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Contemporary Issues: Diseases with a Food Vector†

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INTRODUCTION

The past decade, indeed the last few years, have been interesting times for public health and regulatory officials concerned with foodborne disease. An increase in the incidence of diseases that can be transmitted by food has become apparent not only in the United States, but also in other industrialized nations. The United States has recognized both its largest (207) and, in a separate incident, its deadliest (248) outbreak of foodborne disease. Food has been shown to be a vector for bacteria previously thought to infect humans only by other routes, and newly discovered causes of disease have been associated with particular foods. Some long-recognized pathogens have appeared in foods once believed to be incapable of supporting their growth. The finding that some of these pathogens are far more resistant to long-standing food-processing and storage techniques than expected has caused alarm within the food industry.

The increased recognition of foodborne disease in both foods and clinical specimens is, in part, due to better methodologies for the detection of microorganisms, e.g., the application of recombinant deoxyribonucleic acid (DNA) techniques to epidemiological investigations. It may also be due to awareness by physicians of possible etiologies and public awareness that microbiologically contaminated food can cause illness. The data, however, suggest a true increase in incidence of foodborne disease. In Great Britain, for example, the incidence of human salmonellosis is rising rapidly and alarmingly, yet the reasons for this remain unclear (262). Likewise, salmonellosis caused by specific *Salmonella* serotypes is increasing in the United States (43, 236).

There is a new appreciation for the complexities of microorganisms and their ability to adapt quickly to new circum-

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stances. Despite our best efforts and technologies, we cannot always find a causative agent for some diseases, or if we can, we often find it in clinical specimens but not in the environment. A number of factors, discussed throughout this paper, may contribute to this problem.

The virtual explosion of information and the numerous disciplines involved in investigations of foodborne diseases have created a knowledge gap. The clinician, who is concerned about the patient and the threat a disease poses, may not be aware of how that disease was acquired. Concern usually arises only when diseases cluster or when outbreaks with easily recognizable common sources occur. The clinical microbiologist may isolate and identify the causative agent from clinical specimens, yet may be unaware of its potential pathogenicity or of the updated, improved methods used by food or environmental microbiologists. The epidemiologist tries to pinpoint the source of a disease outbreak, but may be either unaware of the full spectrum of investigational tools available or ill equipped to analyze food. Foodborne disease has posed other problems for the epidemiologist, e.g., unavailability of the suspect food for analysis and poor methods for analyzing the available food. In addition, because many foodborne diseases occur frequently but sporadically, and not in clusters, food sources are nearly impossible to trace.

The food microbiologist, then, must find a few pathogenic microbes (the needle) in a tremendous background load of normal food microflora (the haystack). Clinical methods are seldom applicable to food, and the chemistry and natural flora of each food pose a new set of problems. The food microbiologist can often analyze food by adapting methods developed by the clinical microbiologist, yet may not realize that acute or chronic and sometimes life-threatening diseases can be attributed to a food vector. Given the breadth of the literature, even the clinician may not be aware of the full spectrum of foodborne diseases. Perhaps none of these groups appreciates how frequently such diseases occur in a country as large as the United States, or perhaps the problem seems trivial when compared with others such as heart disease or acquired immunodeficiency syndrome (AIDS).

Regardless of the reasons, a communication and knowledge gap exists. This review discusses a vast group of foodborne diseases and is designed to stimulate microbiologists to apply more effort in this field. Finally, the emphasis on food microbiology and its status should be markedly increased in both the university setting and professional organizations.

DEFINITION AND FREQUENCY OF FOODBORNE DISEASE

The term foodborne disease may cause confusion. Foodborne disease encompasses a variety of clinical conditions whose etiology may be actively or passively transmitted by food. Food may serve as a vehicle for many pathogenic or nonpathogenic organisms and in some circumstances may support the growth of the etiologic agent. In many situations, food probably has a passive role; that is, the etiologic agent(s) does not grow in the food but is merely transmitted to humans through it. Food, then, may act as a vehicle by transmitting viruses, protozoa, and even some bacteria, which are present but unable to replicate on food, to humans who ingest it. Food unquestionably constitutes the bulk of "foreign" material encountered daily by humans and in many circumstances is an unavoidable, ingestible fomite.

Foodborne disease is a subset of enteric disease, a major category of total disease. The list of clinical conditions that comprise foodborne disease includes, but is not limited to, the acute symptoms of food poisoning or food infection (e.g., vomiting and diarrhea) and other gastrointestinal manifestations; respiratory, febrile, and other symptoms may occur independently or may accompany the acute gastrointestinal symptoms.

Chronic diseases that may result from foodborne disease include arthropathies, renal diseases, nutritional and other malabsorptive disorders, and other conditions that may affect virtually every organ system. The evidence that foodborne microorganisms directly or indirectly cause these disorders ranges from circumstantial to very convincing. Foodborne disease may also result in death.

Enteric diseases rank second in prevalence to respiratory diseases in the United States (89). A recent estimate suggests that, on the average, every U.S. citizen has at least one episode of enteric (diarrheal) disease annually (6). Foodborne disease conservatively constitutes one-third of the total, or more than 80 million cases annually (6). This does not indicate that our food supply is inherently unsafe, but rather that people make food unsafe by handling it improperly or by transmitting their diseases via food. The suggested morbidity figures are staggering, and the attendant cost of human suffering and economic burden to society may be in the tens of billions of dollars per year.

MICROBIAL ECOLOGY OF THE HUMAN GASTROINTESTINAL TRACT

The human gastrointestinal tract is a remarkably complex microbial ecosystem, composed of about 10^{14} procaryotic cells, most of which reside in the intestinal tract (146). This huge number is 10-fold larger than the estimated number of eucaryotic cells in the human body (211).

Savage (211) defined the autochthonous (indigenous) flora of the gastrointestinal tract as organisms which can grow anaerobically, are always found in normal adults, colonize particular areas of the tract, colonize successively from infancy, maintain stable populations (climax communities) in normal adults, and associate intimately with the mucosal epithelium. In contrast, allochthonous microbes usually cannot colonize unless abnormal circumstances exist. They may often be "just passing through" and only occupy ecological niches abandoned by autochthonous microbes because of abnormal circumstances (211). The criteria for autochthony are neither absolute nor complete and may be modified as knowledge of the gastrointestinal microecology accumulates.

Each section of the alimentary tract may have its own autochthonous microbial flora, its own microhabitats (niches), and its own microenvironmental conditions, e.g., pH, oxygen tension, and nutrient gradient. The rapid changes that occur in the microenvironment are due to cell turnover, mucus flow along the epithelial surfaces, peristaltic movement, and nutrient availability, each of which differs as various foods are consumed and digested. Autochthonous microbes must adapt to cope with these changes and maintain generally stable populations (212).

Most acute foodborne pathogens are allochthonous microbes that temporarily colonize the epithelial surface at some point along the tract (e.g., *Vibrio cholerae* on the small intestine) or traverse the mucus and invade the epithelium without the need to colonize (e.g., *Shigella* spp.). Colonization by an allochthonous microbe is far more likely to occur

in an abnormal circumstance, e.g., after large oral doses of antibiotics, than during normal circumstances. Mechanisms such as specifically evolved attachment factors and motility that permit potential disease-causing allochthonous microbes to colonize the normal host may be thought of as virulence factors, as may mechanisms that obviate the need for colonization, such as invasiveness. Autochthonous bacteria may possibly gain virulence properties through genetic exchange (e.g., plasmids and transposons) with allochthonous bacteria that harbor transmissible or mobilizable genetic elements containing virulence genes.

Food and water (mostly food in developed countries) are the major source of allochthonous microbes. In the normal host, however, the temporary colonization of the bowel by allochthonous pathogenic microbes will, in time, be reversed and autochthonous microbes will recolonize the gut (211).

"NEW" FOODBORNE PATHOGENS

Are there really new foodborne pathogens? The answer to this question requires a degree of speculation. Some newly recognized clinical conditions that affect humans are caused by seemingly new microorganisms. For example, the current AIDS epidemic has been attributed to a microorganism that crossed a species barrier. Legionellosis may have been present for some time, but perhaps was diagnosed as a variety of clinical syndromes until a common-source outbreak occurred and appropriate studies were conducted to determine its etiology (174). Hemorrhagic colitis, caused by *Escherichia coli* O157:H7, appeared to be a new clinical entity; actually, the condition was accurately described in 1963 but was not linked to the causative serotype of *E. coli* until 1982 (97, 198). Whether *E. coli* O157:H7 simply became better adapted to humans at some time may never be known. In addition to hemorrhagic colitis, this bacterium is now associated with hemolytic uremic syndrome and thrombotic thrombocytopenic purpura.

Microorganisms may acquire virulence traits through in vivo genetic exchange with allochthonous pathogens (88). Guarino et al. (88) detected heat-stable enterotoxin production in strains of *Citrobacter freundii* (considered autochthonous by some) isolated from the feces of children with diarrhea. Heat-stable enterotoxin is produced by other bacteria considered autochthonous, such as *Klebsiella pneumoniae* (127), *Enterobacter cloacae* (128), and *Proteus* spp. (10). Thus, certain plasmidborne virulence genes may readily be exchanged by several bacterial genera; some may be transferred along with antibiotic resistance on resistance transfer factors (204). Frequency and ease of transfer of extrachromosomal genetic material vary greatly; some elements transfer with apparent ease, even among several genera, and others are highly restricted in their movement. Plasmids containing virulence genes may also be lost when the microbe is isolated and cultured, complicating the search for the causative pathogen (95).

Recently, a new clinical condition, linked to raw-milk consumption, was described and named Brainerd diarrhea (183). The point-source outbreak of this chronic diarrheal syndrome occurred in December 1983, when 122 persons were affected. Clusters of cases have been observed since then (155, 183). The 1983 outbreak, however, was probably not the first occurrence of the disease; sporadic outbreaks of a similar illness had earlier affected persons in at least six states (183). Because many of the victims had consumed raw milk from a single dairy, Brainerd diarrhea was recognized as a distinct clinical condition with an infectious etiology. Despite concentrated efforts of many clinicians and microbiologists, however, the etiologic agent of Brainerd diarrhea remains unidentified.

Other factors may contribute to the appearance of new pathogens; an etiologic agent is found in only 50% of diarrheal disease cases (177). In general, recognition of an organism as a pathogen must often await development of appropriate technologies, culture techniques, animal models, or sheer serendipity. Increased consumption of imported, new, or fad foods, the processing or packaging of foods in new ways, and the trend towards consumption of raw or undercooked foods for nutritional and organoleptic qualities may introduce new pathogens or reintroduce old ones that were previously restricted geographically. Better epidemiological and microbiological studies may link single cases or clusters of disease to causative agents acquired by food through cross-contamination during preparation or transferred to food by an ill food handler.

In most instances the pathogens are not truly new, but this does not preclude the possibility of new pathogens arising. Recent experience with AIDS reminds us that microbes evolve rapidly, at rates far faster than humans, and thus can adapt to humans faster than humans can adapt to them. The adaptation of microbes to humans may bring about more chronic disease problems in which microbial antigens mimic human tissue antigens.

ACUTE GASTROENTERITIS-CAUSING AGENTS AND OTHER ACUTE PATHOGENS OF CONCERN

The traditional symptoms of foodborne disease are diarrhea or vomiting or both, but other symptoms may suggest infection or intoxication. Fever, abdominal cramps, nausea, flatulence, constipation, bloating, or other vague symptoms may result from a dose of toxin insufficient to cause diarrhea or from a subacute threshold dose of a pathogenic microorganism. Innate or acquired resistance of the host, or some other factor less well understood, may also contribute to subacute and variable symptoms. Even the definition of diarrhea itself may vary among individuals. For the purpose of this review, discussion is limited to those pathogens of more recent interest and to gastroenteritis and/or other primary, acute symptoms. Other complicating symptoms (e.g., respiratory, bacteremia, or meningitis) and chronic sequelae to acute infection are dealt with separately.

Yersinia spp.

Yersinia enterocolitica has been recognized as a human pathogen for nearly five decades, but its true importance has only begun to be realized. It is an excellent example of global partitioning. Countries of northern Europe and Scandinavia have a far greater problem with yersiniosis than does the United States, possibly because of geographic partitioning of particular *Yersinia* serotypes. *Y. enterocolitica* causes an enterocolitis, usually accompanied by abdominal pain in the lower right quadrant, fever, and diarrhea. Diarrhea is not always present, however, and owing to the pain pattern and fever, yersiniosis may be mistaken for acute appendicitis. Outbreak follow-up studies (61) have reported many needless appendectomies. The largest outbreak of yersiniosis in the United States occurred in the summer of 1982 in Tennessee and adjoining states and was linked to contaminated pasteurized milk; 172 cases were confirmed, and possibly several thousand persons were actually affected. Other foodborne outbreaks have occurred, but none was of this magnitude.

Y. enterocolitica is not uncommon in the environment or in food. In a recent survey of raw milk, 48% of the samples analyzed contained this organism (163). Fortunately, pathogenic *Y. enterocolitica* is rarely encountered. Serotyping of recent isolates from the New York City area indicate that serotype O:3, the predominant European serotype, is beginning to appear on the U.S. east coast (30). Serotype alone, however, does not determine whether *Y. enterocolitica* is virulent or avirulent. The factor that confers pathogenicity on *Y. enterocolitica* remains unknown, except that it is plasmid mediated; chromosomal genes may have to interact with plasmid genes for full virulence to be expressed (167). Pathogenic *Y. enterocolitica* serotypes contain a 40- to 50-megadalton plasmid, but the extent of DNA relatedness among the plasmids varies with serotype (170). Although *Y. enterocolitica* produces a heat-stable enterotoxin, the toxin apparently is not produced at temperatures above 25°C. Pathogenicity has been associated with calcium dependency, autoagglutination, and binding of Congo Red dye. These traits may reflect more meaningful virulence traits, such as the production of fimbriae (116) or outer membrane proteins, which may protect from opsonization (245), phagocytosis by polymorphonuclear leukocytes (141), or intracellular survival (170). It would seem desirable to keep serotypes O:3 and O:9 that are virulent for humans from becoming established in the United States, not because they are more virulent than North American serotypes, but because of their strong association with autoimmune phenomena (discussed later).

The food industry is concerned about *Y. enterocolitica* because it is a psychrotroph and, as such, can grow at refrigeration temperatures. The infectious dose of *Y. enterocolitica* is unknown but probably varies, depending on characteristics of both the host and the organism. *Y. enterocolitica* may produce symptoms limited to acute abdominal pain or sore throat and has been suspected of causing exudative pharyngitis, without diarrhea or abdominal pain, in some victims exposed to foods that have caused large outbreaks of enteritis (203). The severity of the acute enteritis or enterocolitis may be further exacerbated in persons with iron overload (122).

Although not a major cause of gastroenteritis in the United States, *Y. pseudotuberculosis* may be a more common cause of disease than the frequency of clinical isolation would indicate. In a study of 194 patients with acute abdominal pain, Attwood et al. (9) found that 23% had serological evidence of yersiniosis. *Y. pseudotuberculosis* was encountered five times more frequently than *Y. enterocolitica*, with *Y. pseudotuberculosis* type IV accounting for 43% of all *Yersinia* infections (9). Yersiniosis occurred in 31% of patients who were diagnosed as having acute appendicitis. Accurate diagnosis was more likely when sequential rather than only acute serum samples were examined for antibodies to *Yersinia* spp. (9). Abdominal pain may be the major symptom or the only symptom of yersiniosis, and unless great care is taken in serum collection, the illness may be drastically underdiagnosed.

Recently, Isberg et al. (107) discovered a novel means by which *Y. pseudotuberculosis* enters mammalian cells, which may be the way certain other enteric pathogens gain access to host cells. A single chromosomal gene codes for a 103-kilodalton protein, "invasin," which is probably an outer membrane protein of the bacterium. Invasin interacts with the mammalian cell (putative receptor), is actively endocytosed by the binding cell, and is presumably the inducer of internalization (107). The authors suggest that any innocuous bacterium might enter host tissues by acquiring invasin or a similar binding protein (107). In fact, this single gene, when incorporated into *E. coli* K-12, permitted K-12 to enter (invade) cultured cells (106). How or if this chromosomal gene is transmitted to other bacteria is unknown, but it is clear that not all invasive bacteria possess such a simple one-gene product mechanism of inducing their uptake into eucaryotic cells. *Shigella flexneri*, for example, requires interplay between multiple genes, both chromosomal and plasmid, for full virulence expression (158). The invasin gene (*inv*) has recently been found in *Y. enterocolitica*. A second invasion gene, *ail*, also found in *Y. enterocolitica*, is more host specific than *inv* with regard to cell lines invaded (167).

Salmonella spp.

Salmonella spp. are certainly not new foodborne pathogens, but they are of current interest for a variety of reasons. The largest-ever outbreak of foodborne salmonellosis occurred in the Chicago area in 1985, with 16,284 confirmed cases (207). Estimates of the actual number of persons affected are much higher, perhaps one-quarter million. As previously indicated, salmonellosis is on the rise in the United States and other industrialized countries such as the United Kingdom. There is ample evidence that, in many instances, extremely low numbers of *Salmonella* spp. (even a single organism) may cause disease (25, 53, 54). One member of this vast group, *Salmonella enteriditis*, is currently of particular concern. In the past decade, the New England/Middle Atlantic region of the United States has experienced a fivefold increase in reported human isolations of *S. enteriditis*, compared with a 1.7-fold increase for all other serotypes (250). Although several food vehicles have been implicated in outbreaks and sporadic cases, intact grade A eggs in their shells were involved in many of the outbreaks (236). Of 130 confirmed *S. enteriditis* cases in nursing homes in the Northeast from January 1985 to May 1987, 10 persons died (8% mortality) (236).

The salmonellae are also the center of much controversy involving antibiotic resistance. The use of antibiotics as growth promoters in cattle has for some time been suspected of influencing the induction or maintenance of antibiotic-resistant *Salmonella* spp. in animals. Recently, epidemiologists used recombinant DNA technology to trace chloramphenicol-resistant *S. newport*, which had caused a large outbreak of disease, through hamburger and back to the dairy farms where the beef had originated (232). This topic is highly controversial because the levels of antibiotic(s) fed to cattle are subtherapeutic, and evidence exists that the growth-promotional effect may be a "direct" effect, separate from the antimicrobial properties of the antibiotic (204). Evidence derived from studies on *S. typhimurium* and *S. gallinarum* suggests that antimicrobial resistance, or, rather, the possession of antibiotic resistance plasmids, does not confer increased virulence and infectivity on the microbe, but may in fact decrease virulence (226). The problem of quantifying the difference between the health and economic impacts of infections caused by antibiotic-susceptible and antibiotic-resistant bacteria was recently discussed by Holmberg et al. (100). The issue of antibiotic-resistant *Salmonella* spp., the role of these bacteria in human disease, and the changing patterns of resistance are reviewed in detail by MacDonald et al. (148).

Salmonella-induced diarrhea involves a complex interplay of pathogenic mechanisms and is not completely understood. The mechanism(s) involved probably varies among

the many serotypes. Again, recombinant DNA technology is beginning to help unravel the puzzle. Panigrahi et al. (185) developed a simple immuno-dot-blot assay to detect a cholera-related enterotoxin in *S. typhimurium*, the leading cause of salmonellosis in the United States. They question whether choleralike enterotoxins produced within an enterocyte give equivalent or greater effects than those produced in the lumen by noninvasive pathogens (185). In light of the mechanism previously described for *Y. pseudotuberculosis* invasion (107), it may be that all invasive mechanisms of the *Salmonella* group have not yet been uncovered. The invasion of HeLa cells was recently shown to be enhanced by calcium, suggesting that this cation is involved in binding the bacterium to the eucaryotic cell membrane (179). Perhaps an invasinlike mechanism is but one armament of certain *Salmonella* spp. The extraintestinal manifestations of *Salmonella* infections, occurring in as many as 30% of victims, have been reviewed in depth by Cohen et al. (47).

A 20-fold increased annual incidence of *Salmonella* infections in men with AIDS was recently reported by Celum et al. (41) and is the ninth most common diagnosis in San Francisco AIDS patients. Although the possibility of anal-oral transmission of salmonellosis cannot be excluded entirely, food is unquestionably the major vehicle for *Salmonella* spp. (43), and an increase in reported cases in persons with AIDS is anticipated (43).

Campylobacter spp.

Campylobacter jejuni (and probably other *Campylobacter* spp.) is now considered the most frequent cause of bacterial diarrhea in the United States (61). Ingestion of as few as 200 organisms may produce disease (61). Results of a large human volunteer feeding study recently confirmed that low doses of *C. jejuni* may produce infection and illness (23). Acute symptoms vary from mild to severe enteritis with diarrhea, vomiting, abdominal pain, and fever. Occult (and possibly overt) blood in stools occurs in very severe cases. Symptoms may recur, probably because of the demonstrated ability of *Campylobacter* spp. to rapidly undergo phase (surface antigen) variation (discussed later). Many outbreaks of campylobacteriosis have been associated with consumption of raw milk and undercooked poultry. Cross-contamination of cooked food with raw poultry in the kitchen is one likely means of disease transmission. *Campylobacter* spp. are not extremely hardy in food or in the environment and are rapidly killed by cooking temperatures. *C. jejuni*, a complex pathogen that can be invasive, can also produce two or more biologically active toxins, one of which is related to cholera toxin (159).

The *Campylobacter* spp. remain an enigma. Several atypical campylobacters, including *C. coli* and *C. laridis*, were recently isolated from the stools of diarrhea patients (244). Although infrequently reported in patients infected with human immunodeficiency virus (HIV), *C. jejuni* was recently found to cause atypical, cryptic, severe, and persistent infections in HIV-positive individuals. The authors suggest that many *C. jejuni* infections in these patients may be overlooked because of an unusual clinical course or lack of sufficient cultures (188). In prospective studies in which both *Salmonella* and *Campylobacter* species were routinely sought in fecal specimens, *Campylobacter* isolates outnumbered *Salmonella* isolates 10 to 1 in college students, 3 to 1 in members of a health maintenance organization, and 2 to 1 in patients in a multicenter study (243).

Most proven common-source outbreaks of *Campylobacter* enteritis have involved raw milk or contaminated water, yet epidemiological studies consistently implicate poultry as the cause of the majority of cases (27, 48, 60, 109, 243). As pointed out by Schmid et al. (216), reservoirs and vehicles for *C. jejuni* infection vary geographically; in their study, raw milk was a significant risk factor in a Midwest town. However, a large prospective study in King County, Washington, drawing on a 320,000-member health maintenance organization, clearly demonstrated a link between poultry consumption and campylobacteriosis; poultry accounted for 10-fold more cases than did raw milk (48). Istre et al. (109) reported a clear association between campylobacteriosis and undercooked barbecued chicken at a large family cookout. Although other food animals may harbor *C. jejuni* in their intestines, the clearest association of a food source with human disease is poultry (27). Curiously, in the aforementioned raw milk survey conducted in the upper Midwest (163), *C. jejuni* was found in 1 of 237 samples. Given the high incidence of campylobacteriosis in the United States and the highly variable growth requirements for campylobacters, the relative difficulty in isolating them from food and the environment is apparent. Perhaps culture methods for isolating these organisms from their natural habitat(s) are not as good as generally assumed.

Aeromonas spp.

Aeromonas hydrophila is related to the vibrio group, but generally resides in fresh and brackish water. Although its role in diarrheal disease remains controversial, its production of cytotonic and cytotoxic exoprotein argues strongly that it is a potential pathogen (124, 143). In Australia, *A. hydrophila* is considered the leading bacterial cause of diarrheal illness, usually via water, including municipal water supplies (99). Records of diarrhea patients in a large U.S. hospital show that the incidence of *Aeromonas* spp. in stool cultures was the highest of any potential enteric pathogen (80). A human feeding study in which clinical isolates of *A. hydrophila* were used at very high doses failed to elicit diarrhea in healthy volunteers (D. Morgan, P. C. Johnson, H. L. DuPont, T. K. Satterwhite, and L. V. Wood, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, B190, p. 49). Thus, the status of *A. hydrophila* as a human pathogen is still questionable. The cytotonic enterotoxin, like that of *Campylobacter* spp., is similar to cholera toxin, both serologically and genetically (217). Like *V. cholerae*, but unlike *E. coli* heat-labile enterotoxin, the *Aeromonas* cytotonic toxin gene appears to be chromosomal (42). Another member of the genus *Aeromonas*, *A. sobria*, has been reported to cause diarrheal disease (79). *Aeromonas* spp. are of concern because their frequent presence in foods and their psychrotrophic growth capability enable them to reach high numbers in refrigerated foods. A recent survey of stool isolates of *Aeromonas* spp. failed to demonstrate a correlation between enterotoxin production, cytotoxin production, or biochemical profiles of *Aeromonas* spp. and gastroenteritis (Morgan et al., Abstr. Annu. Meet. Am. Soc. Microbiol. 1985). *A. caviae*, now documented as a cause of human enteritis (2), was isolated from 4.7% of ice cream samples in a survey conducted in the United Kingdom (104), suggesting that *Aeromonas* spp. may be postprocessing contaminants of food.

Tetrodotoxin, a sodium channel inhibitor recently shown to be produced by both *Vibrio* spp. and *A. hydrophila* (222, 241), is another potential virulence factor or cofactor that may provide a valuable clue to solving the discrepancies among toxin production, toxin action, and human feeding studies.

Escherichia spp.

E. coli O157:H7 has been well documented as the cause of hemorrhagic colitis, a diarrheal disease, the hallmark of which is the presence of copious, overt blood in the stool. Abdominal cramps are followed by frank blood excretion. Most victims probably seek medical attention because of the alarming nature of the symptoms. Serotype O157:H7 is a strong producer of what was originally described as Vero or Shiga-like toxin, which appeared to account for its ability to elicit intestinal bleeding (117). It is now known that O157:H7 produces two phage-encoded, antigenically distinct cytotoxins called Shiga-like toxin I and Shiga-like toxin II. Both of these toxins kill Vero and HeLa cells (110, 152).

Enteroinvasive, enterotoxigenic, enteropathogenic, and enteroadherent forms of *E. coli* as well as the enterohemorrhagic O157:H7 are now recognized as human enteric pathogens (140). The complexity of *E. coli* pathogenicity mechanisms is readily apparent, and transmission of some pathogenic *E. coli* types through fecal contamination of food is well documented.

Sporadic outbreaks of hemorrhagic colitis affecting 28 persons in 11 states have been reported to the Centers for Disease Control, Atlanta, Ga., and *E. coli* O157:H7 has been isolated from humans in 29 states (P. N. Griffin, personal communication). Most outbreaks have implicated raw or undercooked ground beef; however, recent outbreaks have involved raw milk (29) and person-to-person transmission in daycare (231) and nursing home settings (39, 208). The daycare center outbreak (231) and other person-to-person transmissions strongly suggest a low infectious dose for this pathogen. In an outbreak caused by frozen hamburger patties, microbiological analysis of patties from the same lot yielded 200 *E. coli* O157:H7 cells per gram. This number was probably reduced during cooking; therefore, the dose consumed may have been <200 per gram. In another outbreak, raw milk consumed by children visiting a dairy farm resulted in a high attack rate of illness. Follow-up analysis of the raw milk from the responsible herd was difficult, however, because a unique enrichment procedure was needed to detect the *E. coli* O157:H7 that was being shed by only a few cows in the herd. This again suggests that a low number of organisms was sufficient to cause disease (29).

The U.S. problem with this serotype does not appear to be as severe as that in Canada, where outbreaks in geriatric care facilities have resulted in high mortality; in one Canadian outbreak, 31% of elderly infected persons died (39). In a 12-month stool survey, only 2 of 2,552 stool specimens submitted to a U.S. hospital clinical laboratory were serotype O157:H7 (93). Although O157:H7 and hemolytic uremic syndrome (HUS) have apparently become endemic in parts of the northwestern United States (176, 242), the incidence of HUS in other geographic regions suggests that it is not unique to the Northwest (125). Several reports suggest that O157:H7 disease may exhibit a spectrum of severity, but the reasons for this are not clear.

Other Vero toxin-producing strains of *E. coli* may also cause hemorrhagic colitis or less severe diarrhea. Evidence suggests that dairy cattle are an important reservoir for O157:H7 (29, 182), but humans may also be a reservoir, possibly because of prolonged fecal carriage of O157:H7 (193). This serotype may be far more prevalent in the food supply than previously suspected. Doyle and Schoeni (62) have shown it to be present in 1.5 to 3.7% of fresh pork, lamb, poultry, and ground-beef samples from several grocery stores in Madison, Wis.

Listeria monocytogenes

In the past few years, *L. monocytogenes* has become established as a foodborne pathogen. Surveys suggest that the incidence of listeriosis, considered rare a decade ago (one case per million population) is now at least seven cases per million population (C. V. Broome, personal communication). Although this rate is low in comparison with those of some other foodborne bacterial pathogens such as *Salmonella* and *Campylobacter* species, listeriosis is characterized by a high mortality rate, ranging from 20 to nearly 50% in individual cases, clusters, or outbreaks (31). The epidemiology in other countries suggests an increase in incidence as well. A clear increase has occurred in Britain since the mid-1970s (160, 161) and has been noted again in a recent publication of the first confirmed case of foodborne listeriosis in England (13). The incidence of listeriosis in France is reported to be 11.3 cases per million population (86). A single California hospital reported a perinatal isolation rate for *L. monocytogenes* of 1.5 isolates per 10,000 deliveries (31). Since attention has been drawn to listeriosis, future surveys will probably determine an even higher incidence rate.

Evidence that food may be a major route of transmission for *L. monocytogenes* includes several outbreaks since 1981 that have been linked epidemiologically or by direct isolation to cole slaw, pasteurized milk, and Mexican-style soft cheese (160). The last-named episode, which occurred in the Los Angeles area in 1985, resulted in the largest number of confirmed food-associated deaths in recent U.S. history.

Clinical manifestations of listeriosis include neonatal sepsis or meningitis and puerperal sepsis, which occurs during pregnancy and often results in perinatal sepsis and stillbirth of the fetus. In adults, most of whom are immunologically compromised or have underlying malignancy, the manifestations include meningitis, meningoencephalitis, or septicemia. A recent study reports that meningitis or bacteremia or both were preceded by gastrointestinal symptoms in 13 of 20 patients in eight Boston hospitals involved in a nosocomial outbreak of listeriosis (96). Gastrointestinal symptoms were defined as vomiting, abdominal pain, or diarrhea. An additional 4 of the 20 patients who acquired listeriosis experienced nausea or anorexia and were presumptively positive for gastrointestinal symptoms. This outbreak of nosocomial listeriosis was epidemiologically linked to food, possibly the raw vegetables used in salads (96). Although clinical manifestations of meningitis, meningoencephaltis, and septicemia are considered the "acute" form of the disease, they may actually be later stages of disease; the true acute symptoms may have been overlooked because they were mild or nonspecific.

Listeriosis has also been reported in apparently healthy persons. One study failed to identify underlying disease or other predisposition in 31 of 102 patients (178); likewise, 6 of 11 patients in another study (164) had no identifiable predisposition. *L. monocytogenes*, like *Y. enterocolitica* and *A. hydrophila*, is a psychrotroph, capable of growth at refrigeration temperatures and, therefore, a source of unforeseen problems for the food industry.

The known virulence mechanisms of *L. monocytogenes* include a hemolysin called listeriolysin, without which the bacterium is far less pathogenic for mice (78, 118). Gaillard et al. (77) demonstrated that *L. monocytogenes* induced its own uptake into cultures of Caco-2 cells, which had retained many similarities to native intestinal epithelial cells. Similar bacterium-induced uptake was also demonstrated by Kuhn

et al. (133), who reported that although hemolysin permitted intracellular survival of *L. monocytogenes*, its presence did not enhance uptake into cells. The hemolysin inhibited macrophage-mediated presentation of antigen to T lymphocytes, thus inhibiting cell-mediated immunity directed against the producer bacterium (46). Paradoxically, acquired immunity to *L. monocytogenes* is directed mainly against the hemolysin (17), and intracellular growth is a prerequisite for induction of specific T-cell-mediated immunity (18). A genetic probe for *L. monocytogenes* hemolysin is the basis for a newly developed DNA colony hybridization test for this pathogen (55).

Listeriosis has been transmitted to infants during delivery (223). A survey conducted in Scotland, however, failed to detect *L. monocytogenes* in the cervicovaginal secretions of 54 pregnant women, and fecal carriage rate was as low (2%) in these women as it was in 60 nonpregnant women (3.4%) (135); this study argues against *L. monocytogenes* being either an autochthonous or a ubiquitous allochthonous microbe. Although some studies have suggested that the fecal carriage rate may be as high as 70% (114), it may be sporadic and transient and simply reflect the dietary load (134).

With the vexing problems of *L. monocytogenes*, such as its infectious dose in a defined set of predisposed persons, its widespread occurrence in the environment, and its not yet fully defined virulence mechanisms, this pathogen is likely to remain the subject of intense investigation for the foreseeable future.

Vibrio spp.

Within the genus *Vibrio* is another group of organisms consisting of a dozen or more separate species that are potential foodborne pathogens. Members of this genus display diverse pathogenic mechanisms, which further compounds the problem of identifying these species. *Vibrio vulnificus* is of particular concern for several reasons: (i) it is widely and sporadically distributed in U.S. coastal waters; (ii) normal indices of water and shellfish quality do not ensure its absence; (iii) it is apparently infectious at an extremely low oral dose; and (iv) patients with liver disease or abnormalities of iron metabolism may exhibit a rapid, often fatal sepsis (240). *V. vulnificus* may cause gastroenteritis in healthy persons as well. The pathogenic mechanisms of this bacterium are not yet well understood. The virulence of *V. vulnificus* is associated with its ability to utilize transferrin-bound iron, but not with cytotoxin or protease production (172). A DNA probe for the cytotoxin-hemolysin gene in conjunction with newly developed selective growth media (157, 171), however, has simplified identification of *V. vulnificus* from environmental samples.

There is still much work to be done on the various pathogenic members of the genus *Vibrio*, as underscored by the reports that Kanagawa-negative *V. parahemolyticus* can cause gastroenteritis (102) and that several vibrios, at times essentially unculturable from the environment, can cause human disease (206). As stated previously, several *Vibrio* spp. produce tetrodotoxin (222, 241); however, the importance of this toxin in acute human gastrointestinal disease is unclear.

Bacillus cereus

B. cereus has emerged as a troublesome foodborne pathogen, a sporulating gram-positive organism found in the environment and in many dried foods. Spores are not always killed during the cooking process, and when the food cools, they germinate. The vegetative bacterium then faces little competition because most potential competitors were killed by heat. *B. cereus* may produce a diarrheagenic and/or emetic toxin (81). The diarrheagenic toxin is fairly well characterized and easily destroyed by normal cooking temperatures. In contrast, the emetic toxin is poorly characterized, has a low molecular weight, and is extremely resistant to heat. The onset time to emesis caused by preformed emetic toxin in food is very rapid, about 1 to 5 h, or less. Many of the outbreaks of *B. cereus* food poisoning have occurred in restaurants serving oriental foods. Rice and fried rice seem to be particularly vulnerable.

Shigella spp. and Other Gram-Negative Bacteria

Although generally thought of as waterborne pathogens, the *Shigella* spp. are a foodborne problem as well (227). Several large outbreaks of *Shigella sonnei* gastroenteritis have been traced to contaminated produce (154). The *Shigella* spp. have a low infectious dose (100 or fewer cells), and rapid methods for detecting them in foods are lacking. Of particular concern is the appearance and spread of a multiply resistant strain of *Shigella sonnei* that limits the effectiveness of antimicrobial agents used for treating shigellosis (252). *Shigella flexneri* (various serotypes) and *Shigella boydii* are documented to have been transmitted by food (227).

Incriminated Gram-Negative Bacteria

The causative agent of a diarrheal illness is often difficult to identify. In some instances, Koch's postulates are easily fulfilled, particularly when human volunteer feeding studies can be done. In other instances, studies are restricted to animal models; in still others, toxins, in vitro invasiveness, or virulence-associated genes are detected and the organism becomes a putative pathogen. The detection of an allochthonous microbe, with no other known pathogen present in the stools of a diarrhea patient, has at times been sufficient to incriminate an organism as causative or to initiate the search for virulence factors. For example, a microaerophilic bacterium resembling *Campylobacter* was incriminated as a cause of chronic, mild gastroenteritis in two patients because it was present in patient stools and no other pathogens could be found (201). It should not be forgotten, however, that some microbes now recognized as potential pathogens were considered allochthonous or even autochthonous a decade ago, or perhaps could not be cultured at all.

Among the gram-negative bacteria, many are sporadically associated with food poisoning. Involvement is suspected when the organism is isolated from the ill person's stool or from the suspect food. Toxins or toxin genes may or may not be found in these organisms. *Plesiomonas shigelloides*, *K. pneumoniae*, *Citrobacter freundii*, and other gram-negative bacteria fit this category. *Citrobacter freundii*, capable of producing an *E. coli* heat-stable-like toxin, was recently found in the stools of children with diarrhea (88). Recombinant DNA technology may further unravel some of the mysteries surrounding this diverse group of microbes and facilitate identification of pathogenic strains.

Botulinal Food Poisoning

Although outbreaks of botulism, caused by the gram-positive anaerobic sporeformer *Clostridium botulinum*, are

rare in the United States, this foodborne pathogen remains significant. Between 1976 and 1984, 124 outbreaks of foodborne botulism involving 308 persons were reported (147); the overall fatality rate was 7.5%. As botulism caused by canned and home-canned foods has decreased, other more unusual food types have become a source for the disease (147). Baked potatoes wrapped tightly in foil and held for prolonged periods of time were implicated as the source of toxin in potato salad in two restaurant outbreaks of botulism and as a possible causative food in another outbreak (239). In follow-up studies, potatoes inoculated with varying numbers of *Clostridium botulinum* spores were baked and permitted to remain at 22 or 30°C for various time periods. The higher the starting spore number, the shorter the time until toxin was produced; potatoes inoculated with 10 spores were toxic after 5 to 7 days (239).

In a restaurant outbreak of botulism in 1983, 28 persons became ill, and sauteed onions were the implicated food (149). Analysis of raw onions of the type sauteed for the implicated patty-melt sandwiches showed that some onions contained *Clostridium botulinum* type A spores (149). A laboratory recreation of the sautee process demonstrated that this *Clostridium botulinum* isolate produced toxin more rapidly than isolates from other sources (228). Cooking the onions in butter apparently provided the anaerobic condition necessary for spore germination and toxin production.

An outbreak of type B botulism affecting 36 persons was retrospectively discovered in late 1985 (237). The implicated food, chopped garlic in soybean oil, had not been kept under refrigeration by the restaurant involved. Follow-up laboratory studies confirmed that toxin can form in garlic when it is kept under oil at temperatures above refrigeration (H. M. Solomon and D. A. Kautter, J. Food Prot., in press). In both the sauteed onion and the garlic-in-oil outbreaks, the incriminated foods had been thought to be incapable of supporting the growth of *Clostridium botulinum* (228). These outbreaks demonstrated, however, that no food can be automatically discounted as a potential source of botulinal toxin. The significance of the finding that a *Clostridium botulinum* isolate usually associated with raw onions could grow and produce toxin in cooked onions (228) will be determined in future studies with other raw food-associated strains.

A small international botulism outbreak recently occurred in which ungutted, salted whitefish was shown to be the source of the toxin (251). The remains of partly consumed fish, known as ribyetz or kapchunka, and the serum of one patient yielded type E botulinal toxin. Other sporadic cases of botulism had previously been caused by this type of fish product (251).

Extensive reviews of infant botulism (7, 8, 144) report that food can be a vector for *Clostridium botulinum* spores. Why only certain infants develop botulism or why the toxin is absorbed only from the colons of certain infants has not been determined. Recently, two cases of an infant botulismlike syndrome have been reported, one in a 5-year-old child (191) and another in a 27-year-old adult (229). Both cases involved *Clostridium botulinum* toxin type F, and in both, the original diagnosis was Guillain-Barré syndrome. In the latter, toxin production correlated with numbers of viable *Clostridium botulinum* in the feces, which, in turn, correlated with an increase in serum type F toxin (229). The possibility of misdiagnosing botulism as Guillain-Barré syndrome has been discussed in classical botulism intoxications (147) as well as in mild cases of botulism (139).

Staphylococcal Food Poisoning

Food poisoning caused by *Staphylococcus aureus* has been recognized for several decades. At least five staphylococcal protein toxins, designated A through E, have been characterized and demonstrated to be fairly heat resistant. Many misconceptions about this disease were addressed by Holmberg and Blake (98) in a review of 131 outbreaks between 1977 and 1981, which affected 7,000 persons in the United States. The study showed that in several outbreaks toxin was present despite the absence of large numbers of coagulase-positive staphylococci, that food handlers (carriers) of the implicated *Staphylococcus aureus* bacteriophage type usually did not have obvious skin lesions, and that absence of fever did not exclude staphylococcal food poisoning. Contrary to popular belief, the disease is not necessarily mild; 10% of patients surveyed were admitted to hospitals. Although the overall case/fatality ratio was only 0.03%, in individual outbreaks it was as high as 4.4%. The study also showed no definite pattern of seasonality to staphylococcal food poisoning.

The emetic effects of staphylococcal enterotoxin B have been likened to a pseudoallergic reaction (214). When injected intradermally in monkeys, staphylococcal enterotoxin B elicited a skin response similar to a histamine-induced reaction (215). A selective inhibitor of leukotrienes D_4 and E_4, given orally, abrogated both the skin reaction to staphylococcal enterotoxin B and the emetic response caused by staphylococcal enterotoxin B given intragastrically (214). Staphylococcal enterotoxins were shown to be potent activators of T lymphocytes and inducers of T-lymphocyte-derived lymphokines (136).

The nucleotide sequence of the staphylococcal enterotoxin A gene was recently determined, and a degree of amino acid sequence relatedness was demonstrated with enterotoxins B and C_1 and with toxic shock syndrome toxin 1 (22). A 624-base natural DNA probe to the enterotoxin A gene hybridized to an internal fragment of the enterotoxin E gene. A 17-base oligonucleotide derived from the enterotoxin A gene sequence hybridized with strains producing enterotoxins A, B, C_1, and D. These results strongly suggest that considerable sequence divergence has occurred within the family of staphylococcal enterotoxins (22).

Protozoa

Protozoa may also be foodborne pathogens. They do not replicate in food, but the cyst form of the organism may remain infectious in food for prolonged periods. Very low numbers of these cysts (or oocysts) may cause disease. *Giardia lamblia* is highly infectious in the cyst form, and its transmission by food is well documented (14, 189). Although the mechanism by which *G. lamblia* induces diarrhea is uncertain, a cholera toxin-like protein isolated from *G. lamblia* partially cross-reacted serologically with cholera toxin and activated adenylate cyclase in a manner identical to cholera toxin (B. A. McCardell, personal communication).

Cryptosporidium parvum is infectious in the oocyst form, and its transmission by food has been suggested by epidemiologic investigations (68). The association with homosexual males and AIDS patients has stimulated research on this organism. Both giardiasis and cryptosporidiosis are now recognized as frequent causes of diarrhea in the general population. Improved methodology and increased awareness have certainly contributed to the apparent increase in incidence.

Entamoeba histolytica is more commonly a waterborne pathogen, but because contaminated water frequently becomes part of food, the potential for foodborne transmission exists.

Enterically Transmitted Viruses

Numerous gastroenteritis-causing viruses may be transmitted on food, usually through fecal contamination by food service workers (51) or by fecally polluted water. Rotavirus, Norwalk agent, echo- and coxsackieviruses, enteric adenoviruses, caliciviruses, astroviruses, coronaviruses, and parvoviruses have all been demonstrated to cause gastroenteritis directly, epidemiologically, or serologically (51). Viruses may be seen in intestinal biopsies or in feces by electron or immunoelectron microscopy. It is much simpler to observe or isolate viruses from feces than from foods. Virus particles are generally numerous in feces, which are relatively constant in composition; foods, however, vary greatly in composition, each requiring a different extraction procedure, and only a few virus particles may be present. Many of the gastroenteritis-causing viruses are difficult or as yet impossible to culture in vitro. Nevertheless, viral gastroenteritis, with food acting as a passive vector, is known to occur and may constitute a large part of total foodborne diarrheal disease.

Besides Norwalk agent, a family of structurally similar but antigenically distinct viruses, referred to in the United States as Norwalk-like viruses and in Britain as small, round, structured viruses, has been implicated as a frequent cause of gastroenteritis. One such virus, Snow Mountain agent, has caused at least one waterborne outbreak of gastroenteritis as well as foodborne outbreaks involving various finished foods (34, 90) and raw or baked hardshell clams (247).

Ice was recently implicated as the vehicle for a large, multistate outbreak of viral gastroenteritis (253). The ice had been produced from well water contaminated by flood runoff from a nearby stream. An estimated 5,000 cases of gastroenteritis occurred, with nausea and vomiting as the major symptoms (253). A 27-nm particle in the stool of one patient was observed by electron microscopy. Fortuitously, this stool specimen had not been frozen. In samples that were frozen, viral morphology may have been destroyed (253).

The rotavirus group commonly associated with human gastroenteritis is termed group A rotavirus. Besides group A, other morphologically similar but antigenically distinct rotaviruses exist. A rotavirus known to cause gastroenteritis in rats was associated with human diarrhea (66). Such agents are of particular concern in imported and stored foods, which are frequently contaminated with rodent feces and urine. Methods for detecting foodborne viruses are particularly inadequate. Several important viruses cannot be cultured in vitro, and food presents particular problems in isolating low numbers of viruses. Perhaps recombinant DNA technology will provide tools necessary to overcome some of these problems.

Hepatitis A virus has long been associated with foodborne outbreaks of hepatitis. Recently, an enterically transmitted non-A, non-B hepatitis virus (ET-NANB) that has caused waterborne outbreaks of viral hepatitis in Asia and the Indian subcontinent (32) was described, and the first ET-NANB outbreaks in the Americas were reported in Mexico (249). Although most outbreaks have been associated with contaminated water, ET-NANB has a fecal-oral route of transmission and therefore will probably emerge as a foodborne virus. ET-NANB hepatitis demonstrates a mortality rate as high as 20% for pregnant women (249).

ACUTE RESPIRATORY AND OTHER NONGASTROINTESTINAL EFFECTS OF ENTERIC PATHOGENS

First in order of occurrence in the United States are the respiratory diseases, some of which are caused either directly or indirectly by enteric pathogens. Several of the enteroviruses have been associated with mild upper respiratory illness (165). Coxsackieviruses A2, A10, A21, A24, B2, B3, B4, and B5 are among the causative agents. Echoviruses 1, 11, 19, 20, and 22 have been associated with respiratory symptoms; pneumonia caused by coxsackievirus A7, the echoviruses, and enterovirus has been reported (165) as have respiratory symptoms associated with cryptosporidiosis (92). It is not clear, however, whether these cases were acquired via the respiratory or the orogastric route.

Bacteria may also lead to respiratory illness, but again, the actual portal of entry is often difficult to determine. Substantial evidence shows, however, that group A streptococci can cause streptococcal pharyngitis through ingestion of contaminated food, and several outbreaks have been linked epidemiologically to salads prepared by individuals ill with pharyngitis (103). It is unknown at this time whether *K. pneumoniae* or *C. jejuni* can enter via the gastrointestinal tract, travel through the blood, and ultimately cause infections of the lung, but they can become bloodborne. Although there is ample evidence of infection with *C. jejuni* via food and water, there is no evidence for inhalation infection; yet lung infections with *C. jejuni* have been reported (26).

Infections with enteric pathogens have produced symptoms in virtually all organ systems. A few examples are circadian urticaria associated with *Campylobacter* infections (33); thrombotic thrombocytopenic purpura (173), hemorrhagic balanitis, and cystitis (87) associated with *E. coli* O157:H7-induced hemorrhagic colitis; erythema nodosum associated with *Salmonella* gastroenteritis (49); endocarditis associated with *Y. enterocolitica* infection (3); and meningitis caused by enteroviral infection (21).

FATALITIES ASSOCIATED WITH FOODBORNE DISEASE-CAUSING MICROORGANISMS

Although the case/fatality ratio of foodborne disease or enteric disease in general is low, the actual number of deaths is probably quite high when the large number of annual cases is considered. In addition, most deaths resulting from bacteremia are classified as just that, with little concern for the original portal of entry of the pathogen.

Cancer patients and other immunocompromised individuals are subject to bacteremia (28). Beebe (16), whose report addresses the source/portal of infection, has placed this problem in a new perspective, listing the following bloodborne bacteria as reflectors and indicators of cancer: *S. typhimurium*, *L. monocytogenes*, *A. hydrophila*, and *C. fetus*. He identifies the gastrointestinal tract as the portal of entry for all of these organisms except *L. monocytogenes*, whose source of entry he lists as unknown (16). (As previously stated, the orogastric route is now believed to be a major portal of entry for this bacterium.)

These organisms have a prediliction for persons with specific tumor types, for example: *S. typhimurium* and *L. monocytogenes* for those with Hodgkin's disease, leukemia, and lymphoma; *C. fetus* for those with leukemia; *A. hydrophila* for those with leukemia or carcinoma (16). The relationships are quite reliable, and when these organisms are isolated from the blood of seemingly normal persons, they

often signal the presence of an undetected neoplasm (16). In three separate hospital-based studies (137, 145, 209), 16 of 18, 10 of 64, and 11 of 18 listeriosis patients had demonstrable underlying malignancy. Nieman and Lorber (178) reported that *L. monocytogenes* was the leading cause of bacterial meningitis in cancer patients.

Other more common causes of bacteremia in cancer patients, particularly those receiving antineoplastic chemotherapy with its attendant immunosuppressive side effects, are *K. pneumoniae*, *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (16), all of which are encountered in or on food. *K. pneumoniae*, for example, reaches very high numbers in shellfish from warm waters; this observation forced consideration of a change in the coliform standards for shellfish. *P. aeruginosa* is a common food spoilage organism on many foods that contain proteins, and both *K. pneumoniae* and *P. aeruginosa* have gained access to hospitalized persons via food (40, 129). In nosocomial outbreaks of *K. pneumoniae* pneumonia, mortality rates may reach 50%, even with antibiotic therapy. The virulence of gram-negative bacteria such as *K. pneumoniae* involves more than capsular polysaccharides and lipopolysaccharides. Straus (238) recently characterized an extracellular toxic complex, composed of capsular polysaccharide, lipopolysaccharide, and protein, which is released from bacteria during all phases of growth. The toxicity of the complex may be responsible for the extensive lung damage caused by *K. pneumoniae*.

Elting et al. (69) reviewed the polymicrobial septicemias often lethal to cancer patients and listed the relative frequency of isolation of many bacteria from these patients. Most of these organisms are often encountered in foods. The same review (69) details the most frequent combinations of organisms encountered simultaneously in cancer patients, and, again, one is struck by the high probability that these organisms would be encountered in food.

Cancer patients are not the only group that may suffer fatal infections from foodborne pathogens. Mortality of non-cancer patients is associated with many of these organisms, particularly the gram-negative enteric bacteria. In some instances, predisposing factors are identifiable; in others, they are not. Wilson (260) reviewed the immunologic basis for increased susceptibility of the neonate to infection, which is related to the maturation times of specific immunologic mechanisms during neonatal development. Watson and Yunis (258) reviewed the immunologic effects of aging; the very old suffer a disproportionate number of fatal infections from foodborne pathogens. In their review, Torrey et al. (246) concluded that the incidence of bacteremia among urban infants following *Salmonella* gastroenteritis is 5 to 10%.

L. monocytogenes meningitis is often fatal in neonates, but in general the infection is actually perinatal, i.e., acquired in utero. If the fetus survives to term and is not prematurely aborted, it is born infected. The same can be said for *C. jejuni*, *C. fetus*, and *C. coli* infections (224), although in utero infection with these bacteria is far less common than with *L. monocytogenes*. The *Campylobacter* spp. can establish other potentially life-threatening infections in various organ sites, including the gallbladder and lung (26). The fatalities associated with *C. jejuni* infections were discussed by Smith and Blaser (225), who noted that fatal outcomes were associated with the use of antimotility drugs taken for gastroenteritis.

Viruses may cause death in certain individuals. In a case of fatal coronavirus infection in a 15-month-old infant, Rettig and Altshuler (196) reported evidence of coronavirus replication in the small intestine. Rotavirus infection remains the leading cause of childhood hospitalization for dehydration; fortunately, in the United States few children succumb to dehydration because proper rehydration therapies are quickly applied. Children in other parts of the world may not be as fortunate.

The clinical literature contains numerous scattered reports of death associated with microorganisms commonly found in food. The underlying, predisposing conditions of the victims are varied and unproven; they are merely observed or supported by epidemiologic evidence. For example, a young woman with a history of bulimia reportedly succumbed to a fatal *A. sobria* and *Plesiomonas shigelloides* dual infection following diarrhea that ensued after she inadvertently drank contaminated water (213).

People with AIDS are at elevated risk for fatal infections with several microorganisms frequently encountered in the food supply. Enteric infections by bacteria, viruses, protozoa, and fungi are common features of AIDS (70, 162, 210). Bacteremias caused by *Campylobacter*-like organisms are generally treatable (187). *S. typhimurium* bacteremia is sometimes observed before diagnosis of AIDS (82, 111), often without the usual gastrointestinal symptoms (230). Cytomegalovirus colitis may lead to death in AIDS patients, as do infections with the protozoan *Cryptosporidium* spp. and *Isospora belli*. *L. monocytogenes* infection was thought to be rare in AIDS patients, as discussed in a 1986 commentary (112). Subsequently, several cases of listeriosis in AIDS patients have been reported in the literature. Listeriosis occurs over 300-fold more often in AIDS patients than in the general public (156).

Finkelstein et al. (74) suggest that research be directed at determining the role of iron in microbial virulence. To be successful, a commensal or pathogen must compete for iron with iron-binding host proteins. The effects of increased free iron on the growth and pathogenicity of bacteria cannot be summarized here. However, one might ask which diseases involve mobilization of bound iron stores and whether these are the same diseases that predispose humans to fatal infections with foodborne pathogenic microorganisms. Bacteria have evolved elaborate mechanisms to acquire iron, particularly when the iron is sequestered in the body by transferrin or lactoferrin. The ability of *V. vulnificus*, with an associated mortality rate of 50% or more in persons with liver disease, to mobilize transferrin-bound iron was previously cited (172). Bacteria may produce special chelators (siderophores), special transport mechanisms, or special pathways to acquire iron. *L. monocytogenes* must acquire iron intracellularly to survive and, as such, has a reductive pathway to free iron from transferrin. Other intracellular parasites, such as *Mycobacterium paratuberculosis*, can directly utilize iron from intracellular ferritin stores (169). A high-affinity iron transport system for *L. monocytogenes* has not yet been demonstrated (50).

During any bacteremic situation, endotoxic shock may occur and cause death, and antibiotic treatment may actually exacerbate endotoxin release from gram-negative bacteria (220). The pharmacology of endotoxic shock, however, is complex and beyond the scope of this review.

The factors that may predispose humans to death from enteric infection are numerous and complex and, in some instances, cannot be readily identified. Although this discussion has dealt mainly with death as a direct result of enteric infection, death may also occur indirectly, that is, as a result

of the known or suspected sequelae to enteric infections. Some of these aspects are discussed later.

The number of deaths attributable to enteric infections (and the subset at issue, foodborne infections) is far larger than statistics would suggest. Characteristics of the strains of *C. jejuni* and *C. coli* involved in extraintestinal infections have been studied by Blaser et al. (26); however, in other extraintestinal infections involving enteric pathogens (e.g., *K. pneumoniae* in cancer patients), it is assumed that the acute pathogenic attributes of the organism dictate the risk to the predisposed person. This may not be the case. Therefore, the mechanisms by which usually innocuous microorganisms attack certain populations must be characterized to separate the truly harmless from the harmful environmental microbes. It is impractical to have a sterile food supply, but a better understanding of the nature of the entire spectrum of pathogens, whether for the general population or special population groups, is desirable and achievable.

CHRONIC DISEASES DIRECTLY OR INDIRECTLY CAUSED BY FOODBORNE PATHOGENS

A growing body of medical literature strongly suggests that foodborne pathogenic microorganisms may directly or indirectly cause or predispose humans to chronic diseases. In many instances, the microbes function as "environmental triggers." A complex interaction between the microbe or its products and the host immune system can lead to autoimmune reactions and resultant tissue damage in organ systems. The genetic makeup of the host also plays a role in the response of the host to a microbe or its products. In other instances, the organism or its products may disrupt intestinal integrity and permit entry of normally excluded substances, or disrupt nutrient transport, which may lead to nutritional deficits and, in turn, to immunologic deficits or autoimmune disease.

The immune system is clearly one of the body's most complex systems; it is composed of sets and subsets of cells with discrete functions and a complex system of chemical signals, which interact specifically with other cells of the immune system or affect organs in other body compartments. The immune system of the intestine, although related to the systemic immune system, is in many ways quite distinct, which is not surprising, given the enormous load of foreign material with which it must deal. Two recent reviews (67, 71) elaborate elegantly on the complexities of the intestinal immune system. Another study (255) of the murine immune system revealed that 80% of all immunoglobulin-secreting cells were located in the small intestine. The integrity and optimal functioning of both of these immune systems are crucial for survival.

The Microbe's Perspective

The pathogenicity or nonpathogenicity of a microorganism should not be viewed from the microbial aspect alone; a more holistic approach must be taken to fully understand the host-parasite interaction (108). The comprehensive review by Gotschlich (85) summarizes host defenses, immunologic and nonimmunologic (physical) barriers, and the evasive mechanisms attributed to various microorganisms. The list of mechanisms is impressive, and its variety emphasizes the difficulty of generalizing about the pathogenic traits of microorganisms. Recent developments in biomedical research suggest that two of the mechanisms are important in triggering several diseases of unknown etiology. These mechanisms are antigenic heterogeneity and molecular mimicry.

Antigenic heterogeneity (or phase variation) is the ability of some microorganisms to change their antigenic profile. This mechanism has been recognized for some time in such organisms as *Borrelia recurrentis* (relapsing fever), *V. cholerae*, and *C. jejuni*, which sometimes causes a recurring diarrheal syndrome (24). It was once thought that when an infection occurred, a population of pathogens with the same surface antigen was slowly eliminated by immunologic defense mechanisms, followed by expansion of a population of pathogens with different surface antigens (originally a minority population). It has since been demonstrated that many microorganisms are genetically programmed to produce a wide array of antigens but to express them in a deliberate, genetically triggered manner.

In the concept of molecular mimicry, major antigens of a particular microorganism cross-react with human tissue antigens. This concept was stated by Ebringer (64) in the context of a particular disease, ankylosing spondylitis, as the "cross tolerance hypothesis." From this hypothesis, one would predict that if a microorganism totally shared identical antigens with a host, the host would be destroyed, since no "foreignness" would be present to trigger an immune response. If host and microbe shared no antigens, the microbe would be rapidly eliminated. The middle ground, where microbes share some antigenic determinants, permits host and microbe to coexist, but in some persons the shared antigens, or shared epitope(s), may lead to problems. A good example of molecular mimicry is the long-recognized connection between group A beta-hemolytic streptococci and rheumatic heart disease (259).

Fujinami and Oldstone (76) have tested the hypothesis. Using the experimental allergic encephalomyelitis rabbit model, which closely mimics human multiple sclerosis, they studied the gene sequence of myelin basic protein and with computer-assisted analysis searched for common sequences with known viral proteins. A site within the hepatitis B virus polymerase gene which shared six identical amino acids was found. Synthetic octa- and decapeptides were constructed; they contained the six-amino-acid sequence common to myelin basic protein and two or four nonidentical amino acids to confer foreignness. Rabbits injected with these octa- or decapeptides demonstrated antibody that reacted with myelin basic protein. After a period of time, myelin sheath lesions similar to those in experimental allergic encephalomyelitis were seen. Lymphocytes from injected rabbits also reacted with myelin basic protein in vitro. Thus, molecular mimicry was demonstrated experimentally. One striking feature of the Fujinami and Oldstone work is that as little as a single shared epitope was sufficient to trigger a pathological process. Oldstone (181) refers to molecular mimicry as a hit-and-run event, wherein the immunogen is removed before the occurrence of immunologic injury. Norden and Kuller (180) discuss the ability of infectious agents, particularly those causing persistent infections, to trigger destructive immunologic processes in genetically susceptible individuals.

Although there are many examples of immunological cross-reactivity between microbial antigens and human tissue antigens, cross-reactivity alone does not necessarily indicate the potential to cause chronic disease; it may, in fact, reflect a normal mechanism by which long-lasting immunity to certain pathogens develops (11). Molecular mimicry is actually quite common (233). Obviously, for

chronic disease to be triggered, other factors must come into play.

Genetic susceptibility predisposes humans to some diseases. For example, the link between certain chronic diseases and specific human leukocyte antigen (HLA) types is well established, and a connection between ABO blood groups and certain gastrointestinal diseases has been suggested. The predisposition of persons with blood groups B and AB for gram-negative enteric pathogens other than *Shigella* spp. (200) and of persons with blood group O for *V. cholerae* (83) has been reported. In studies of urinary tract infections caused by gram-negative organisms, persons with blood group B had more infections with *K. pneumoniae*, *Pseudomonas* spp., and *Proteus* spp. than did those with blood group A (194). Susceptibility was related to the presence or absence of certain classes and types of isoagglutinins.

Joint Diseases

Two types of arthritis, septic and aseptic, may result from foodborne (and other) pathogens. Septic arthritis is a common and serious problem, arising from the ready accessibility of blood to the synovial space (84). Viable microorganisms can be recovered and cultured from the synovial fluid in this form of arthritis. Generally, the risk factors for septic arthritis are the same as those for sepsis. Treatment consists of joint aspiration, antibiotic therapy, and joint rest; response to treatment depends on host factors and the causative organism. Permanent joint damage may occur, even with successful treatment.

Aseptic arthritis is now known as reactive arthritis (119). Many rheumatologists accept reactive arthritis as a clinical entity, not merely one of the triad of symptoms associated with Reiter's syndrome. Reactive arthritis, Reiter's syndrome, and ankylosing spondylitis are seronegative spondyloarthropathies. All three are linked, to greater or lesser degrees, to the HLA-B27 gene. A person who suffers an enteric infection with any of a certain group of intestinal pathogens and is HLA-B27 positive has an 18-fold-greater risk of developing reactive arthritis, a 37-fold-greater risk of developing Reiter's syndrome, and an 87- to 126-fold-greater risk of developing ankylosing spondylitis than an HLA-B27-negative individual who suffers from the same enteric infection (4). Other genes, either related to or in concert with B27, may dictate which disorder is acquired.

The implicated triggering organisms for reactive arthritis include *Y. enterocolitica*, *Y. pseudotuberculosis*, *Salmonella* spp., *C. jejuni*, *Shigella dysenteriae* and *Shigella flexneri*, *E. coli*, and *K. pneumoniae*, the last-named organism being more associated with ankylosing spondylitis. The outlook for reactive arthritis is usually favorable, with symptoms lasting for <1 year in most persons, although 5 to 18% may have symptoms that last more than 1 year and 15 to 48% may experience multiple episodes of arthritis (119). *Y. enterocolitica* serotypes O:3 and O:9, of the particular biotypes and phage types endemic to parts of Scandinavia, Finland, and parts of Europe, seem to be highly arthritogenic, with some persons experiencing debilitating symptoms for years and an unfortunate fraction of those developing rheumatoid arthritis (153). Generally, 1 to 2% of persons infected with an arthritogenic strain of one of the aforementioned bacteria will suffer reactive arthritis (119); this case frequency takes into account the distribution of B27-positive individuals in the general population.

Following the 1985 Chicago milk outbreak of *S. typhimurium* gastroenteritis, Ike et al. (R. Ike, W. Arnold, C. Simon, G. Eisenberg, M. Batt, and G. White, Clin. Res. **34**:618A, 1986) reported an incidence of reactive arthritis of 2.3%, as judged by physical examination. Only patients who were confirmed positive for *S. typhimurium* in their stools were scored in the reactive arthritis study. Reiter's triad occurred approximately 10-fold less often than reactive arthritis (Ike et al., Clin. Res. **34**:618A, 1986). Curiously, only one patient was HLA-B27 positive, but that patient was the most seriously affected. The lack of correlation with HLA-B27 was also observed in reactive arthritis patients after an *S. typhimurium* outbreak in Canada (R. D. Inman, M. E. A. Johnston, B. Chin, J. Falk, and S. Vas, 51st Annu. Meet. Am. Rheum. Assoc. 1987, S25), in which 4 of 19 were HLA-B27 positive and 6 of 19 were HLA-B7 positive. In a 1-year follow-up of the Chicago study patients, Ike et al. (R. W. Ike, W. J. Arnold, and G. M. Eisenberg, 51st Annu. Meet. Am. Rheum. Assoc. 1987, S24) found that 20 of 29 reported persistent symptoms of reactive arthritis, and 6 had actually worsened. They noted a tendency towards chronicity and progression of the arthropathy associated with the Chicago salmonellosis outbreak (Ike et al., 51st Annu. Meet. Am. Rheum. Assoc.).

The mechanism by which bacteria trigger reactive arthritis is not yet clear, although the leading theory involves molecular mimicry of the B27 antigen by bacterial surface proteins; this theory has been supported experimentally by Chen et al. (45), who demonstrated a cross-reaction of a 19-kilodalton *Y. pseudotuberculosis* surface protein with HLA-B27.1 antigen. Whether an antibody- or cell-mediated immune reaction with resultant damage is triggered is still an open issue.

K. pneumoniae has been connected with ankylosing spondylitis for several years; victims of this disease frequently carry larger numbers of *K. pneumoniae* in their feces than do healthy individuals (65). Ankylosing spondylitis is similar to reactive arthritis, but symptoms are confined to the vertebrae. Keat (120) reviewed the current theories of what triggers spondylitis and the mechanisms involved. Molecular mimicry is a major theory; *K. pneumoniae* does, in fact, cross-react with B27 in a manner identical to that of other gram-negative enteric pathogens (254). Homology was demonstrated between six consecutive amino acids on the HLA-B27.1 subtype-coded protein and the nitrogenase enzyme of *K. pneumoniae* (218). The shared sequences are hydrophilic, suggesting that the molecules may be exposed on the surface of their respective cell and therefore accessible to the immune system. The presence of cross-reactive antibodies to the HLA-B27.1 and *K. pneumoniae* nitrogenase common sequences was demonstrated in the sera of ankylosing spondylitis and Reiter's syndrome patients. This finding suggests that a *Klebsiella*-triggered autoimmune mechanism may be involved in the disease of a subset of these patients (218). The report of the co-occurrence of Reiter's syndrome and AIDS may shed light on the immunologic mechanisms responsible for joint damage in Reiter's and other seronegative arthropathies (261).

A second theory that arose from molecular mimicry involves the active, plasmid-mediated synthesis of a "modifying factor," which alters the B27 antigen so that cells demonstrating modified B27 are susceptible to immune destruction (120). Whichever theory proves correct, there is no dispute about the bacterial triggers involved. Konttinen et al. (130) presented evidence that diseased joints in reactive arthritis are a site for active, but normally down-regulated, cell-mediated immunity. This implies that the loss of a

regulatory lymphocyte function may be involved in the pathologic process of reactive arthritis.

Although rheumatoid arthritis has been associated with a variety of microorganisms for decades, no specific microbe has been identified as the cause of the disease. Stanforth (234) reviewed the possible role of abnormal thiol-reactive immunoglobulin A (IgA) in rheumatoid arthritis and noted that much of the serum IgA is complexed with alpha-1-antitrypsin, a major antiprotease. High levels of similarly abnormal IgA are observed in ankylosing spondylitis patients, many of whom also demonstrate raised levels of IgA directed against *K. pneumoniae*. In the future, discovery of the mechanism of one of the arthropathies may lead to the solution of several disease mechanisms. Holoshitz et al. (101) reported that the reactivity of T lymphocytes from rheumatoid arthritis patients was augmented by a fraction of *M. tuberculosis* that cross-reacts with human cartilage. In an accompanying article, Ottenhoff et al. (184) provided evidence for an HLA-DR4-associated immune response gene for *M. tuberculosis* and suggested that this was a clue to the pathogenesis of rheumatoid arthritis. The capacity of mycobacteria to persist in phagocytic cells and become degraded very slowly would account for the chronicity of an immunologic (or inflammatory) stimulus (202).

Autoimmune Thyroid Diseases

Genetic and immunologic factors have been suspected of participating in the pathology of Graves' disease for nearly 40 years. Bech et al. (15) reported that antibody titers to *Y. enterocolitica*, serotype O:3 only, were found in Graves' disease patients significantly more often than in controls. This observation suggests that the O:3 serotype may trigger autoimmune thyroid disorders, particularly Graves' disease. Since that report, other evidence has suggested that receptor autoantibodies, which mimic the biological effects of thyroid-stimulating hormone, play a causal role. Heyma et al. (94) reported that thyroid-stimulating hormone binding sites were present on *Y. enterocolitica* and were recognized by immunoglobulins from Graves' disease patients. Thus, a causal role for molecular mimicry in the pathology of thyroid disorders is supported by scientific evidence. Further evidence, presented by Davies and Platzer (57), suggests that, in addition to autoantibody, a suppressor cell dysfunction may be involved in the pathology of Graves' disease.

Neural/Neuromuscular Disorders

The occurrence of Guillain-Barré syndrome following campylobacteriosis is an example of an empirical association of a chronic sequela with an acute gastrointestinal infection. Guillain-Barré syndrome is a rare sequela to Reiter's syndrome (6) and can be triggered by any enteric pathogen capable of causing Reiter's syndrome, including *C. jejuni*. The results of one retrospective study suggested that *C. jejuni* is the most common single, identifiable pathogen associated with Guillain-Barré syndrome and that persons with campylobacteriosis develop a significantly more serious form of the syndrome (113). Although considered rare, the actual incidence rate of Guillain-Barré syndrome is 1.7 cases per 100,000 population, making it one of the most common neurologic causes for hospital admission (166). It is a potentially life-threatening syndrome with principal symptoms of acute weakness, autonomic dysfunction, and respiratory insufficiency, generally striking those under 40 years of age. The report of Budka et al. (35) of the association of IgA2 subclass antibodies with the myelin of neural tissue from Guillain-Barré patients was the first demonstration of IgA antibodies in the nervous system. IgA2, a subclass of IgA, is preferentially synthesized in the gastrointestinal tract. The finding that IgA2 antibodies specifically react with myelin suggests that a selective antigen-dependent triggering of these antibodies precedes demyelination in Guillain-Barré and that an enteric antigen may be the triggering immunogen (35). In the same study, IgA2 antibodies were specifically associated with the myelin of neural tissue from adrenoleukodystrophy patients.

Koski et al. (131) discovered that immunoglobulin isotypes directed against peripheral nerve myelin were, in some instances, IgM, an antibody capable of fixing complement component 1. A cross-reactivity between *C. jejuni* outer membrane proteins and the peripheral nerve myelin has not been demonstrated. Koski et al. (132) further identified the terminal attack components of the complement cascade (C5b to C9) to be associated with antiperipheral nerve myelin antibody and observed that the terminal complement complex and IgM localized in the affected peripheral nerves of a Guillain-Barré patient. However, antibodies with antilymphocyte cytotoxicity have also been detected in Guillain-Barré patient sera (219); thus, generalizations about the mechanism of nerve damage seem premature.

Both adult and pediatric cases of *Campylobacter*-associated Guillain-Barré syndrome have been reported (58). For the inflammation to become chronic, the antigenic stimulus must persist. This may occur for mycobacteria, as previously mentioned, but persistence of antigen without the presence of viable bacteria has been suggested for *Y. enterocolitica* (256) and *C. jejuni* (123) infections.

A large body of evidence supports the role of autoantibodies to the acetylcholine receptor in the pathology of myasthenia gravis. Stefansson et al. (235) reported that the antigenic determinants were shared between the acetylcholine receptor and surface proteins of certain strains of *E. coli*, *Proteus vulgaris*, and *K. pneumoniae*. This represents another example of the possible involvement of molecular mimicry in a pathologic process.

Two other observations of autoimmunity relate to foodborne pathogens: acceleration of the onset of systemic lupus erythematosus symptoms by prolonged polyclonal stimulation of mouse B cells with bacterial endotoxin (91), and the greater expression of protooncogenes, c-*myc* and N-*ras*, by lymphocytes from human systemic lupus erythematosus patients than from normal controls (126). Bacterial products mitogenic for both T and B cells of humans have been shown to activate protooncogenes.

Nutritional Disturbances, Gastrointestinal Permeability, and General Immunity

Enteric pathogen-induced diarrheal episodes may lead to malabsorption of nutrients, anorexia, and efflux of fluids, electrolytes, and nutrients, all of which result in some degree of malnutrition. The enteric pathogens known to cause malabsorption and specific nutrient losses were reviewed by Rosenberg et al. (205). Even the nutrient stress of diarrhea of short duration may cause subtle changes in immunologic status. Chandra (44) pointed out that marginal malnutrition, such as occurs during diarrheal episodes, may cause subtle defects in immunity. Thus, diarrhea may indirectly contribute to overall morbidity by reducing nutritional status, which then compromises immunity. More severe situations have been reported. *C. jejuni* was shown to be the cause of an

infant's prolonged diarrhea and malabsorption, which led to failure to thrive (190).

Aside from nutrient loss, enteric pathogens may alter gut permeability so that normally excluded proteins are taken into the systemic circulation, where they may activate components of the immune system, possibly with deleterious consequences, such as induction of atopy and autoimmunity, particularly immune-complex-mediated diseases (257). Such permeability alterations have been reported in studies in which radiolabeled molecules of known size were administered during acute gastroenteritis in children (75). Using a piglet model and the porcine equivalent of human rotavirus infection, transmissible gastroenteritis virus, Keljo et al. (121) demonstrated that a significant quantity of intact bovine serum albumin traversed the virus-damaged gut. The peak uptake, reflected by circulating bovine serum albumin, coincided with maximal diarrhea.

The destruction of brush border enzymes such as lactase may contribute to prolongation of diarrhea (1). Research and controlled human studies are needed to delineate the pathologic processes that occur in the intestinal tract during diarrheal episodes.

Diseases of the Heart and Vascular System

Several known foodborne pathogens have been associated with endocarditis and myocarditis either directly (bacteria and viruses) or indirectly. Heart damage induced by either means may be permanent. Persons with ankylosing spondylitis (linked to gastrointestinal pathogens as triggering agents) demonstrate a high incidence of cardiac conduction disturbances (19), which may be sequelae to other seronegative arthropathies as well (20). Aortic insufficiency and mitral valve damage in ankylosing spondylitis patients have been observed in patients in the United States (199). Kandolf et al. (115) demonstrated enteroviral ribonucleic acid in myocardial cells by in situ hybridization with a coxsackievirus B3 complementary DNA probe. Probably because of the high degree of nucleic acid sequence homology among the enteroviruses, the complementary DNA probe reacted with several enteroviruses, including coxsackieviruses B1 to B6 and A9, echoviruses 11 and 12, and poliovirus 1 (115). This approach may facilitate identification of etiologic agents of various forms of heart disease.

Davies (56) outlined the events that occur during early atherogenesis. Two of the three early steps involve focal mononuclear leukocyte infiltration and accumulation and large intracellular cholesterol ester deposits in macrophages. Monocytes or macrophages accumulate within the blood vessel, where they interact with both smooth-muscle cells and endothelial cells. In the first step of this process, monocytes attach to the endothelium, but the chemoattractant signal for adhesion has only recently been ascertained. Libby et al. (142) have shown that cultured endothelial cells produce interleukin-1-beta in response to bacterial endotoxins; this chemoattractant signal may lead to monocyte adherence and, ultimately, to recruitment of more monocytes and granulocytes, which in turn produce more interleukin-1. Libby et al. (142) postulated that this novel autocrine mechanism and amplification loop, resulting from hematogenous infection with endotoxin-containing pathogens, could contribute significantly to the vasculitides and arteriosclerosis. Duncan et al. (63) demonstrated that the immunologic activity of lipopolysaccharide (endotoxin) released from macrophages that have taken up intact *E. coli* is potentiated 10- to 100-fold in its ability to induce interleukin-1. Thus, gram-negative bacteria engulfed by macrophages on or near endothelial tissues may provide a potentiated interleukin-1-inducing signal. The possible connection between foodborne gram-negative bacteria and atherosclerosis was recently reviewed (5).

Renal Diseases

IgA glomerulonephritis, a probable immune-complex-mediated disorder, may be caused by any prolonged stimulation of the IgA-producing lymphoid tissues (e.g., by enteric pathogens) or may result from the formation of IgA immune complexes in response to an influx of dietary antigens (e.g., in instances when intestinal integrity is compromised). Genetics evidently plays a role, because certain persons produce high levels of IgA and others do not (186). Any large load of antigenic material entering the general circulation, including infectious agents, can lead to glomerulonephritis. IgA nephropathy appears to be increasing in the United Kingdom (12).

Hemorrhagic colitis, caused by *E. coli* O157:H7, is frequently followed by HUS in both children and adults (175). HUS is characterized by a triad of symptoms: acute renal failure, thrombocytopenia, and microangiopathic hemolytic anemia. The Vero (Shiga) toxin produced by certain enteric pathogens is presumed to play a key role in the syndrome (38). As previously mentioned, at least two distinct Vero toxins exist (152). Invasive Vero toxin-producing organisms lead to HUS with poor prognosis, whereas the prognosis of HUS associated with noninvasive Vero toxin-producing organisms is generally good. Episodes of HUS following gram-negative pathogen-induced diarrhea are well documented. *Shigella*, *Salmonella*, *Campylobacter*, *E. coli*, and *Y. pseudotuberculosis* infections have all been linked to the disorder. Vero toxin directly damages vascular endothelium (38). In addition, Butler et al.(36) showed that patients who had shigellosis when admitted to a hospital were far more likely to develop HUS if inappropriate antibiotic treatment (antibiotics to which the bacteria are resistant) was given on admission. The authors suggest a role for endotoxin in HUS; in fact, a rabbit model demonstrated renal cortical necrosis in response to injected endotoxin from *Shigella dysenteriae* 1 and *Shigella flexneri* (37). Thus, more than one causative mechanism, or in some circumstances a combination of mechanisms, may be required for HUS. Perhaps some organisms produce either a toxin similar to Vero toxin or toxins with peptide sequences similar to the active site of Vero toxin.

As previously mentioned, thrombotic thrombocytopenic purpura, a syndrome first described in 1925 (197), was recently linked to *E. coli* O157:H7 (173). Thrombotic thrombocytopenic purpura, which is composed of the same triad of symptoms as HUS, also includes fever and neurologic symptoms (197). Several enteric bacterial pathogens cause both fever and neurologic symptoms by various mechanisms, including endotoxin. Thrombotic thrombocytopenic purpura has a high mortality rate.

CONCLUDING THOUGHTS

Knowledge Gaps

Even though the application of recombinant DNA technology has greatly increased our understanding in a short time, a great deal remains unknown about the virulence mechanisms of most enteric pathogens. The best animal

models do not adequately mimic the intestinal tract of humans, and although in vitro tests are valuable, they are not always definitive.

Roszak and Colwell (206) point to an area of potentially great concern in foodborne disease research. To directly link a disease-causing agent with a food vector or any other environmental source, the agent must be culturable from that source. Roszak and Colwell (206) force the microbiologist to question the supposition that if a bacterium is there, it can be cultured by existing methods. They presented evidence that marine vibrios may be unculturable, yet may cause disease. Brayton et al. (P. R. Brayton, R. R. Colwell, B. D. Tall, D. Herrington, and M. M. Levine, 23rd Joint Conf. Cholera 1987, p. 21) rendered an attenuated strain of *V. cholerae* unculturable by incubation in phosphate-buffered saline at 4°C and then fed the cultures to human volunteers. Viability (52) was ascertained by acridine orange staining, and unculturability was verified throughout. Culturable *V. cholerae* cells were isolated from the stools of two volunteers (Brayton et al., 23rd Joint Conf. Cholera 1987), suggesting that human passage effectively triggers the growth of otherwise unculturable (dormant) *V. cholerae*.

Culture methods developed for isolating a particular bacterium, e.g., *Campylobacter* spp., from clinical material are often applied to finding the organism in other environments. Some microbiologists believe that if a microorganism will not grow on standard plating media, an enrichment is required. Again, Roszak and Colwell (206) question whether that is always the appropriate approach. Their work may answer many questions regarding the whereabouts and true numbers of pathogens in the environment.

The approach taken by Falkow et al. (72) on bacterial invasion, if applied to other poorly understood pathogens, may advance our understanding of how secretory diarrheas are caused. Their findings imply that, besides attachment to epithelial cells, direct invasion of the epithelium, or traversal of the M cell of Peyer's patch, bacteria may induce their own entry into epithelial cells by a very simple, single gene-mediated mechanism. This implies that the cholera-coli family of toxins may cause secretory diarrhea from inside the epithelial cell as well as from without. Investigations into the cholera-coli family of enterotoxins by Finkelstein et al. (73) suggest that a related family of toxins exists within a large spectrum of bacteria.

Many major virulence determinants may still have been overlooked; others have not been fully studied. Endotoxins, for example, are potent pharmacologically active compounds that may play a role in both reactive (195) and rheumatoid (105) arthritis. In some animal models, endotoxin promotes the translocation of bacteria from the gut to the mesenteric lymph node, another means by which bacteria may gain entry into the host (59). This increased permeability of the gut to bacteria and their products is important to our understanding of how systemic infections arise. Viral disease of the gastrointestinal tract was previously discussed in the context of increased permeability to large protein molecules (121). Thus, gastrointestinal pathogens may contribute greatly to systemic antigenic challenge during active disease. It is now generally accepted that the progression of HIV infection to AIDS is, at least in part, accelerated by antigenic activation of the CD4+ lymphocyte (151). Mock and Roberts (168) hypothesized that a variety of agents, infectious and noninfectious, may affect progression of HIV seropositivity to clinical AIDS. Ranki et al. (192) presented evidence that HIV infection may be present long before seropositivity by existing methods is apparent. Although this finding is still unconfirmed, it underscores the importance of understanding the relationships between pathogenic microorganisms and gastrointestinal integrity and physiology.

The subject of molecular mimicry will no doubt expand along with our knowledge of the genetic makeup of humans and microorganisms. The advantage(s) to the microorganism that can mimic the tissues of its host has been discussed, but the significance goes beyond survival in the host. For example, both human cells and bacteria produce heat shock proteins in response to environmental stress. Numerous genera of bacteria produce structurally related heat shock proteins, demonstrating a high degree of evolutionary conservation (221). A human heat shock protein and the Lon protein of *E. coli* share at least one antigenic determinant (138). Besides the evolutionary implications and the molecular mimicry involved in this relationship, the heat shock phenomenon may have additional importance. Heat shock proteins (produced in response to stresses other than just heat) may affect our ability to culture some microorganisms from "stressful" environments. If present during food processing, they may afford heat resistance to bacteria and permit their survival through normally lethal processes. Sublethal heat shock of *S. typhimurium* increases its resistance to thermal inactivation by 2.6- to 20-fold (150). This aspect of foodborne pathogens has not been adequately researched and is of potential importance in food-processing plants. Heat shock may also play a role in expressing virulence factors in several prominent foodborne pathogens, including *Shigella* spp. (158).

There are great opportunities and challenges in all subdisciplines involved in foodborne disease research. The basic findings are ultimately providing answers or asking relevant questions that affect more practical, applied areas of food safety.

Communication Gaps

There appears to be a communication gap among workers in the area of foodborne disease research. The field is becoming so vast, with subdiscipline involvement of epidemiology, molecular biology, clinical sciences, basic pathogenic and environmental microbiology, immunology, and virology, that it becomes an enormous task to see the broad picture. This review points out the problem by examples, but solutions can be provided only by research and integration of the fields. Better communication between the major subdisciplines would focus research and give investigators a clearer understanding of their place in the broader scheme.

Food is becoming recognized as a major vector of acute enteric disease and other significant diseases, both acute and chronic. The evidence that foodborne pathogens play a role in chronic diseases "of unknown etiology" varies from preliminary to convincing. Foodborne disease is a major problem, in terms of both morbidity and mortality, and cannot be overlooked. Proof that it may play a role in modulating other disease processes, e.g., HIV-related diseases and respiratory tract diseases, further underscores the need for integrated research and expanded communication.

Foodborne diseases are, for the most part, preventable. Knowledge of hygienic practices, processing techniques, and epidemiology gives the public health community the investigative tools necessary to prevent such disease. To do so, however, will take commitment. What is needed is a full understanding of the impact of foodborne disease. That some diseases are transmitted by food is not an indictment of the food supply. There is an inherent risk associated with

consuming certain uncooked foods such as meats, poultry, eggs, fish, and shellfish. When adequately cooked, and unless recontamination occurs, the food is safe. Even raw vegetables present a small but real risk unless they are washed sufficiently to remove soil and other extraneous material. The safety record of commercially processed foods in the United States is also remarkably good; home-processed foods have far more frequently led to diseases such as botulism.

Food also becomes a vector of disease when it is contaminated by people who use poor or unhygienic food-handling practices. The public must be aware of the ways in which harmful microorganisms enter the food chain and how they can be avoided. This will require public education programs such as those adopted by several other industrialized nations. Most importantly, the message must reach the homemaker.

Foodborne disease is part of a cycle that includes waterborne disease, person-to-person transmission, and environmental contamination; these factors contribute to foodborne disease, which in turn contributes to total disease. If ignored, the cycle becomes self-sustaining; attacking only one part of the cycle will not achieve maximum results. A concerted effort to prevent foodborne disease is needed to produce a significant and positive effect on public health in the United States.

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