacent cells and, via the blood circulation, to the liver. During the preicteric (prejaundice) stage of the disease there is loss of appetite, fatigue, malaise, abdominal discomfort, and fever. The virus is shed in the feces until the onset of jaundice or slightly longer. With the appearance of jaundice the patient feels better, but the liver remains tender and palpable. Jaundice persists for 1 to 3 weeks. The disease is rarely fatal and recovery usually occurs gradually over a period of 2 to 6 weeks.

Diagnosis of type A hepatitis is based on (1) detection of the virions in the patient's feces by immune electron microscopy (a technique by which virions which have reacted with specific antibody become clearly distinguishable from

other particles when observed with an electron microscope), (2) serologic tests for viral antigens, or (3) demonstration of a rising titer of antibodies in the patient's serum. No vaccine is presently available for active immunization against HAV, but passive immunization using pooled gamma globulin can often be protective.

Viral Hepatitis Type B. This disease is also known as serum hepatitis, homologous serum jaundice, and long-incubation hepatitis. The virus is transmitted by transfusion of blood or blood products, by contaminated hypodermic syringes, and possibly by saliva and by sexual contact. HBsAg appears in the blood during the incubation period, 2 to 8 weeks before the onset of jaundice, but disappears during convalescence.

Tumorigenic activity of HBV has been suggested by a correlation between hepatitis B and primary hepatocellular carcinoma, a type of cancer which is rare in the United States but common in certain parts of Africa, Mozambique, and Southeast Asia and in certain regions bordering the Mediterranean.

A hepatitis B vaccine was licensed in 1981 for immunization of persons who are at high risk of exposure. The vaccine consists of highly purified HBsAg obtained from the blood serum of apparently healthy carriers.

Viral Hepatitis, Non-A, Non-B. A form of viral hepatitis not caused by type A or type B viruses (i.e., non-A, non-B hepatitis, or NANB) was first described in 1974 and is now recognized as the most frequent form of post–blood-transfusion hepatitis in the United States. However, little is known of the fundamental nature of the NANB viruses. Moreover, there are no satisfactory tests for identifying NANB antigens or antibodies, and diagnosis of NANB hepatitis is based on exclusion of other types of viral hepatitis.

Slow Viruses

The classic slow virus diseases (kuru and Creutzfeldt-Jakob disease in humans, scrapie and transmissible mink encephalopathy in animals), and the unusual nature of the agents that cause them are described in Chap. 21.

VIRAL PATHOGENS OF PLANTS

Most plant viruses consist of single-stranded RNA surrounded by a protein capsid. Some, such as the wound tumor virus, have double-stranded RNA, and others, such as the cauliflower mosaic virus, have double-stranded DNA. In general, the shapes of plant viruses fall into two categories: rod-shaped and spherical. The rod-shaped viruses are commonly referred to as helical, and they vary from the rigid type to long, flexuous rods. The small spherical viruses are usually icosahedral.

Viroids constitute another group of agents that are pathogenic for plants. They are unusual in that they are composed of infectious RNA without any protein coat (see Chap. 21).

Pathogenic plant viruses are spread by insects, by infected vegetative parts of plants used for propagation, occasionally by infected seed, and by various other means. Many plant viruses are transmitted only by insects, including aphids, leafhoppers, whiteflies, and mealybugs, which carry infected plant juices from plant to plant. As an example, curly top of sugar beets is a highly destructive

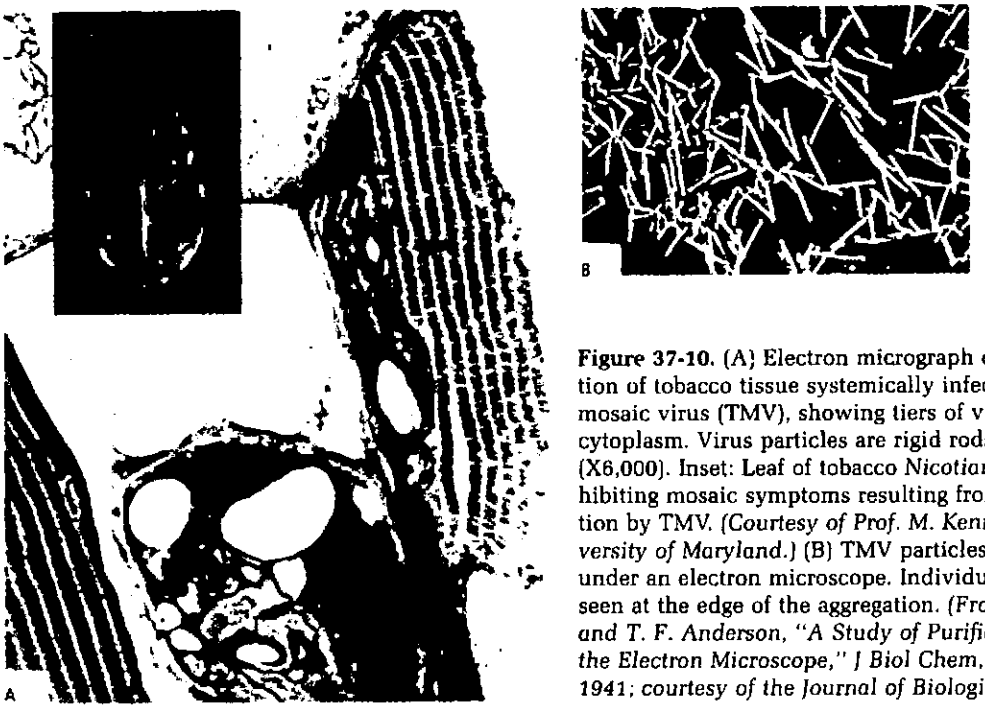

Figure 37-10. (A) Electron micrograph of an ultrathin section of tobacco tissue systemically infected with tobacco mosaic virus (TMV), showing tiers of virus particles in the cytoplasm. Virus particles are rigid rods 300 nm long (X6,000). Inset: Leaf of tobacco *Nicotiana tabacum)* exhibiting mosaic symptoms resulting from systemic infection by TMV. *(Courtesy of Prof. M. Kenneth Corbett, University of Maryland.)* (B) TMV particles photographed under an electron microscope. Individual particles are seen at the edge of the aggregation. *(From W. M. Stanley and T. F. Anderson, "A Study of Purified Viruses with the Electron Microscope," J Biol Chem,* **139**:339–344, *1941; courtesy of the Journal of Biological Chemistry.)*

virus disease which is transmitted naturally by a leafhopper (or experimentally by inoculation with infected sap). When plants are propagated by grafting or budding or by tubers, roots, shoots, or other vegetative means, any virus present in the parent plant is carried over to the new plant. This important factor in the spread of virus diseases of plants can be controlled by the use of virus-free nursery stock for all new plantings. About 10 percent of plant viruses are seed-borne, and this mode of transmission can be important in certain diseases such as bean mosaic.

There are two major types of virus diseases of plants; these are differentiated according to the type of injury produced. The first group, the so-called mosaic diseases, causing mottling or spotting of leaves, is the largest and most important. These diseases are characterized by the production of yellowish spots or blotches and necrotic spots on the leaves and sometimes on the blossoms of plants. Cucumber and tobacco mosaic diseases and tomato spotted wilt are economically important examples of such infections (Fig. 37-10). The effects of some infections have been exploited in ornamental horticulture to produce unusual variegations on foliage or blossoms, examples of which are seen in certain tulips, in which the variegation is called **breaking.**

The second group of virus diseases of plants causes curling of leaves, yellowing, dwarfing, and sometimes excessive branching as well. This is the leaf curl and yellows group and includes such diseases as sugar-beet curly top, peach yellows, and strawberry stunt. Formation of tumors on roots and stems can also occur, as in wound tumor disease.

Many plant diseases caused by viruses result in considerable economic losses to the agricultural economy. High on the list of economic importance are virus diseases of sugarcane, sugar beets, stone fruits, tomatoes, potatoes, and other fruits and vegetables.

VIRAL PATHOGENS OF INSECTS

More than 250 viruses that are pathogenic for some insects have been isolated. Viral pathogens of insects affect the levels of insect populations in nature; moreover, some have economic importance, such as those which cause disease in silkworms or honeybees. Insect viruses usually cause latent infections and probably are transmitted vertically, to the offspring of the insect host, as well as horizontally, by ingestion of infectious virus. The latent infections seem to be activated to cause overt disease by certain environmental factors and also by various chemical and mutagenic agents. Some insect viruses are occluded (the virions are embedded within a protein matrix); others are nonoccluded (the virions are free within the infected cell).

Occluded Viruses

Polyhedrosis Viruses. Many insect diseases are **polyhedroses**, which are characterized by the formation of polyhedral inclusions in the infected cells. These inclusions, which can be seen with the light microscope, consist of a matrix of protein molecules that occur in a lattice arrangement and numerous virions which are embedded within the protein matrix. In **nuclear polyhedroses** the polyhedra occur within the nucleus of the infected host cell (Fig. 37-11). The virions within the polyhedra are rod-shaped, contain double-stranded DNA surrounded by a protein coat, and are enclosed within an envelope. The polyhedra enlarge to the point where they rupture the nucleus of the host cells and cause the cell to disintegrate. Nuclear polyhedroses occur mainly in the larvae of *Lepidoptera*, *Hymenoptera*, and *Diptera*. In **cytoplasmic polyhedroses** the polyhedra occur in the cytoplasm. The virions within the polyhedra are icosahedral and contain double-stranded RNA. Cytoplasmic polyhedroses occur mainly in *Lepidoptera* larvae.

Figure 37-11. Electron micrograph of a thin section through a polyhedral inclusion body occurring in the nucleus of a cell of the southern armyworm, *Spodoptera eridonia*, infected with a nuclear polyhedrosis virus. The enveloped virions can be seen embedded within the polyhedral protein matrix. The bar represents 0.5 μm. *(Courtesy of Jean R. Adams, Insect Pathology Laboratory, USDA, Beltsville, Maryland.)*

Some nuclear polyhedrosis viruses have been used for biological control of insects. A virus preparation called Elcar (Sandoz, Inc., Crop Protection, San Diego, Calif.) has been used commercially in California for control of the cotton bollworm. Other preparations have been used experimentally for control of the gypsy moth in the northeastern United States and the spruce budworm in Canada.

Granulosis Viruses. Granuloses caused by DNA-containing viruses occur in *Lepidoptera* larvae and are characterized by small oval inclusions, each consisting of a protein matrix in which are embedded one or two rod-shaped virions. These inclusions occur mainly in the nucleus of the host cells.

Entomopox Viruses. Infected host cells contain spherical inclusions composed of a protein matrix and numerous virions that have a shape and structure resembling that of the classical poxviruses such as vaccinia. Entomopox viruses have been isolated from *Coleoptera*, *Lepidoptera*, *Orthoptera*, and *Diptera*.

Nonoccluded Viruses

Iridescent viruses are icosahedral viruses containing double-stranded DNA. Infected tissues acquire an iridescent appearance due to occurrence of microcrystals of the virus within the cytoplasm of the infected cells. Iridescent viruses occur in *Coleoptera*, *Diptera*, and *Lepidoptera*. One example is tipula iridescent virus (TIV), a DNA-containing virus that causes a disease of crane-fly (*Tipula paludosa*) larvae.

Another example of a nonoccluded virus is the *densonucleosis virus*, which is icosahedral, contains single-stranded DNA, and causes an infection of the greater wax moth, *Galleria mellonella*. Some examples of nonoccluded RNA-containing viruses are the *sigma virus* of fruit flies, the *bee paralysis viruses*, the *sacbrood virus* of honeybee larvae, the *Wassersucht virus* of *Coleoptera*, and several viruses that infect *Lepidoptera*, such as the virus of Flacherie—a fatal disease of silkworm larvae.

Other Viruses

In addition to these insect viruses, it must be remembered that many viral diseases of humans, animals, and plants are transmitted by arthropods. However, even when the arthropod is a biological vector (i.e., when the virus multiplies within the arthropod), it is not usually harmed by its viral infection.

QUESTIONS

1 For immunization against poliomyelitis, give advantages and disadvantages of (a) the Salk vaccine, and (b) the Sabin vaccine.
2 Why is it impractical to prepare a vaccine against the common cold?
3 Explain how secretory IgA can act to prevent infection by some specific viruses.
4 What role might recombinant DNA techniques play in the development of vaccines against viral diseases? Give examples.
5 List some antiviral drugs and explain their mechanisms of action.
6 Indicate the features of smallpox that made eradication of this disease possible. Give the essential features of the grand strategy that was used for eradication of this disease.

7 Rhinoviruses and coronaviruses can cause the common cold. How do these two kinds of viruses differ?
8 Could the following viruses easily be eradicated: (a) togaviruses, (b) orthomyxoviruses, (c) rabies viruses, and (d) adenoviruses? Explain your answer in each case.
9 What recent developments in rabies immunization have led to a safer yet effective vaccine?
10 Why is a live vaccine rather than an inactivated vaccine required for immunization against smallpox?
11 Contrast infections caused by herpes simplex virus type 1 (HSV-1) with those caused by HSV-2.
12 Contrast hepatitis virus type A with hepatitis virus type B.
13 Briefly define the following items:

Negri bodies	Guarnieri bodies
Variola minor	HBsAg
Adenovirus-associated virus	Reverse transcriptase
Polyhedroses	Latent infection
Horizontal transmission	Vertical transmission

REFERENCES

See also references for Chaps. 31, 35, and 36.

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Marx, J. L.: "Strong New Candidate for AIDS Agent," *Science*, **224**:475–477, 1984. *A succinct summary of the evidence linking HTLV-III to AIDS. The four original papers to which the summary refers are included in the same issue.*

Smith, K. M.: *Plant Viruses*, 5th ed., Chapman and Hall, London, 1974. *A textbook which covers the entire field of plant viruses in a concise and readable manner.*

Chapter 38 Microbial Agents of Disease: Fungi and Protozoa

OUTLINE

In addition to the bacteria and viruses, there are two groups of eucaryotic protists with members that are agents of disease. These are the fungi and the protozoa. We have discussed the biology of these microorganisms in Chaps. 17 and 19.

Fortunately, even though there exist over 100,000 species of fungi, only about 50 of these are known to be pathogenic for humans. Of these, only a few are pathogens; most of them are **opportunists** (i.e., able to cause infection in a compromised or weakened host). Fungal or mycotic diseases are assuming new importance because of the use or abuse of antibiotics in the treatment of bacterial infections. These antibiotics eliminate the natural microbiota which would otherwise suppress the growth of opportunistic fungi. The latter can then grow unrestrained and become pathogenic. Upon infection, initially there is acute inflammation with the accumulation of many polymorphonuclear leukocytes. Damage to the tissues is not caused by toxins but by development of allergic necrosis due to hypersensitivity of the host immune system to the fungi (see Chap. 33 on type IV hypersensitivity reactions). A compromised state in patients can also result from chemotherapy for cancer as well as from immunosuppressive therapy for organ transplantation, and opportunistic fungi can cause systemic infections in these circumstances. A few mycotic diseases are caused by fungi of **endogenous origin** (i.e., are part of the normal host microflora), such as candidiasis. Others are due to fungi of **exogenous origin** (i.e., coming from outside the body, such as the soil or bird droppings); an example of such disease is histoplasmosis.

Even though more than 65,000 species of protozoa have been described, only a few cause disease in humans. But these few protozoa have engendered untold misery for millions of people, especially in the rural areas of tropical countries (Table 38-1). The reason is that the control of many of these infections is

Table 38-1. Some Epidemiological Data on Protozoan Diseases

Disease	Thousands infected/yr	Thousands of cases/yr (Symptoms)	Death in thousands/yr
Malaria	800,000	300,000 (Fever, coma)	3,000
South American trypanosomiasis	12,000	1,200 (Heart disease)	60
Amoebiasis	400,000	1,500 (Dysentery, liver abscess)	30
Leishmaniasis	12,000	12,000 (Sores)	5
African trypanosomaisis	1,000	10 (Sleeping sickness)	5
Giardiasis	200,000	500 (Diarrhea)	Very few

dependent on an increased standard of living, as manifested by improved sanitation and better education, nutrition, and medical care. Furthermore, no vaccine exists for any of these diseases; preventive measures exist only for one, malaria. For most of these protozoan diseases, even diagnostic procedures are quite rudimentary and laborious. However, within the last few years, many commercial reagents and tests have become available for the **serodiagnosis** of major parasitic diseases.

DISEASES CAUSED BY FUNGI

Fungal diseases may be conveniently grouped into two types: (1) the superficial mycoses (diseases caused by fungi) or dermatomycoses and (2) the systemic mycoses.

Early concepts of the dermatophytic diseases attributed such infections to insects. Thus the Romans called these infections *tinea*, meaning "small insect larva." This term is still used as part of the clinical terminology for some dermatophytic diseases.

The fungi that cause superficial mycoses frequently are spread from animals to humans, a notable exception being athlete's foot, or ringworm of the feet, which is spread from person to person in locker rooms, swimming pool areas, and other locations. Fungi that cause systemic infections generally come from soil, vegetation, or bird droppings and are transmitted by air movements. Thus infection often starts in the lungs and then spreads to other organs.

Dermatomycoses

Fungus diseases that occur on the nails, skin, hair, and mucous membranes are referred to as superficial mycoses (see Fig. 38-1). Many of these fungi cause various forms of ringworm, or tinea, and the organisms that cause them are commonly called the dermatophytes, or ringworm fungi (Figs. 38-2 and 38-3). These fungi spread radially in the dead keratinized layer of the skin by means of branching hyphae and occasional arthrospores. Inflammation of the living tissue below is very mild and only a little dry scaling is seen. Usually there is irritation, erythema, edema, and inflammation at the spreading edge; this pinkish circle gave rise to the name *ringworm* (Figs. 38-4 and 38-5). These diseases are widespread and difficult to control, but fortunately they are often more

Figure 38-1. Black piedra is a fungus infection of hair, characterized by dark brown or black nodules on the hair shaft. It is caused by *Piedraia hortai*. *(Courtesy of Everett S Beneke and the Upjohn Company.)*

Figure 38-2. Some ringworm of the scap is caused by various species of *Microsporum*, such as (A) *M. canis* and (B) *M. gypseum*. The large spindle-shaped conidia (spores) attached to the hypae are characteristic of species of this fungus. Such conidia are formed in artificial culture and are used to differentiate between the genera of dermatophytes. *(Courtesy of Everett S. Beneke, Michigan State University.)*

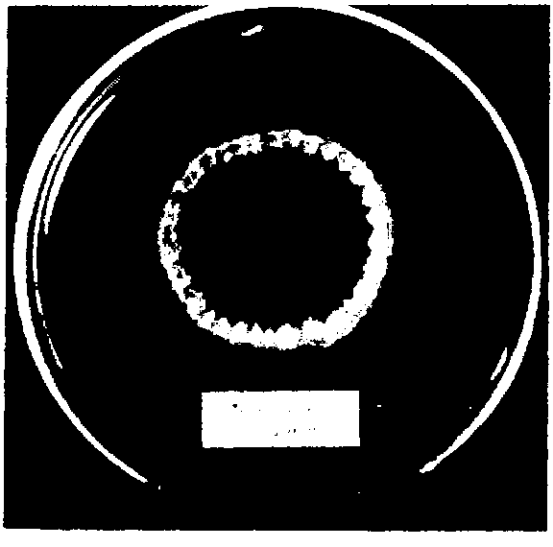


Figure 38-3. A colony of *Trichophyton violaceum*, a dermatophyte fungus. *(Courtesy of Centers for Disease Control, Atlanta, Ga.)*

Figure 38-4. Ringworm lesions on the back of a patient caused by *Trichophyton verrucosum*. *(Courtesy of Centers for Disease Control, Atlanta, Ga.)*

Figure 38-5. Ringworm of the scalp caused by a species of *Trichophyton*. *(Courtesy of Centers for Disease Control, Atlanta, Ga.)*

annoying than serious. The causative microorganisms are sometimes present in the epidermal tissues without producing symptoms. They rarely, if ever, cause fatal infections. Transmission is commonly by direct contact with infected people or animals and by fomites. Dry skin is a fairly effective barrier against such diseases, but a "waterlogged" skin is vulnerable. This is why the sweat-laden, moist feet of athletes get infected with tinea, giving rise to the term *athlete's foot*. The most common dermatophytes are listed in Table 38-2.

The correlation of a dermatophyte species with a characteristic disease entity has been difficult because a single species can cause a variety of clinical symptoms in different parts of the body. Further, the same clinical manifestation can be caused by different species of dermatophytes. Thus dermatologists frequently have used a terminology based on the part of the body involved: For example, tinea capitis is ringworm of the scalp (Fig. 38-5); tinea unguium, or onycho-

Table 38-2. The Dermatophytes

Group	Organisms	Occurrence and Disease
Epidermophyton	*E. floccosum*	Causes infections of the skin and nails on fingers and toes
Microsporum	*M. audouinii*	Causes epidemic ringworm of the scalp in children
	M. canis	Common cause of infection of skin and hair on cats, dogs, and other animals; causes tinea capitis of children
	M. gypseum	Occurs as a saprophyte in the soil and as a parasite on lower animals; occasionally found in ringworm of the scalp in children
Trichophyton	Gypseum subgroup	
	T. mentagrophytes	Primarily a parasite of the hair
	T. rubrum	Causes ringworm on many parts of the body; infects hair and scalp
	T. tonsurans	Infects hair and scalp
	Faviform subgroup	These fungi cause ringworm of the skin, scalp, and glabrous skin in humans; *T. verrucosum* causes ringworm in cattle also
	T. schoenleinii	
	T. violaceum	
	T. ferrugineum	
	T. concentricum	
	T. verrucosum	
	Rosaceum subgroup	
	T. megnini	Causes ringworm of the human scalp
	T. gallinae	Causes an infection in chickens
Miscellaneous	*Piedraia hortae*	Causes an infection of the hair and scalp characterized by hard, black concretions; black piedra
	Trichosporon beigelii	Causes an infection similar to above, except that the concretions are white; white piedra
	Malassezia furfur	Causes tinea versicolor, a generalized fungus infection of the skin covering trunk and sometimes other areas of the body
	Candida albicans	Causes candidiasis of skin, mucous membranes, and nails

mycosis is ringworm of the nails (Figs. 38-6 and 38-7); and tinea pedis is ringworm of the feet (Fig. 38-8).

Systemic Mycoses

The systemic, or deep, mycoses are mainly fungus diseases that often are serious or fatal. The organisms invade subcutaneous tissues or the lungs, from which they may spread to other organs of the body where they become established and produce disease. Many of them are airborne and enter the body through the respiratory tract; they may, however, enter by other portals.

Systemic mycoses appear to be increasing in importance, but their greater apparent incidence may actually be due to improved diagnostic methods and a greater appreciation of their importance. As a result of the great mobility of

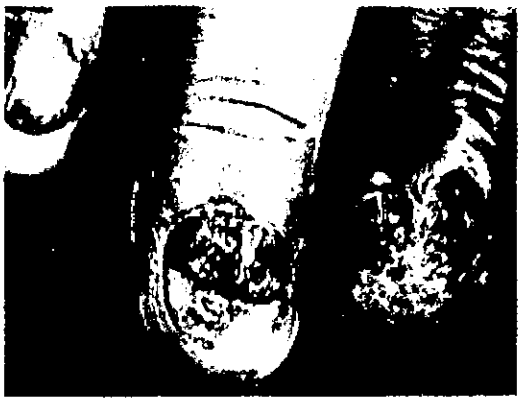


Figure 38-6. Onychomycosis is a disease of the nails caused by fungi. The infection pictured here was caused by *Trichophyton rubrum*. *(Courtesy of J. D. Schneidau, Jr.)*

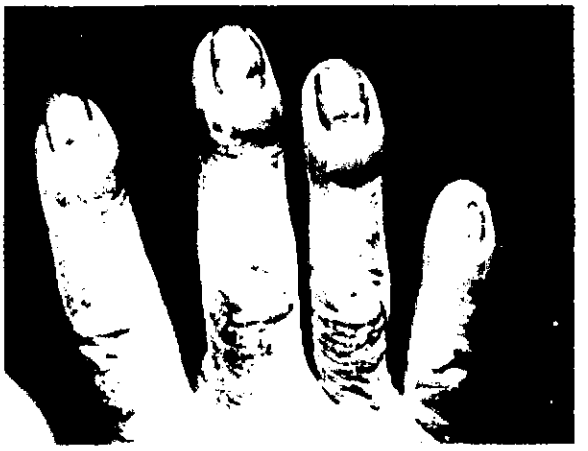


Figure 38-7. Onychomycosis caused by *Candida albicans*. *(Courtesy of J. D. Schneidau, Jr.)*

Figure 38-8. Tinea pedis, also known as athlete's foot, is caused by *Trichophyton rubrum*, *T. mentagrophytes*, and *Epidermophyton flocossum*. The chronic infection shown here is also manifested by the appearance of vesicles and is caused by *T. rubrum*. *(Courtesy of J. D. Schneidau, Jr.)*

Americans, more of them are exposed to fungi that are only prevalent in and endemic to specific, limited areas of the world.

Factors that predispose an individual to become more susceptible to systemic fungus infections include the following:

1 The presence of chronic debilitating diseases such as cancer, diabetes, leukemia, and tuberculosis
2 The use of newer types of drugs, such as antibiotics and hormones, which cause changes in the metabolism of the body or upset the normal relationships among the microorganisms on or in the body

Table 38-3. The Systemic Mycoses

Disease	Causative Organism	Characteristics of the Organisms	Characteristics of the Infection
Cryptococcosis	*Filobasidiella (Cryptococcus) neoformans*	These are yeastlike capsule-producing organisms that reproduce by budding; no hyphae or spores are formed; the cells grow well on ordinary culture media	This organism may infect any part of the body but usually starts in the lungs and spreads through the bloodstream; infections of the brain and meninges usually cause death; mode of transmission is not known, and spread from known cases in humans or other animals has not been established
Moniliasis	*Candida albicans* (order *Moniliales*)	*C. albicans* cells are yeastlike with pseudohyphae; they produce large, thick-walled, spherical chlamydospores; on ordinary culture media, pasty, smooth colonies having a yeasty odor develop	Monilia may infect any body tissue; it is found on mucous membranes of intestinal tracts of many healthy persons; infection with *C. albicans* in the mouth is called thrush; may also cause a mycotic endocarditis, pulmonary moniliasis, and vaginitis; may be spread by contact
North American blastomycosis	*Blastomyces dermatitidis*	These are large round cells with a single bud; intercalary and terminal chlamydospores appear in old cultures; optimum growth temperature is 37°C; on infusion-blood agar the colonies resemble *M. tuberculosis*; typical cells are found in body exudates and in cultures	Infection with *B. dermatitidis* resembles pulmonary tuberculosis with involvement of the lungs and pleura; it is characterized by chronic, granulomatous, suppurative lesions of any body tissue; it occurs only in the United States and Canada, most commonly among rural males aged 30 to 50; systemic infections are often fatal; it is not transmitted from humans or other animals to humans
South American blastomycosis	*Paracoccidioides (Blastomyces) brasiliensis*	These are yeastlike cells that are larger than *B. dermatitidis*; cells range from 6 to 30 μm in cultures and to 60 μm in exudates; parent cells give rise to many buds; smooth, waxy, yeastlike colonies appear on blood or meat medium after several days' incubation at 37°C	South American blastomycosis is clinically similar to North American blastomycosis; it also resembles coccidioidomycosis; it occurs most frequently in Brazil; lesions are most commonly found in the mouth and gastrointestinal tract and in the lymph nodes of the neck
Histoplasmosis	*Histoplasma capsulatum*	Small, oval cells found intracellularly in tissues; colonies on blood agar resemble *Staphylococcus aureus*; cells have single buds; at room temperature on Sabouraud's glucose agar, delicate branching, septate hyphae appear, with chlamydospores present in old cultures	This may occur as an acute or chronic, localized or disseminated infection of the reticuloendothelial system; clinically it may be confused with carcinoma of the nose, tongue, or pharynx, or with tuberculosis, Hodgkin's disease, or aplastic anemia; most infections regress spontaneously, but fulminating cases are usually fatal
Coccidioidomycosis	*Coccidioides immitis*	In cultures on Sabouraud's glucose agar these organisms develop as typical white- to buff-colored mold colonies that sporulate by arthrospore formation; in body exudates they are single-celled, thick-walled spherical organisms filled with endospores; chlamydospores are present in old cultures	This disease goes by many other names, such as valley fever, San Joaquin fever, and desert rheumatism; the fungus is highly infectious and widely distributed in the soil of certain areas of the United States; most cases are mild and transitory, but a few terminate fatally

Table 38-3. (*continued*)

Disease	Causative Organism	Characteristics of the Organisms	Characteristics of the Infection
Sporotrichosis	*Sporothrix schenckii*	Organisms are rarely found in tissues, but in experimentally infected rats the organisms are Gram-positive, resembling fusiform bacilli, in polymorphonuclear leukocytes; on Sabouraud's glucose agar incubated at room temperature, delicate, branching, septate hyphae with spherical or pyriform microconidia in clusters on lateral branches are found; on brain-heart-infusion agar, colonies are soft and composed of budding, yeastlike, cigar-shaped cells with a few mycelial elements	This is a chronic infection that usually begins as a subcutaneous nodule at the site of an injury; initial lesions resemble warts, boils or chancres; the organisms are disseminated in the body through the lymph channels to various lymph nodes; pulmonary involvement is infrequent; in rare cases involving spread of the organisms throughout the body, the patient may die

3 Local lesions caused by vitamin deficiency, irradiation, peptic ulcers, or other factors, which allow the fungi to get into the deep tissues

The fungi that cause the deep mycoses are important not only because the diseases they cause can be very serious, but also because the symptoms produced by some of them resemble tuberculosis or other diseases. It is essential that accurate diagnostic procedures be used in order that the most suitable treatment be used.

The principal systemic mycoses, their causative agents, the characteristics of these organisms, and the characteristics of the infections they cause are given in Table 38-3. Figure 38-9 shows the morphology in line drawings of some of these causative organisms. Note that many of them have a dimorphic growth habit, for example, a parasitic yeastlike phase and a saprophytic filamentous phase. Figure 38-10 shows the clinical symptoms of some of these diseases. Figures 38-11 and 38-12 illustrate the morphology of some of these etiologic organisms.

DISEASES CAUSED BY PROTOZOA

In adapting to their hosts, protozoa, like other animal parasites, have evolved many life-cycle patterns. While some species are parasitic during only one phase of their life cycle, others have adapted to more than one host during the different phases of their life cycle.

The host in which a parasite reaches sexual maturity and reproduction is termed the **definitive host**. If no sexual reproduction occurs in the life cycle of a protozoan, such as a trypanosome or an amoeba, the host that is believed to be the most important is arbitrarily identified as the definitive host. An **intermediate host** is one in which the other stages of the life cycle occur. For example, for the malaria protozoa, the mosquito is the definitive host, and humans or other vertebrates are the intermediate hosts. An animal (or human) that is

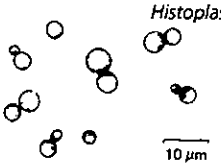
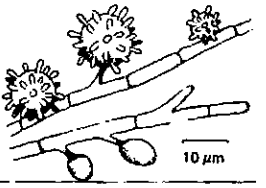
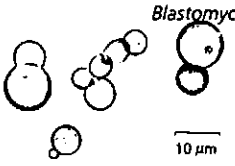
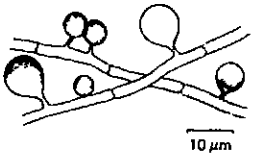
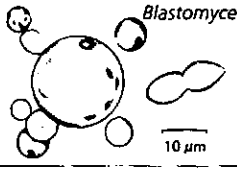
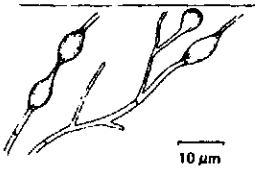
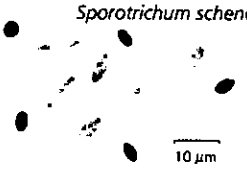
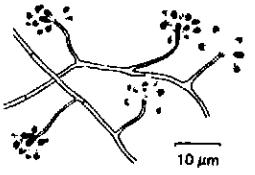
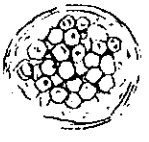
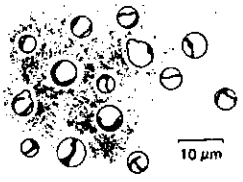
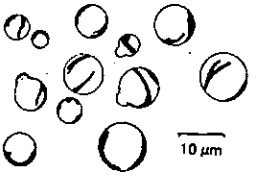
PARASITIC PHASE in the host and in cultures at 37°C		SAPROPHYTIC PHASE in soil and in cultures at 25°C (room temperature)	
Histoplasma capsulatum			
Yeast-like Short oval, very delicate budding cells 2 to 3 by 3 to 4 μm in size. Typically intracellular in the host.		*Mycelium* Septate hyphae 2 to 3 μm wide. Conidia tuberculate or smooth, short oval to spherical, 2-4 to 8-14 μm in diameter.	
Blastomyces dermatitidis			
Yeast-like Nearly spherical thick-wall budding cells 8 to 15 μm in diameter. Buds produced on broad base.		*Mycelium* Septate hyphae 2 to 3 μm wide. Conidia smooth, nearly spherical 2 to 10 μm in diameter.	
Blastomyces brasiliensis			
Yeast-like Spherical cells 10 to 60 μm in diameter. Budding single or in multiples; buds varying in size from 1-2 to 10 μm in diameter.		*Mycelium* Fine septate hyphae producing intercalary or terminal chlamydospores. Conidia rarely found.	
Sporotrichum schenckii (mostly subcutaneous)			
Yeast-like Budding cells, up to 10 μm in diameter or elongated cigar bodies (also budding) 1 to 3 by 3 to 10 μm in size.		*Mycelium* Very fine septate hyphae 1 to 2 μm wide. Conidia 2 to 3 by 3 to 6 μm, occasionally larger, in bouquetlike arrangement on sterigmata.	
Coccidioides immitis			
Spherules Thick-walled cells 15 to 90 μm in diameter, packed with small (2 to 5 μm in diameter) endospores.		*Mycelium* Septate hyphae 2 to 3 μm wide in nonfertile portions. Arthrospores alternating with empty cells. No conidia.	
Filobasidiella neoformans			
	Monophasic growth. A *yeast* at *both* temperatures, in the host *and* in cultures. Spherical budding cells, typical of true yeasts, 4 to 20 μm in diameter. Buds produced singly on a narrow neck. Encapsulated. The capsule varies in thickness in different strains.		

Figure 38-9. Some pathogenic fungal species causing systemic mycoses. (*Erwin F. Lessel, illustrator.*)

routinely infected with a protozoan or parasite which can also infect humans is termed a **reservoir host.** The major human diseases caused by protozoa are summarized in Table 38-4. Some of the important protozoan diseases are discussed below.

Figure 38-10. Clinical manifestations of some fungal diseases. (A) Blastomycosis of the hand. (B) Candidiasis on tongue and lips of a 19-year-old patient. *(A and B Courtesy of Centers for Disease Control, Atlanta, Ga.)* (C) Cryptococcosis on the skin of the face. (D) Coccidioidomycosis in a lesion of the arm. *(C and D Courtesy of Armed Forces Institute of Pathology.)*

Amoebiasis

Several protozoa cause intestinal diseases and are transmitted from person to person in infected food and water, by flies, and by direct contact. The most important of these, because of the great incidence of the disease and the fact that some fatalities occur, is *Entamoeba histolytica*. The most serious outbreak of amebiasis in many years occurred in 1933, when an epidemic originating in Chicago during the Century of Progress Exposition spread across the nation, and 1,400 cases and 4 deaths were reported.

Amebiasis occurs more commonly than is generally supposed. Authorities estimate that 10 million persons in the United States harbor *E. histolytica* and 2 million have symptomatic cases of the disease. People with amoebiasis may have few clinical indications of the infection, or they may have symptoms ranging from abdominal discomfort with slight diarrhea alternating with constipation to severe dysentery with blood and mucus in the stools. Abscesses may be formed in the liver or lungs or even in the brain.

Laboratory diagnosis depends on identification of *E. histolytica* in the stools. Search for the amoeba should be made as soon as possible after the stool is

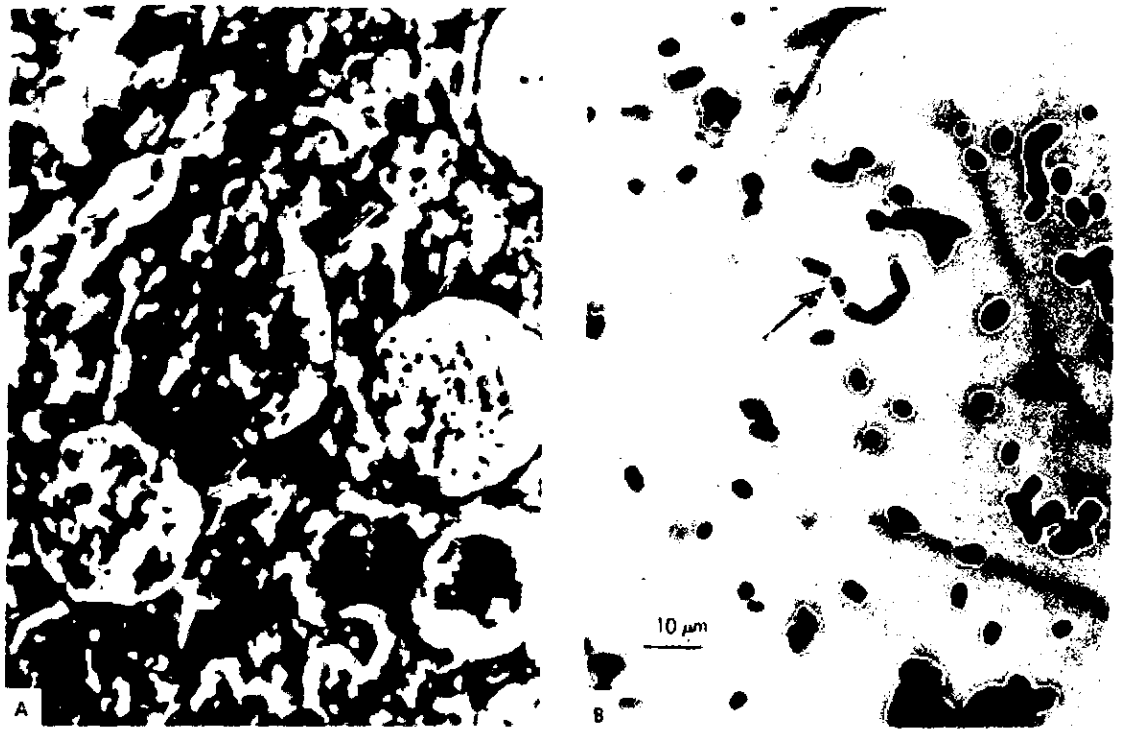


Figure 38-11. *Coccidioides immitis* is the fungus causing the disease coccidioidomycosis. (A) In the tissue phase it forms spherules containing endospores. (B) In culture the saprophytic filamentous phase forms arthrospores alternating with empty cells. *(Courtesy of L. Kapica and E. C. S. Chan, McGill University.)*

Figure 38-12. (Opposite page, top). Common airborne fungi can cause systemic mycoses. (A) *Candida albicans* in slide culture. (B) *Histoplasma capsulatum* in slide culture. (C) *Sporotrichum schenckii* from a culture (X1,200). *(Courtesy of Everett S. Beneke and the Upjohn Company.)*

passed to be sure of the presence of living, motile forms. Diagnosis requires differentiation from the nonpathogenic types of amoebas often found in the human intestinal tract.

Amebiasis can be controlled by the recognition of chronically infected persons, from whom infective cysts of the amoeba may be transmitted, and by proper sanitation and personal hygiene. The life cycle of the etiologic agent is shown in Fig. 38-13.

Malaria

The disease malaria has been known from antiquity and is aptly described as the single greatest killer of the human race. On a global scale, malaria is one of the most common infectious diseases of humans, causing much morbidity and significant mortality. As indicated earlier, each year more than 300 million people are gravely ill with malaria; about 3 million of these victims die of it.

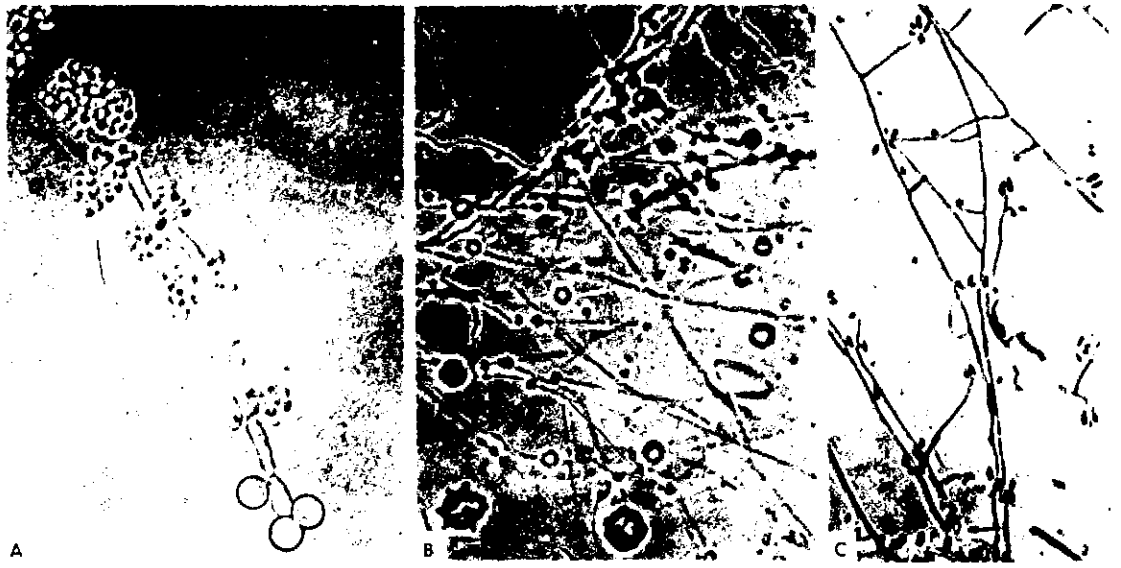


Table 38-4. Major Human Diseases Caused by Protozoa

Disease	Protozoan	Mode of Transmission to Humans	Definitive Hosts	Intermediate Hosts	Reservoir Hosts
Amoebiasis	*Entamoeba histolytica*	Ingestion (mature cyst)	Humans	None	Humans
Balantidiasis	*Balantidium coli*	Ingestion (mature cyst)	Hogs, humans	None	Hogs, humans
Giardiasis	*Giardia lamblia*	Ingestion (mature cyst)	Humans	None	Humans
Vaginitis	*Trichomonas vaginalis*	Contact (flagellate)	Humans	None	Humans
African sleeping sickness	*Trypanosoma gambiense* *T. rhodesiense*	Fly bite	Humans, animals	Tsetse flies (*Glossina* spp.)	Humans, animals
Chagas' disease	*T. cruzi*	Feces of bug	Animals, humans	Reduviid bugs	Armadillos, opossums, humans
Kala azar	*Leishmania donovani*	Fly bite	Humans, dogs	Sandflies (*Phlebotomus* spp.)	Dogs, humans
Oriental sore	*L. tropica*	Fly bite	Humans, dogs	Sandflies	Dogs, humans
Espundia	*L. brasiliensis*	Fly bite	Humans	Sandflies	Humans
Malaria	*Plasmodium vivax* *P. falciparum* *P. ovale* *P. malariae*	Mosquito bite	Anopheline mosquitoes	Humans	Humans

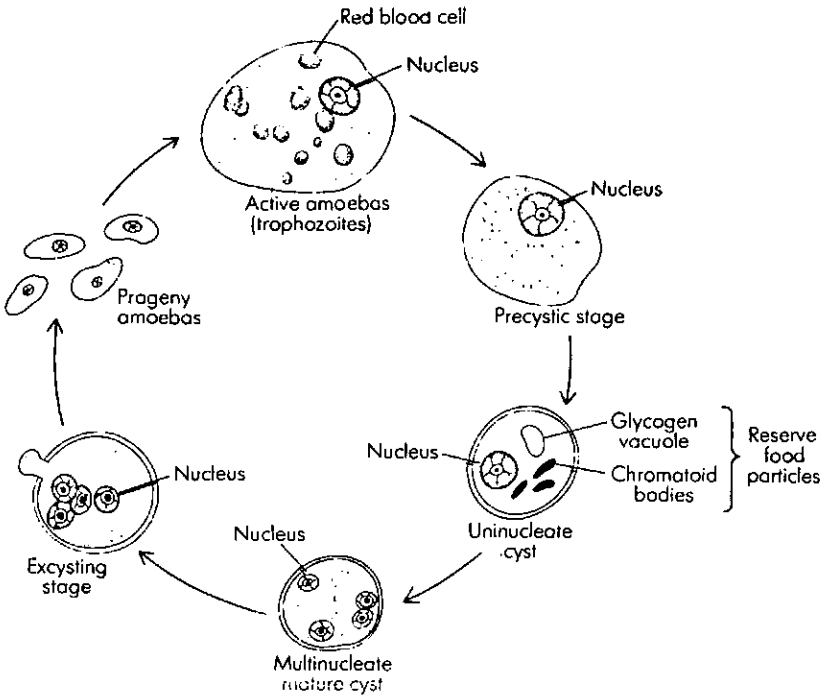


Figure 38-13. Life cycle of *Entamoeba histolytica*, the parasitic amoeba of humans that causes amoebic dysentery. Food or water contaminated with infective mature cysts is ingested. Excystation occurs in the host, releasing progeny amoebas, which become active amoebas called trophozoites in the intestine. Penetration of the intestinal mucosa by the trophozoites, with subsequent invasion of the portal circulation, may result in infection of the liver and other organs. Continued multiplication by binary fission and tissue destruction combine to result in abscesses. Thus the accompanying diarrhea is often tinged with blood. Cysts are passed in the feces and can infect other humans.

Figure 38-14. Malaria-infected youngster with enlarged spleen (area outlined on belly). *(Courtesy of Centers for Disease Control, Atlanta, Ga.)*

The disease has been virtually eliminated from the United States by control of its insect vector, the anopheline mosquito. But in these times of global travel, there is always the threat of contracting the disease in some country where it is prevalent and having it expressed only after returning to the United States.

Of the four species of *Plasmodium* protozoa that cause malaria in humans, *P. falciparum* and *P. vivax* are the two that most commonly cause infections. Symptoms usually occur 10 to 16 days after infection by mosquitoes. Paroxysms frequently begin with bed-shaking chills that are followed by high fevers, sweating, headache, and muscular pain. Fever cycles vary according to the species causing the infection. The symptomatic periods usually last less than 6 h. The spleen becomes enlarged and tender (Fig. 38-14); eventually the patient becomes weak and exhausted, and an anemia develops. The pattern of paroxysmal (periodic) illness interspersed with periods of well-being is characteristic of benign malaria, such as is caused by *P. vivax*, *P. ovale*, and *P. malariae*. In malignant falciparum malaria, the fever and symptoms are usually more persistent and also include edema of the brain and lungs and blockage of kidney activity.

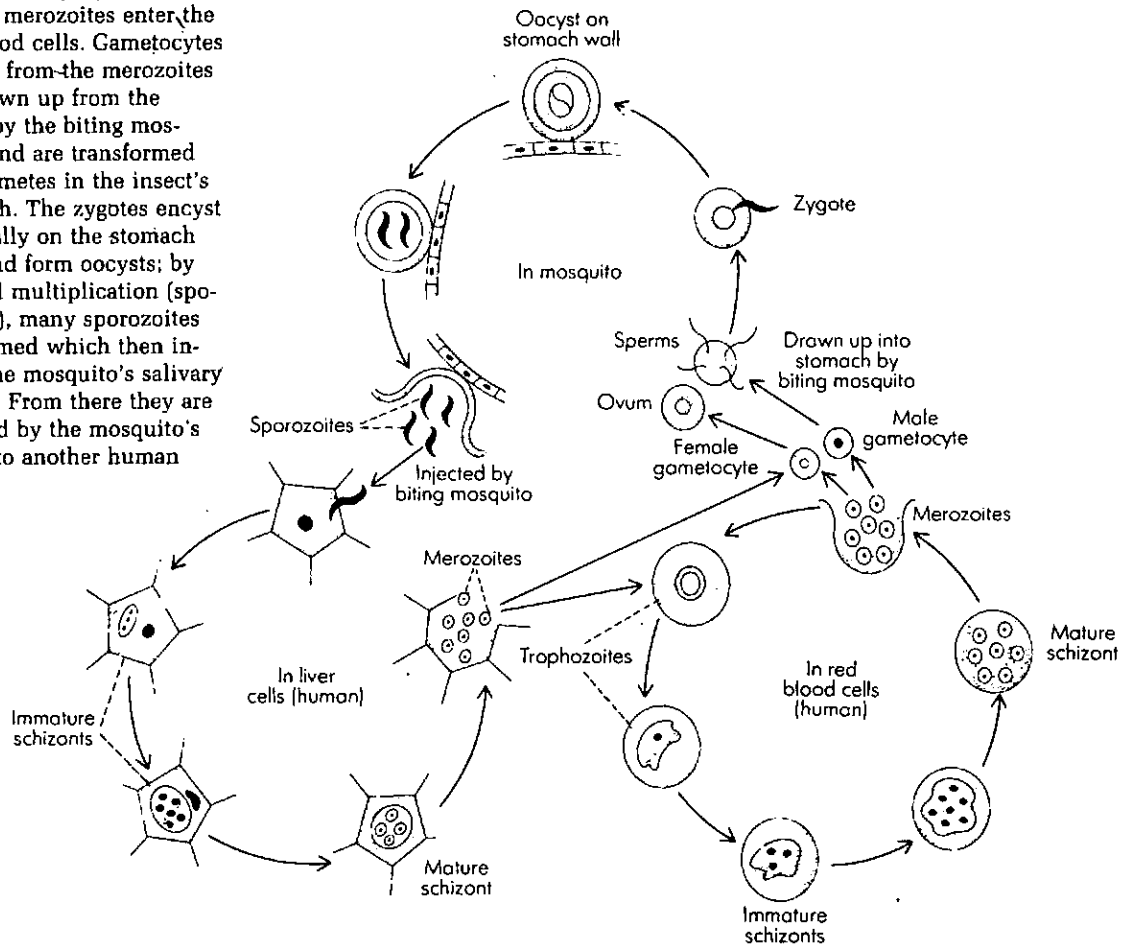


Figure 38-15. Life cycle of *Plasmodium* species that cause malaria. Sexual reproduction takes place in the mosquito. Asexual reproduction takes place in the human host in liver cells as well as in the red blood cells. Sporozoites injected by the mosquito's bite enter liver cells via the blood stream and multiply asexually (schizogony). The resulting merozoites enter the red blood cells. Gametocytes formed from the merozoites are drawn up from the blood by the biting mosquito and are transformed into gametes in the insect's stomach. The zygotes encyst externally on the stomach wall and form oocysts; by asexual multiplication (sporogony), many sporozoites are formed which then invade the mosquito's salivary glands. From there they are injected by the mosquito's bite into another human victim.

If not treated, benign malaria usually subsides spontaneously and recurs at a later date. Malignant malaria caused by *P. falciparum* has a high fatality rate if not treated promptly.

The malaria parasites are protozoa belonging to the group known as *Sporozoa*; their genus name is *Plasmodium*. Malaria is caused by more than fifty different species of *Plasmodium*; only four of them attack humans. The rest attack several hundred other animal hosts. The four species that cause human disease are *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. *P. falciparum* causes the most serious form of the disease.

Plasmodium species have a complex life cycle, as shown in Fig. 38-15. When an *Anopheles* mosquito bites, its saliva, which contains the protozoan at the sporozoite stage of its life cycle, is injected into the bloodstream of the victim. The sporozoites quickly enter the liver, where they divide and develop into multinucleated forms known as **schizonts**. Within 6 to 12 days, the schizonts rupture and release the form known as **merozoites** into the bloodstream. These merozoites invade the host red blood cells, where they grow and divide to form more schizonts. These schizonts also rupture, destroying the erythrocytes and releasing more merozoites into the bloodstream to invade more red blood cells. The major symptoms of malaria are associated with rupture of the schizonts.

Some of the asexual merozoites in the patient's bloodstream develop into male and female gametocytes. When a mosquito bites, the gametocytes enter the mosquito's stomach where they become free male and female gametes. After fertilization occurs, the zygote passes to the outside of the stomach lining, where it develops into an oocyst containing many sporozoites. The mature sporozoites migrate to the salivary glands, from which they can be injected into the bloodstream of another victim to begin the cycle all over again.

Disease in malaria is caused specifically by the asexual erythrogenic cycle. The rupture of infected erythrocytes at the completion of schizogony occurs every 48 h with *P. vivax* and *P. ovale* and every 72 h with *P. malariae*, producing coincident chills and other symptoms. Synchronized, or coincident, schizogony and paroxysms of fever and chills are not, however, common with *P. falciparum* infection. The release of an endogenous pyrogen from injured cells may be the cause of the paroxysms of fever.

The high mortality rate of falciparum malaria is due in part to the high rate of reproduction of the asexual erythrocytic form of the parasite. The small veins and capillaries of the heart are clogged with parasitized erythrocytes; effective coronary blood flow and cardiac function are diminished.

The typical symptoms of malaria mimic a large variety of other human infections. Therefore the definitive diagnosis of the disease is made in the laboratory by the demonstration of the parasite in blood smears from patients (Fig. 38-16).

The indirect fluorescent-antibody and indirect hemagglutination tests are used in serologic diagnosis of malaria; however, antibodies are usually not detectable until after the second week of infection.

In North America, Europe, and probably northern Asia, malaria is largely a disease of the past. The cases that occur in North America and Europe have been contracted by persons visiting Asia, Africa, or Latin America. The influence of military involvement and of control measures on the number of cases reported in the United States is shown in Fig. 38-17. There were 1,103 cases of malaria

Figure 38-16. Malaria worker taking a blood sample from a child in a malaria eradication program in Thailand. The blood sample will be examined microscopically for the presence of parasites. *(Courtesy of Centers for Disease Control, Atlanta, Ga.)*

in the United States in 1981. In the tropical and subtropical areas of the world, however, malaria is still the single most severe health problem today (Fig. 38-18).

Many African and American blacks are resistant to infection by *P. vivax*. It is suggested that this is due to the high frequency of a lack of a specific binding factor (Duffy determinants) on their erythrocytes, to which the protozoan must bind to infect. Also, the presence of the sickle cell trait (a genetic anomaly in the formation of the hemoglobin molecule), which is largely confined to blacks, confers some resistance to malaria.

The control of malaria depends on the elimination of the insect vector which transmits the disease. Eradication of the mosquito requires destruction of its breeding areas and killing of the larval stages and adults. These are not easy tasks because some mosquitoes develop resistance to insecticides, the behavioral patterns of others prevent their contact with insecticides, and in certain areas it is physically impossible to eliminate the breeding of mosquitoes.

At the individual level of control, netting can be used around sleeping areas; houses can be screened; and mosquito boots, insecticides, and mosquito repellents can be used. This type of control, of course, can be used for the prevention of all arthropod-borne diseases.

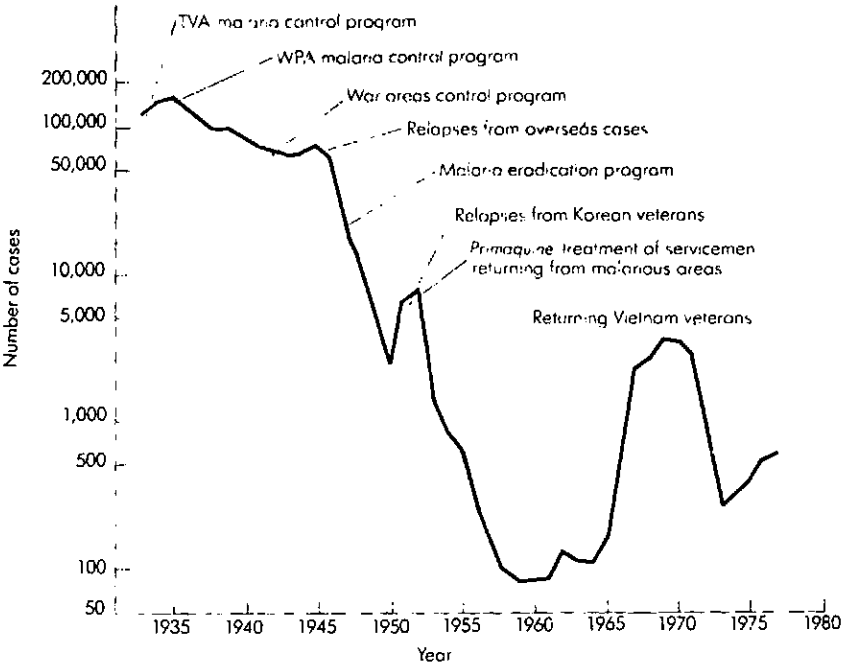


Figure 38-17. Reported cases of malaria by year in the United States, 1933–1977. The total number of cases each year has fluctuated with the application of control measures and the return of military personnel from areas of world where malaria is common. *(Centers for Disease Control: "Reported Morbidity and Mortality in the United States," Annual Summary 1977, issued September 1978.)*

Drug prophylaxis for the prevention of malaria can also be employed. For over 100 years, quinine was the only drug available. In World War II it was replaced by quinacrine, which in turn was supplanted by chloroquine and primaquine, the current drugs of choice. Chloroquine destroys merozoites in the blood, while primaquine destroys schizonts located in the liver. The combination of these two drugs is very effective against susceptible malaria parasite strains found in Africa, India, and Central America. Travelers to endemic areas are advised to take chloroquine phosphate.

Beginning in 1959, chloroquine-resistant strains of *P. falciparum* have appeared in many countries. An effective chemoprophylactic and therapeutic regimen for the chloroquine-resistant falciparum malaria is a combination of antagonists of folate metabolism in the parasite: sulfonamides and pyrimethamine.

There is no commercial vaccine available at the present time against malaria. A vaccine against the mosquito-borne infective form (the sporozoites) is currently in the advanced stages of development. But since an immune response to sporozoites apparently occurs naturally only after many exposures to infected mosquitoes, this vaccine must artificially immunize the host with antigens that the immune system may not readily respond to under natural conditions. It appears that the antibody formed prevents sporozoites from attaching to host cells.

Cultures of the asexual form of *Plasmodium* (merozoites) have been grown in vitro and serve as good sources of protozoa for vaccine development. Vaccina-

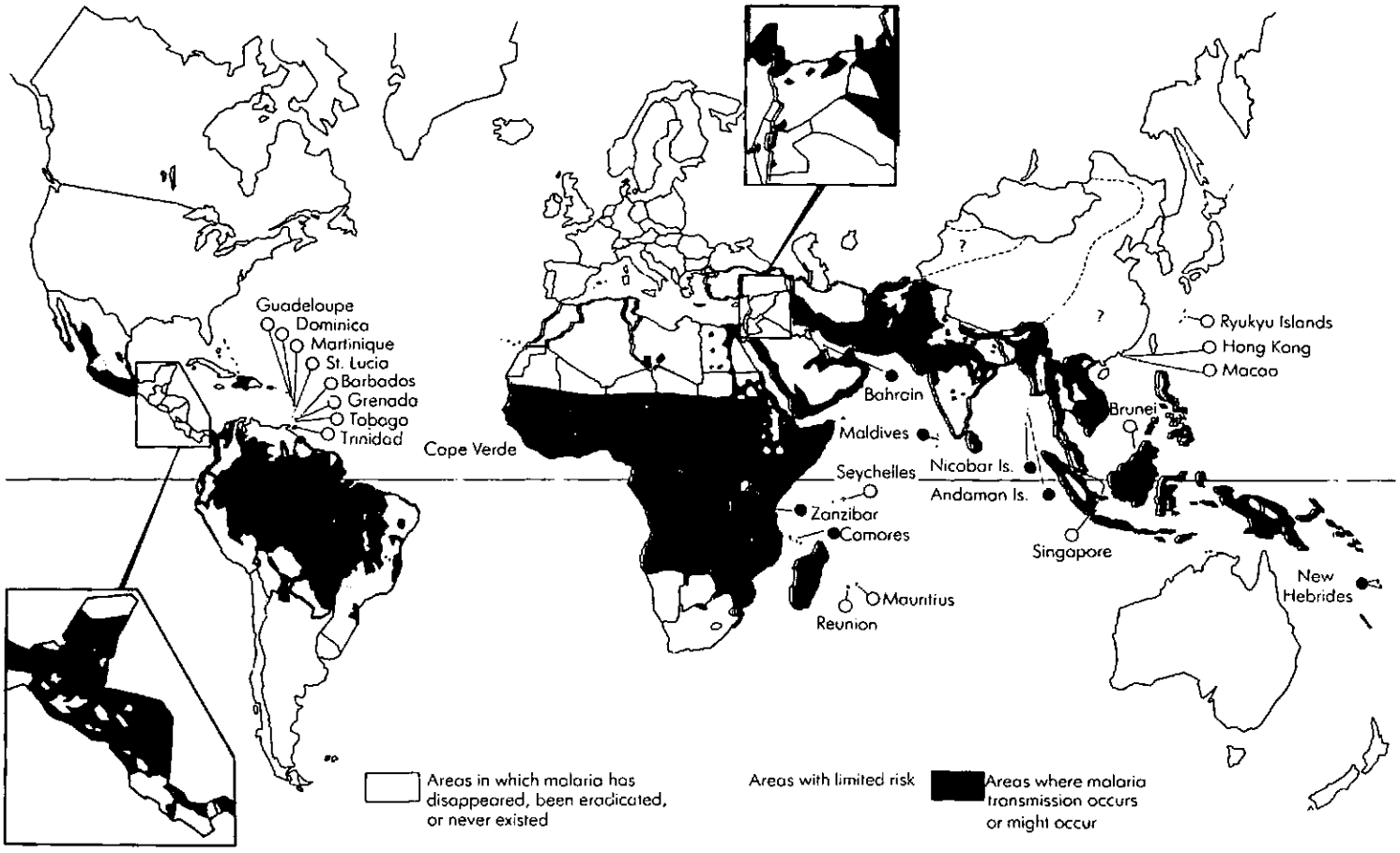


Figure 38-18. Epidemiological assessment of status of malaria, December 1976. *(WHO Chronicle, 32:9–17, 1978.)*

tion has been successful in simian malaria caused by isolated merozoites of *P. knowlesi*. The protection in rhesus monkeys was species-specific, stage-specific, and long-lasting. Owl monkeys that were immunized with *P. falciparum* merozoites were similarly protected. Unfortunately, optimal protection required the use of adjuvants that are unacceptable for human use. In the future, a malaria vaccine for human use may include a variety of stage-specific antigens. Although the prospects for a malaria vaccine have never been as good as in recent years, there are many obstacles yet to overcome.

Hemoflagellate Infections

Flagellated protozoa that are transmitted to humans by the bites of infected bloodsucking arthropods are referred to as hemoflagellates.

Leishmaniasis

Leishmaniasis is a parasitic disease caused by protozoa belonging to the genus *Leishmania*. The disease is encountered in India, China, Africa, the Mediterranean basin, Brazil, and other Central and South American countries.

Clinical forms of leishmaniasis are caused by infection with three different species: *L. donovani* in visceral leishmaniasis, commonly called kala azar; *L. tropica* in oriental sore (cutaneous leishmaniasis) and mucocutaneous leishmaniasis; and *L. brasiliensis* in the disease called espundia. These organisms are transmitted to humans by the bites of sandflies (genus *Phlebotomus*) harbored by dogs and other animals that serve as reservoirs for the parasites

Clinically, kala azar has a variable incubation period, usually 2 to 4 months. Onset may be gradual or sudden, and the source of the disease may be acute or chronic. Symptoms often resemble malaria, with irregular, recurrent fever and leukopenia, with enlargement of spleen and liver. When the parasites invade the intestine, ulcers, secondary infections, and dysentery ensue. Relapses often occur, the disease becomes chronic, and the patient has a persistent fever, emaciation, and pigmented skin. Untreated cases become complicated with secondary infections and usually terminate in death. Patients who recover may have a permanent immunity to subsequent infections with *L. donovani* and *L. tropica*.

Cutaneous leishmaniasis begins with one or more small papules at the site of the bite. These develop into crusted ulcers. Spontaneous healing with scarring occurs within a year, leaving the patient immune to subsequent infections with *L. tropica*. In *mucocutaneous* leishmaniasis, the lesions are more extensive than in the cutaneous type and may involve mucous membranes of the mouth, nose, and throat, as well as the skin. The lesions result in extensive scarring and mutilation.

Diagnosis in the laboratory is dependent upon the finding of Leishman-Donovan bodies in stained smears from lesions or infected organs. Leishmaniasis is controlled by elimination of the insect vector, destruction of dogs or other animals known to harbor the parasites, isolation of patients, and treatment of all human cases with various pentavalent antimony compounds.

Trypanosomiasis

Two distinctly different diseases are caused by protozoan parasites belonging to the genus *Trypanosoma*. African trypanosomiasis, or sleeping sickness, is caused by *T. gambiense* and *T. rhodesiense*, whereas *T. cruzi* is the causative organism of Chagas' disease (American trypanosomiasis), which occurs in South

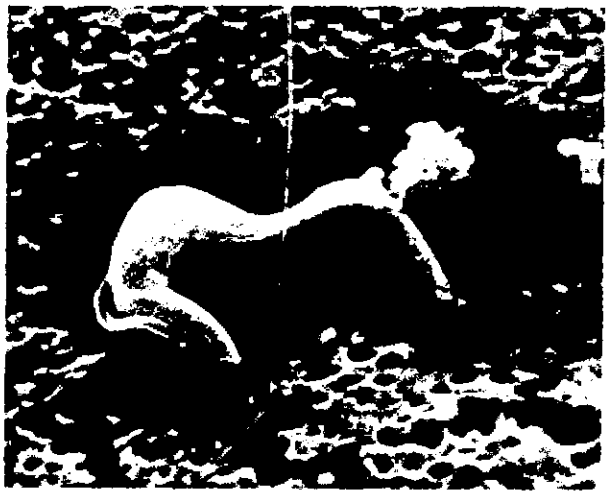


Figure 38-19. Scanning electron micrograph of an infective form of *Trypanosoma brucei* (X13,500.) Many parasitologists consider *T. gambiense* and *T. rhodesiense* to be subspecies of *T. brucei*. *(Courtesy of Peter R. Gardiner, International Laboratory for Research on Animal Diseases, Nairobi, Kenya, and the Journal of Protozoology.)*

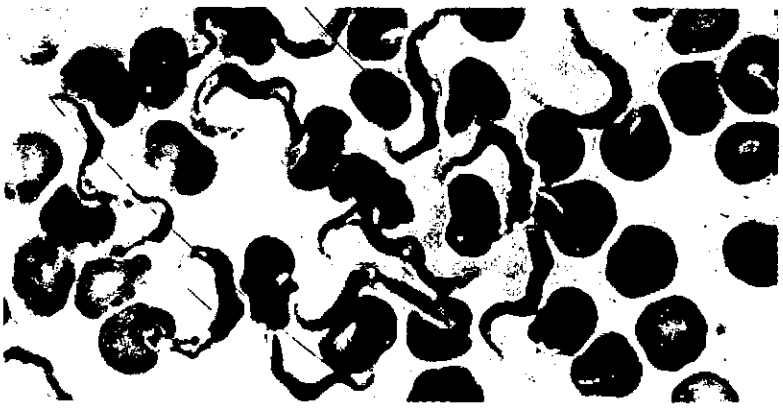


Figure 38-20. *Trypanosoma cruzi*, the causative agent of Chagas' disease (X960). *(Courtesy of A. Packchanian.)*

America and Mexico. Other species produce diseases in domestic and wild animals (see Fig. 38-19).

African sleeping sickness is transmitted to humans by bites of the tsetse fly (genus *Glossina*), and control of the disease depends upon control of these vectors and prompt treatment of human cases with tryparsamide (an organic arsenical preparation) or other drugs. Diagnosis may be made in the laboratory by demonstrating the flagellates in smears from blood, spinal fluid, or lymph nodes. In the early stages, African trypanosomiasis is characterized clinically by fever, headache, insomnia, lymphadenitis, anemia, and rash. Later, the central nervous system becomes involved, and symptoms include tremors, delusions, emaciation, lethargy, and somnolence. Untreated cases usually terminate in death.

T. cruzi (Fig. 38-20), the causative agent of Chagas' disease, is transmitted to humans by reduviid bugs (*Triatoma, Rhodinas,* and *Panstrongylus*), which are intermediate hosts as well as vectors for the parasites and in which the trypanosomes pass through a stage of their life cycle. As the insect feeds on an infected

human or on animal hosts such as an armadillo or opossum, it ingests the trypanosomal forms, which multiply in the gut of the insect and pass into an infective stage of their life cycle. These parasites are excreted by the reduviid bugs on the human skin as the insect feeds. They enter the body through scratches and abrasions rather than by bites as the malaria plasmodia do.

Chagas' disease is an acute febrile disease more common in children than adults. During the acute stage it is characterized by fever and general discomfort, with enlargement of the liver and spleen. The face and eyelids become swollen and inflamed. Fatality is due to a meningoencephalitis or myocardial failure. *T. cruzi* is present in blood during the acute stage of Chagas' disease. Motile forms can be seen in coverslip preparations and smears stained with Wright's or Giemsa's stain. Animal-inoculation and complement-fixation tests also are used.

There is no known means of immunization. Prevention depends upon the avoidance of contact with the insect vector. Transmission from person to person has not been demonstrated, and there is no specific treatment for Chagas' disease.

The animallike flagellates, *Leishmania* and *Trypanosoma*, which cause leishmaniasis and trypanosomiasis may occur in four morphologically distinct stages representing consecutive developmental forms, as shown in Figs. 38-21, 38-22, and Table 38-5. The **amastigote** stage is an intracellular form that appears as a small round cell that has a distinct basal body but no flagellum. The **promastigote** is an elongated cell with a terminal flagellum. The **epimastigote** has a flagellum originating from the midportion of the cell. The **trypomastigote** form is the most mature stage, with a flagellum that begins in the caudal (tail) portion of the cell and forms an undulating membrane. In addition to the morphological forms, both genera manifest striking similarities in their life cycles. Both have two hosts, one being the human (or some other vertebrate) and the other being an insect vector (an invertebrate).

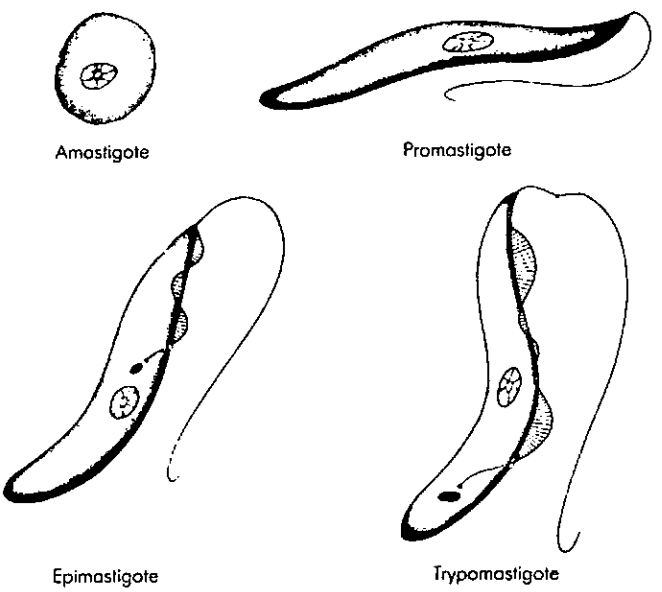


Figure 38-21. Morphological forms of the animallike flagellates. *(Erwin F. Lessel, illustrator.)*

Figure 38-22. Some morphological forms of *Trypanosoma cruzi*. (A) Amastigote or intercellular form (bar represents 10μm.) (B) Epimastigote form. (C) Trypomastigote form, the most mature stage of the flagellate. Note the presence of red blood cells. *(Courtesy of Madeleine Faucher, McGill University.)*

Other Protozoan Infections

Balantidiasis

Balantidiasis is a disease caused by *Balantidium coli*, the only species of the ciliated protozoa known to cause disease in humans. *B. coli* is the largest of the parasitic protozoa that may be found in the human intestine; it can be as long as 200 μm. It resides primarily in the lumen of the intestine and obtains food by the ingestion of bacteria. Occasionally, however, *B. coli* may cause a bloody diarrhea similar to that present in amoebic dysentery.

B. coli is routinely present in hogs. Humans become infected by consumption of water or food contaminated by cysts present in swine feces. Most infections manifest no demonstrable symptoms.

Giardiasis

The etiologic agent of the disease giardiasis is *Giardia lamblia*. This is the only flagellated protozoan reported to date that causes intestinal disease. Infection in adults may be asymptomatic. The majority of overt cases of giardiasis are

Table 38-5. Host Sites of the Various Morphological Forms of Species of *Trypanosoma* and *Leishmania*

	Forms			
Species	Amastigote	Promastigote	Epimastigote	Trypomastigote
L. tropica	Human (skin)	Sandfly (midgut)	—	—
L. brasiliensis	Human (skin, mucosa)	Sandfly (midgut)	—	—
L. donovani	Human (liver spleen, lymph nodes)	Sandfly (midgut)	—	—
T. cruzi	Reduviid bug (gut); human (skin, liver, spleen, myocardium, CNS)	Reduviid bug (hindgut)	Reduviid bug (midgut)	Reduviid bug (feces); human (blood)
T. rhodesiense	—	Tsetse fly (salivary glands)	Tsetse fly (salivary glands)	Tsetse fly (proboscis); human (blood, lymph nodes, CNS)
T. gambiense	—	Tsetse fly (salivary glands)	Tsetse fly (salivary glands)	Tsetse fly (proboscis); human (blood, lymph nodes)

manifested by diarrhea and abdominal cramps. Clinical signs may include abdominal distension and tenderness, weight loss, anemia, and even protein malabsorption. *G. lamblia* has been proven recently to invade and destroy the epithelial lining of the duodenal mucosa.

G. lamblia is distributed worldwide, but it causes outbreaks in small local endemic foci. Both trophozoite and cyst stages can be found in feces. The infectious cysts are usually transmitted in contaminated water supplies, although person-to-person transmission of the disease has been reported.

Trichomoniasis

The sexually transmitted disease trichomoniasis, a type of vaginitis, is caused by *Trichomonas vaginalis*. The organism is found mainly in vaginal secretions and infects both men and women. Only the trophozoite stage of the organism is known. Thus its life cycle is presumably direct. The organisms reproduce within the vagina (in females) or the urethra (in males) by longitudinal binary fission. Infection in males is usually asymptomatic except in cases involving the seminal vesicles and prostate. In women, a thin, watery vaginal discharge is the most prominent symptom, usually accompanied by itching and burning. Both male and female partners should receive treatment with an effective antimicrobial agent such as metronidazole. The prevalence of trichomoniasis is estimated to be about 5 to 30 percent in the United States. The parasite is cosmopolitan in its distribution.

Toxoplasmosis

Toxoplasmosis is a disease caused by the protozoan *Toxoplasma gondii*. The disease in adult humans is frequently mild or asymptomatic. Mild symptoms include fatigue, fever, sore throat, malaise, rash, and headache. However, the disease in the immunocompromised patient is usually a severe, life-threatening one; the symptoms manifested are encephalitis (inflammation of the brain), myocarditis (inflammation of the myocardium or muscular tissue of the heart), and pneumonia. The disease can also be acquired congenitally by the human embryo or by newborn infants infected late in fetal development. The consequences of these infections are very severe. There is central nervous system involvement resulting in mental retardation and severe visual impairment or blindness; convulsions, fever, and an enlarged liver also may be present.

Toxoplasmosis is worldwide in distribution and is one of the most common infections of humans. Even in the U.S., serologic tests have indicated that over 50 percent of adults have been infected. The organism also infects all orders of mammals as well as many birds and some species of reptiles.

Toxoplasma gondii is a protozoan of the *Sporozoa* group. It exists in three forms: trophozoite, cyst, and oocyst. The cyst and oocyst are the principal forms in which the protozoan is transmitted. The crescent-shaped trophozoite form invades mammalian cells (except erythrocytes) and is the form found in acute human infections. The organism is spread by the ingestion of oocysts that are excreted in the feces of cats and by ingestion of undercooked pork and mutton containing cysts.

Diagnosis may be made by isolation of the parasite, by histologic demonstration of the organism in tissues, or by serology. A number of serologic tests are

available. In the Sabin-Feldman dye exclusion test, living *Toxoplasma* cells are stained with methylene blue in the presence of the patient's serum. In the absence of specific antibodies, the cells are stained with the dye. In the presence of antibodies, the dye is excluded from the cells.

Pneumocystosis

As discussed in Chap. 33, *Pneumocystis carinii* is the infectious agent that causes the most common life-threatening secondary infection in AIDS (acquired immunodeficiency syndrome) victims. This microorganism is usually regarded as a protozoan belonging to the *Sporozoa* group although some workers consider it a fungus. The disease is called pneumocystosis and is an infection of alveolar spaces of the lung (pneumonia). The organism appears in vivo as aggregates of thick-walled cysts. The cysts are 6 to 9 μm in diameter, and each contains two to eight nucleated pear-shaped cells produced by binary fission. The organism has not been propagated in vitro. The source of human infections is unknown, although the microbe is suspected to be transmitted by fomites or respiratory droplets. Individuals susceptible to the disease are compromised persons, e.g., undernourished infants, immunosuppressed patients, and of course AIDS victims.

IMMUNE RESPONSE TO FUNGAL AND PROTOZOAN DISEASES

Humoral antibodies seem to play little or no role in host resistance to fungal infections. Cell-mediated immunity is the primary defense mechanism. Thus in patients with T-cell defects, opportunistic fungal infections are common.

Immediate or delayed-type hypersensitivities to fungi can be expressed in various ways. Inhalation of fungal spores may produce asthma. Other allergic reactions are manifested by eruptions of the skin which are associated with cutaneous infections due to dermatophytes (fungi causing skin infections) or species of *Candida*. In other cases an allergic reaction such as **erythema nodosum** (an eruption of pink to blue, tender nodules) may be part of the symptoms with systemic fungal disease, as in coccidioidomycosis. Hypersensitivity reactions may also be demonstrated by an intradermal test with a specific fungal extract, e.g., coccidioidin or histoplasmin. (Such tests are described in Chap. 34.)

In the case of protozoan infections, both cellular and humoral immunity to various protozoal antigens often can be demonstrated. Both sensitized T cells and antibodies arise during infection, but the exact role of each of them in combating and resolving the infection is unclear.

IgE antibodies are produced commonly, if not always, during protozoan infections. IgG and IgM antibodies are found frequently as well. Such antibodies are useful in the serodiagnosis of infections. Secretory IgA is also produced to prevent parasites from attaching to mucosal cells in the intestines. Not much is known at the present time about the exact role of cell-mediated immunity in recovery from protozoan infections, but it is thought to be an important mechanism for the elimination of these parasites. But unlike many viral and bacterial infections, parasitic infections rarely confer lifelong or complete immunity after primary infection.

THERAPEUTIC DRUGS FOR TREATMENT OF FUNGAL AND PROTOZOAN DISEASES

Most antibacterial antibiotics are ineffective in the treatment of fungal infections because the eucaryotic fungal cells lack the target sites of the procaryotic cells. Likewise, the few antifungal agents available are not effective against bacteria. One group of effective antifungal agents is the polyene antibiotics; the two most useful of these are nystatin and amphotericin B. Both act on the plasma membrane of the fungus, combining with membrane sterols, and cause leakage of intracellular potassium and other metabolites. Nystatin is effective in topical *Candida* infections but not in deep mycoses or even dermatophyte infections. Amphotericin B is effective against the deep mycoses, such as cryptococcosis and histoplasmosis.

Unfortunately, polyene antibiotics also have an affinity for human cells, lysing erythrocytes and causing leakage of potassium and other low-molecular-weight intracellular substances. Side effects from these antibiotics vary from a mild headache, chills, and fever to severe hemolytic anemia and acute nephritis (kidney inflammation). Another effective group of antifungal agents is the pyrimidine antimetabolites; the best example is 5-fluorocytosine, which is especially effective in the treatment of yeast infections. Because it is an analogue of pyrimidine, it interferes with pyrimidine metabolism or DNA synthesis.

The imidazole compounds are another group of antifungal agents. They have a very broad spectrum, high activity, and mild side effects. Miconazole reacts with cytoplasmic membranes and causes them to leak. It is very active against coccidioidomycosis and paracoccidioidomycosis. Ketoconazole is an oral imidazole with few side effects. It is active against several fungi, including *C. albicans*. Its mechanism of action is inhibition of ergosterol biosynthesis, an important step in synthesis of fungal cell walls and membranes.

The antibiotic griseofulvin is active against the dermatophyte infections (superficial skin infections) but not against the deep mycoses (systemic diseases caused by fungi). It interferes with protein and nucleic acid synthesis.

The drug treatment of parasitic (or protozoan) diseases varies widely and depends on the kind of disease organism implicated. Often all that is required is prevention of reinfection. In such cases the healthy host is capable of expelling the parasite. The use of drugs in therapy is complicated by the fact that most of the drugs used to eradicate protozoa are toxic to the host also. Thus the

Table 38-6. Some Drugs Used in Treating Protozoan Diseases

Disease	Drugs
Leishmaniasis	Sodium stibogluconate
Trypanosomiasis, early	
West African	Pentamidine isethionate
East African	Suramin
Amoebiasis, dysenteric, intestinal	Metronidazole + iodoquinol
Falciparum malaria, acute	
Chloroquine-sensitive	Chloroquine phosphate + primaquine phosphate
Chloroquine-resistant	Quinine sulfate + pyrimethamine + sulfadiazine

SOURCE: *Guide to Antimicrobial Therapy*, J. P. Sanford, West Bethesda, MD, 1984.

effectiveness of the drug must be weighed against its side effects and the necessity of treating a given patient. Unfortunately, for some parasitic diseases no effective drugs are available. Table 38-6 shows some drugs used in the treatment of protozoan diseases. It is apparent that the antibiotics effective against bacterial infections are not active against protozoa. This is because protozoa, like fungi, being eucaryotic cells, do not have the target structures for antibiotic activity, such as a peptidoglycan cell wall, 70S ribosomes, or a nucleus without a nuclear membrane, all characteristics typical of procaryotic cells.

QUESTIONS

1 Define the following terms: (a) opportunists, (b) microbes of endogenous and exogenous origin, (c) histoplasmin, (d) nephritis, (e) mycosis, and (f) dermatophytes.
2 Describe the factors that contribute to a rising incidence of mycotic diseases.
3 Why do protozoan diseases remain such a scourge to humans?
4 What is known about the role of cell-mediated immunity in fungal and protozoan diseases?
5 Describe the mode of action of these fungal antibiotics: (a) griseofulvin, (b) ketoconazole, (c) miconazole, (d) nystatin, and (e) amphotericin B.
6 What complication is encountered in using drugs in the therapy of protozoan infections?
7 Why are antibiotics that are effective against bacteria inactive against protozoa?
8 Are fungal infections transmitted by air or by contact? Explain.
9 What diseases are referred to as superficial mycoses?
10 Outline the factors that predispose a person to systemic fungus infections. Name three of these infections and identify their causative agents.
11 Describe the differences between a definitive host, an intermediate host, and a reservoir host in parasitic or protozoan diseases.
12 Describe the life cycle of *Entamoeba histolytica*.
13 Why is malaria such an important disease? How was the disease virtually eliminated in the United States?
14 Name the four species of protozoa that cause malaria in humans and identify the one that causes the most serious form of the disease.
15 Write an account of the life cycle of a *Plasmodium* species that causes malaria.
16 Chloroquine phosphate has been used as a prophylaxis for travellers to areas with endemic malaria. With the development of chloroquine-resistant strains of *Plasmodium falciparum* in many countries, what alternative drugs are recommended?
17 What difficulties have been encountered in the development of a vaccine against malaria?
18 Identify the specific etiologic agents of leishmaniasis and the clinical forms caused by each of them. Comment on their transmission and reservoir hosts.
19 What is Chagas' disease? Comment on its transmission, reservoir and intermediate hosts, and vector.
20 Discuss the similarities in the life cycles and morphological forms of *Leishmania* and *Trypanosoma* species.

21 Give a brief description of the diseases caused by the following protozoa: (a) *Balantidium coli*, (b) *Giardia lamblia*, and (c) *Pneumocystis carinii*.

REFERENCES

The references cited in Chaps. 17 and 19 are also applicable to this chapter.

Schmidt, G. D., and L. R. Roberts: *Foundations of Parasitology*, 2d ed., Mosby, St. Louis, 1981. *A clearly written and well-organized text intended for use in introductory courses in parasitology. In addition to the host-parasite relationship, pathogenesis, and epidemiology of the parasites, their biology, morphology, ecology, biochemistry, and immunology are also described. The first 10 chapters are of direct relevance to protozoology.*