Annual Report
August 1978
(for the period 1 December 1977 to 31 August 1978)

by

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Supported by
US Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701
Contract No. DAMD 17-78-C-8011

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents
Enterotoxigenic Escherichia coli in Adult Volunteers; Immunity to Enterotoxigenic Escherichia coli; Traveler's Diarrhea; Enteropathogenic Escherichia coli in Adult Volunteers; Antibacterial Immunity Against E. coli; Antitoxic Immunity Against E. coli.

Enterotoxigenic Escherichia coli (ETEC) represent the most frequent etiologic agent of Traveler's Diarrhea. Studies of immunity to ETEC were undertaken in volunteers in order to evaluate the feasibility of immunoprophylaxis against ETEC. Seventeen students and other community volunteers were given $10^6$ or $10^7$ organisms of E. coli B7A (0148:H28) which produces heat-labile (LT) and heat-stable (ST) enterotoxins. Ten individuals developed diarrheal illness closely resembling natural Traveler's Diarrhea; of these ten, rises in titer of circulating antitoxin and antisomatic (See next page for continuation)
antibody occurred in eight (80%). Eight B7A "veterans" and 12 controls were challenged nine weeks later with 10^7 B7A organisms. Only one of eight "veterans" developed diarrhea vs. seven of 12 controls (p=0.05). Despite clinical protection, all "veterans" excreted B7A after rechallenge.

Four controls who developed diarrhea during the homologous B7A rechallenge were rechallenged nine weeks later with 10^7 organisms of E. coli strain E2528-C1 (O25:NM) which produces only LT and which shares no other common antigens with B7A. Three of four "veterans" and two of six controls developed comparable diarrhea.

These studies demonstrate that prior illness due to ETEC can confer significant homologous immunity against subsequent challenge and the operative mechanism apparently is not bactericidal. Heterologous protection was not conferred where the only common antigen was LT, indicating that LT antitoxin also is not protective. These data suggest that local anti-adhesive mechanisms deserve further study as the possible operative mechanism of immunity against ETEC diarrhea.
# ANNUAL REPORT

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PREFACE

Acute intestinal infections represent one of the most important health problems of military importance. Such infections are of particular concern when contingents are sent from areas with high levels of sanitation and bacteriologically clean water supply, such as in the U.S.A., to less developed areas of the world. The Travelers' Diarrhea that occurs in personnel of such contingents, acquired by ingestion of contaminated food and water, significantly impedes the function and productivity of individual soldiers as well as of entire units. Enterotoxigenic Escherichia coli have been found to be the most frequent agents of acute Travelers' Diarrhea, accounting for approximately 50% of cases.

Our U.S. Army Medical Research and Development Command - supported work during the past year has focused on two main areas:

1) Studies of immunity to enterotoxigenic Escherichia coli in man.

2) Studies of the virulence of enteropathogenic E. coli (EPEC) strains that are epidemiologically associated with diarrhea yet do not give evidence of production of heat-labile or heat-stable enterotoxins when examined by standard, sensitive assays.

A summary of our studies in these areas is contained in the ensuing pages.
PART A: STUDIES OF IMMUNITY TO
ENTEROTOXIGENIC ESCHERICHIA COLI IN
VOLUNTEERS
Enterotoxigenic Escherichia coli (ETEC) represent the most frequent etiologic agent of Travelers' Diarrhea. Studies of immunity to ETEC were undertaken in volunteers in order to evaluate the feasibility of immunoprophylaxis against ETEC. Seventeen students and other community volunteers were given $10^6$ or $10^8$ organisms of E. coli B7A (0148:H28) which produces heat-labile (LT) and heat-stable (ST) enterotoxins. Ten individuals developed diarrheal illness closely resembling natural Travelers' Diarrhea; of these ten, rises in titer of circulating antitoxin and antisomatic antibody occurred in eight (80%). Eight B7A "veterans" and 12 controls were challenged nine weeks later with $10^8$ B7A organisms. Only one of eight "veterans" developed diarrhea vs. seven of 12 controls ($p=0.05$). Despite clinical protection, all "veterans" excreted B7A after rechallenge.

Four controls who developed diarrhea during the homologous B7A re-challenge were rechallenged nine weeks later with $10^9$ organisms of E. coli strain E2528-C1 (025:NM) which produces only LT and which shares no other common antigens with B7A. Three of four "veterans" and two of six controls developed comparable diarrhea.

These studies demonstrate that prior illness due to ETEC can confer significant homologous immunity against subsequent challenge and the operative mechanism apparently is not bactericidal. Heterologous protection was not conferred where the only common antigen was LT, indicating that LT antitoxin also is not protective. These data suggest that local anti-adhesive mechanisms deserve further study as the possible operative mechanism of immunity against ETEC diarrhea.
INTRODUCTION:

As modern high-speed transport and changing social customs have encouraged the movement of persons from one part of the globe to another, Travelers' Diarrhea has emerged as a vexing consequence. Enterotoxigenic Escherichia coli (ETEC) are the most frequent etiologic agents of short-incubation Travelers' Diarrhea (17,28,40) and in some areas also appear important as a cause of endemic infantile diarrhea (8,18).

Prophylactic antibiotics have shown some efficacy in diminishing the risk of development of Travelers' Diarrhea, including that due to ETEC (21,37,43). However, control of this problem via chemoprophylaxis with antibiotics has multiple drawbacks including:

1) Daily doses must be taken throughout the travel period; 2) adverse drug reactions may occur; 3) development of antibiotic-resistant bacterial strains may be encouraged; 4) normal intestinal flora are altered perhaps leading to enhanced susceptibility to other enteropathogens such as shigella and salmonella (3,16,20).

Because of the limitations of chemoprophylaxis with antibiotics, the concept of immunoprophylaxis to control Travelers' Diarrhea due to ETEC is appealing. However, a vaccine to prevent diarrhea due to ETEC will have to protect against multiple serotypes, often possessing several distinct virulence properties. For example, numerous E. coli serotypes involving many O, H and K antigens, have been incriminated as pathogenic enterotoxigenic strains (11,15,28,31,36). Furthermore, most ETEC elaborate heat-stable enterotoxin (ST) as well as heat-labile enterotoxin (LT) (28,36). LT, which resembles cholera toxin pharmacologically and immunologically, is of large molecular weight, immunogenic and neutralizable by LT or cholera antitoxin (12,19,29). ST, in contrast, is of low molecular weight, is not (or is minimally) immunogenic, and is not neutralized by LT or cholera antitoxin (1,12,19,29);
nevertheless, *E. coli* that elaborate only ST are virulent for man (24,35).

Because of the multiplicity of serotypes and toxinogenic types, studies were carried out to investigate several aspects of immunity to ETEC, including:

1) To determine if diarrheal infection due to a particular ETEC strain that produces both LT and ST would confer protection against subsequent challenge with the identical *E. coli* strain.

2) To investigate whether individuals who experience diarrheal illness due to an *E. coli* strain that produces both LT and ST, and who develop an immunologic response (documented by rises in circulating LT antitoxin and antisomatic antibody), will be protected against subsequent challenge with a heterologous *E. coli* strain of different antigenic composition that elaborates only LT.

3) To quantitate the circulating immune response to various ETEC antigens.

4) To determine the relative importance of antibacterial versus antitoxic immunity against *E. coli*.

**MATERIALS AND METHODS:**

**Volunteers:** Volunteers were college students and other free-living healthy adults (mean age 25 years, range 18 to 41). Challenge studies were carried out in the 22-bed Isolation Ward of the Center for Vaccine Development. The protocol was approved by the University of Maryland Hospital Human Volunteer Research Committee. The studies were explained to volunteers in detail and signed, witnessed consent was obtained. The informed nature of consent was documented prior to inoculation by requiring that all
volunteers pass a written examination containing multiple choice and true-false questions on all aspects of the study including purpose, hazards, procedures, and pertinent bacteriology and immunology (23). The pre-inoculation health status of volunteers was ascertained from medical history, physical examination, chest radiograph, electrocardiogram, complete blood count, negative pregnancy test, urinalysis, blood chemistries (including serum glucose, urea nitrogen, and electrolytes), and tests for liver function, syphilis and hepatitis B surface antigen.

**Challenge Strains:** *E. coli* strain B7A (0148: H28), which elaborates LT and ST (LT+/ST+), was isolated from a U.S. soldier with diarrhea in Vietnam (11). *E. coli* strain E2528-C1 (025:NM) was responsible for an outbreak of diarrhea which involved several hundred persons on a cruise ship; this strain produces only LT (LT+/ST-) (5).

Neither challenge strain exhibited mannose-resistant hemagglutination when grown on solid agar or in broth (14,32). Both strains, after 48 hour stationary culture in Mueller-Hinton broth, manifested mannose-sensitive hemagglutination of human type A and guinea pig erythrocytes, indicative of the presence of 'common' type 1 somatic pili (9,10,30). Antibody to colonization factor antigen I (CFA/I) of *E. coli* H10407, prepared according to the method of Evans et al (13), failed to agglutinate either challenge strain after cultivation on solid or in broth media. *E. coli* B7A was strongly agglutinated by antibody to H10407 type 1 somatic ('common') pili (kindly provided by C. C. Brinton, Jr., Pittsburgh, Pa.).

The challenge strains were non-invasive (negative in the guinea pig eye test) (39) and were sensitive to multiple antibiotics including ampicillin, neomycin, trimethoprim/sulfamethoxazole, kanamycin, gentamicin and nalidixic acid.
Inocula and Challenge: Inocula were prepared as previously described (5, 24). Briefly, 18 hour trypticase soy agar cultures of the challenge strains were harvested with saline and appropriate dilutions in saline were made. All volunteers drank 240 ml. of water containing two grams of NaHCO₃. One minute later the inoculum (10⁶, 10⁸, or 10⁹ organisms), which was suspended in 45 ml. of phosphate-buffered saline (pH 7.2) was ingested. Fifteen minutes later, the volunteers (except three in the initial challenge group) drank another 240 ml. of water containing one gram of NaHCO₃. Volunteers were allowed nothing orally for 90 minutes before or after challenge. Inoculum size was quantitated by replicate pour-plate technique.

Clinical Observations: Volunteers were examined daily beginning two to three days prior to inoculation. Oral temperatures were taken every six hours and repeated within five minutes if 100°F (circa 37.8°C) or above. All stools and vomitus were collected in plastic cholera seats, examined, graded and the volume measured by a nurse or physician. Stools were graded on a five point scale (25) in which grades 1 and 2, representing fully formed and soft stools, respectively, are considered gradations of normal. Grade 3 describes thick liquid stool; grade 4 denotes opaque watery and grade 5 rice water stools. Diarrhea was defined as two or more loose (grade 3-5) stools in 24 hours or at least one voluminous loose stool (>200 ml.). Prior to discharge, all volunteers received a five day course of oral antibiotics (tetracycline or neomycin) to eradicate stool excretion of the pathogen.

Typing Sera: Specific antisera for agglutination of the E. coli strains were prepared in rabbits as previously described (24).

Stool Culture: Stool specimens or rectal swabs were inoculated on Levine's Eosin-Methylene-Blue (EMB) agar with and without appropriate anti-
biotic. Streptomycin, or chloramphenicol was incorporated into EMB agar to facilitate recovery of organisms from volunteers who ingested B7A, or E2528-C1, respectively; the strains were resistant to these respective antibiotics. Fifteen colonies (at least five from the agar plate without antibiotics) possessing a typical *E. coli* metallic sheen were picked and subcultured to slants of trypticase soy agar in screw top tubes. After 18 hours of incubation at 37°C, the *E. coli* were tested for agglutination with specific antiserum; the challenge *E. coli* served as a positive control and saline as a negative control. Slants were overlaid with mineral oil and stored at 4°C until tested for LT within four weeks.

**Enterotoxin Assays:** *E. coli* from slants were inoculated into synctase media (0.5 ml. in vials) and incubated overnight at 37°C. 0.05 ml. of the culture was then added to monolayers of Y-1 adrenal cells in 96-well tissue culture plates. The cells were examined for rounding (evidence of LT enterotoxin) after 20 hours of incubation at 37°C in 5% CO₂. Positive control strains included stock *E. coli* H10407.

**Serology:** Sera were collected pre-challenge and 10 and 21-28 days post-challenge. After heat inactivation (56°C, 30 min.) and absorption with unsensitized sheep erythrocytes, antisomatic antibody was measured by microtiter passive hemagglutination technique (22) using glutaraldehyde-treated sheep erythrocytes (2) coated with lipopolysaccharide antigen from *E. coli* B7A or E2528-C1. Antitoxin to *E. coli* LT was assayed by adrenal cell neutralization technique (7,38).

**RESULTS:**

**Dose Response Studies with *E. coli* B7A:**

Two challenges were undertaken to identify the dose of ETEC organisms
of strain B7A that would induce a clinical attack rate of approximately 60-70% (ID_{60}-ID_{70}) in healthy adult volunteers. As seen in Table 1, three of six volunteers who ingested 10^6 organisms (50%) and seven of 11 who received 10^8 organisms (64%) developed diarrhea. The clinical and immunologic responses of the 10 volunteers who developed illness are presented in Table 2 and demonstrate that the clinical spectrum of the volunteer disease model resembles that of naturally acquired "Travelers' Diarrhea".

Prior to challenge, no volunteers had E. coli in their stools that were agglutinated by B7A antisera. Within 24-48 hours post-ingestion, all volunteers developed positive stool cultures and E. coli B7A was the predominant coliform. Approximately one dozen E. coli clones that agglutinated with B7A antisera were randomly selected from each volunteer's stool cultures and tested for LT in the adrenal cell assay; 167 of 192 tested were positive (87%). Volunteers were not given antibiotics until the eighth day post-challenge in order to ensure an adequate antigenic stimulus.

Homologous B7A Re-challenge:

The ten volunteers who developed diarrheal illness during the dose response studies were asked to participate in a homologous re-challenge study and eight agreed. Nine weeks after the initial B7A challenge, these eight B7A "veterans" and 12 control volunteers ingested 10^8 E. coli B7A organisms. The results of this challenge are shown in Table 3. Only one of eight "veterans" (13%) developed diarrheal illness versus seven of 12 controls (58%). Despite the small numbers, this difference in attack rates was statistically significant (p=.05, Fisher's Exact Test) (4). The paucity of diarrheal illness in the eight veterans upon re-challenge was in distinct contrast with their clinical response to initial challenge (Table 3).
Despite the clinical protection evident in the veteran group upon re-challenge, positive stool cultures were encountered in this group with the same frequency as the control group. Quantitative cultures were not done. Onset of excretion occurred one day later in the re-challenge group but the percentage of \textit{E. coli} colonies picked that were agglutination-positive (i.e. B7A) were the same in the stool cultures of the 'veteran' and control groups.

The titers of circulating antitoxic and antisomatic antibody in the eight veteran volunteers in relation to the two challenges are displayed in Table 4. Clear-cut correlations between these antibody levels and protection or susceptibility cannot be made from the data. The single "veteran" who developed illness upon re-challenge had demonstrated (Table 4) a complete immunologic response after initial challenge, with significant rises in antitoxic (8-fold) and anti-somatic (8-fold) circulating antibody titers. These titers diminished four-to-eight fold from their peak to the time immediately prior to re-challenge.

**Heterologous Re-Challenge Study:**

Four volunteers who developed diarrhea while serving as controls in the B7A homologous re-challenge agreed to return ten weeks later to participate in a heterologous re-challenge study. These four "veterans" and six control volunteers were fed $10^9$ organisms of \textit{E. coli} strain E2528-C1; this strain (O2S:NM) produces LT but not ST and possesses somatic pili that are antigenically distinct from \textit{E. coli} B7A. Dose response studies with E2528-C1 and other LT+/ST- \textit{E. coli} are reported elsewhere (M. M. Levine, D. R. Nalin, E. J. Bergquist, R. B. Hornick, submitted for publication). Three of four "veterans" and two of six control volunteers developed diarrhea of comparable
Following their initial challenge with *E. coli* B7A, all four "veterans" developed four-fold or greater rises in circulating antitoxin (Table 6). However, by the time of re-challenge only two veterans still had serum antitoxin levels of eight units/ml or above. Following ingestion of *E. coli* E2528-C1, four of six controls developed rises in circulating antitoxin versus only one of four veterans. All volunteers, veterans and controls, had positive stool cultures.

**DISCUSSION:**

*E. coli* B7A was selected as the main challenge organism because enterotoxigenic O148:H28 strains have been associated with Travelers' Diarrhea in several geographically dispersed areas (11,13). The selection was well justified; within 24-48 hours after challenge, *E. coli* B7A became the predominant coliform flora in stool cultures, the clinical illness mimicked natural Turista and the volunteers had prominent serologic responses to both toxin and somatic antigen (Tables 1 and 2).

The exact mechanism responsible for the immunity conferred by initial *E. coli* B7A infection is not clear. Possibilities include anti-LT, anti-somatic or flagellar antibody and anti-adhesion factor antibody, either local or circulating. Since ETEC do not invade the mucosal surface, it is assumed that local, intestinal antibody of secretory IgA variety is probably highly important but serum IgG and IgM antibody that leak onto the mucosal surface may also be involved in protection.

We consider it unlikely that anti-LT mediated the homologous immunity, since B7A also elaborates non-immunogenic ST, which is not neutralized by LT antitoxin (12,19,29), and ST is a recognized virulence factor in human *E. coli*...
strains (24,35). The role of antisomatic or antiflagellar antibodies in immunity is not clear at the present time. We favor the hypothesis that local antibody directed against pili (or other organelles of attachment) was the operative mechanism of immunity and prevented attachment of E. coli to epithelial cells of the proximal small intestine, which is the anatomic site of enterotoxic diarrhea. This mechanism would explain clinical protection in the face of positive stool cultures and is also compatible with a role for secretory IgA antibodies which are believed incapable of bactericidal activity in the gut (41,42).

For the heterologous challenge study, E. coli E2528-C1 was selected because it shares no common antigens with B7A except production of LT. This strain did not produce ST, lacked H antigen, and possessed a different O antigen. Although E. coli E2528-C1 has common type 1 somatic pili, they are antigenically distinct from B7A pili; antibody against E. coli H10407 type 1 somatic pili, which strongly agglutinates B7A, failed to agglutinate E2528-C1. Antibody produced in rabbits by inoculation with living B7A organisms also failed to agglutinate E. coli E2528-C1.

After recovery from B7A diarrhea, volunteers were not protected against diarrhea caused by E2528-C1. Since LT was the only common antigen between the two strains and all four veterans had developed significant rises in antitoxin following B7A diarrhea, the lack of protection implies that antitoxic antibodies were not an effective mode of immunity. However, this interpretation must be tempered by the observation that serum antitoxin levels in the four "veterans" involved in the heterologous rechallenge had declined from their peak to much lower levels by the time of challenge with E2528-C1 (Table 6). Local intestinal antitoxin may also have decreased to non-protective levels.

Alternatively, LT of LT+/ST+ strains may differ from that of LT+/ST-
strains and may not be neutralized by antitoxin against the former. In volun-
teers, the diarrheal syndrome associated with LT+/ST- *E. coli* is distinct from
that due to LT+/ST+ strains (M.M. Levine, D.R. Nalin, E.J. Bergquist, R.B.
Hornick, submitted for publication). The incubation period of LT+/ST- strains
is shorter and diarrhea volume and duration are less. Against this hypothesis
is the fact that volunteers challenged with E2528-C1 had rises in neutralizing
antitoxin to LT derived from an LT+/ST+ strain.

The observations reported here lend support to the concept that ETEC
infections may eventually be preventable by immunologic methods. The existence
of homologous immunity in the absence of bactericidal mechanisms suggests that
the mechanism of immunity could be local intestinal and anti-adhesive in nature.
Cholera toxoid vaccines that stimulate purely antitoxic immunity have been
highly disappointing with respect to providing notable protection against cholera
in field trials (6) or in volunteer challenge studies (26,27). The failure
of purely antitoxic immunity to prevent diarrhea following challenge with
a heterologous LT+/ST- strain infers that antitoxic immunity also is
incapable, by itself, of conferring notable protection in man against ETEC
diarrhea.
REFERENCES


12. Evans, D.G., Evans, D.J., Jr., and Pierce, N.F. 1973. Differences in the
response of rabbit small intestine to heat-labile and heat-stable enterotoxins

Plasmid-controlled colonization factor associated with virulence in Escherichia

group A erythrocytes by enterotoxigenic Escherichia coli isolated from adults
with diarrhea: Correlation with colonization factor. Infect. Immun. 18:
330-337.

Patterns of loss of enterotoxigenicity by Escherichia coli isolated from
adults with diarrhea: suggestive evidence for an interrelationship with sero-

16. Freter, R. 1956. Experimental enteric shigella and vibrio infections in

17. Gorbach, S.L., Kean, B.H., Evans, D.G., Evans, D.J., Jr., and Bessudo, D.
292:933-936.

of toxigenic and invasive bacteria in acute diarrhea of childhood. N. Engl.


Only nine paired sera were available for testing.

<table>
<thead>
<tr>
<th>Size</th>
<th>Anti-Somatol</th>
<th>Anti-Toxin</th>
<th>Fever</th>
<th>Diarrhea</th>
<th>Volunteers</th>
<th>Pre-Treatment NADCO</th>
<th>Incuclum</th>
</tr>
</thead>
<tbody>
<tr>
<td>I06</td>
<td>8 (89%)</td>
<td>2 (33%)</td>
<td>7 (84%)</td>
<td>II (100%)</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I08</td>
<td>8 (89%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
<td>6 (100%)</td>
<td>+</td>
<td></td>
<td></td>
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</table>

Following oral challenge with E. coli strain 87A, clinical and immunologic response of volunteers.

Table 1
Table 1
CLINICAL AND IMMUNOLOGIC RESPONSE OF VOLUNTEERS
FOLLOWING ORAL CHALLENGE WITH E. COLI STRAIN B7A

<table>
<thead>
<tr>
<th>INOCULUM SIZE</th>
<th>NaHCO₃ PRE-TREATMENT</th>
<th>VOLUNTEERS</th>
<th>DIARRHEA</th>
<th>FEVER</th>
<th>POSITIVE STOOL CULTURE</th>
<th>4x OR &gt; RISE IN CIRCULATING ANTIBODY ANTI-TOXIN</th>
<th>ANTI-SOMATIC</th>
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<td>10⁶</td>
<td>+</td>
<td>6</td>
<td>3 (50%)</td>
<td>1 (17%)</td>
<td>6 (100%)</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>10⁸</td>
<td>+</td>
<td>11</td>
<td>7 (64%)</td>
<td>2 (18%)</td>
<td>11 (100%)</td>
<td>8 (89%)*</td>
<td>8 (89%)*</td>
</tr>
</tbody>
</table>

* Only nine paired sera were available for testing
<table>
<thead>
<tr>
<th>Incubation (Hrs.)</th>
<th>Duration of Illness (Days)</th>
<th>Total # of Loose Stools per Volunteer</th>
<th>Peak # of Loose Stools per Volunteer per 24 Hrs.</th>
<th>Total Stool Volume per Volunteer (ml)</th>
<th>Fever</th>
<th>Nausea</th>
<th>Malaise</th>
<th>Cramps</th>
<th>Antibody ** Rises</th>
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<tr>
<td>41.8*</td>
<td>3.6</td>
<td>8.9</td>
<td>3.7</td>
<td>1124</td>
<td>50%</td>
<td>30%</td>
<td>70%</td>
<td>80%</td>
<td>80%</td>
</tr>
<tr>
<td>(14-124)*</td>
<td>(1-6.5)</td>
<td>(1-25)</td>
<td>(1-9)</td>
<td>(300-2150)</td>
<td>(100^2</td>
<td></td>
<td></td>
<td></td>
<td>10^1*</td>
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</table>

* mean
+ (range)

** 4-fold or greater
Table 3

CLINICAL, SEROLOGIC AND BACTERIOLOGIC RESPONSE OF 8 VOLUNTEERS UPON INITIAL CHALLENGE WITH E. COLI B7A AND UPON SUBSEQUENT RECHALLENGE 9 WEEKS LATER IN COMPARISON WITH 12 CONTROL VOLUNTEERS

<table>
<thead>
<tr>
<th>Group</th>
<th>Volunteers</th>
<th>Diarrhea</th>
<th>Incubation (hrs.)</th>
<th>Total Diarrheal Stool Volume (ml)</th>
<th>Total Loose Stools</th>
<th>Nausea or Vomiting</th>
<th>Malaise</th>
<th>Anti-toxin (%)</th>
<th>Anti-toxin (%)</th>
<th>Positive Stool Culture (%)</th>
</tr>
</thead>
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<tr>
<td>Initial Challenge of Veterans</td>
<td>8</td>
<td>8</td>
<td>44* (100%) (19-124)</td>
<td>7,035</td>
<td>30</td>
<td>3 (38%)</td>
<td>6 (75%)</td>
<td>75</td>
<td>75</td>
<td>100</td>
</tr>
<tr>
<td>Rechallenge</td>
<td>8</td>
<td>1</td>
<td>52</td>
<td>557</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>13</td>
<td>100</td>
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<tr>
<td>Controls</td>
<td>12</td>
<td>7</td>
<td>45</td>
<td>6,355</td>
<td>33</td>
<td>2 (17%)</td>
<td>7 (58%)</td>
<td>92</td>
<td>83</td>
<td>100</td>
</tr>
</tbody>
</table>

*p = 0.002

*p = 0.05

*Mean (Range)
Table 4
SEROLOGIC RESPONSE OF VOLUNTEERS FOLLOWING INITIAL AND RE-CHALLENGE WITH E. COLI B7A

<table>
<thead>
<tr>
<th>VOLUNTEERS</th>
<th>INITIAL B7A CHALLENGE</th>
<th>B7A RE-CHALLENGE</th>
<th>DIARRHEAL ILLNESS</th>
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<tbody>
<tr>
<td></td>
<td>ANTI-TOXIN * Pre-titer</td>
<td>ANTIMOTIATIC ** Pre-titer</td>
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<tr>
<td></td>
<td>Peak titer</td>
<td>Peak titer</td>
<td>Peak titer</td>
</tr>
<tr>
<td>&quot;Veterans&quot;†</td>
<td>16 128 64 128</td>
<td>128 128 128 64</td>
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<td>2005-21</td>
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<td>32 16 16 16</td>
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† = all volunteer experienced diarrheal illness on initial challenge with E. coli B7A
** = antitoxin units per ml. of serum
*** = reciprocal hemagglutination titer
<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Volunteers</th>
<th>Number with Diarrhea</th>
<th>Incubation (hrs.)</th>
<th>Mean Total Diarrheal Stool Volume (ml) per Ill Volunteer</th>
<th>Mean Total Number Loose Stools per Ill Volunteer</th>
</tr>
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<tr>
<td>B7A Veterans</td>
<td>4</td>
<td>3</td>
<td>19.7* (13-24)†</td>
<td>514 (301-622)</td>
<td>2.7 (1-5)</td>
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<td>Controls</td>
<td>6</td>
<td>2</td>
<td>16</td>
<td>482 (415-549)</td>
<td>4.5 (4-5)</td>
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</table>

* = mean  
† = (range)
Table 6
SEROLOGIC RESPONSE OF VOLUNTEERS FOLLOWING INGESTION OF
ESCHERICHIA COLI STRAINS B7A AND E2528-C1

<table>
<thead>
<tr>
<th>VOLUNTEERS</th>
<th>B7A CHALLENGE</th>
<th>E2528-C1 CHALLENGE</th>
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<tr>
<td></td>
<td>ANTI-TOXIN *</td>
<td>ANTIMONOCYTE † †</td>
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<td></td>
<td>Pre-titer</td>
<td>Peak titer</td>
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</tbody>
</table>

†= all veterans experienced diarrheal illness on initial challenge with E. coli B7A
*= antitoxin units/ml. of serum
††= reciprocal hemagglutination titer against B7A antigen
**= reciprocal hemagglutination titer against E2528-C1 antigen
PART B: *Escherichia coli* strains
that are epidemiologically incriminated
as pathogens yet are non-invasive
and give negative tests for
heat-labile and heat-stable enterotoxins
ABSTRACT:

Three enteropathogenic Escherichia coli (EPEC) strains (0127:K63:H6, 0128:K67:H2, and 0142:K86:H6) isolated from outbreaks of infantile diarrhea and one strain from the "normal" colonic flora (E. coli HS) of a healthy adult were fed in doses of $10^6$, $10^8$, and $10^{10}$ organisms in NaHCO$_3$ to adult volunteers. The strains, which had been stored for 7-9 years, gave negative results in sensitive tests for heat-labile (LT) enterotoxin (Y-1 adrenal-cell test), heat-stable (ST) enterotoxin (infant mouse assay), invasiveness (guineapig eye test), and gross fluid accumulation (infant rabbit assay). Two strains (0142 and 0127) caused diarrhea. LT or ST enterotoxins were not found in E. coli stool isolates from individuals with diarrhea and no one had a rise in LT antitoxin titre; the findings suggest that LT and ST enterotoxins were not involved in pathogenesis of the diarrhea. Non-invasive EPEC strains probably induce diarrhea by a mechanism (presumably an enterotoxin) distinct from LT or ST enterotoxins.

INTRODUCTION:

In the 1940's and 1950's, some strains of Escherichia coli were shown by epidemiological studies to be important causes of infantile summer diarrhea. Specific E. coli serotypes were often cultured from infants with diarrhea, whereas their isolation from control infants was uncommon. The most frequently incriminated serotypes were called "enteropathogenic E. coli" (EPEC). At the time that these E. coli serotypes were recognized as probable pathogens, there were no laboratory tests which distinguished virulent from non-virulent strains of a particular E. coli serotype. Since then, it has been found that the E. coli strains often responsible for travellers' diarrhea in adults usually possess either the ability to produce heat-labile (LT) or heat-stable (ST) enterotoxins or the capacity to invade epithelial cells.
Several investigators found that EPEC strains from infantile diarrhea neither produced LT or ST enterotoxins nor had invasive properties.\textsuperscript{11-16} Suggestions have been made that the strains either were not virulent or had lost their enterotoxin plasmids during storage.\textsuperscript{11-13,15,17-20} Another interpretation was that virulence may depend on factors other than invasiveness or the production of LT or ST enterotoxins.\textsuperscript{14}

We aimed to determine whether EPEC strains isolated from outbreaks of infantile diarrhea can cause diarrhea in adults although they are non-invasive, do not produce LT or ST enterotoxins, and had been stored for 7-9 years before the study.

\textbf{MATERIALS AND METHODS:}

\textbf{Volunteers:} The forty-three volunteers were college students and other healthy adults (mean age 24 years). Challenge studies were carried out in the 22-bed isolation ward of the Center for Vaccine Development. The protocol was approved by the University's Human Volunteer Research Committee and the Clinical Research Sub-Panel of the National Institute of Allergy and Infectious Diseases. Informed consent was obtained; the informed nature of consent was confirmed before inoculation by requiring all volunteers to pass a written examination\textsuperscript{21} containing multiple-choice and true-false questions on all aspects of the study including its purpose, hazards, procedures, and bacteriology and immunology. The pre-inoculation health of volunteers was determined by medical history, physical and psychological examination, chest x-ray, E.C.G., full blood-count, urinalysis, blood-chemistries (including serum glucose, urea nitrogen, electrolytes, and tests for liver-function), and tests for syphilis and hepatitis-B surface antigen.

\textbf{Challenge Strains:} \textit{E. coli} strains E851/71, E74/68 and E2348/69 were obtained from the Central Public Health Laboratory, Colindale. These strains had been shown by epidemiological methods to be responsible for outbreaks
of infantile diarrhea in the United Kingdom—strain E851/71 (0142:K86:H6) for several episodes of gastroenteritis in infectious-disease wards in two hospitals in Glasgow; strain E74/68 (0128:K67:H2) for an outbreak affecting three hospitals in Teesside; and strain E2348/69 (0127:K63:H6) for an epidemic among babies in a residential nursery in Taunton. E. coli HS, a non-pathogenic strain isolated from a healthy laboratory scientist, has been a negative control strain in laboratory and clinical studies.

Laboratory characterization of E. coli challenge strains:

Before challenge in volunteers strains E851/71, E74/68, E2348/69, and HS were tested for heat-labile enterotoxin by the Y-1 adrenal-cell assay, for heat-stable enterotoxin by the infant mouse assay (with unconcentrated and 100X concentrates of supernatants), for fluid accumulation by the infant rabbit test, and for Vero-cell toxin by the Vero-cell technique. Fresh cultures were examined for invasiveness by the guineapig eye test.

Inocula and Challenge: Inocula were prepared by harvesting 18-hour trypticase-soy-agar cultures of the challenge strains with saline and making dilutions in saline. 2 g of NaHCO₃ were dissolved in 150 ml. of distilled water of which the volunteers drank 120 ml. One minute later, they drank the remaining 30 ml. in which the E. coli inoculum (10⁶, 10⁸, or 10¹⁰ organisms) was suspended. Volunteers were allowed no other food or drink for 90 min. before and after challenge. Each strain was given to groups of four to five volunteers, starting with the smallest dose of 10⁶ organisms. Inoculum size was quantitated by replicate pour-plate technique before and after challenge.

Clinical Observations: Volunteers were examined daily starting 3 days before inoculation. Oral temperatures were taken every 6 h and repeated within 5 min. if they were 100°F (37.8°C) or above. All stools and vomitus were collected in plastic cholera seats, examined by a nurse or physician, and volumes measured. Stools were graded on a five-point scale—grades 1 (fully
formed) and 2 (soft) were considered normal; grade 3 denoted thick liquid stool, grade 4 opaque-watery, and grade 5 rice-water stools. Diarrhea was defined as two or more loose stools (grades 3-5) in 24 hours and making up a total volume at least 300 ml. After 96 h of post-inoculation clinical observation, oral neomycin (500 mg/6 h) to which all strains were sensitive, was given for 5 days. Volunteers were discharged only when stool cultures were free of challenge strains for 3 consecutive days.

**Typing sera:** Specific antisera for agglutination of the *E. coli* strains were prepared in rabbits as previously described.²⁶

**Stool culture and enterotoxin assays:** Stool specimens or rectal swabs collected daily were inoculated on Levine's eosin-methylene-blue (E.M.B.) agar. Ten colonies with a typical *E. coli* metallic sheen were subcultured on slants of trypticase-soy agar in screw-top tubes. After 18 h of incubation at 37°C, the *E. coli* were tested for agglutination by specific antiserum (the challenge *E. coli* served as positive control, saline as negative control). Slants were overlaid with mineral oil and stored at 4°C for 1-7 days until tested for LT and ST enterotoxins. To identify LT, *E. coli* from slants were inoculated into vials of syncase medium (0.5 ml.) and incubated overnight at 37°C; 0.05 ml. of the culture was then added to monolayers of Y-1 adrenal cells in 96-well tissue-culture plates.²⁷ The cells were examined for rounding (evidence of LT) after 20 h of incubation at 37°C in 5% CO₂. To detect ST, supernatants of *E. coli* isolates were prepared and tested in the infant mouse assay,²⁸ a gut:remaining-body ratio of 0.083 or greater was considered positive.²⁶ *E. coli* H10407 and B7A which produce LT and ST acted as positive control strains; *E. coli* HS served as the negative control.

**Serology:** Sera were collected before challenge and 12-28 days after challenge. After heat inactivation (56°C, 30 min) and absorption of sera with
unsensitized sheep erythrocytes antisomatic antibody was measured by passive hemagglutination using glutaraldehyde-treated sheep erythrocytes coated with lipopolysaccharide antigen from the challenge strains.\textsuperscript{32} Antitoxin to \textit{E. coli} LT was assayed by adrenal-cell neutralization technique.\textsuperscript{33-35}

\textbf{RESULTS:}

\textbf{Laboratory characterization of the challenge strains:} The three challenge strains (E851/71, E74/68, and E2348/69) and the control strain (\textit{E. coli} HS) were negative in all the assay systems for enterotoxins, Vero-cell toxin, and invasiveness.

\textbf{Volunteer studies:} \textit{E. coli} strains E74/68 and HS did not cause diarrhea or other adverse effects in volunteers in doses of up to \(10^{10}\) organisms (table 1). Strain E851/71 induced clear-cut diarrhea at all dose levels; the attack rate was 100\% with \(10^{10}\) organisms (table 1). One of four volunteers who took \(10^6\) organisms of strain E2348/69 had a non-diarrheal illness consisting of abdominal cramps, malaise, and low-grade fever, while three of five volunteers had diarrhea after ingestion of \(10^{10}\) bacteria. Both strains E851/71 and E2348/69 induced a similar illness characterized by a short incubation (mean 18.7 and 8.5 h respectively) and duration (mean 1.9 and 1.3 days), table II. In nine out of ten ill volunteers the onset of diarrhea occurred 9-16 h after inoculation in the tenth volunteer, who received \(10^6\) organisms, the incubation period was 63 h. Symptoms caused by either strain consisted of multiple loose stools, abdominal cramps, nausea, and vomiting (table II). Several volunteers passed voluminous, cholera-like rice-water stools and one individual had 19 watery stools (total of 5.6 l) and a fever of 102°F. Blood and mucus were not seen in stools and sporadic examinations of diarrheal stools (by methylene-blue stains) did not reveal leucocytes.\textsuperscript{36}
**Bacteriology:** All but two of the forty-three persons who ingested the challenge strains had positive stool cultures. Multiple isolates of *E. coli* E851/71 and E2348/69 cultured from volunteers with diarrhea were tested for production of LT and ST enterotoxins; all were negative. Excretion of the organisms stopped within 48-72 hours of starting neomycin.

**Serology:** No volunteers had a significant rise in LT antitoxin titre (table I). Seven of nine individuals (78%) who ingested strain E2348/69 had significant rises in antisomatic antibody, as did four of fifteen who received strain E851/71 and one of fifteen volunteers who ingested strain E74/68. None who ingested strain HS had rises in antisomatic antibody.

**DISCUSSION:**

The suggestion that certain *E. coli* serotypes could cause infantile diarrhea was widely-accepted for more than two decades. These enteropathogenic *E. coli* included serotypes within serogroups 0111, 086, 055, 0127, 0128 and 0142.3,6,22,23,37-39 The evidence for their pathogenicity was epidemiological1-4, 25 subsequent volunteer studies in the U.K.,40 the U.S.A.,41-43 and Japan44 carried out in the early 1950's clearly showed that EPEC strains from cases of infantile diarrhea caused diarrhea when fed to volunteers in high doses, while "normal flora" control strains did not. These studies were carried out at a time when laboratory tests could not identify pathogenetic mechanisms or assess virulence. The observation that *E. coli* strains that cause adult travellers' diarrhea usually produce LT or ST enterotoxins or are invasive7-10 prompted an examination of EPEC strains for virulence properties. With few exceptions, sensitive tests failed to show that classic EPEC serotypes isolated from sporadic cases or from outbreaks of infantile diarrhea possessed invasive properties or produced enterotoxins.11-16 The issue was further confused when it was seen that LT and ST producing *E. coli*
from adults and children with diarrhea\textsuperscript{45,46} (and from some nursery outbreaks)\textsuperscript{46,47} rarely were of "classic" infant diarrhea EPEC serotypes. These observations stimulated a debate. Some workers suggested that the classic EPEC serotypes were pathogenic but only when they possessed enterotoxin plasmids, which tended to be lost during storage.\textsuperscript{14,17-20} Other workers said that failure to demonstrate enterotoxigenicity or invasiveness in classic EPEC strains indicated that E. coli may cause diarrhea by mechanisms distinct from LT or ST production or invasiveness.\textsuperscript{14,46}

Our study was designed to help resolve this controversy. Three E. coli strains isolated from infants during outbreaks of diarrhea, stored for several years and negative in the usual tests for enterotoxins and invasiveness,\textsuperscript{14} were fed to volunteers. Two of the strains, E851/71 (0142:K86:H6) and E2348/69 (0127:K63:H6), which caused diarrhea in healthy adults, are "classic" EPEC serotypes associated with infant diarrhea. The large inoculum (10^{10} organisms) required to induce a high attack-rate, the short incubation, and the diarrheal syndrome encountered, closely resemble the findings reported in volunteer studies employing EPEC strains (including O127:K63)\textsuperscript{43} carried out in the early 1950's.\textsuperscript{40-44}

The failure to identify ST or LT enterotoxins in isolates from volunteers with diarrhea due to E. coli E851/71 and E2348/69 shows that these enterotoxins were not involved in the pathogenesis of the illness. The absence of rises in LT antitoxin titres further suggests that LT enterotoxin was not produced in vivo. The Vero-cell toxin recently shown to be present in some EPEC strains\textsuperscript{30} was not present in strains E851/71 or E2348/69 when tested in our laboratory or in that of J. I. Speirs in Ottawa, Canada. It has not yet been shown whether this toxin can produce diarrhea.

Our study firmly supports the contention that certain virulent EPEC strains cause diarrhea by a mechanism which does not depend on LT
or ST enterotoxin production or on invasiveness. Gurwith et al.\textsuperscript{16,48} also found that, with few exceptions, EPEC strains isolated from children under two years of age with diarrhea (not that due to shigella or non-bacterial gastroenteritis\textsuperscript{16}) did not elaborate LT or ST enterotoxins and were non-invasive.\textsuperscript{16,48} Cantey et al.\textsuperscript{49} have reported an analogous lapine \textit{E. coli} strain that induces diarrhea in infant rabbits yet is neither enterotoxigenic nor invasive.

How EPEC strains E851/71 and E2348/69 produce diarrhea is being investigated. We have found that highly concentrated supernatants of \textit{E. coli} E851/71 and E2348/69 cultures significantly decrease absorption in the canine acute jejunal-loop\textsuperscript{50} test, which suggests the presence of an uncharacterized enterotoxin. Concentrated supernatants of strains E74/68 and HS did not alter absorption. Work is also underway to determine if this toxin is of the Shiga-like variety reported by O’Brien et al.\textsuperscript{51}
REFERENCES


# TABLE I

**CLINICAL, BACTERIOLOGICAL, AND SEROLOGICAL RESPONSE OF VOLUNTEERS TO INGESTION OF ESCHERICHIA COLI**

<table>
<thead>
<tr>
<th>CHALLENGE STRAIN</th>
<th>SOURCE</th>
<th>INOCULUM SIZE (NO. OF ORGANISMS)</th>
<th>DIARRHEA (NO. ILL/NO. CHALLENGED)</th>
<th>VOLUNTEERS WITH POSITIVE STOOL CULTURE (NO. POS./NO. CHALLENGED)</th>
<th>CIRCULATING ANTIBODY (4-fold rise or more)</th>
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<tr>
<td></td>
<td></td>
<td>$10^{10}$</td>
<td>5/5</td>
<td>5/5</td>
<td>1/5</td>
</tr>
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<td>0/5</td>
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<td>STRAIN</td>
<td>NO. ILL VOLUNTEERS</td>
<td>INCUBATION (h)</td>
<td>DURATION OF ILLNESS (days)</td>
<td>PEAK NO. OF LOOSE STOOLS PER 24 H PER VOLUNTEER</td>
<td>TOTAL STOOL VOLUME (ml) PER VOLUNTEER</td>
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<td>---------------------------</td>
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<tr>
<td>E851/71 (0142:K86:H6)</td>
<td>7</td>
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<td>4* (2-8)</td>
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<td>8.5* (6.5-12)</td>
<td>1.3* (1-2)</td>
<td>8* (2-19)</td>
<td>2700* (894-5612)</td>
</tr>
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* Mean. Range given in parentheses.
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