NOTES
Pathogenic Mechanisms of a Non-Agglutinable Vibrio cholerae Strain: Demonstration of Invasive and Enteroinvasive Properties


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A non-agglutinable Vibrio cholerae strain isolated from the blood of a child with kwashiorkor and fever was shown to have the potential to invade as well as to produce a toxin resembling cholera toxin.

Pathogenic mechanisms of gastroenteritis have been closely studied during the past few years. Generally, bacteria capable of causing diarrhea have been divided into two broad groups: enterotoxin-producing and enteroinvasive (4). Vibrio cholerae is considered to be the prototype of an enteroinvasive organism that adheres to the intestinal mucosa but does not invade the epithelial cell layer (8). Certain non-agglutinable (NAG) V. cholerae strains have also been shown to be capable of causing diarrhea in man, and a recent study has demonstrated that these strains produce an enterotoxin which biologically and immunologically resembles cholera toxin (12).

We have recently investigated a NAG V. cholerae strain (A3057/76) isolated from a blood culture of a 6-year-old male black child with kwashiorkor and fever of uncertain origin, but without gastroenteritis. The isolate gave the characteristic biochemical reaction of V. cholerae (1) but failed to agglutinate with cholera O-antiserum (Wellcome). As it agglutinated with anti-10, -16, and -17 NAG V. cholerae antisera, it could not be assigned to any specific serotype (A. L. Furniss, personal communication).

Several investigations were performed to determine the enteropathogenicity of this strain. In the ligated rabbit ileal loop model, fluid accumulation was determined after injection of 10^7 bacteria into 15-cm ligated segments of rabbit ileum. The volume-to-length ratio increased with time: from 0.12 ± 0.03 ml/cm (mean ± standard error of the mean, n = 6) at 6 h to 0.48 ± 0.14 ml/cm (mean ± standard error of the mean, n = 6) at 18 h.

Tests for enterotoxin production were performed as described previously (6). The suckling mouse assay (3), used for the detection of the heat-stable toxin of Escherichia coli, was repeatedly negative (gut weight to total body weight ratio: 0.053 ± 0.002) (9). The Chinese hamster ovary tissue culture assay is used to detect enterotoxins, such as cholera enterotoxin and the heat-labile toxin of E. coli (7), that act on the adenyl cyclase/cyclic adenosine 5'-monophosphate system. When filtrates of the test strain, cultured overnight in Synase broth (5a), were examined in this model, 18 to 20% of the cells were seen to have elongated, indicating a positive result (Fig. 1, Table 1). Tests for skin permeability factor were performed by using Synase broth culture filtrates, concentrated by dialysis, and ultrafiltration through a Diaflo UM 10 membrane (Amicon Corp.) (12). The protein content of the concentrate was adjusted to 100 µg/ml, and the skin bluing test was performed as described by Craig (2). The results (Table 1) suggest the presence of a toxin similar to, but less potent than, cholera enterotoxin. The activity of culture filtrates in both the Chinese hamster ovary assay and the skin bluing test was lost after heating at 65°C for 10 min.

Tests for the invasive ability of the NAG V. cholerae were performed in the guinea pig keratoconjunctivitis test (10) and ligated rabbit ileal loops. The NAG vibrio failed to invade the guinea pig cornea, but did invade the rabbit ileal mucosa (Fig. 2). This finding was confirmed on five separate occasions. Moreover, cultures of peripheral venous blood, liver, and spleen of two rabbits enterally infected with NAG V. cholerae...
FIG. 1. Phase-contrast photomicrograph (×250) of Chinese hamster ovary tissue culture incubated overnight with culture filtrates of (A) NAG V. cholerae A3057/76 and (B) a non-enterotoxigenic E. coli E 357.
yielded the organism after 18 h. Repeated tests for invasion of various agglutinable *V. cholerae* strains as well as a *NAG V. cholerae* recovered from the stool of a patient with diarrhea gave consistently negative results.

A recent re-examination of the organism stored for 8 months on a semisolid medium revealed an apparently complete loss of enterotoxigenicity, suggesting that this property is plasmid mediated. Despite this, the invasive ability had remained intact.

Earlier reports of *NAG V. cholerae* septicemia have been published (5, 9), but this is the first in vitro demonstration of a *V. cholerae* strain with invasive as well as enterotoxigenic potential. The presence of this organism in the peripheral blood of a patient was probably the result of depressed host immunity associated with malnutrition (11), compounded by the invasive ability of the strain.

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A. L. Furniss, Public Health Laboratory, Maidstone, Kent, England, typed the *NAG V. cholerae*.

**LITERATURE CITED**


6. Freiman, I., E. Hartman, H. Kassel, R. M. Robins-

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**TABLE 1. Effect of filtrates of *NAG V. cholerae* A3057/76 and a positive control (*V. cholerae* Inaba strain 35A3) in Chinese hamster ovary tissue culture and skin permeability factor tests**

<table>
<thead>
<tr>
<th>Determination</th>
<th>NAG V. cholerae</th>
<th>Classical V. cholerae</th>
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<tbody>
<tr>
<td>Elongation of Chinese hamster ovary cells (%)</td>
<td>20 (3)</td>
<td>54 (4)</td>
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<tr>
<td>Area of skin bluing (mm²)</td>
<td>24 (0)</td>
<td>38 (0)</td>
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* Mean of six experiments. Values in parentheses indicate results after heating of filtrates at 65°C for 10 min.


