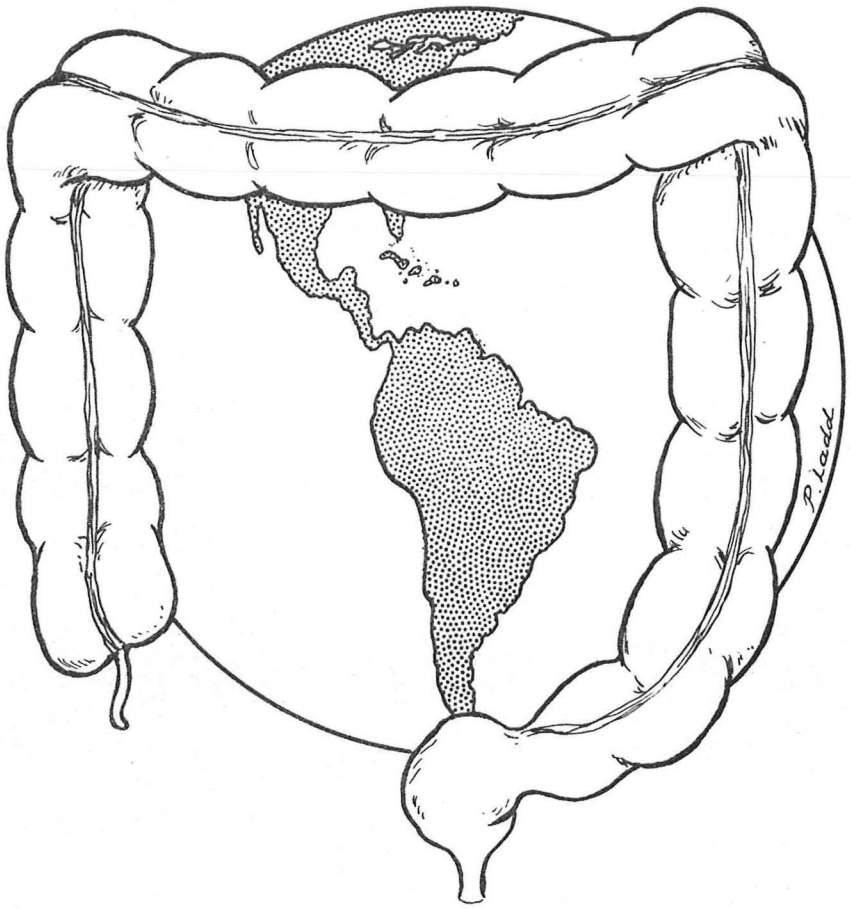
Infectious Disease

MEDICAL GRAND ROUNDS

TRAVELER'S DIARRHEA



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"To travel hopefully is a better thing
than to arrive"

Robert Louis Stevenson (1850-1894).

INTRODUCTION

Three hundred million people, mostly tourists, participate in international travel each year. The development of an acute diarrheal syndrome abroad, while returning home, or shortly after arriving home is often referred to as traveler's diarrhea, which I will abbreviate as TD. TD is not a specific diagnosis but, rather, is a clinical syndrome with multiple etiologies, much like pneumonia or disseminated intravascular coagulopathy. In this Medical Grand Rounds, I will review the clinical and epidemiological features of TD, specific etiologies and their pathogenesis, as well as current means of diagnosis, treatment, and prevention.

"Dysentery of this Arabian Coast sort used to fall like a hammer blow, and crush its victims for a few hours, after which the extreme effects passed off; but it left men curiously tired."

Seven Pillars of Wisdom, Chapter 31
T.E. Lawrence (1888-1935)

CLINICAL FEATURES OF TD

Definition of TD. Most experts agree that the diagnosis of TD can be made when a patient develops acute diarrhea while abroad or shortly after returning home. Diarrhea is usually defined as 3 or more loose or watery bowel movements per day. In most cases the diarrhea does not contain blood or pus. Some physicians will diagnose TD if the patient has 2 loose stools per day plus other symptoms. These associated symptoms and their approximate frequency in TD are shown in Table 1. These data were derived from three separate studies of TD in Mexico (11,60,133) but are similar to symptom frequency in other countries.

TABLE 1

SYMPTOM	FREQUENCY (%)
ABDOMINAL PAIN OR CRAMPS	68-73
FEVER	7-76
NAUSEA	46-65
VOMITING	8-29
BODY OR JOINT ACHES	23-25
HEADACHE	21-39
WEAKNESS, FAINTING, DIZZINESS	12-74
CHILLS	29-52
ANOREXIA	46-53

The particular symptom complex will depend upon many factors, including the specific etiology of TD (see below).

Synonyms for TD. TD probably has more synonyms than any clinical syndrome in medicine, and some of these are listed in Table 2.

TABLE 2

TURISTA (MEXICO)	HONG KONG DOG
MONTEZUMA'S REVENGE (MEXICO)	POONA POOHS (INDIA)
AZTEC TWO-STEP (MEXICO)	MALTA DOG
GI TROTS	RANGOON RUNS (BURMA)
GYPPY TUMMY	TOKYO TROTS
SPANISH FLUX	TROTSKY'S (USSR)
CASABLANCA CRUD (MOROCCO)	BOMBAY RUNS (INDIA)
ADEN GUT (YEMEN)	HO CHI MINHS
BASRA BELLY (IRAQ)	EMPORIATRIC ENTERITIS
TURKEY TROT	VIATOR'S DIARRHEA

The term "emporiatric enteritis" was coined by Dupont from the Greek word, emporion which means traveler. Having been a student of Latin rather than Greek, I decided to add my own term to the list - viator's diarrhea, since viator means traveler in Latin. However, I have refrained from using my new term in this Grand Rounds because it does not abbreviate satisfactorily.

Who Gets TD? There are five major categories of travelers who are at risk for TD. These are listed in Table 3.

TABLE 3

1. VACATIONERS, HONEYMOONERS, CONVENTIONERS
2. MILITARY PERSONNEL, PEACE CORPS VOLUNTEERS
3. STUDENTS
4. BUSINESS PEOPLE
5. ATHLETIC CONTESTANTS (E.G. OLYMPIC ATHLETES)

Most research on the frequency, etiology, and treatment of TD has utilized conventioners, military (4) and Peace Corps personnel, and students. It has been stated that military battles have often been decided more by dysentery affecting one country's troops than by military strategy. Also, outcomes of some summer Olympic contests in Mexico City were affected by TD in some of the athletes. Development of TD by the Canadian National Hockey Team touring in the Soviet Union (probably due to giardiasis) led to the term "Trotsky's". Even the Yale Glee Club was afflicted with TD (6).

Men and women are affected by TD equally. There is some evidence that young adults are more likely to develop TD than older adults, perhaps because the older people have developed immunity. Most research studies of TD have utilized students aged 16-28. There is little information on risk of TD in children, although it certainly does occur and may lead to a chronic, life-threatening condition (8).

The TD Syndrome. By definition, diarrhea is the hallmark of the TD syndrome. Onset of diarrhea is uncommon before the third day of the visit. Most cases begin during the second week of the visit. Kean studied 208 foreign (mostly American) students newly arrived in Mexico; 68 developed TD within 21 days (33%). Only 1 of these 68 students had bloody diarrhea. Stool frequency ranged from 2 to 39 per day, and 13 students had $\geq$10 stools per day. Thirty of the 68 students were confined to their rooms for 1 to 3 days (11).

Diarrhea lasts, on the average, for 2-3 days (range, 1 to 10 days). It is not uncommon for a traveler to have 2 or 3 discrete attacks of diarrhea during their visit with symptom-free intervals. In one survey conducted at the Los Angeles airport, 33% of 1265 travelers returning from Mexico had had TD. Of those with TD, 58% had had only one attack; 23% had had 2 distinct attacks; and 19% had had 3 or more attacks (10). Obviously, the frequency of TD and the

number of attacks of TD is a function, at least in part, on duration of stay in the foreign country (see below).

Although there is considerable information on stool frequency in TD, there is little information on stool weight or stool electrolytes in TD. In one experimental study in which TD was induced by feeding volunteers an enterotoxigenic strain of E. Coli (ETEC), stool weight averaged only 500 gm per 48 hrs (116), which is only slightly above normal. However, the illness induced in this study was quite mild. In another study (90), stool water was 68% of stool weight before TD and 86% of stool weight during TD (actual stool weight was not given). Banwell found that stool output ranged from 192 to 3000 ml per 24 hr in patients from Calcutta with ETEC infection. Stool electrolytes were compatible with a secretory diarrhea in that there was a very small osmotic gap in stool water (Na^+ = 128; K^+ = 10; Cl^- = 86; HCO_3^- = 38 mmol/liter; stool osmolality = 290 mOsm/ liter). Intestinal perfusion studies in most of these patients indicated net intestinal secretion (jejunum > ileum). Recovery of normal fluid and electrolyte absorption in the jejunum and ileum was usually present by 6 to 8 days after the onset of the disease (21).

Other symptoms associated with TD have been discussed already (see Table 1). These symptoms are quite non-specific. High fever and bloody stools usually imply an entero-invasive organism (e.g. Shigella, Salmonella). Watery, non-bloody diarrhea without fever suggests an infection with enterotoxigenic E. Coli (ETEC), but this may be seen in shigellosis, salmonellosis, and other "invasive" infections.

The major complication of TD, irregardless of etiology, is depletion of fluid and electrolytes. On rare occasions, more serious complications may ensue. For example, in patients with TD due to salmonella species, bacteremia may develop. Amebiasis may result in amebic abscess in the liver. Giardiasis may lead to a chronic illness with weight loss and malabsorption. In general, however, TD is a self-limited syndrome without serious sequellae. Despite this good overall prognosis, TD can ruin a long-planned vacation, honeymoon or business trip, as well as interfere with military operations and international athletic events.

Geography. The risk of contracting TD is to a large extent a function of the country visited. In one study, Kendrick questioned Americans returning from abroad to Honolulu, Miami, New York, and Los Angeles (13). Incidence of TD varied with the country visited: Mexico 39%, Latin America 30%, Spain 26%, Columbia 25%, Israel 15%, France 13%, and United Kingdom 2.5%. Tropical and subtropical countries, especially those with poor sanitation conditions, tend to be high-risk areas, but there are exceptions. From the synonyms for TD listed in Table 2, it is apparent that TD occurs all over the world. Low-risk areas for TD include the United States, Canada, Northern and Western Europe (5), Australia, New Zealand and Japan. Areas of moderate risk include Southern Europe, the Soviet Union, and China. High-risk areas are listed in Table 4. With regard to the Caribbean islands, one survey found a 33% incidence of TD in Swiss and German tourists visiting Haiti, compared to an incidence of 15-19% in Antigua, Barbados, Guadeloupe, Martinique, and St. Lucia. This same study found only a 5% incidence of diarrhea in visitors to the United States and/or Canada (126).

TABLE 4

HIGH RISK AREAS FOR TRAVELER'S DIARRHEA

MEXICO
CENTRAL AMERICA (E.G. EL SALVADOR)
CARIBBEAN ISLANDS (E.G. HAITI)
SOUTH AMERICA (E.G. PERU)
AFRICA (E.G. MOROCCO, KENYA)
MIDDLE EAST (E.G. EGYPT, IRAN, YEMEN)
SOUTHERN ASIA (E.G. PAKISTAN, INDIA, BANGLADESH)
SOUTHEAST ASIA (E.G. THAILAND, VIETNAM)
MEDITERRANEAN (E.G. SPAIN, GREECE, MALTA)

The country of origin also influences risk of TD. Risk is maximal when visitors from low-risk countries travel to high risk areas; there are approximately 12 million such visits made by travelers from highly industrialized nations annually (87). Students visiting Mexico from other Latin American countries have a lower risk of contracting TD (primarily enterotoxigenic E. Coli infection) than do American-born students visiting Mexico (3,84). This is probably because of some degree of immunity in the Latin American students due to previous infection. Such students do have higher titers of baseline serum antibodies directed against the heat-labile enterotoxin of E. Coli than American students. Kean found that only 2% of Iranians and 8% of visitors from "tropical" countries (e.g. Brazil, India, Pakistan, Egypt) who were attending an international congress in Teheran, Iran developed diarrhea, compared to 41% of visitors from temperate-climate countries (mainly the United States and the United Kingdom) (12). These results and other studies emphasize that development of TD in a high-risk country is related to country of origin.

At one time it was thought that people visiting "low-risk" areas such as the United States from "high-risk" areas such as Latin America would also develop TD. This "syndrome" of Latins developing diarrhea in New York has been referred to as Rockefeller's revenge. However, foreigners visiting the United States appear to be at low risk. In one study (17), only 2 of 96 foreign visitors to Miami for an international congress developed diarrhea (2%), compared to 1 of 51 Americans attending the same congress (2%). In another study of foreign and American college freshmen just entering the University of California at Los Angeles, 30 of 215 foreign students developed diarrhea within one month of arrival (14%) compared to 14% of American students who were not from Los Angeles or Orange Countries in California (2). Whether diarrhea in these students was due to infection or to some other factor such as emotional stress was not studied. Nevertheless, there was no excessive risk in foreigners.

In summary, geographical factors (country of origin, country visited) are important factors determining the incidence of TD. In one controlled study (10), 33% of 1265 Americans returning from Mexico had TD compared to only 7.6% of 210 Americans returning from Hawaii ($P < 0.0001$). Approximately 3 million Americans visit Mexico each year. Thus, there are approximately 1 million cases of TD annually in Americans visiting Mexico.

Length of Stay. Many studies estimating risk of TD have not taken into account length of visit in the foreign country. It seems obvious that the shorter the stay, the lower the risk of developing TD. Moreover, many field studies of TD only lasted 7, 14, or 21 days. Such studies will underestimate the true incidence of TD if cases occur after the period of surveillance (1). Figure 1 demonstrates the cumulative risk of developing TD in one 21-day study conducted in Mexico (112).

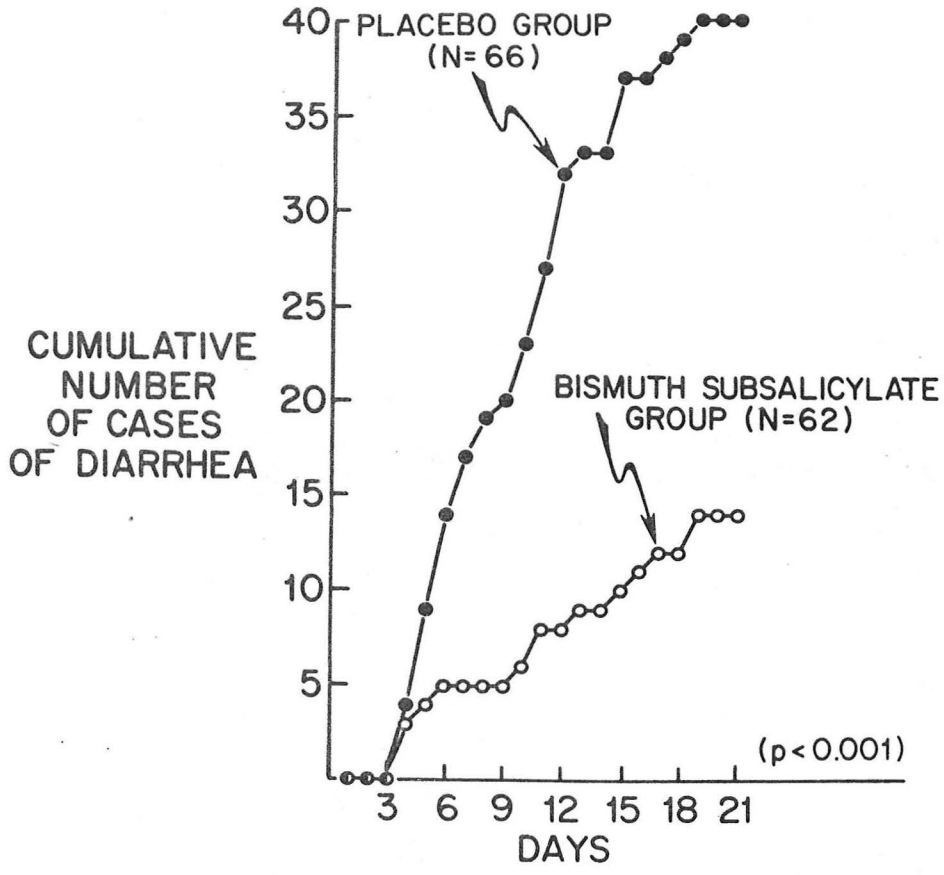

FIGURE 1

It is apparent from Figure 1 that no cases occurred before the fourth day of the visit. By the end of this study, 40 of the 66 patients at risk (placebo group) had contracted TD (61%). From day 3 to day 21, the incidence of TD was approxi-

mately 3.4% per day. As will be discussed below, prophylactic therapy with oral bismuth subsalicylate reduced the cumulative incidence of TD from 61% to 23%, or 1.3% per day from day 3 to day 21. After reviewing the literature, I expressed the risk of TD as % of patients at risk for developing TD per day at risk. Accordingly, the chance of developing TD after day 3 is approximately 1.5-4% per day in high risk nations, at least during the first 3-4 weeks of travel. There is also some evidence to suggest that the risk of TD is greater if one travels in the summer months than in winter months.

"He who drinks a tumbler of London water has literally in his stomach more animated beings than there are men, women, and children on the face of this globe."

Syndey Smith
Letter to the Countess Grey
November 19, 1834

ETIOLOGIES OF TD AND PATHOGENESIS

TD has many different etiologies, although almost all are considered to be infectious. The relatively low incidence of TD when visiting certain places such as the continental United States, Hawaii, or England makes stress-related diarrhea an unlikely explanation for the TD syndrome.

Table 5 lists the infectious agents most commonly associated with TD in Mexico and their relative frequences according to Dupont. In most cases, the incubation period is short (hours to a few days), so that diarrhea usually occurs while traveling, on the trip home, or shortly after arriving home.

TABLE 5

AGENT	PERCENT CONTRIBUTION
BACTERIAL	
ENTEROTOXIGENIC E. COLI (ETEC)	40
SHIGELLA SPECIES	15
SALMONELLA SPECIES	7-10
CAMPYLOBACTER SPECIES	3
VIRAL	
ROTAVIRUS, NORWALK AGENT	10
PARASITIC	
GIARDIA LAMBLIA	3
ENTAMEBA HISTOLYTICA	1
UNKNOWN	22

Although these figures vary somewhat from study to study, enterotoxigenic E. Coli (ETEC) are the most common cause of TD in most studies. The relative proportions of agents may vary with the country visited or the country or origin. For example, in Americans traveling to the Soviet Union, Giardia lamblia is a more important cause of TD than is G. lamblia in Mexico (26). Furthermore, Panamanian tourists visiting Mexico have far less infection with ETEC than American tourists visiting Mexico, possibly because of natural immunity to ETEC. The major causes of TD in Panamanians visiting Mexico are rotavirus, Norwalk agent, and Campylobacter species (18).

The relative proportions of different agents is also, in part, a function of the types of cultures and serologic tests which are carried out in a particular study. For example, until recently subjects with the TD syndrome were subjected only routine bacterial stool culture and a stool examination for ova and parasites. Infection with ETEC, Campylobacter, and viral agents would not be diagnosed using these methods. Although cultures for Campylobacter species are now routine in most clinical microbiology laboratories, tests for ETEC and viruses are still performed only in special laboratories.

It is also important to point out that a large percentage of the "unknown" category in Table 5 probably represents bacterial infections with as yet unrecognized agents. This is because such patients in placebo-controlled clinical trials have responded to antibiotic therapy (e.g. trimethoprim/sulfamethoxazole

(TMP/SMX) or bicozamycin. For example, Dupont found that 100% of patients with TD of unknown etiology responded to TMP/SMX and that 89% responded to TMP alone, whereas only 40% "responded" to a placebo (response = no further unformed stools by 72 hrs) (92).

I also wish to stress that demonstration of a particular organism in the stool does not necessarily mean that the organism identified is the cause of the diarrheal syndrome. This is because (a) potential pathogens are sometimes isolated from stools of asymptomatic subjects participating in field studies in "high-risk" countries and (b) some patients with diarrhea have 2 or more "pathogens" in their stool, making it impossible to be certain which agent is causing the illness.

Agents other than those listed in Table 5 have been also implicated as causes of TD. These include Vibrio parahemolyticus (particularly in the Far East), enteroinvasive strains of E. Coli, Yersinia enterocolitica, Aeromonas hydrophila, Plesiomonas shigelloides, Vibrio cholera, Legionella pneumophila, and Metagonimus yokogawa (an intestinal fluke in the Orient) (48). Vibrio parahemolyticus appears to be an increasingly important etiologic agent. Below, I will review each of the bacterial and parasitic agents listed in Table 5, as well as Vibrio parahemolyticus infection. Special emphasis will be placed on enterotoxigenic E. Coli because of their high incidence.

Escherichia coli (136). E. Coli in intestinal contents or stool are either non-enteropathogenic or enteropathogenic. Non-enteropathogenic E. Coli are often part of the normal stool flora. Enteropathogenic E. Coli can be divided into at least three types, based upon the pathogenetic mechanism by which they produce disease (Table 6).

TABLE 6

ENTEROPATHOGENIC E. COLI (EPEC)
ENTEROTOXIGENIC E. COLI (ETEC)
ENTEROINVASIVE E. COLI (EIEC)
ENTEROADHERENT E. COLI (EAEC)

Enterotoxigenic E. Coli produce disease by elaborating toxin(s) which either retard small intestinal absorption of water and electrolytes or actually induce secretion of water and electrolytes. Enterotoxigenic E. Coli do not invade the small intestinal mucosa or lead to an inflammatory response. Hence, there is usually no blood or pus in the stool which, instead, is usually watery and persistent during fasting. Moreover, the Na^+ and K^+ concentrations of stool water, when added together and multiplied by 2, are roughly equal to the osmolality of stool and plasma, reflecting the secretory nature of this process. ETEC-induced diarrhea is similar pathogenetically to diarrhea produced by cholera toxin, although ETEC usually produces a milder illness. ETEC is the commonest cause of

the TD syndrome (73), is a major cause of severe diarrhea in children, and is an important cause of acute diarrhea in children and adults in developing countries (e.g. Bangladesh) (61). Enterotoxin production is not limited to certain E. Coli strains and to Vibrio cholera, having been reported with Shigella, Salmonella, Klebsiella, Citrobacter, Enterobacter, Pseudomonas, Proteus, Serratia, Aeromonas, and Yersinia species. However, the role of the enterotoxin in diseases produced by the latter organisms is uncertain. Enterotoxins are also produced by Staphylococcus aureus, Clostridium perfringens and Bacillus cereus, important causes of acute food poisoning (59,82,139).

Certain strains of E. Coli penetrate the epithelium of the colon, multiply within epithelial cells, pass laterally from cell to cell and enter the lamina propria, producing inflammation and exudation of pus and/or blood from the bowel. Enteroinvasive E. Coli infections thus resemble shigellosis. These types of organisms are uncommon causes of the TD syndrome.

E. Coli can also produce diarrhea by adhering to the small bowel mucosa without invading the mucosa or elaborating a toxin (51). Diarrhea is thought to be due to bacterial penetration of the glycocalyx and disruption of the microvillous brush border with reduction in brush border enzymes (e.g. disaccharidases). This has been reported as a sporadic cause of diarrhea in children, and, in a recent study, enteroadherent E. Coli were implicated as cause of TD in Mexico in 6-9% of cases (57).

Besides the 3 mechanisms listed in Table 6, there is increasing evidence that enteropathogenic E. Coli may act via other mechanisms. For example, some EPEC may produce a Shigella-like toxin that induces secretion. Other pathogenic mechanisms are likely to be discovered in the future.

Because non-enteropathogenic E. Coli are found in the stool of healthy subjects, special tests are needed to document that an E. Coli which is isolated in the stool is enteropathogenic. E. Coli which are enterotoxigenic produce either a heat-labile toxin (LT), a heat-stable toxin (ST), or both. LT is a protein with antigenic similarities to cholera toxin; they both activate intestinal adenylate cyclase leading to increased intracellular cyclic-AMP and to intestinal secretion. To demonstrate LT, it is customary to determine whether an extract of the test strain induces fluid secretion in a ligated ileal loop of an adult rabbit or other animal. LT also produces cyclic AMP-mediated morphologic changes in certain types of cells grown in vitro in tissue culture, such as Y-1 adrenal tumor cells from mice or ovary cells from Chinese hamsters (67). ST is a low molecular weight polypeptide and activates guanylate cyclase (increasing intracellular cyclic GMP). ST is unrelated antigenically to LT or cholera toxin. ST is detected by injecting the E. Coli extract into the milk-filled stomach of a newborn suckling mouse and, four hours later, by examining the small intestine for distention and then weighing the small intestinal contents. LT does not induce intestinal secretion in this suckling mouse model (32). There is some debate whether E. Coli strains which produce ST but not LT are capable of producing diarrhea in man, although most evidence indicates that they can. There is no controversy over the fact that strains that produce LT but not ST can produce diarrhea in man.

Because tests for LT and ST are not simple and are expensive, only 5 to 10 strains of E. Coli isolated from the stool are customarily tested for toxin production. Since stool contains 10^8 or more aerobic coliforms per gram, it is

possible that a toxigenic strain will be missed if only 10 strains or less are screened for toxin production, unless the toxigenic strain has overgrown and accounts for a high percentage of the coliforms present in the stool. Thus, negative tests for toxin production do not totally exclude a toxigenic E. Coli infection.

Enteroinvasive E. Coli infection is most often diagnosed by the ability of these stains to invade the conjunctival membrane of the guinea pig (Sereny test). This test is only available in specialized laboratories. Finally, enteroadherent E. Coli which neither invade or produce a toxin can be diagnosed by small bowel biopsy in which the organisms can be demonstrated adhering to the mucosa microscopically or in vitro by their ability to adhere to HEp-2 cells grown in tissue culture (ETEC do not adhere to HEp-2 cells) (30,57).

At one time, it was widespread to try to detect enteropathogenic E. Coli by their somatic antigenic constituents (serotyping). It is now evident that pathogenicity of E. Coli is often related to pieces of non-nuclear DNA called plasmids (41). DNA sequences coding for enterotoxin production, enteroadherence and enteroinvasion are often found on these plasmids, which can be transferred from toxigenic strains to non-toxigenic recipients (see below). Genes for resistance to many antibiotics can also be transferred by plasmids (42). Thus, serotyping is rarely carried out today in the study of potentially enteropathogenic E. Coli, except in epidemiological studies.

There are several pathogenetic factors that are involved in infections with EPEC.

(1) Inoculum size. Several volunteer studies have shown that the chance of developing diarrhea after oral challenge with a given strain of EPEC is a function of the number of organisms ingested (34).

(2) Gastric pH. At pH 3 or less, most bacteria are killed in the normal stomach. Patients who are achlorhydric or hypochlorhydric are much more susceptible to enteric infections such as cholera, salmonellosis, and possibly shigellosis (45,47,63,68). One study in healthy prisoners showed that ingestion of 2 grams of sodium bicarbonate prior to challenge with enteropathogenic E. Coli lowered the inoculum size necessary to produce illness by 100-fold (34). Thus, an elevated gastric pH at the time of exposure to EPEC may reduce the inoculum size neccessary to produce TD. Some studies have suggested that patients who have had gastric surgery or are receiving antacids or histamine H_2-receptor antagonists (and possibly regular users of cannabis) are at higher risk for contracting TD because of an elevated intragastric pH and that such patients should be considered for TD prophylaxis (62).

(3) Immunity. I have already mentioned that travelers from "high-risk" areas develop ETEC less commonly than travelers from "low-risk" areas, presumably because of a natural immunity to this type of infection. Because there are no vaccines for ETEC as yet, immunity must have been acquired only via infection. It is likely that natural immunity to ETEC is mediated by secretory IgA, although circulating antibodies to LT can be detected after ETEC infection. The duration of protection after ETEC infection is unknown. In one volunteer study, rechallenge with an LT^+ ETEC 9 weeks after recovery from an illness produced by an initial challenge with the same strain resulted in diarrhea in only 1 of 8 individuals, compared to in 7 of 12 controls being challenged with this

strain for the first time. However, these "immune" volunteers were not immune to challenge with a different strain of LT^+ ETEC, indicating that serum antibody to LT which had developed in these patients was not cross-protective against infection with other LT-producing strains (56).

(4) Normal Flora. This may play an important role in preventing infections with colonic pathogens, but there are no studies specifically relating risk of ETEC infection to changes in normal bowel flora (e.g. induced by antibiotic therapy). Since ETEC infect the small bowel, an area which normally has a sparse bacterial flora, the protective effect of a normal bacterial flora appears to be less important than in other causes of TD, such as salmonella and shigella.

(5) Bacterial Colonization. If an inoculum of ETEC of suitable size is ingested and if a sufficient number of organisms survive passage through the acidic stomach, E. Coli must first be able to attach to the mucosa and colonize there before inducing a toxin-mediated secretion. If an ETEC does not possess the ability to adhere to and colonize the upper small intestine, the organism will remain in the bulk intestinal fluid and be excreted by peristalis without harm to the host. Attachment of bacteria to tissue surfaces is the initial event in many bacterial infectious diseases, including ETEC infection. Other examples include cholera, gonorrhea, E. Coli genitourinary infections, and streptococcal infections of the throat and endocardium (22).

Adhesive molecules on the surface of bacteria are called adhesins. A series of investigations by Evans and her associates (35-40) have elucidated the role of certain types of adhesins, called colonization factor antigens (CFAs), in the pathogenesis of ETEC-induced human diarrhea. These antigens are present as fine fimbriae which project from the cellular surface of the bacterial cell. Two types of CFA (CFA/I and CFA/II) have been identified. Each CFA is composed of a heat-labile hydrophobic protein of relatively low molecular weight (around 25000 daltons). These low molecular weight subunits polymerize into thin pilus structures of regular size and shape (fimbriae) which radiate from the cell. These fine fimbriae are not to be confused with the flagellae of the bacterium which are much thicker than fimbriae.

It is now recognized that CFAs have affinity for receptors on epithelial cells of the human small intestine, although such receptors remain to be isolated. Current evidence suggests that the receptor is a ganglioside on the luminal membrane of the enterocyte. CFAs can be detected in the laboratory in vitro by their ability to adhere also to receptors on erythrocytes, producing hemagglutination. Unlike fimbriae of ordinary Enterobacteriaceae whose binding to erythrocytes is inhibited by mannose, hemagglutination by ETEC-associated CFAs is not inhibited by mannose. CFA-I agglutinates human, bovine, and chicken erythrocytes; CFA-II also agglutinates bovine and chicken erythrocytes, but not human erythrocytes. With regard to small intestinal mucosa, binding of CFAs is specific for humans (29). A closely related adhesin, K88, is present on an ETEC strain which is pathogenic for pigs but not for man. It is of interest that certain pigs are genetically immune to E. Coli K88 infection because they lack the gene which codes for production of the receptors for E. Coli K88 on brush borders of intestinal epithelial cells (72). Whether susceptibility to ETEC infections in humans is likewise influenced by genetic factors

is unknown, but this seems likely. CFAs for enteroinvasive E. Coli have not yet been identified. Enteroadherent E. Coli (detected by their ability to bind to Hep-2 cells in tissue culture) do not possess CFA/I or CFA/II. Current evidence suggests that these E. Coli bind to the enterocyte via non-fimbrial adhesins.

Genes encoding for ETEC-related CFA/I and CFA/II reside on plasmids (41). A plasmid is a covalently-closed circle of extra-chromosomal double-stranded DNA. Most plasmids have molecular weights of a few million to 100 million daltons. Thus, plasmids are usually about 1% as large as the E. Coli chromosome, which has a molecular weight of approximately 3 billion daltons. Plasmids code not only for CFAs, but also for enterotoxins (LT, ST), resistance to several antibiotics, hemolysins, and other bacterial products. Plasmids represent a mechanism by which microorganisms may acquire temporarily useful genes without changing the bacterial chromosomal DNA. Plasmids are not essential to the viability of the host cell and can be gained or lost.

Plasmid DNA can be transferred from one organism to another by a process called conjugation (Fig. 2). During conjugation, a donor and a recipient bacterium are joined by a special structure, the sex pilus, which is synthesized by the donor cell. The pilus attaches to the surface of the recipient cell and serves as a conduit through which one strand of the double-stranded plasmid DNA passes from donor to recipient. This DNA strand is then copied in a complementary fashion, reproducing the double-stranded plasmid DNA in both donor and recipient.

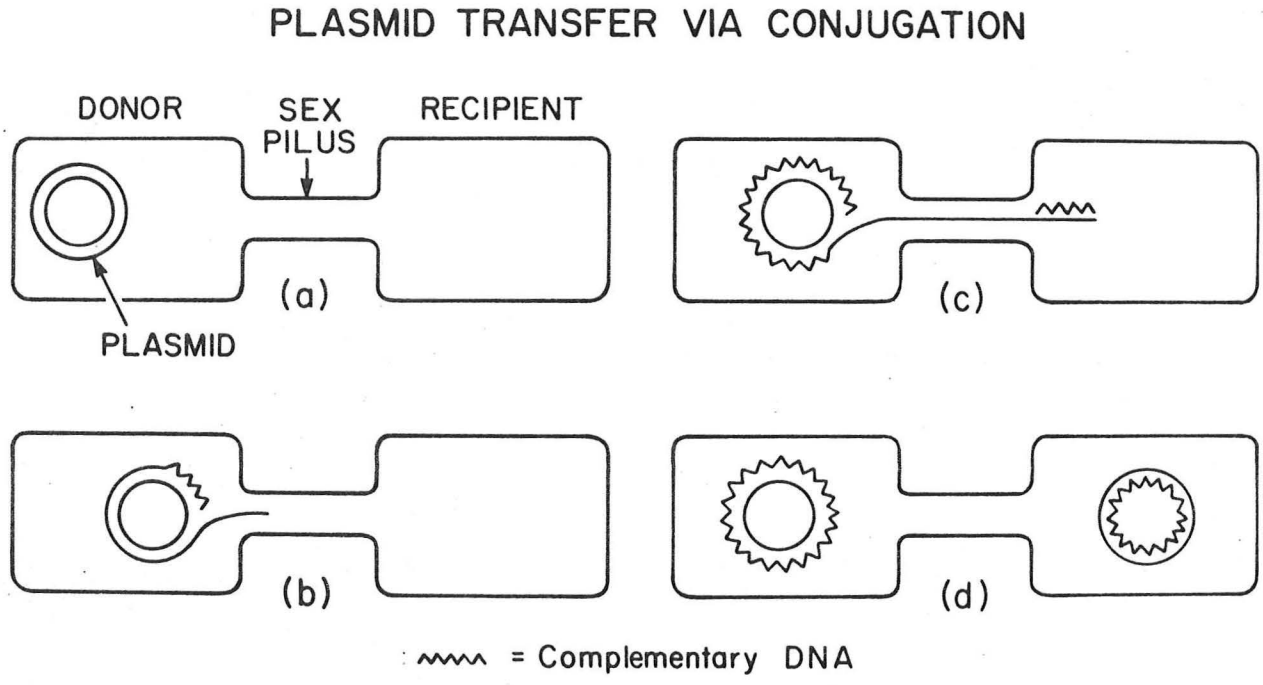

FIGURE 2

Large plasmids (> 20 million daltons) usually contain the genetic information to synthesize the sex pilus proteins and other proteins required for conjugation. Smaller plasmids require a larger, conjugative plasmid to co-exist in the donor cell in order for the smaller plasmid to be mobilized into the recipient bacterial cell (sometimes without co-transfer of the larger, conjugative plasmid). Bacteria usually contain one or a few copies of large plasmids, whereas small plasmids may exist in multiple copies (sometimes, hundreds of copies per cell). Because plasmids can be "lost" or transferred so readily from one type of bacteria to another (bacteria are quite sexually promiscuous), plasmids appear to be relatively unstable.

One type of ETEC isolated from patients with diarrhea in Dacca, Bangladesh contains a 60,000,000 dalton plasmid which produces both CFA/I and ST. This wild strain of ETEC also produces LT, but LT production is controlled by a separate plasmid. During repeated laboratory passage, spontaneous derivatives of this wild LT^+ ST^+ CFA/I^+ strain appear ("P-strains") which lack the CFA/I-ST plasmid. Some volunteers were challenged with either 10^6 or 10^8 of the wild strain (LT^+ ST^+ CFA/I^+); others were given 10^6 or 10^8 of the P-strain (LT^+ ST^- CFA/I^-). Only volunteers challenged with 10^8 of the wild CFA/I^+ strain developed watery diarrhea; they excreted this strain for the duration of the 7-day study; and they often developed circulating antibody responses to LT, 078 antigen (a somatic antigen), and CFA/I. None of the volunteers challenged with the CFA/I^- "P strain" developed diarrhea. This strain was cleared quickly from the stool, and no antibody responses developed (39,71).

TABLE 7

QUANTITATION OF ETEC, BOTH WILD STRAIN (CFA/I^+) AND "P" STRAIN (CFA/I^-), 5 DAYS AFTER ORAL CHALLENGE WITH 10^8 BACTERIA

	WILD STRAIN (CFA/I^+) PER GRAM OF STOOL		"P" STRAIN (CFA/I^-) PER GRAM OF STOOL
SUBJECT 1	2.1×10^9 (100%)	SUBJECT 5	0
2	8.0×10^8 (88%)	6	0
3	7.8×10^9 (80%)	7	0
4	2.0×10^8 (3%)		

(Numbers in parenthesis refer to percentage of all E. Coli in stool represented by the wild strain)

As shown in Table 7, 5 days after oral challenge with 10^8 CFA/I$^+$ wild strain, 2.0 X 10^8 to 7.8 X 10^9 ETEC were present per gram of stool, and in 3 of the 4 subjects this strain had become the predominant E. Coli in the stool. On the other hand, none of the "P" strain devoid of CFA/I antigen was present in stool 5 days after 10^8 of this strain had been given. Thus, pathogenicity of ETEC depends upon CFA, allowing the organism to adhere to and colonize in the upper small intestine rather than being swept away and excreted in the feces.

In one study of 31 students who developed TD in Mexico, all 20 isolates of ETEC which were LT$^+$ and ST$^+$ were either CFA/I$^+$ (N=14) or CFA/II$^+$ (N=6). In the same study, most but not all LT$^-$ ST$^+$ strains were CFA/I$^+$ (4 of 6). On the other hand, none of 5 LT$^+$ ST$^-$ strains possessed CFA/I or CFA/II. This latter finding may suggest that ETEC may produce diarrhea on occasion without CFA. However, Evans has presented *in vitro* data to suggest that antibody to CFA/I favors the overgrowth of LT$^+$ variants that have lost the CFA/I-ST plasmid. If such an antibody is secreted into the intestinal lumen *in vivo*, it remains possible that LT$^+$ ST$^-$CFA/I$^-$ strains in some patients with TD may, in fact, have been derived from LT$^+$ ST$^+$ CFA/I$^+$ wild strains *in vivo* via antibody-induced plasmid loss.

In addition to showing that diarrhea-producing ETEC are usually CFA$^+$, Evans also demonstrated that ETEC isolated from the stool of students without diarrhea are usually CFA negative. Results for 18 such isolates were as follows: LT$^+$ ST$^-$ CFA$^-$ (N=12); LT$^-$ ST$^+$ CFA$^-$ (N=4); and LT$^+$ ST$^+$ CFA/I$^+$ (N=2). Thus, students with toxigenic E. Coli which lacked CFA were usually asymptomatic. The latter two cases indicate that an E. Coli may, on rare occasions, produce LT, ST, and CFA/I and not produce disease, possibly because of natural immunity.

I do not wish to leave the impression that CFAs are the only bacterial factor required for attachment of ETEC to the mucosa. In the case of Vibrio cholera, for example, bacterial motility via flagellae and chemotactic substances in the mucosa or in the mucus gel layer on its surface are also important (52). An enteropathogenic bacterium must overcome a number of non-specific local defenses before adhering to its target cell.

(6) Enterotoxin Production and Action. Once ETEC adhere to and colonize the upper small intestine via CFA-mediated attachment, toxin(s) can act on target cells. Receptors for enterotoxins are located on the luminal aspect of the cell membrane, close to the receptors for CFAs. Adhesion to the cell membrane allows toxin to be delivered to the target receptor at high concentration. Toxins secreted by unattached, luminal organisms might be inactivated by enzymes and chemicals in intestinal secretions.

As already mentioned, ETEC produces two types of toxins, LT and ST. Differences between these two enterotoxins are summarized in Table 8.

Table 8

	E. Coli Enterotoxins	
	LT	ST
Effect of heating to 60°C	Labile	Stable
Mol. Weight (daltons)	102,000	1900-5000
Chemical Structure	Protein	Polypeptide
Homologies with cholera toxin	Yes	No
Receptor for Toxin	GM_1 Ganglioside	?
Onset of Action	Delayed	Immediate
Intracellular Mediator	Cyclic-AMP	Cyclic-GMP (?)
Antigenicity	Strong	Weak
Bioassay Model	Rabbit Ileal Loop	Infant suckling mouse intestine

LT and cholera toxin (CT) have similar structures and modes of action (23,31,33, 43,46,65,69,74,76). Because of these similarities, I will first review recent knowledge regarding the biochemistry of CT (28,53,83).

CT is a protein which consists of A and B subunits (Fig. 3). The B subunit is responsible for CT binding to a receptor on the epithelial cell membrane which is an oligosaccharide component of ganglioside (GM_1). The B subunit of CT is antigenic and patients with cholera develop antibodies in the serum to B. These antibodies are thought to be truly protective, probably by preventing binding of B to GM_1 receptor. The B subunit surrounds the A subunit and actually consists of 5 identical peptides, each of about 11,600 daltons. Once B subunits bind to GM_1, a conformational change in CT occurs which allows hydrophobic regions of the A subunit to interact with and enter the plasma membrane of the epithelial cell. The A subunit is responsible for activating, through a series of complex steps to be reviewed below, the enzyme adenylate cyclase. The A subunit of CT is a polypeptide consisting of 2 portions: an A_1 portion (M.W. = 22,000 daltons) and an A_2 portion (M.W. = 5,000 daltons). A_1 and A_2 portions are linked by a disulfide bond (Fig. 3). Soon after B subunits bind to the GM_1 receptor, A is nicked by a proteolytic enzyme, the disulfide bond is reduced (S-S --> SH + SH) and A_1 thus becomes separated from A_2 (Fig 3). A_1 then passes through the membrane to its interior surface, destined to activate adenylate cyclase. Thus, the action of CT is quite distinct from peptide hormones, since CT must enter the cell to activate adenylate cyclase, whereas peptide hormones operate from outside the cell. Necessity for cell entry may explain the delay of 15-60 minutes observed experimentally between the time of CT administration and the onset of biologic effect. In contrast, peptide hormones act almost instantly in the same systems.

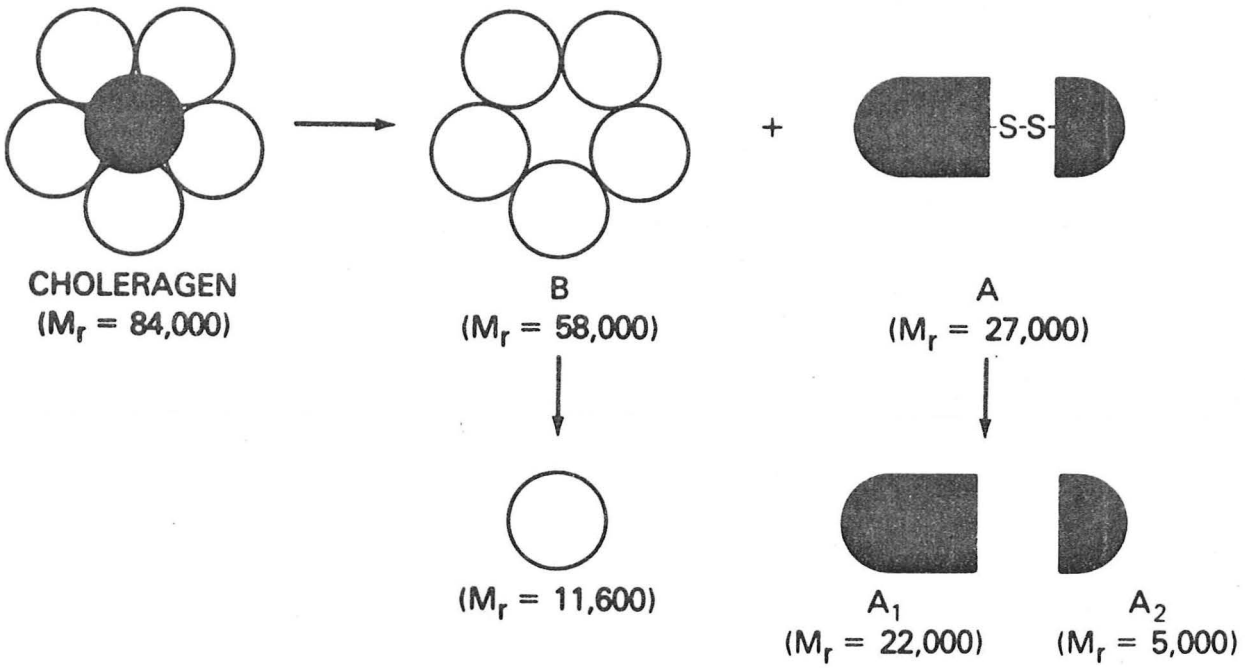

FIGURE 3. Structure of Cholera Toxin (CT, Choleragen). LT from E. Coli has a similar structure.

The A_1 peptide of CT does not activate adenylate cyclase directly. Current evidence suggests that the following steps occur:

1. A_1 interacts with nicotinamide-adenine dinucleotide (NAD), converting it to nicotinamide and adenosine diphosphate - ribose (ADP-ribose).

2. ADP-ribose then interacts with (or ribosylates) a 42,000 dalton protein called guanyl nucleotide binding protein or GTP-regulatory protein. This regulatory protein is one of the two major components of adenylate cyclase, the other being the actual catalytic site of the enzyme. However, the catalytic component of adenylate cyclase will increase production of cyclic AMP from ATP only when the GTP-regulatory protein is bound to GTP. The GTP-regulatory protein has a low affinity for GTP in the absence of CT; when this protein is ADP-ribosylated, its affinity for GTP increases. Although the bound GTP can be converted to GDP by cytosolic GTPase, there is evidence that ADP-ribosylated GTP-regulatory protein not only has a high affinity for GTP but has a lower than normal affinity for GDP, so that GDP produced from GTP by GTPase rapidly dissociates from the regulatory protein, allowing additional GTP to bind to the adenylate cyclase complex. Moreover, transfer of the ADP-ribose to the regulatory protein may actually inhibit GTPase.

3. Cytosolic GTP binds to the ADP-ribosylated GTP-regulatory protein, thus fully activating the catalytic site of adenylate cyclase.

4. Adenylate cyclase then converts cytosolic ATP to cyclic-AMP. Calmodulin, a low molecular weight, heat-stable, calcium-dependent cytosolic protein, also appears to be required for maximal activation of adenylate cyclase by CT.

5. By steps not completely understood (23,43), increased intracellular cyclic AMP inhibits intestinal sodium chloride absorption and stimulates active anion secretion by the intestine. There is evidence to suggest that epithelial cells at the tips of the villi are absorptive and that cyclic AMP's effect on NaCl absorption is on these cells, whereas the epithelial cells deep in the crypts are normally secretory and that cyclic AMP induces active anion secretion from these cells. In vitro and in vivo studies disagree as to whether cyclic AMP induces active chloride secretion (favored by most in vitro studies) or active bicarbonate secretion (favored by some in vivo studies). Irregardless, the potential difference in the lumen of the intestine becomes more negative after CT due to active anion secretion. Sodium and water secretion occur passively in cholera. In some patients, the small intestine may secrete more than a liter of fluid per hour. Although CT interferes with neutral NaCl absorption, CT and cyclic-AMP have no effect of the other major sodium uptake mechanism - glucose-mediated sodium absorption, a fact which can be taken advantage of when treating cholera patients with oral replacement fluids.

I wish to emphasize two points regarding the above schema. First, CT has these same biochemical effects on many different cells in vitro (e.g. erythrocytes); however, in vivo CT only is exposed to intestinal epithelial cells since the organism is not invasive. Second, stimulation of intestinal secretion via increased intracellular cyclic AMP is not unique to CT or LT of E. Coli. Any agent which increases cyclic AMP in these cells will induce secretion and, perhaps, diarrhea (e.g. vasoactive intestinal peptide, prostaglandins).

Diarrhea produced by ETEC, although generally less profuse than in cholera, is responsible for far greater morbidity and mortality world-wide. Electrolyte composition of stool water is similar in ETEC and cholera (Table 9). The LT of E. Coli has striking immunological cross-reactivity with cholera toxin. Like CT, LT binds to GM_1 ganglioside and also participates in an NAD-dependent ADP-ribosylation reaction, leading to increases in intracellular cyclic AMP and intestinal secretion (46). Both LT and CT have an A subunit and five B subunits which contain many homologous amino acid sequences, although homology between LT and CT is not complete. It is possible to produce in the laboratory hybrid toxins that are fully active in vivo. For example, the A subunit from LT can be hybridized with the B subunit from CT to produce a fully active toxin, and visa versa (79).

TABLE 9

	ENTEROTOXIGENIC E. COLI	VIBRIO CHOLERA
Stool Na^+ (mmol/L)	128	126
Stool Cl^- (mmol/L)	86	94
Stool K^+ (mmol/L)	10	19
Stool HCO_3^- (mmol/L)	38	47
(Reference Number)	(21)	(43)

However, there are differences in LT and CT. First, the genetic information for LT production is carried on an E. Coli plasmid (41,70), whereas the gene for CT is part of the chromosomal DNA of Vibrio cholera. Second, there is some evidence to suggest that the A subunit of LT is proteolytically nicked to A_1 and A_2 less readily than the A subunit of CT (83). A_1 from LT, produced in vitro by incubation with trypsin, is as potent as A_1 from CT, suggesting that full conversion of A to A_1 in vivo occurs less easily with LT than CT. Moreover, CT is primarily found as an extracellular protein secreted by Vibrio cholera, whereas LT may remain tightly associated with the membrane of the E. Coli cell. There is also some evidence that Vibrio cholera may produce more toxin per cell than ETEC. Finally, CT may bind to the GM_1 receptor on the epithelial cell membrane more avidly than LT (33). Any or all of these differences may explain why, in general, LT produces less severe disease than CT.

ST is a small peptide or peptides believed to stimulate intestinal secretion by a mechanism separate from LT and CT (Table 8). ST has no effect on adenylate cyclase and cyclic AMP. In contrast, CT activates guanylate cyclase, converting GTP to cyclic GMP. ST production has been identified not only in ETEC but also in Yersinia enterocolitica. However, the role of cyclic GMP in small intestinal secretion is unclear and it is uncertain whether the action of ST is mediated by cyclic GMP. For example, some agents which increase cyclic GMP concentrations in the small intestinal mucosa (e.g cholecystokinin, insulin) have no effect on small intestinal secretion. Thus, the mechanism by which ST elicits intestinal secretion is unknown.

(7) Gut Motility. Normal motor function is an important protective mechanism for clearing bacteria from the small intestine (78). If ETEC do not possess adhesins such as CFA to allow binding to the epithelium, normal propulsive activity will usually eliminate these agents in the feces.

The role of gut motility in the pathogenesis of ETEC infection has not been studied extensively. ETEC or a filtrate containing LT induces in the ileal smooth muscle of rabbits action potential complexes which migrate aborally and which result in strong contractions which propel intraluminal contents in an aboral direction (27). This contratile activity does not occur with non-enterotoxigenic E. Coli. Similar electrical and motor changes occur in rabbits given live Vibrio cholera or CT (58). These studies suggest that, in addition

to inhibiting salt and water absorption and inducing secretion, ETEC may induce a motor response which propels fluid through the small intestine more rapidly. Whether these motor events are due to the toxins per se or are secondary to distention of the gut with intraluminal fluid is uncertain, although one study suggests that sudden distention of the small bowel with fluid per se does not induce these migrating action potential complexes (58).

Abnormalities in gut motility in certain chronic diseases may predispose to bacterial overgrowth in the upper small intestine. This sequence has been described in scleroderma, diabetes mellitus with gastrointestinal autonomic neuropathy, and idiopathic intestinal pseudo-obstruction. The usual sequellae of bacterial overgrowth in the small intestine are vitamin B_{12} deficiency and the malabsorption syndrome.

(8) Colonic Function. The colon plays an important role in conservation of salt and water by the gut. Normally, around 1.5 L of fluid enters the colon per day, of which approximately 1.4 L is absorbed (the remaining 0.1 L is excreted in the stool). However, when presented with excessive amounts of salt and water for absorption, the normal colon can absorb up to 5 L per day. Thus, in a patient with ETEC infection, the severity of diarrhea will depend partly upon the extent to which the colon can reabsorb salt and water. The colon is not affected by E. Coli enterotoxins.

Shigellosis (64,102,103,134). This is probably the second most common cause of TD. Shigella flexneri and Shigella sonnei are the most common species in tropical countries, whereas in the United States endemic infection with Shigella sonnei is more common than S. flexneri. Shigella dysenteriae is less common but produces a more severe illness. Shigella is an invasive organism and primarily affects the colon, although the small bowel may also be involved. Symptoms include fever, rectal urgency, tenesmus, and bloody mucoid diarrhea. The stool contains large numbers of polymorphonuclear leukocytes and red cells and the rectal mucosa is friable, inflamed, and ulcerated on sigmoidoscopy. The organism also produces an enterotoxin and watery diarrhea without blood may be seen early or even exclusively. Unlike CT or ETEC-LT, Shigella enterotoxin does not activate adenylate cyclase. The overall importance of enterotoxin in this disease is unclear. It is likely that Shigella species also produce a cytotoxin which destroys colonic epithelial cells. Enteroinvasive E. Coli produces a disease similar to shigellosis (64).

Salmonellosis (66,139). There are >1700 serotypes of Salmonella and many are pathogenic for humans. As with shigellosis, the clinical picture can vary from asymptomatic infection to a febrile illness with bloody diarrhea or a cholera-like illness with non-bloody, watery diarrhea without fever. Polymorphonuclear leukocytes in the stool are often absent. The major site of infection is the terminal ileum, although the colon may be involved as well. Infection with S. typhosa (typhoid fever) does not ordinarily produce diarrhea. Instead, patients with typhoid fever usually have constipation or no intestinal symptoms at all. S. typhosa has a longer incubation period than most other Salmonella (7-21 days). Thus, typhoid fever usually presents some time after the traveler has returned home. Typhoid fever is uncommon in travelers. Salmonella is an invasive organism inducing an acute and later a chronic inflammatory response in the gut mucosa, and bloodstream invasion is not uncommon (unlike Shigella and invasive E. Coli which rarely enter the bloodstream). Some species of Salmonella have been shown to produce an enterotoxin, but its role in the disease is uncertain.

Campylobacter Species (24,75,132). Human infection with Campylobacter, formerly called Vibrio fetus, was first described in 1947. However, it was not until 1972 that Campylobacter was first cultured from the stool because it had not been recognized that selective media and microaerophilic conditions (e.g. 5% O_2) at 42°C were necessary when culturing stool, whereas blood isolates grow readily in standard media. In the last decade, it has become evident that infection with Campylobacter is the commonest bacterial cause of acute diarrhea in the United States and in many other countries, being more common than shigellosis or salmonellosis. Infection with Campylobacter can also cause TD. As shown in Table 5, Campylobacter species (predominately C. jejuni) cause approximately 3% of cases of TD in Mexico. However, in a study from Bangladesh, C. jejuni was isolated from 41 of 269 foreigners with TD (15%), equal in frequency to Shigella species. Table 10 compares clinical features of TD due to Campylobacter and TD due to Shigella species in the Bangladesh study (75).

TABLE 10

	C. Jejuni	Shigella species
Watery Diarrhea	76%*	54%
Bloody Diarrhea	24%*	46%
Abdominal Cramps	89%	86%
Fever	65%	57%
Nausea and/or Vomiting	43%	40%
> 20 pmn's per high power field on stool exam	38%	63%

(* $P < 0.05$)

Diarrhea in patients who had infection with C. jejuni was less often bloody and purulent and more often watery. However, in an individual patient, it is impossible to distinguish infection with C. jejuni, Shigella, Salmonella, on Yersinia clinically.

Campylobacter means a curved rod. This organism is a motile, S-shaped gram negative rod which, to a trained eye, can be detected using a dark-field or phase-contrast microsope because of its shape and "darting motility". Sometimes, the organism can be recognized in a fresh fecal smear using the Gram stain. C. jejuni is part of the intestinal flora of most animals used for food production, such as cattle, sheep, swine, and poultry and is also part of the intestinal flora of dogs, cats, birds, and rodents. C. jejuni is transmitted to humans by (a) consumption of contaminated foods, primarily unpasteurized milk and undercooked poultry or beef; (b) drinking water which has been fecally contaminated by wild animals. Person-to-person transmission may also occur. The disease can present as an enteritis, colitis, or both and may simulate ulcerative colitis. The illness is usually self-limiting in a week or so, but symp-

toms last more than 1 week in 10-20% of patients seeking medical attention. In addition, relapses of the disease are not uncommon. The organism responds to erythromycin in most cases.

Vibrio Parahaemolyticus (55,80). This is a marine organism, preferring a high salt environment. V. parahaemolyticus colonizes fish and shellfish and causes illness when these foods are eaten raw or only partially cooked, a common practice in Japan and the Far East (77). In travelers, V. parahaemolyticus has caused outbreaks on cruise ships and on airplanes. One recent study from Bangkok, Thailand (a high-risk area) found that 45 of 146 patients with TD between October 1, 1978 and September 30, 1979 had infection with Vibrio parahaemolyticus (31%), with a peak risk in June and July. 87% of infected patients had a history of ingestion of seafood within the prior 48 hours, compared to 61% of patients with TD due to other agents ($P < 0.01$). The incubation period is usually 12-24 hours, after which watery diarrhea, abdominal cramps, and often vomiting ensue. Some patients develop a bloody, mucoid diarrhea, and colitis may occur. It is uncertain whether the organism is mainly invasive, enterotoxigenic, or both. The disease is invariably self-limited after 72 hours or so and bacteremia is extremely rare. The disease is also preventable because the organism is easily destroyed by heating the contaminated seafood prior to ingestion.

In the laboratory, pathogenic Vibrio can be separated from the many murine non-pathogenic Vibrio strains by their production of a zone of hemolysis when cultured on a high-salt blood agar plate (positive "Kanagawa reaction"). 97% of isolates from patients with diarrhea are Kanagawa positive, whereas only 1% of all seafood and marine isolates in Japan are Kanagawa positive.

In addition to V. parahaemolyticus, there is evidence that Plesiomonas shigelloides, Aeromonas hydrophila, and other types of Vibrio can cause TD.

Parasitic Infections (54). Amebiasis is a colonic infection. There is little evidence that amebiasis is a major risk for travelers to other countries. Even in Mexico, where Entameba histolytica is endemic, 1% or less of cases of TD are due to infection with this organism. Frachtman et al studied 326 American students who lived in Guadalajara for an average of 4 weeks. Of the 180 students who developed TD (55%), none had E. histolytica in their stools (44). Moreover, none of the 326 students underwent during their visit a seroconversion (using an indirect hemagglutination assay to detect antibodies to E. histolytica).

G. lamblia infects the upper small intestine primarily. Giardiasis is also an uncommon cause of TD, although it appears to be more common than amebiasis. It is well publicized that travelers to the Soviet Union are especially at risk for developing giardiasis, but this disease has been reported in travelers to more exotic destinations, such as Tahiti and the Mediterranean (25,26,81). The major source of Giardia lamblia cysts is fecally-contaminated water. In one study of 306 American tourists returning from the Soviet Union during 1969 to 1971, stool specimens were examined for cysts of G. lamblia. Of 153 specimens from patients who had diarrhea, 83 were positive for G. lamblia (54%). Only 9 stool specimens from 153 tourists who were not ill contained cysts of G. lamblia (6%). The authors found that 317 of 1211 tourists visiting Leningrad developed giardiasis (26%) compared to only 7 of 208 who had not visited Leningrad (3%), and they implicated the Leningrad water supply.

The major difference between a parasitic cause of TD (e.g., due to giardiasis) and a bacterial or viral etiology is that parasitic infections often do not resolve in a week or less. It is not uncommon for a patient with giardiasis to be ill for a month or two. Thus, a patient with prolonged diarrhea which has developed while abroad or shortly after returning home should be carefully investigated for parasitic infection. In the case of giardiasis, it may not be sufficient to examine the stool only; in some cases, a duodenal-jejunal aspirate and/or small intestinal biopsy may be necessary to demonstrate the organism. Giardiasis and amebiasis usually respond to metronidazole (Flagyl).

Other causes of slowly-resolving or chronic TD besides amebiasis and giardiasis include (a) persistent infection with Salmonella, Shigella, Campylobacter, and Yersinia (thus, stool cultures are mandatory); (b) acquired, temporary disaccharidase deficiency (especially, lactase deficiency); and (c) tropical sprue (96). The latter syndrome is associated with partial or total villous atrophy and usually occurs in foreigners who have lived in tropical countries for a long time (especially in the Indian subcontinent). Tropical sprue is very rare in tourists. Thus, the relationship between tropical sprue and TD is unclear, although the former usually responds to broad-spectrum antibiotics such as tetracycline. Another cause of persistent diarrhea is that an attack of TD may have exacerbated an underlying, pre-existing condition such as inflammatory bowel disease or celiac sprue. Finally, if the patient had received antibiotics either prophylactically or therapeutically, antibiotic-induced colitis due to infection with Clostridium difficile should be considered.

"A dirty cook gives diarrhea quicker than rhubarb."

Tung-su Pai

EPIDEMIOLOGY OF TD

Epidemiology is the scientific study of factors that influence the frequency and distribution of infectious diseases in man. We have already discussed some of these factors in TD. Other epidemiologic factors will be discussed in more detail.

1. Age of traveler. Younger people are more likely to contract TD than older people (11).

2. Country of origin of traveler. Travelers from highly industralized nations in temperate climates are at highest risk; travelers from tropical countries have a lower risk (3,84).

3. Destination of traveler (see Table 4).

4. Food and water consumption (85,89). There is little evidence that contaminated water is a common source of enteric infection in travelers visiting larger cities, although viral infection and giardiasis are exceptions to this statement. Studies in Mexico have shown that tap water and bottled water do not contain enteric pathogens, although water may be more likely to be contaminated in rural areas or in cities after heavy rains. There is no evidence that TD (e.g. ETEC infection) can be transmitted by person-to-person contact (86).

Most evidence suggests that food, with its inherent capacity to support bacterial growth, is the major source of enteric pathogens in TD. Food may contain pathogens that are not killed during food preparation or food may become fecally contaminated by food handlers who have poor sanitary habits. In fact, because of the proximity of Texas to the Mexican border and the high rate of immigration into the United States, it is quite possible without leaving Texas to contract "traveler's" diarrhea of the Mexican type via fecally-contaminated food. Similar cases of "TD" have reported elsewhere in the United States (e.g. from a Mexican restaurant in Superior, Wisconsin) (88).

Foods that are especially likely to be contaminated include leafy green vegetables, meats and other foods served buffet style when they are not steaming hot, hot sauces sitting on the table in open containers, desserts, fresh cheese, and milk served in questionable eating establishments. Bread and tortillas, packaged butter and jelly, fruit that can be peeled by the traveler and fresh citrus fruit and fruit juices, as well as anything boiled or served steaming hot, are of low risk.

The location of food consumption has been shown to have a significant impact on the incidence of TD in American students living in Mexico. Tjao et al found

that 13 of 29 newly-arrived students who had more than half of their meals in the school cafeteria or public restaurants developed TD during their first 30 days in Chalula, Puebla, Mexico (45%). On the other hand, only 3 of 20 students who ate most of their meals in a private home developed TD (15%). There was a significant increase in both ETEC and shigella infections in the former group. Students living in a private home had fewer people preparing their food (in fact, many of these students prepared their own food). Tjao also found that 40-50% of food samples purchased from the school cafeteria, various restaurants, street vendors, open markets, and a butcher shop were contaminated with enteric pathogens. Ten of 21 students who ate any food from street vendors developed TD (48%) compared to only 7 of 33 who did not eat food from vendors (21%, $P < 0.05$) (89).

Ericsson carried out a similar epidemiological study of U.S. summer students in Guadalajara, Mexico (85). Students were asked to keep a diary of where they ate their meals. They found that the incidence of TD correlated positively with the percentage of meals eaten outside of the "home" or apartment and, to a lesser extent, with the percentage of meals eaten at restaurants. This study could not incriminate the school cafeteria or street vendors, although too few students purchased food from vendors to allow meaningful conclusions.

I have already mentioned that TD may begin on the trip home. While this may represent infection acquired in the country visited, this may also be due to ingestion of contaminated food prepared by airport catering services. In one study of 25 international airport catering sources, 13 had toilets without sinks and 14 had toilets without soap and towels for the employees. Eight did not have adequate facilities for food refrigeration and cooking took place in an area just adjacent to the toilet facility in 5.

5. Purpose of travel. It has been noted in several studies that the risk of TD is related to purpose of travel. Travelers who visit their relatives have the lowest risk of TD; business people have an intermediate risk; and students, conventioners, and tourists have the highest risk. It is most likely that these differences are related primarily to location of food consumption (private homes versus restaurants). One study found that tourists from two low-risk countries (Switzerland and Germany) were significantly more likely to develop TD in tropical and subtropical countries if they participated in a multidestinational-adventure tour than if they took a beach vacation and stayed in only one hotel (87).

"A good reliable sett ov bowels iz wurth more
tu a man than enny quantity ov brains."

Josh Billings
His Sayings
1865

DIAGNOSTIC APPROACH TO THE PATIENT WITH TD

In most cases, diagnostic procedures are unnecessary in patients with TD. In fact, only approximately 10% of patients with TD consult a physician for treatment. Because the syndrome is usually self-limited, it is not necessary to launch into an expensive work-up. Moreover, laboratory tests to diagnose ETEC and viruses, two common causes of TD, are not available in most laboratories. Another reason for not carrying out early investigation is that even infections with Salmonella, Shigella, and Campylobacter species usually resolve spontaneously without specific therapy.

Investigation of TD is warranted only if it will influence therapy. This usually means a patient who has a severe illness (e.g. severe bloody diarrhea with high fever) or has a protracted illness lasting > 1-2 weeks. In these situations, some (or all) of the tests listed in Table 11 are indicated. If the patient has received antibiotics, a stool sample should be sent to a laboratory to look for Clostridium difficile toxin. In some cases, a therapeutic trial can be attempted (e.g. with antibiotics or metronidazole) while waiting for laboratory results or before instituting an expensive workup.

TABLE 11

TEST	LOOKING FOR
Stool examination under the microscope	Polymorphonuclear leukocytes, ova and parasites, Campylobacter morphology
Stool culture and sensitivity	Salmonella, Shigella, Campylobacter, Yersinia, other pathogens
Proctosigmoidoscopy with rectal swab and biopsy	Entameba histolytica Evidence for colitis
Duodenal aspirate and biopsy	Giardiasis, tropical sprue (very rare in travelers)
Serologic tests	Amebiasis
Blood culture	Salmonella, other invasive pathogens

"Accuse not Nature. She hath done her part;
do thou but thine."

John Milton
Paradise Lost
VIII: 561

TREATMENT OF TD

In most patients, TD is such a mild illness that no specific therapy is required. In some patients, however, watery diarrhea is voluminous and accompanied by electrolyte depletion. Decreased oral intake due to accompanying nausea, or even vomiting, may exacerbate fluid and electrolyte deficiency. Also, some patients become quite toxic, with bloody diarrhea and high fever, often along with prostration. Some individuals are evacuated back to the United States urgently because of severe disease. In these types of patients, therapy of TD is essential.

Fluid and Electrolyte Replacement (101,106,107). Patients can try to replace their losses with readily available fluids such as milk or fruit juices. In some instances, oral rehydration solutions (ORS) are needed. Such solutions are endorsed by the World Health Organization and are provided as UNICEF Oralyte anhydrous packets which contain glucose, NaCl, $NaHCO_3$, and KCl. When mixed with one liter of boiled drinking water, solute concentrations in the ORS are as indicated in Table 12.

TABLE 12

	O.R.S. SOLUTION
Glucose (gm/L)	20.0
Na^+ (mmol/L)	90.0
K^+ (mmol/L)	25.0
Cl^- (mmol/L)	85.0
$Base^-$ (mmol/L)	30.0

The O.R.S. takes advantage of the fact that glucose normally enhances sodium absorption in the jejunum and ileum and that TD due to ETEC or other agents leaves this coupled glucose-sodium absorptive mechanism unaffected. O.R.S. can

be used to successfully treat patients with cholera. It is apparent that the electrolyte composition of O.R.S. resembles that of stool water in patients with cholera or ETEC-infection (compare Table 12 and Table 9). Fresh O.R.S. should be made daily.

If O.R.S. packets are not available, a "home-made" solution can be prepared as follows (Table 13):

TABLE 13

5 TEASPOONS GLUCOSE OR 10 TEASPOONS SUCROSE
3/4 TEASPOON TABLE SALT
1/2 TEASPOON BAKING SODA (NOT BAKING POWDER)
1/4 TEASPOON KCl
BOILED DRINKING WATER Q.N.S. 1 LITER

If KCl is not available, fruit juices should be added to the diet. In adults, 250-500 ml of the O.R.S. or the home-made version should be ingested hourly. Coffee and tea should probably be avoided (as should certain cola drinks) because the caffeine may inhibit phosphodiesterase, increasing intracellular cyclic AMP levels further. In children above age 2, 125-250 ml per hour is given hourly.

In some patients, oral fluids may not be tolerated due to nausea and/or vomiting. Other patients may not be able to drink these solutions rapidly enough. In these circumstances, it may be necessary to administer these fluids via nasogastric tube or to administer fluids and electrolytes intravenously.

Antimicrobial Therapy. Because TD represents a syndrome usually produced by infectious agents, it is reasonable to consider antimicrobial therapy. In this setting, because antimicrobial therapy is usually instituted without a specific etiologic diagnosis, and because TD has several causes, a "broad-spectrum" antibiotic is necessary.

In vitro antibiotic sensitivites of ETEC, the most common cause of TD, are shown in Table 14. Study 1 was carried out in adults developing ETEC infection in Mexico or Bangladesh (91). Study 2 was carried out in physicians and their family in Mexico City (60). Study 3 was carried out in American adults and children in Southeast Asia (Phillipines, Korea, Taiwan, and Indonesia) (93).

TABLE 14

	PERCENT OF ETEC ISOLATED SENSITIVE TO VARIOUS ANTIMICROBIALS		
	STUDY 1 (N=88)	STUDY 2 (N=30)	STUDY 3 (N=176)
Antimicrobial			
Streptomycin	73	70	49
Tetracycline	80	77	55
Ampicillin	100	93	70
Kanamycin	100	-	95
Gentamicin	-	100	100
TMP/SMX	94	100	-
Sulfa Drugs	-	70	56
Chloramphenicol	94	83	60
Nalidixic Acid	100	100	-
Neomycin	-	100	95
Cephalothin	98	100	52

Results from study 1 and 2 were quite similar. A sizable minority of ETEC were not sensitive to streptomycin and tetracycline, whereas almost all ETEC were sensitive to ampicillin, aminoglycosides (kanamycin, gentamicin), trimethoprim/sulfamethoxazole (TMP/SMX), nalidixic acid and cephalothin. In study 3, conducted in Southeast Asia, antimicrobial resistance was more common, although most ETEC were sensitive to kanamycin and gentamicin.

It is not feasible to evaluate all of the promising agents in Table 14 as therapeutic agents in TD. Some of these agents are very expensive, some are only given parenterally, and some have significant side effect profiles. It should be emphasized that resistance to an antibiotic *in vitro* does not necessarily mean that this drug will be ineffective *in vivo*. In one study of TD due to shigellosis, patients almost invariably responsed to tetracycline with clinical improvement and eradication of shigella from the stool despite the fact only 41% of the shigella were sensitive to tetracycline *in vitro* (103). Conversely, sensitivity to an antibiotic *in vitro* does not guarantee sensitivity *in vivo*. This is because bacteria may acquire plasmid-derived antibiotic resistance factors (42) by conjugating with other bacteria (Fig. 2). There is evidence that enterotoxin can also be co-transferred from bacteria to bacteria via plasmids

along with antibiotic resistance factors (41,42). Thus, widespread use of antimicrobial agents in TD could select out resistant bacteria which are toxigenic (see below). With the above reservations in mind, I would like to review two placebo-controlled double-blinded clinical studies of the role of antibiotic therapy in TD.

TMP/SMX (92). In the summer of 1981, Dupont et al treated 110 American students in Guadalajara, Mexico who had developed TD. Patients were randomly allocated to 160 mgm TMP/800 mgm SMX p.o. b.i.d. for 5 days; 200 mgm TMP p.o. b.i.d. for 5 days, or placebo b.i.d. for 5 days. Mean daily stool frequencies after beginning therapy in the three groups are shown in Figure 4.

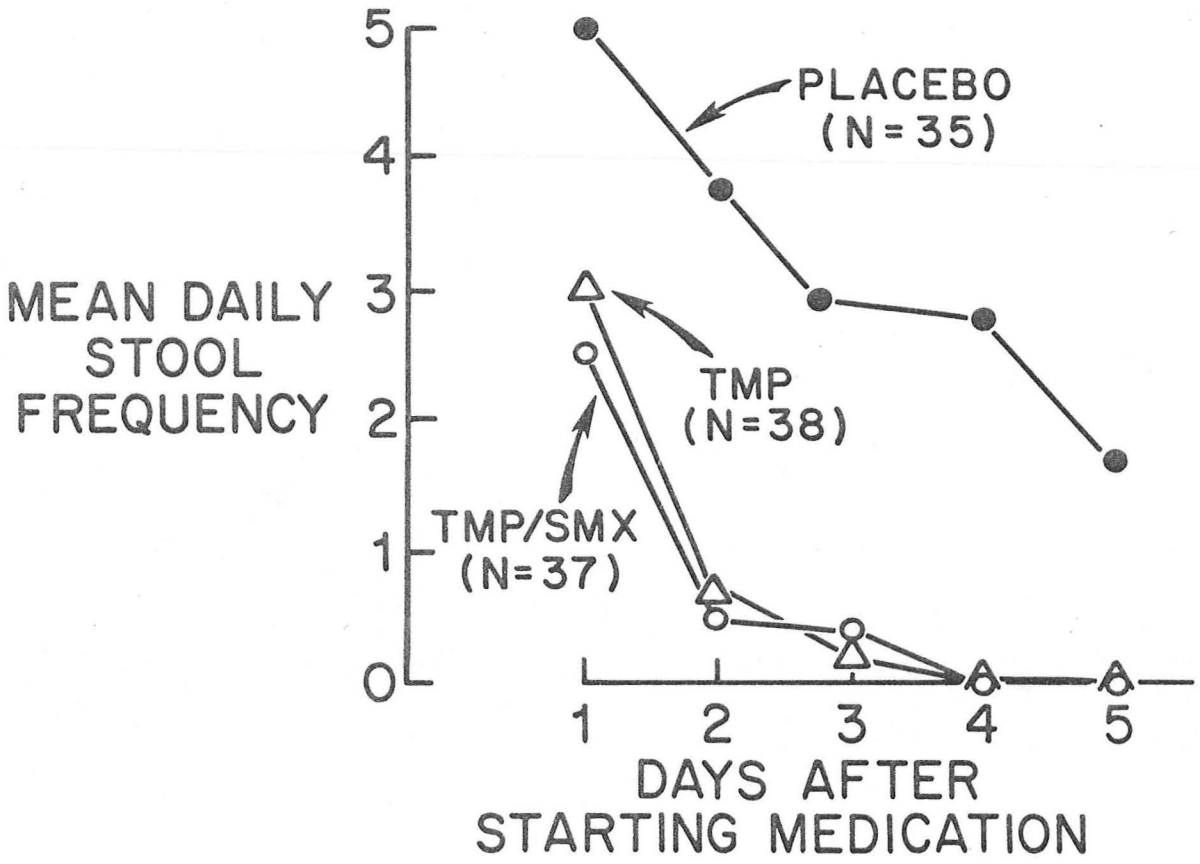

FIGURE 4

The placebo curve indicates that the illness was gradually improving during the 5-day study period. However, both TMP alone and TMP/SMX accelerated the resolution of diarrhea ($P < 0.001$). Relief from abdominal cramps and nausea was also more common in patients treated with active drugs. Causes of TD in this study were: ETEC (58%), Shigella (22%), Salmonella (4%), Campylobacter (3%), and "no pathogen" (24%). TMP/SMX and TMP were significantly effective in all 3 major sub-groups (ETEC, Shigella, "no pathogen"). Clinical deterioration during the trial or an illness which persisted beyond 5 days occurred in 49% of placebo-

treated patients, in 8% of TMP-treated patients, and in 5% of TMP/SMX-treated patients. 1 of 38 TMP-treated patients developed a rash, compared to none of 37 on TMP/SMX and none of 35 on a placebo. This study therefore showed that TMP, with or without SMX, was an effective and safe therapy for TD.

Bicozamycin (95). Ericsson et al evaluated a new, poorly absorbable antibiotic, bicozamycin, in 140 U.S. summer students in Guadalajara in 1980. This is an experimental drug not yet available commercially. The antimicrobial spectrum of bicozamycin includes many gram-negative rods and most enteropathogens such as ETEC, Shigella, and Salmonella. Seventy-two students with TD received 500 mgm bicozamycin four times daily for 3 days and 68 students receive a placebo four times daily for 3 days. Bicozamycin significantly improved diarrhea, abdominal cramps, and nausea. As in the TMP/SMX study, bicozamycin was effective in all 3 major etiologic subgroups of TD: ETEC, Shigella, and "no pathogen". As shown in Table 15, bicozamycin hastened elimination of Shigella species from the stool in those with TD due to this organism.

TABLE 15

	NO. WITH SHIGELLA IN STOOL	
	BICOZAMYCIN GROUP*	PLACEBO GROUP
Day 1	13	12
Day 3	1	9
Day 7	0	4
Day 14	0	1

*Therapy given days 1-3 only; P $<$0.001 bicozamycin vs. placebo group.

Only 1 of 72 patients in the bicozamycin group developed a rash, which was a macular, erythematous pruritic rash involving the foot and thigh.

These two controlled studies indicate that patients with TD, in general, respond more quickly to TMP/SMX or to bicozamycin than to no treatment and that both regimens are safe.

Bismuth Subsalicylate. Bismuth compounds have been used to treat non-specific diarrhea for many years. Pepto-Bismol contains 1.75% bismuth subsalicylate as well as flavoring, coloring in a suspending vehicle of magnesium-aluminum silicate and methylcellulsoe. This compound will turn the stools black and may lead to salicylate toxicity if taken in excess. Dupont et al explored a possible therapeutic role of bismuth subsalicylate in 111 American summer students in Chalula, Mexico. Fifty-six students with TD received 1 ounce of the active drug every 30 min for a total dose of 240 ml over 3.5 hours. Fifty-five students received a placebo liquid of identical appearance and taste. A second study was carried out in 58 other students. The second study was identical in design

except that the doses were doubled (i.e. 2 ounces every 30 min for 8 doses) (90). In both studies, there was a modest but significant reduction in stool frequency, but not in stool weight, in the bismuth subsalicylate group. Nausea, vomiting, and abdominal cramps also responded somewhat better in the active drug group, but only after patients with Shigellosis were excluded from analysis. The drug only appeared effective in the subgroup with ETEC infection and not in the Shigella group or the "no pathogen" group. On the other hand, a recent study of experimentally-induced ETEC infection in man was unable to confirm a therapeutic benefit for bismuth subsalicylate (116).

Experimental studies (94,97) have shown that bismuth subsalicylate and Pepto-Bismol are capable of inhibiting E. Coli enterotoxin - or cholera toxin-induced intestinal secretion in pigs and rabbits. There is also evidence that bismuth subsalicylate can inhibit activity of CT and LT in vitro (94). In rabbits, bismuth subsalicylate was more effective in inhibiting intestinal secretion when the drug was given before exposure to toxin than when the drug was given after toxin had bound to the mucosa. Thus, bismuth subsalicylate may be more effective when given prophylactically than after secretory diarrhea has already been initiated (see below).

The mechanism of action of bismuth subsalicylate is uncertain. Bismuth subcarbonate does not prevent LT-induced fluid accumulation in ligated loops of rabbit ileum. Thus, the salicylate moiety may be the active component and may act by blocking intestinal mucosal prostaglandin synthesis and hence prostaglandin-dependent stimulation of adenylate cyclase activity.

Antidiarrheal Agents. Drugs used to slow intestinal transit are widely used in diarrheal disease, including TD. Many experts are cautious about slowing intestinal peristalsis in TD because this may delay excretion of a pathogen and allow further replication and damage. For example, Shigellosis may be exacerbated by antidiarrheal agents. A recent study showed that an anticholinergic agent, in a dose sufficient to produce anticholinergic side effects, had no benefit over a placebo in TD occurring in Mexico (105).

A more recent study found that loperamide (Imodium), a peripehrally acting opiate agonist, was more effective in relieving diarrhea than bismuth subsalicylate in American students with TD visiting Latin America. Patients with bloody diarrhea or with a temperature $>102^{0}$ F were excluded from this experiment (98). Loperamide was safe in this study. At the present time, there is insufficient evidence regarding the role of antidiarrheal agents in the treatment of mild to moderate TD. They should be avoided in severe cases associated with fever and bloody diarrhea.

"Now to perform a true physician's part,
And show I am a perfect master of my art,
I will prescribe what diet you should use,
What food you ought to take, and what refuse."

The Remedies of Love, v. 796
Ovid (43 B.C. - A.D. 17?)

PREVENTION OF TD

Nearly everyone who is planning a trip to Mexico or some other "high-risk" area wishes to know how to prevent TD. Some people are willing to dedicate a suitcase to allow an entire pharmaceutical armamentarium to accompany them to their destination. Others are more interested in dietary advice, although most have already been told by a friend or relative "Don't drink the water". There are several options when confronted with prophylaxis of TD.

Dietary Measures. As reviewed above, there is usually little evidence to incriminate water as a source of infection in TD. Nevertheless, many tourists avoid water and instead drink wine, beer or Coca-Cola. If desired, water can be purified by boiling it or by adding a few drops of chlorine bleach or iodine per quart at least 30 minutes prior to drinking the water. However, most evidence suggests that tap water is safe and these "extra-effort" measures are unnecessary. Ice cubes may become contaminated by food handlers, however.

Certain foods should be avoided. These include raw vegetables, raw meat, raw fish and shellfish, food which has not been refrigerated or has been left out at room temperature for several hours, and food which is undercooked. In general, food served "piping hot" will contain no viable pathogenic bacteria. Food from street vendors should be avoided as should food in restaurants and hotels with a bad local reputation. Airport and airplane food are a decided gamble.

Do these recommendations really work? Steffen et al carried out an extensive survey of more than 16,000 Europeans who were travelling to "high-risk" areas (126). The overall incidence of TD was 34% in this study. This incidence was not changed by the 1500 travelers who only avoided tap water to try to prevent TD (33% got TD). However, 38% of the 9500 travelers who restricted their diets to avoid TD developed the syndrome ($P < 0.001$ versus entire group), whereas in the 3400 travelers who had not used any water or dietary restrictions, the incidence of TD was only 30% ($P < 0.01$ versus the entire group). The authors concluded that "diarrhea seemed to occur more frequently the more a person tried to elude it". Thus, there is no compelling evidence in the literature that one can prevent TD by dietary modifications, although a certain degree of caution by the traveler seems prudent.

Modification of the Intestinal Flora. *In vitro* and *in vivo* animal studies suggested that yogurt, buttermilk or other agents which contain live Lactobacilli might prevent ETEC from colonizing the intestine and also might produce antitoxins locally. Lactinex is a product marketed for the treatment of non-specific

diarrhea, consisting of dried Lactobacillus acidophilus and Lactobacillus bulgaricus. Two studies have recently been published evaluating Lactobacillus preparations in the prophylaxis of TD (one in travelers to Mexico and another in volunteers exposed to ETEC). Lactobacilli were ineffective in both of these placebo-controlled studies (109,110).

Antimicrobial Drugs. Tetracycline can prevent cholera in family members of cholera patients (120). The concept of antibiotic prophylaxis of TD is not new (117). Several antimicrobial agents have been shown, in placebo-controlled studies, to be effective in preventing TD. Some of the drugs studied and the country in which they have been tested are listed in Table 16.

TABLE 16

DRUG	COUNTRY
Sulfa drugs	Mexico
Streptotriad	Italy, Mexico
Neomycin	Mexico
Doxycycline	Kenya, Morocco, Mexico
TMP ± SMX	Mexico
Erythromycin	Mexico

Streptotriad (streptomycin, sulfadimidine, sulfadiazine, and sulfathiazole) was used successfully by the British Olympic Team in the 1960 Summer Olympics in Rome and again in 1968 during the Summer Olympics in Mexico City. According to Turner, "the incidence of diarrhea in the U.S.A. and Australian teams, who were eating under similar conditions, was much higher than in the British Team" (128,129). The two most effective and adequately tested regimens in Table 16 are doxycycline and TMP/SMX (125). I will therefore review these in some detail. A recent study evaluating erythromycin will also be discussed.

Doxycycline (121). Sack et al studied Peace Corps volunteers traveling to Kenya in 1975 and found that 69% developed TD within 5 weeks (2% per day at risk) (19). The most common pathogen was ETEC. Because most ETEC were sensitive to tetracycline, Sack reasoned that doxycycline might prevent TD in these volunteers. Doxycycline, rather than tetracycline, was chosen for his prophylaxis study because of (a) its long duration of action; (b) its unique excretion by small intetinal mucosa and (c) no need for dosage adjustments in patients with renal or liver disease (130).

In 1976, Sack et al treated 18 volunteers with 100 mgm doxycycline once daily for 3 weeks beginning shortly after arrival in Kenya compared to 21 subjects

given placebo (124). During the 3-week treatment period, TD developed in 9 of the placebo-treated patients (43%, or 2.2% per day at risk) compared to in only 1 doxycycline-treated patient (6%, or 0.3% per day at risk). This one patient had Shigella sonnei infection which was resistant to tetracycline and ampicillin in vitro. The placebo-treated patients who developed TD had primarily ETEC infection. Three subjects developed TD 8,9, and 10 days after discontinuation of doxycycline chemoprophylaxis. No side effects of doxycycline were mentioned. Nearly identical results were reported in another study in Peace Corps volunteers in Morocco (123).

One uncontrolled study suggested doxycycline was ineffective in preventing TD in Mexico (7). However, Freeman et al studied 145 crew members of the U.S.S. Belleau Wood during a 2.5 day visit to Mexico (115). Subjects received either 200 mgm doxycycline on arrival and 100 mgm daily thereafter while in Mexico or placebo capsules. TD occurred in 15 placebo-treated patients and 3 in the doxycycline group ($P = 0.002$). The major pathogen isolated was ETEC in both groups. Nausea with or without vomiting occurred in 5 of 75 doxycycline-treated patients, usually following the 200 mgm dose. None developed skin photosensitivity. There was no rebound effect after discontinuation of doxycycline, unlike in earlier studies in Kenya and Morocco, probably because the ship personnel remained in Mexico for a short time, whereas the Peace Corps volunteers continued to live in Africa after doxycycline was discontinued.

In summary, doxycycline appears to be a safe and effective agent for preventing TD in Africa and possibly in Mexico. Except for gastrointestinal side-effects in some patients, the drug seems safe.

Trimethoprim/Sulfamethoxazole. Dupont et al have carried out two studies in Guadalajara, Mexico evaluating TMP/SMX in the prophylaxis of TD. In the first study, students received either 160 mgm TMP/800 mgm SMX b.i.d. for 21 days or placebo (111). TD occurred in 44 of 80 students receiving placebo (55%, or 2.6% per day at risk) compared to in 11 of 67 students taking TMP/SMX (16%, or 0.8% per day). Bacteriologic studies indicated significant protection against ETEC and possible protection against Shigella (4 cases of Shigella in placebo group with TD, none in TMP/SMX group with TD). Within 9-16 days after beginning TMP/SMX, a generalized rash occurred in 12 subjects in the TMP/SMX group (18%) compared to only 1 in the placebo group, and the rash necessitated discontinuation of the drug in these patients. Some patients also developed mouth soreness. Within 8 days after discontinuation of TMP/SMX in the entire group, a rebound effect was observed: 14 students developed TD (20.9%) compared 2.5% in the placebo-group ($P < 0.05$). Thus, the drug was only protective during administration.

In the second study (114), subjects received either a placebo once daily, 200 mgm TMP once daily, or 160 mgm TMP/800 mgm SMX once daily for 14 days. Significantly less TD occurred in subjects given TMP (8%) or TMP/SMX (2%) than in subjects receiving placebo (33%). TMP/SMX was superior to TMP alone ($P < 0.05$). These agents reduced ETEC infection and prevented infection with Salmonella and Shigella strains. Again, a rebound effect was observed after drug discontinuation. Rash occurred in 3% of TMP/SMX patients and in 7% of TMP patients and sometimes led to discontinuation of medication. Mouth soreness also occurred infrequently in both active drug groups.

Erythromycin. Andremont recently reported that 1000 mgm erythromycin per day prevented TD in American tourists visiting Acapulco, Mexico (108). Seven of

24 placebo-treated subjects compared to none of 24 erythromycin-treated subjects developed TD. This study is interesting for the following reasons: (1) Erythromycin is better known for its activity against gram positive organisms, but is also effective in reducing fecal Enterbacteriacaea; (2) erythromycin is usually well-tolerated and is not expensive; (3) plasmid-mediated resistance to erythromycin has not been described in Enterobacteriacaea; and (4) unlike TMP/SMX, erythromycin is active against Campylobacter species, an important cause of TD in some areas. More studies are needed to confirm the efficacy of erythromycin in preventing TD.

Bismuth Subsalicylate. In rabbits with ligated ileal loops, this compound was able to prevent enterotoxin-induced fluid accumulation. Thus, this compound was evaluated as a prophylactic agent in human TD (112). Sixty-two American students in Guadalajara received 60 mL bismuth subsalicylate four times daily for 21 days, whereas 66 patients took a placebo liquid that looked and tasted like active drug. Thus, during the entire test period these heroic subjects ingested more than 5 liters of a Pepto-Bismol like fluid. The salutary effect of active drug in preventing TD has already been demonstrated in Figure 1 on page 6. Moreover, enteric pathogens were less commonly isolated from the stool in bismuth subsalicylate-treated subjects. Thus, this drug may inhibit intraluminal bacterial proliferation as well as reduce toxin-mediated secretion. It is also possible that bismuth subsalicylate prevents adhesion of pathogens to the mucosa. A recent study in which volunteers were challenged with ETEC also found that bismuth subsalicylate prevented diarrheal illness (50% in placebo group versus 13% in bismuth subsalicylate group, $P < 0.05$) (116).

The cost of some therapeutic and prophylactic regimens discussed above are summarized in Table 17.

Table 17

TREATMENT OF TD	DOSE	COST TO TRAVELER*
TMP/SMX	One twice daily for for 5 days	$7.80 ($6.05)
Pepto-Bismol+	30 mL every 30 min for 8 doses	$2.39
PREVENTION OF TD	**DOSE**	**COST TO TRAVELER***
Doxycycline	100 mgm daily	$17.20 ($12.25)
TMP/SMX	One daily	$7.80 ($6.05)
TMP alone	200 mgm daily	$6.87
Erythromycin	1000 mgm daily	$8.19 ($7.19)
Pepto-Bismol+	60 mL four times daily	$23.90

*Numbers in parenthesis refer to cost of generic drug, when available. Costs of prevention regimens are based upon a 10 day stay. Cost data kindly provided in March, 1984 by Anna Djardjevic, Eckerd's Pharmacy, Preston and Royal, Dallas, Texas.
+Active ingredient, 1.75% bismuth subsalicylate.

Immunopropylaxis. There is considerable research on attempts to develop a vaccine to prevent TD, as summarized nicely by Levine (119). With regard to ETEC, both the LT and CFA are antigenic and could possibly be used as antigens for a vaccine (113,127).

"Travel is the road to nothingness."

John S. Fordtran, M.D.
(1931-)

RECOMMENDATIONS FOR TRAVELERS FROM "LOW-RISK" TO "HIGH-RISK" COUNTRIES

The most reliable way to avoid traveler's diarrhea is to adopt John Fordtran's philosophy and stay home. However, if the trip is deemed necessary or perhaps even desirable, it is reasonable to anticipate questions from patients regarding prophylaxis and/or early treatment.

In my opinion, routine antibiotic prophylaxis is not justified for the average traveler. Widespread use of antimicrobial agents in "high-risk" countries may lead to the emergence, due to selection pressure, of bacteria that are now resistant to these drugs. Moreover, because resistance to antibiotics is plasmid-mediated as are genes for virulence factors (enterotoxin production, CFA production), the emerging, resistent strains may prove to be quite poisonous. Theoretically, TMP/SMX should be less likely to lead to development of resistance because these drugs interfere with bacterial folic acid synthesis by inhibiting two different, but sequential, enzymes in the folate pathway: dihydrofolate reductase and dihydropterate synthetase, respectively. Thus, development of resistance would be unlikely because either two independent mutational events would be required in the bacterial chromosome or the bacteria would have to acquire two separate resistance genes which happen to reside on the same plasmid. Despite these theoretical considerations, an experiment in U.S. students in Mexico demonstrated that, during prophylactic therapy with TMP/SMX, the fecal flora is partially or totally replaced by Enterobacteriaceae (mainly E. Coli) which are resistant to both TMP and SMX (100). Fortunately, almost all of the newly-acquired E. Coli in that study were non-enteropathogenic, but this will not necessarily be true in the future.

Another reason for not recommending routine antimicrobial prophylaxis in travelers is that this approach subjects many individuals to an unnecessary and

costly treatment. For a 10-day visit to Mexico, 15-40% of subjects will develop TD. If each had been treated prophylactically, 60-85% of individuals would be receiving a drug without need. Moreover, the most effective prophylactic agents, doxycycline and TMP/SMX, have gastrointestinal and/or cutaneous side effects which may limit their usefulness.

Certain individuals probably should be considered for prophylaxis. These include patients who might not easily tolerate a major diarrheal illness with associated loss of fluid and electrolytes (e.g. patients on diuretics and/or digitalis); patients with inflammatory bowel disease; patients who may be at high risk because they have had ulcer surgery or are receiving acid antisecretory drugs or antacids which increase gastric pH; or businessmen or politicians in whom an severe episode of TD could have serious consequences (e.g. an important business meeting or speech could be cancelled). Some individuals who fit into none of these categories will ask to receive prophylaxis in order to "be sure" they will not get ill on their long-planned vacation or honeymoon. If prophylaxis is used, my choice is TMP/SMX or TMP alone due to their low cost, although erythromycin may be a viable alternative (Table 17). Travelers are sometimes under the misconception that, if they are receiving any type of prophylactic, they have license to devour all types of food indiscriminately.

If prophylaxis is not used (and it usually should not), the most rational approach to therapy, in my opinion, is <u>early</u> treatment of TD when it develops. If diarrhea is mild and there are minimal associated symptoms, no medication may be required and attention should be focused on satisfactory fluid and salt intake. In mild to moderate cases of TD, bismuth subsalicylate can be given (30 ml q 30 min X 8 doses). Although results with Pepto-Bismol are not always dramatic and relapses may occur, the regimen appears to be effective and is inexpensive. Moderate to severe cases of TD should be treated promptly by TMP/SMX taken b.i.d. for 5 days (Table 17). If bicozamycin becomes commercially available, this may be a reasonable alternative. If TD persists after treatment with TMP/SMX, especially into the second week, further bacteriologic and parasitic evaluation is indicated so that specific antimicrobial therapy can be prescribed after a specific diagnosis is made. The evaluation of the persistent or non-responding TD patient is described in Table 11 of this protocol.

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