Cholera toxin, zonula occludens toxin and accessory cholera enterotoxin gene-negative *Vibrio cholerae* non-O1 strains produce the new cholera toxin

*Vibrio cholerae* non-O1 have been reported to produce cholera toxin (CT), although certain strains did not do so. In recent years, several other extracellular products such as a heat stable toxin (NAG-ST), a thermostable direct haemolysin, El Tor-like haemolysin, a shiga-like toxin, haemagglutinin and zot2 produced by *V. cholerae* non-O1, have been reported to play some role in causing disease. However, none of these virulence factors alone was considered...
as the cause of enteropathogenicity. Moreover, the recent observation of Kurazono et al. that the majority strains of V. cholerae non-O1 of clinical and environmental origin lack the virulence genes cassette comprising CT, zonula occludens toxin (zot) and accessory cholera enterotoxin (ace), indicates that these strains may possess another unidentified virulence gene encoding an yet unrecognized secretogen.

Earlier we demonstrated that CT or CT strains of V. cholerae O1, biotype El Tor or classical, serotype Ogawa or Inaba of clinical or environmental origin or genetically engineered in the laboratory produce a new cholera toxin (NCT) and, the disease cholera may be caused either by CT or NCT or both. However, the production of NCT by V. cholerae non-O1 has not yet been reported, although these organisms cause diarrhoea in humans and a secretory response in experimental animals. In this study, an attempt was therefore, made to examine the production of NCT by V. cholerae non-O1, isolated from the River Ganga in Varanasi, India. These strains of V. cholerae non-O1 were cdx, zot and ace negative as tested using specific DNA probes and were, therefore, devoid of the core dynamic region of V. cholerae O1 (G.B. Nair, pers. commun.).

Live cells and one of the five isolates of V. cholerae non-O1 tested in ligated ileal loops of adult albino rabbits (Belgian strain), following the method of De and Chatterjee, caused accumulation of fluid in the initial test (0.7-1.2 ml/cm of RIL). The other four isolates did so after one to four consecutive passage(s) through rabbit gut in the range of 0.5-1.1 ml/cm of RIL, and thereafter outpouring of fluid by every strain increased with each passage. Culture filtrates (CFs) of these strains prepared in AKI medium, only after their live cells caused fluid accumulation in RILs, when tested in the same assay showed a similar secretory response (0.5-1.2 ml/cm of RIL), although slightly less than that of toxigenic V. cholerae strain 569B (1.1-1.4 ml/cm of RIL) but did not cause lysis of sheep erythrocytes when tested by conventional method.

These observations indicate that the non-O1 V. cholerae strains that lack gene for all known toxin factors such as CT, zot and ace, except NCT, produce a secretogen. Enhancement of secretory response upon passage through the gut of a susceptible host suggests that if such strains circulate in the community, because aquatic life is the reservoir of non-O1 V. cholerae strains, its virulence may increase further.

In RIL assays, the enterotoxic activity of non-O1 V. cholerae strains was completely neutralized by the antiserum against purified NCT diluted in 1:32 (Figures 1 and 2) prepared with CT strain of V. cholerae X-392, which was shiga or shiga-like toxin gene-negative as tested using specific DNA probes (P. Echeverria, pers. commun.) and was non-haemolytic when tested by conventional method.

The observation that CF of one isolate showed complete identity and neutralization by anti NCT indicates that this strain produces a secretogen antigenically similar to NCT. However, the other four isolates that showed partial identity with NCT and complete neutralization of enterotoxic activity in RIL by its antiserum in the same dilution may suggest that these strains also produce NCT but differ in some weaker epitopes, as has been observed in CTB subunits produced by classical and El Tor biotype strains. Tamplin et al. also observed five shared and one unshared epitope between classical and El Tor CFs as well as some variation in the extent of cross reactivity between different El Tor CT-B preparations with some of the anti-classical monoclonal antibodies. Although the subunit structure of NCT could yet be determined, its
molecular weight being as large as 61,000 Da (unpublished data) there is every likelihood that this toxin possesses some subunits, the epitopes of which may differ slightly from strain to strain. This difference, however, is minor and does not affect the neutralizing capability of the antitoxin against X-392.

In gel-diffusion test, 10 times concentrated CF of CT$^+$ V. cholerae X-392 that produces NCT$^+$ and V. cholerae non-OI strains gave a precipitation band against anti-NCT. Only one isolate showed reaction of identity (Figure 3) and the other four showed reaction of partial identity (Figure 4).

The results of this study suggest that the strains of V. cholerae non-OI can produce NCT in the absence of ctx, zot and ace or when these genes are deleted. They, thus possess the potential to cause diarrhoea. These observations are of importance in understanding the pathogenesis of diarrhoea caused by V. cholerae non-OI strains as this toxin seems to play an important role in the causation of diarrhoea. However, further study with a large number of isolates is needed to strengthen this conclusion.


ACKNOWLEDGEMENTS. This study in part was supported by the Council of Scientific and Industrial Research, New Delhi in the form of Fellowships to D.V.S. and A.T. We thank Dr G. Balakrish Nair, NICED, Calcutta for providing laboratory facility.

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Erratum

Effect of foetal exposure to low-dose X-rays on the postnatal growth of mouse

K. S. Bisht and P. Uma Devi
[Curr. Sci., 1995, 69, 496-498]

The table appearing on page 497 contains some mistakes. The correct table is printed below.

Table 1. Observations on postnatal development of mice exposed to 0.05 Gy of X-rays at day 14.5 post-coitus

<table>
<thead>
<tr>
<th>Observations</th>
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<th>3</th>
<th>4</th>
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<tr>
<td>Number of offsprings</td>
<td>C 122</td>
<td>C 121</td>
<td>C 120</td>
<td>C 119</td>
<td>C 117</td>
<td>C 115</td>
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<td>E 98</td>
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<td>E 95</td>
<td>E 94</td>
<td>E 92</td>
<td>E 88</td>
<td>E 87</td>
</tr>
<tr>
<td>Growth-retarded offsprings (%)</td>
<td>E C 1.64 (2)</td>
<td>E C 1.65 (2)</td>
<td>E C 2.50 (3)</td>
<td>E C 3.36 (4)</td>
<td>E C 3.42 (4)</td>
<td>E C 3.48 (4)</td>
<td>E C 3.51 (4)</td>
</tr>
<tr>
<td>Body weight (mean ± SE, g)</td>
<td>E C 1.67 ± 0.014</td>
<td>E C 4.76 ± 0.054</td>
<td>E C 7.27 ± 0.081</td>
<td>E C 11.59 ± 0.159</td>
<td>E C 17.57 ± 0.367</td>
<td>E C 24.43 ± 0.273</td>
<td>E C 28.05 ± 0.303</td>
</tr>
<tr>
<td>Body length (mean ± SE, mm)</td>
<td>E C 32.11 ± 0.115</td>
<td>E C 46.78 ± 0.235</td>
<td>E C 54.91 ± 0.227</td>
<td>E C 68.06 ± 0.366</td>
<td>E C 70.65 ± 0.447</td>
<td>E C 87.83 ± 0.366</td>
<td>E C 91.64 ± 0.417</td>
</tr>
<tr>
<td>Head length (mean ± SE, mm)</td>
<td>E C 31.88 ± 0.133</td>
<td>E C 46.19 ± 0.317</td>
<td>E C 54.79 ± 0.417</td>
<td>E C 64.68 ± 0.670</td>
<td>E C 71.62 ± 0.780</td>
<td>E C 83.95 ± 0.949</td>
<td>E C 89.89 ± 0.814</td>
</tr>
<tr>
<td>Head width (mean ± SE, mm)</td>
<td>E C 8.73 ± 0.056</td>
<td>E C 15.83 ± 0.091</td>
<td>E C 15.85 ± 0.103</td>
<td>E C 20.85 ± 0.146</td>
<td>E C 22.38 ± 0.160</td>
<td>E C 24.12 ± 0.115</td>
<td>E C 24.70 ± 0.125</td>
</tr>
<tr>
<td>Tail length (mean ± SE, mm)</td>
<td>E C 8.58 ± 0.061</td>
<td>E C 16.60 ± 0.119</td>
<td>E C 16.19 ± 0.146</td>
<td>E C 20.59 ± 0.122</td>
<td>E C 21.13 ± 0.185</td>
<td>E C 23.58 ± 0.176</td>
<td>E C 24.36 ± 0.114</td>
</tr>
</tbody>
</table>

Note: Figures in parentheses are the actual numbers.
1C: Sham-irradiated animals, number of mothers 15.
E: Exposed to 0.05 Gy X-rays, number of mothers 12.
Difference from respective control (C): $p < 0.05$, $p < 0.01$, $p < 0.001$ (Mann-Whitney test), $* p < 0.05$ (Fisher's exact test).