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BUSINESS OFFICE: H. Thompson Price, Jr., Secretary-Treasurer
Room 900, 2nd & Chestnut St.
Philadelphia, Pennsylvania 19106

EDITORIAL OFFICE: Orlen J. Wiemann, Editor
6890 So. Clarkson St.
Littleton, Colorado 80122

THE "NEW" PATHOGENS: AN UPDATE OF SELECTED EXAMPLES¹

Robert L. Buchanan
Microbiological Safety Group
Food Safety Laboratory
Eastern Regional Research Center
ARS, USDA
Philadelphia, PA 19118

INTRODUCTION

The past decade has seen a tremendous increase in available scientific information, with the biological science having been one of the particularly active areas for scientific advances. Among the various subdivisions of biological research, food safety microbiology has been one of the most active, and there has been a dramatic increase in our understanding of bacterial disease associated with foods. This has included both increased understanding of the types of microorganisms that are capable of causing food poisoning, and the mechanisms by which these bacterial species can elicit gastroenteritis.

Until approximately ten years ago, almost all fully confirmed foodborne outbreaks of gastroenteritis were traced to *Staphylococcus aureus*, *Clostridium perfringens* or *Salmonella*, the major etiologic agents of bacterial food poisoning in the United States. These bacteria have been well characterized as foodborne pathogens, and considerable information is available concerning the foods they are associated with, the diseases they cause, and the food sanitation protocols needed to prevent their presence in foods and food products.

While these microorganisms are the major foodborne pathogens in the United States, it was often not fully appreciated that these organisms accounted for only 60%-65% of the reported food poisoning outbreaks. The majority of other reported incidences of food poisoning were simply listed under the category of "unknown etiology." Recent improvements in methods for isolation and identification of bacteria, and a better understanding of how bacteria produce gastroenteritis-type diseases, have led to significant progress in determining the agents that cause food poisoning outbreaks previously attributed to unknown causes. As a result of this progress, the list of known or suspected foodborne pathogens has almost doubled. Furthermore, some of the basic precepts of food safety microbiology are being modified as a result of this new information.

For the purposes of the current discussion, food poisoning bacteria have been subdivided into two groups: those that are well known and those that have come into prominence as potential foodborne pathogens during the past decade (Table 1). The objective of this paper is to summarize information on the current status of four of these "new" pathogens, *Campylobacter fetus* subspecies *jejuni*, enteropathogenic *Escherichia coli*, *Yersinia enterocolitica*, and *Aeromonas hydrophilia*. These species have been selected because either initial assessments indicate that the microorganism may be major food

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poisoning bacteria, or the species possess unique characteristics of interest to individuals charged with assuring the safety of the U.S. food supply.

TABLE 1.

WELL-KNOWN FOOD POISONING BACTERIA	THE "NEW" PATHOGENS
<i>Staphylococcus aureus</i> <i>Salmonella spp.</i> <i>Shigella spp.</i> <i>Clostridium perfringens</i> <i>Bacillus cereus</i>	<i>Campylobacter fetus</i> subsp. <i>jejuni</i> <i>Aeromonas hydrophilia</i> enteropathogenic <i>Escherichia coli</i> <i>Vibrio parahaemolyticus</i> <i>Vibrio cholera</i> NAG <i>Vibrio fluvialis</i> <i>Plesiomonas shigelloides</i> <i>Treponema hydroysenteriae</i>

CAMPYLOBACTER FETUS SUBSPECIES JEJUNI:

One of the microorganisms that has received a great deal of attention in the last several years is *Campylobacter fetus* subspecies *jejuni* (also designated in the literature as *Campylobacter jejuni* or as *Vibrio fetus* in the earlier literature). This is not a newly identified species. It was originally isolated by McFadyean and Stockman from aborted cattle in 1909, and as early as 1957, King suggested that it might play a role in the etiology of human gastroenteritis (5, 86). However, it was not until the early 1970's that adequate methods (14) began to be developed for its efficient recovery from stool, food, and water sample; thereby allowing a systematic assessment of the bacterium as a human enteric pathogen. Since that time a substantial body of information has been gathered concerning this microorganism, and it is now generally accepted that *C. fetus* subsp. *jejuni* is as important as a causative agent for human gastroenteritis as *Salmonella* (4, 26, 57, 62). These data further suggest that *C. fetus* subsp. *jejuni* should be considered among the most prevalent of the foodborne and water-borne pathogens in the United States.

The symptoms of *Campylobacter* enteritis (Table 2) are similar to those observed with other gastroenteritis syndromes, with diarrhea, containing blood and mucus, being the predominant feature in severe cases (5). Other common symptoms include vomiting, nausea, headache, and abdominal cramps. Fever may or may not be evident. The severity of the symptoms varies greatly among infected individuals, and is likely to be dependent on the initial inoculum size, the virulence of the specific strain contracted, and the resistance of the host. Typically, the onset of symptoms occurs within 2 to 5 days after ingestion of the microorganism, and persist for 48 to 72 hours. In some individuals symptoms can persist for several weeks, and reports of relapses are relatively common. After the acute phase of the disease, *Campylobacter* is generally cleared from the intestinal tract within 4 to 7 weeks (40). However, a portion of the individuals will continue to harbor the

bacterium for up to a year (66), and the possibility of asymptomatic carriers working as food handlers is of potential significance. In addition to the symptoms already described, recent studies by Kosunen (45) have indicated that a small proportion of infected individuals will also experience episodes of reactive arthritis.

TABLE 2.

SYMPTOMS OF ENTERIC *Campylobacteriosis* (50).

INCUBATION:	2 - 5 days post-ingestion
SYMPTOMS:	Mild to Severe Dysenteric Syndrome -Diarrhea (with blood and mucus in severe cases) -Abdominal Cramps -Nausea -Vomiting -Headache -With or Without Fever
RECOVERY:	Generally 2 - 3 days, though in some cases symptoms may persist for several weeks. Fatalities Rare
TREATMENT:	Electrolyte Replacement + Antibiotics

The mechanism of pathogenicity associated with *Campylobacter fetus* is not fully known. Generally polymorphonuclear leukocytes are present in the stools of infected individuals, this being indicative of an invasive-type disease (4). Likewise, intestinal biopsy studies with test animals indicate an invasive disease process. However, several research groups (47, 55, 68) have recently reported that *C. fetus* subsp. *jejuni* also produces an enterotoxin having a molecular weight of 68,000 daltons and resembling the enterotoxin produced by *Vibrio cholera*. The ability of the microorganism to produce this enterotoxin appears to be correlated with the presence of a 44 megadalton plasmid. Clarification of the exact roles of both invasiveness and enterotoxin formation in *Campylobacter gastroenteritis* awaits further research studies.

C. fetus subsp. *jejuni* is ubiquitous, and has been isolated worldwide. One characteristic of the microorganism that makes it unique is its rather strict atmosphere requirements. It grows best in a microaerophilic environment composed of 5% O₂, 8% CO₂, and 87% N₂ (54), and will grow in air only in conjunction with large inocula. This is the prime reason why *C. fetus* was not recognized as a major food poisoning bacteria until the 1970's.

A second unique characteristic of this organism is the rather narrow temperature range that will support growth. Most strains of the *C. fetus* subsp. *jejuni*, which account for more than 99% of the isolates associated with human gastroenteritis (5), grow over a temperature range of 32° to 45°C, with optimal growth occurring from 42° to 45°C (15). While this represents a rather limited temperature range, the microorganism can survive up to several weeks in refrigerated foods, and for extended periods in frozen products (9, 32).

A third important determinant of *C. fetus* subsp. *jejuni* growth is the sodium chloride content of the environment. The microorganism is salt sensitive: it does not grow in 2.5% NaCl, and only grows poorly at 2.0% (33). Those food products containing 2% NaCl in their aqueous phase would not be expected to support the growth of the pathogen.

The natural habitat of this bacterium is the intestinal tract of a variety of mammalian and avian species (25, 34, 78, 82). It is particularly prevalent in birds, possibly due to their elevated body temperatures. The organism is commonly isolated from a variety of raw animal products including poultry, pork, milk, and veal (25, 53, 61, 77, 85). To date, sources implicated in outbreaks of *Campylobacter* gastroenteritis have included water, poultry, unpasteurized milk, clams, and food handlers (5, 86). There are also indications that pets, particularly puppies and kittens, may represent a potential source of the organism in household-limited outbreaks.

Because of its ubiquitous nature, it is unlikely that *C. fetus* subsp. *jejuni* could be completely removed from raw foods. However, proper food handling and sanitation practices should virtually eliminate the risk of food poisoning from this microorganism. It is heat sensitive, and heating to 60°C for 15 minutes assures its elimination (15). This means that normal pasteurization and most types of cooking would destroy the bacterium. However, very mild heat treatments may not be sufficient. For example, it is likely that viable *Campylobacter* would survive in the center of a rare hamburger. The microorganism does not appear to have any significant resistance to sanitizing agents, and normal sanitation practices should ensure control of the pathogen. As previously mentioned, an area of possible concern is transmission from infected food handlers, and adequate personal hygiene is mandatory. Likewise, individuals known to harbor the microorganism should not be in contact with food products, particularly those that will receive no further heat treatment. In general, *C. fetus* subsp. *jejuni* has resistance characteristics equivalent to *Salmonella*. One of the reasons we have probably not seen more cases of *Campylobacter* enteritis is that current food handling practices have been largely designed to eliminate hazards associated with *Salmonella*, and these practices and procedures would also be expected to eliminate hazards associated with *Campylobacter fetus* subspecies *jejuni*.

ENTEROPATHOGENIC *ESCHERICHIA COLI*:

Another well known bacterium that has only relatively recently been implicated as a foodborne pathogen is *Escherichia coli*. In fact, *E. coli* has been the most intensely studied microorganism, and has become the prime biological system for studying biochemical and genetic processes. It had been generally assumed that this bacterial species was nonpathogenic, and the basis for the coliform test used extensively in food and water microbiology was that *E. coli* is a nonpathogenic indicator organism for *Salmonella*. However, over the years there continued to be isolated reports of diarrheal disease outbreaks where the only bacterial species isolated in unusually high numbers was *E. coli*. This was particularly evident in individuals suffering from travelers diarrhea and infantile diarrhea.

It was ultimately determined that a percentage of *E. coli* strains existing in nature are capable of producing either a dysentery-like or a colera-like gastroenteritis, with the latter being the more prevalent (44, 70). During the past several years, these enteropathogenic *E. coli* strains have been studied intensely, and the results of this research have greatly enhanced and altered our understanding of how foodborne bacteria act as enteropathogens. It has also been established during this period that enteropathogenic *E. coli* represents one of the major causes of both travelers and infantile diarrhea in this country and throughout the world (3, 63, 70), and it appears that one of the prime vectors for the organism can be foods (44).

TABLE 3.

CHARACTERISTICS OF ENTEROTOXINS ASSOCIATED WITH ENTEROTOXIGENIC *Escherichia coli*.

HEAT-LABILE ENTEROTOXIN: Molecular Weight = 91,440	HEAT-STABLE ENTEROTOXIN: Molecular Weight = 1,972; 18
Cross-React with Cholera Toxin	Amino Acid Peptide
Stimulates Adenylate Cyclase	Stimulates Guanylate Cyclase
Plasmid-Linked	Plasmid-Linked

The induction of cholera-like symptoms in humans by specific *E. coli* isolates is associated with their ability to synthesize a heat-stable enterotoxin and/or a heat-labile enterotoxin (Table 3) (70, 90). The heat-labile enterotoxin is a protein that has a molecular weight of approximately 91,440 daltons (11), though there are indications that there may actually be a family of closely related heat-labile enterotoxins, since enterotoxins from human and porcine isolates have been reported to differ immunologically (23, 35). The heat-stable enterotoxin is a 18-amino acid peptide (molecular weight + 1,972 daltons) (8), and it appears that the heat-stable enterotoxins from *E. coli* strains isolated from humans, cattle, and swine are identical (71).

Several factors concerning these enterotoxins have stimulated the interest of a large number of microbiologists and molecular biologists. The first is that the genetic material encoding the enterotoxins is not part of the normal complement of *E. coli* DNA. Instead, the genes for the enterotoxins are generally associated with 55-61 megadalton plasmids that can be transferred among *E. coli* strains (18). Furthermore, enterotoxin synthesis is not the sole determinant of virulence in enterotoxigenic *E. coli*. To be fully pathogenic, strains must also be capable of producing an appropriate adhesion factor, with some the the better known being designated the K88, K99, and 987P antigens. These adhesion factors are proteins located on the surface of the bacterium which allow it to adhere to and colonize the intestinal tract (22). Like the enterotoxins, the adhesion factors of enterotoxigenic *E. coli* are associated with plasmid-linked genes (18).

A second factor that has stimulated interest in the enterotoxigenic *E. coli*

has been the identity of the heat-labile enterotoxin. Physiologically and serologically, it produces responses almost identical to those observed with cholera toxin (10, 56, 84). Studies of this type have indicated that the two toxins are very similar both biochemically and genetically, and have led to speculation that at some point in time normally nonpathogenic strains of *E. coli* may have acquired the gene for enterotoxin synthesis from the cholera organism.

A third characteristic of *E. coli* that has become of interest to food safety microbiologists is the temperature range which supports growth and enterotoxin synthesis. While *E. coli* is classified as a mesotrophic species and grows best at approximately body temperature, it is capable of slow growth in pure cultures at temperatures just above the freezing point of water. Initial assessments (52) indicated that heat-labile and heat-stable enterotoxins are synthesized in conjunction with temperature ranges of 25°-40°C and 30°-35°C, respectively. However, a recent report (60) indicated that *E. coli* produced enterotoxin in culture medium at 4°C, but that synthesis did not occur in sterile milk. Just as important, the microorganism grew slowly at 4°C, and potentially could reach the minimum infectious dose level (3×10^4 /g) within a reasonable timeframe. The exact public health significance of enteropathogenic *E. coli* growth at refrigeration temperatures awaits adequate assessment, and will undoubtedly be a research area of interest during the next several years.

YERSINIA ENTEROCOLITICA:

One of the basic precepts of food sanitation is that while refrigeration only retards microbiological spoilage, it does prevent the growth of foodborne pathogens. This country relies heavily on the use of refrigeration as a means of controlling bacteria of public health significance at all levels of food production, distribution, and consumption. As would be expected, the identification of pathogens capable of growth at normal refrigeration temperatures would be of extreme interest both to food safety microbiologists and public health officials charged with assuring the safety of our food supply. Potentially, identification of such a microorganism could mean that a substantial review and possibly a revamping of present food sanitation protocols employing refrigeration would be necessary to insure that current procedures are adequate for controlling a psychrotrophic pathogen. During the last several years this possibility has been given serious consideration as a result of *Yersinia enterocolitica* and *Aeromonas hydrophilia* having been identified as potential foodborne pathogens.

The possibility that adequate refrigeration may not totally guarantee control of foodborne pathogen growth began to be seriously considered in 1976 after a highly publicized outbreak of *Y. enterocolitica* food poisoning occurred among grade school children in a small community in New York (2, 73). While it has been suspected earlier that *Y. enterocolitica* could produce a food poisoning syndrome (79), the New York outbreak definitively established the microorganism's role as a foodborne pathogen, and brought it to the attention of the scientific community. In this outbreak 220 children were affected, with

36 hospitalized with apparent acute appendicitis. Before it could be established that the patients were suffering from *Yersinia*-mediated pseudoappendicitis, 16 of the children had already undergone emergency appendectomies.

TABLE 4.

A CASE STUDY OF A MAJOR FOODBORNE OUTBREAK of
Yersinia enterocolitica GASTROENTERITIS (73).

WHO:	35% of 455 campers and staff attending summer camp, July 1981, Liberty, NY
SYMPTOMS:	Abdominal Pain Fever Diarrhea Vomitting Appendectomies performed on 5 of 7 hospitalized campers
INVESTIGATION:	Campers were positive for serotype 0:8 <i>Y. enterocolitica</i> Same serotype isolated from milk dispenser, turkey chow mein, and the cook. Was not isolated from non-rehydrated dry milk powder. Food handler likely source of infection.

Since this initial outbreak, there have been several additional small outbreaks of *Y. enterocolitica* food poisoning, plus a second major outbreak that occurred in a summer camp (74). The particulars of that outbreak are summarized in Table 4. The general symptoms of *Y. enterocolitica* gastroenteritis are similar to those of most food-related gastroenteritis syndromes. A characteristic unique to *Y. enterocolitica* is that some infected individuals display abdominal pains localized in the region of the appendix. This pain, in conjunction with an accompanying fever, can be easily misdiagnosed as acute appendicitis. It appears that unneeded appendectomies are a relatively common consequence of *Y. enterocolitica* outbreaks. It has also been found that a small percentage of the population which are genetically predisposed will respond to *Y. enterocolitica* with transitory rheumatoid arthritis-like syndrome (75).

The recent outbreaks of *Y. enterocolitica* gastroenteritis stimulated a great deal of interest in the microorganism. While this bacterium displays general characteristics similar to those of most pathogens (83), a relatively unique feature is its ability to grow well at refrigeration temperatures (29, 31, 60, 80, 81). The microorganism will grow at temperatures as low as 0°C, and at the slightly higher temperatures associated with adequate refrigeration, the microorganism's growth is relatively rapid. For example, it has been shown that the *Y. enterocolitica* can reach infectious levels in 4-14 days in milk, beef,

and pork stored at 2.5°-7°C (29, 31, 60, 80, 81).

Y. enterocolitica is ubiquitous, and has been isolated from a variety of food and environmental sources (46, 83). Swine and poultry appear to be major sources of the organism, although it has been isolated from a variety of animals and animal products. Pets also appear to be potential sources of the organism, and rats (39) and flies (21) may be important vectors for its dissemination. Like *E. coli*, most food and environmental isolates of *Y. enterocolitica* do not appear to be pathogenic (83).

The pathogenicity of *Y. enterocolitica* appears to be dependent on two virulence factors: synthesis of a heat-stable enterotoxin and the presence of an invasiveness factor. The enterotoxin is a small (9,700 dalton) protein (59) that appears to be encoded by a chromosomal gene (92). Ability to synthesize the enterotoxin appears common among isolates of *Y. enterocolitica*, including strains considered nonpathogenic (83). Instead, the key determinant of virulence appears to be associated with adhesion and/or invasiveness, which allows the organism to colonize or invade the epithelium of the intestinal tract. Invasiveness has been correlated with the presence of a 41-48 megadalton plasmid (24, 46, 92) that codes for the synthesis of a 140,000 dalton protein located on the surface of pathogenic strains (6). It is possible that the mechanism of pathogenicity in *Y. enterocolitica* is similar to that observed with enterotoxigenic *E. coli*, where pathogenicity is dependent on an initial attachment or invasion of the intestinal mucosa, and only then is the relative concentration of enterotoxin high enough to result in an emetic response.

As already indicated, with the exception of its ability to grow at refrigeration temperatures, *Y. enterocolitica* resembles other enteric pathogens such as *Salmonella* in terms of its growth requirements and thermal resistance. It is readily destroyed by heating to 60°C for several minutes (30), and pasteurization readily eliminates the microorganism (20, 30). The microorganism has also been shown to be quite sensitive to inactivation by irradiation (17). Those food sanitation practices designed to eliminate *Salmonella* would be expected to be effective against *Yersinia*. Based on the characteristics of the microorganism, it would appear to be of greatest concern in products that are refrigerated for extended periods and then consumed without further cooking.

AEROMONAS HYDROPHILIA:

Aeromonas hydrophilia is another example of a bacterial species only recently recognized as a potential mediator of food-associated gastroenteritis outbreaks. In the last several years sufficient epidemiological, clinical, and microbiological data have been accumulated to warrant its inclusion as a food poisoning organism. However, its food poisoning potential is not widely known even among food safety specialists. Currently relatively little is known concerning the presence and behavior of this bacterium in foods.

Like the previously discussed foodborne pathogens, *A. hydrophilia* has been known for a long time. It is an important pathogen of fish and frogs (67, 76), and causes various infections in humans; the best known being septicemia in patients that are immunologically compromised (13, 89). Between 1974-1980

a number of reports appeared in the literature pertaining to *A. hydrophilia* as a potential cause of food poisoning and travelers diarrhea (64, 65, 69, 72). Additional reports have subsequently implicated *A. hydrophilia* as an agent for human gastroenteritis on a worldwide basis (16, 27, 38, 91). Recent surveys of individuals suffering from gastroenteritis episodes have indicated that *A. hydrophilia* can be isolated at a rate comparable to that observed with *Salmonella* and *C. fetus* (27, 89). Estimates suggested that as much as 13% of reported incidences of gastroenteritis may be attributable to this microorganism; however, more complete assessments will be needed to evaluate the microorganism's true public health significance.

A. hydrophilia is a common bacterial species, and can be readily isolated from a variety of sources. The best known source is contaminated water supplies; however, it can also be routinely isolated from apparently unpolluted waters (1). As would be expected, the microorganism is also routinely isolated from fish (7) and shellfish (43). While *A. hydrophilia* is commonly thought of as an aquatic bacterium, it is by no means restricted to that environment. It can be isolated with relatively high frequency from the intestinal tracts of a variety of domestic animals, though it has not been determined if it is part of the normal microbiota of these animals. The genus *Aeromonas* is also commonly associated with meats, poultry, and raw milk (19, 28, 41, 42, 58, 88), and appears to be ubiquitously associated with the spoilage of refrigerated animal products. A final potential source for *Aeromonas hydrophilia* is individuals that have low-grade or asymptomatic infections. These persons could serve as vectors for the bacterium, particularly if working as food handlers.

The mechanism by which *A. hydrophilia* strains produce gastroenteritis has not been fully elucidated. Some pathogenic strains produce a heat-labile enterotoxin that has a mode of action similar to that of enterotoxigenic *E. coli* heat-labile enterotoxin (48, 49, 50, 51). Other investigators (12, 36) have attributed the enterotoxigenicity to a cytotoxic (cell death), rather than a cytotoxic (cell rounding) enterotoxin. Jiwa (37) reported that both cytotoxic and cytotoxic enterotoxigenicity could be detected in enterotoxigenic *A. hydrophilia* strains. In addition to enterotoxin, the microorganism also produces hemolysins, proteases, and endotoxins that may contribute to its virulence. The subject of *A. hydrophilia* virulence factors has been recently reviewed by Ljungh and Wadstrom (48). To date, the pathogenicity of isolated *A. hydrophilia* strains has not been correlated with the presence of a specific plasmid (87).

Like *Y. enterocolitica*, *A. hydrophilia* is able to grow at low temperatures. The effect of temperature and other food-system parameters on the growth of this species has not been examined extensively; however, it appears that the organism will grow at a reasonably rapid rate at 0°-5°C. In fact some of the initial interest in *Aeromonas* came about because it interfered with the detection of *Y. enterocolitica* by low-temperature enrichment (58). The microorganism has been shown to grow in chicken, beef, pork, and milk stored at 4°-5°C (19, 28, 41, 88), and can reach reasonably high population densities

within 7-10 days. Growth can occur in both air and vacuum packaged meats. The public health significance of *A. hydrophilia* in meats has not been assessed; however, based on a limited number of surveys of *Aeromonas* species isolated from fish and other sources (7, 36), it is likely that a significant portion of the strains isolated from meats are potentially enteropathogenic.

Probably one of the reasons that *A. hydrophilia* has only been recently identified as a potential foodborne pathogen is that it appears reasonably easy to control. Like *Y. enterocolitica* and *C. fetus*, *A. hydrophilia* appears to have resistance characteristics similar to those of *Salmonella*. Food hygiene procedures designed to eliminate *Salmonella* should also control *A. hydrophilia*. Again, the primary concern with this microorganism would be foods refrigerated for a significant period of time, and then consumed without further cooking. Cross contaminations of raw cooked products, and possible carriers among food handlers would also be of concern.

CONCLUDING REMARKS:

The examples of "new" pathogens of food safety significance discussed are by no means inclusive. There are a number of other species that are being studied as possible etiologic agents of human gastroenteritis. With the improved techniques that have been developed for identifying and characterizing enteric pathogens, there should continue to be a rapid accumulation of information concerning these and other as yet unidentified foodborne pathogens. How this wealth of new data will ultimately affect individuals charged with safeguarding the United States' food supply is unclear, but it is unlikely to make the job any simpler. The high state of flux that currently is characteristic of the field will require food safety officials to continually update their practices in order to take advantage of our increased understanding of foodborne pathogens. The need for an integrated effort by inspectors and laboratory personnel will become increasingly critical.

While the research activities of scientists throughout the world have greatly increased the complexity of assuring the safety of our food supply, the end result of the current research effort will be that the consumer will receive even safer foods and food products. It is now up to us to integrate our new knowledge into the current food safety practices so that this goal can be realized.

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