# Studies on Enterotoxigenic *E. coli*

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A large percentage of cases of acute, watery diarrhea cannot be attributed to a recognized etiologic agent. Studies in Mexico, Brazil, India, the United States and Bangladesh have now shown that enterotoxigenic *E. coli* (EEC) can be isolated from a number of such cases. EEC have been shown to produce two enterotoxins. One is a heat-stable toxin (ST) which is non-antigenic and rapidly causes fluid accumulation when introduced into the gut of various animals. The other is a heat-labile toxin (LT) which causes a delayed onset diarrhea. LT is similar to cholera toxin in its mode of action and immunogenicity.

Several systems are available for the detection of the enterotoxins produced by *E. coli*. Animal loop models are used for the detection of LT and ST and the infant-mouse is now widely used for ST assay. Y-1 adrenal cells and the Chinese hamster ovary (CHO) cells are tissue culture systems used for the detection of LT.

In the latter part of 1974, Dr. Richard Guerrant brought CHO cells to CRL. Because it is difficult to keep a $CO_2$ incubator going here, the cells were adapted to growth in a tissue culture medium containing HEPES buffer, thus obviating the need for a $CO_2$ incubator. At about the same time, Dr. Dave Sack, also interested in EEC came out from Johns Hopkins and a collaborative study was set up. This study had three objectives :

1) To determine the incidence of EEC diarrhea in patients at CRL.

2) To compare the Y-1 adrenal and CHO cell systems for the detection of *E. coli* enterotoxin.

3) To evaluate the feasibility of running the CHO cell assay at CRL.

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The bulk of the data from that study was reported at last year's T.C. meeting. I want to briefly review that data and also discuss some further work that has been done with the isolated strains.

During a one week period in November 1974, 65 patients from whom Shigella, Salmonella or V. cholerae were not isolated were chosen for this study. Each patient was admitted to the CRL hospital with acute, watery, cholera-like diarrhea. Ten typical E. coli colonies were picked from MacConkey agar plates streaked with rectal swabs from each patient and a pool of these colonies was also made.

Enterotoxigenic E. coli were isolated from 20 of these patients. From 18 of the 20 patients with tissue culture positive E. coli, all ten isolates picked were toxigenic. Nine of 10 and 4 of 10 isolates picked were toxigenic in the other two cases. All pooled cultures were positive in the tissue culture assays if that pool contained any toxigenic isolates.

The isolates were tested in the Y-1 adrenal cells by Dr. Sack in Baltimore and by myself here. A comparison of the results of the two tissue culture assays is shown in table 1. The two assays systems were in agreement with 640 of the isolates. The CHO cells gave a positive result with three isolates which were negative in the adrenal cells. Culture filtrates from these three E. coli were negative in rabbit ileal loops at 18 hours.

One isolate from each of the positive patients was confirmed to be toxigenic in the rabbit ileal loop. Seven of seven pools positive in the tissue culture assays were confirmed to be positive in dog jejunal loops by Dr. Nalin and six negative pools were likewise confirmed.

Pools were tested for ST in the infant-mouse assay. Strains from 17 of the 20 patients were positive in this assay. Thus, 3 of the pools contained LT only EEC. Using the same assay, it was also found that E. coli producing only ST were isolated from three patients. Until recently it was thought that human strains of EEC produced both LT and ST. Althought the mode of action of ST has not been determined there are several reports now of E. coli producing only ST in association with diarrhea in humans.

Enterotoxin production in E. coli is related to a plasmid and any serotype is potentially enterotoxigenic. The results of serotyping done by Dr. Fritz Ørskov and shown on table 2 are, therefore, particularly interesting.

The serogroups 015 : H11 and 078 : H 11, 12 were isolated from almost half of the patients with EEC in this study. Enterotoxigenic strains with these serogroups have also been frequently detected in India and other geographic areas. A serologic test to detect EEC would be very useful and this finding warrants further investigation

## Prevalence of EEC in normal individuals

Although several studies have shown that EEC is associated with human diarrhea, little is known about the prevalence of EEC in normal individuals. In February 1975, Kenneth Steinberg came to CRL and examined a group of 44 asymptomatic children in Sardarkandi village in the Matlab field surveillance area.

Rectal swabs were obtained from children in families which had no history of any diarrhea for the previous month. The family was checked three days later and if any diarrhea developed during this time, the family was dropped from the study. Swabs were plated on MacConkey's agar and after overnight incubation, ten typical E. coli colonies were picked and streaked onto individual blood agar base slants. These were later confirmed as E. coli and examined for toxigenicity using the CHO cell assay.

Forty-four children were included in the study. No EEC were isolated from 35 of these. However, EEC were isolated from nine children. Four had one positive, four had two positive and one had five out of ten positive colonies (Table 3). This is in contrast to the study of diarrhea patients done by Dave Sack and I where 18 out of 20 cases yielded 10 of 10 positive colonies and nine of 10 and four of 10 were positive in the other two. Three of the nine children, including the one with five toxigenic colonies, were from the same family.

The children were followed for three weeks, after obtaining the rectal swab, for any history of diarrhea. Four of the children with no EEC developed diarrhea within 8 to 11 days and one child from whom EEC was isolated developed diarrhea four days after the sample was taken.

The significance of this little study is that because 9 of 44 of these asymptomatic children harbored EEC, one has to be careful when analysing the data of any epidemiologic study examining only pools of E. coli isolates. Presumably, although it was not investigated in this study, a pool of ten E. coli colonies containing even one toxigenic strain would give positive results in the C.H.O. or other assay. Because the present assay systems are tedious, large epidemiological studies dictate that pools rather than individual colonies be assayed and I am not trying to discourage that. When toxigenic E. coli are isolated from an individual with diarrhea, particularly when the individual shows an antibody rise to LT, I think it is safe to say that EEC is the etiologic agent.

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It has recently been reported by Evans et al that an E.coli colonization factor may be an essential virulence factor in E. coli diarrhea in man. Perhaps the toxigenic E. coli isolated from these asymptomatic children did not contain the colonization factor. We plan to look at some of our enterotoxigenic E. coli to determine how widespread this virulence factor is.

The CHO cell assay is alive and well at CRL. The results obtained here have compared favorably with the Y-1 adrenal cell assay and been confirmed in rabbit and dog loop studies. We have used the CHO cell assay for the studies I've reviewed and those which Dr. Curlin will now discuss.

TABLE 1

COMPARISON OF ADRENAL CELL CHINESE HAMSTER OVARY CELL ASSAYS IN THE DETECTION OF HEAT-LABILE ENTEROTOXIN PROTECTION IN 643 *E. coli* ISOLATES FROM DACCA, BANGLADESH

| | | Adrenal Assay | |
|---|---|---|---|
| | | Positive | Negative |
| CHO Assay | Positive | 193 | 3 |
| | Negative | 0 | 447 |

TABLE 2

SEROTYPES OF ENTEROTOXIGENIC *E. COLI* STUDIES

| O:H SEROTYPE | NUMBER OF PATIENTS |
|---|---|
| O6:H16 | 3 |
| O8:H9 | 3 |
| O25:H42 | 2 |
| O15:H11 | 5* |
| O78:H11 | 2 |
| O78:H12 | 4* |
| O115:H40 | 2 |
| (O48)°:H26 | 1* |
| O8,O60:H? | 1 |
| | 23[s] |

* ONE PATIENT WITH LT ONLY.

[s] TOTAL INCLUDES STRAINS FROM 3 PATIENTS WITH OTHER BACTERIAL PATHOGEN SUBSEQUENTLY ISOLATED.

TABLE 3

NUMBER OF ENTEROTOXIGENIC COLONIES
FROM 9 OF 44 CHILDREN SAMPLED

| | NUMBER OF EEC (OF 10 TESTED) PER CHILD | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| NUMBER OF ASYMPTOMATIC CHILDREN | 4 | 4 | - | - | 1 | - | - | - | - | - |