

This Week's Citation Classic

Konowalchuk J, Speirs J I & Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect. Immunity* 18:775-9, 1977.

[Bureau of Microbial Hazards, Food Directorate, Health Protection Branch, Health and Welfare Canada, Ottawa, Canada]

This paper describes the use of Vero cells for detecting a heat-labile cytotoxin produced by some strains of enteropathogenic *Escherichia coli*, for which pathogenic markers had not previously been identified. This marked the discovery of verotoxin. [The SC[®] indicates that this paper has been cited in more than 320 publications.]

Discovery of Verotoxin

S. Stavric and J. Speirs
Bureau of Microbial Hazards
Food Directorate
Health Protection Branch
Health and Welfare Canada
Ottawa, Ontario K1A 0L2
Canada

In our studies on developing cell-culture procedures for *Escherichia coli* toxin detection, we first used Y-1,¹ mouse adrenal cells, and CHO,² Chinese hamster ovary cells, which measure the cytotoxic effects of heat-labile enterotoxin (LT). However, we found that these assays were not too convenient for our practical use, as Y-1 cells were slow-growing and the test with CHO was somewhat temperamental. We therefore proceeded to search for other cell lines more suitable for detecting *E. coli* toxins.

Vero, a continuous line of African green monkey kidney cells, responded cytotoxically to LT,³ and its morphological response was accompanied by an increase in the intracellular level of cyclic AMP.⁴ These cells could be cultured quickly in quantity and required little maintenance. The LT effect was noted as early as 2 hours, and, as with Y-1 and CHO cells, no

increase in the effect was discernible after 18 hours. Vero cells were less sensitive than CHO, but more sensitive than Y-1.

In addition to *E. coli* enterotoxigenic strains, we tested a number of enteropathogenic isolates obtained from children with diarrhea. Some of these induced a distinctive cytotoxic effect on Vero, but not on Y-1 or CHO cells. Affected cells appeared round but shriveled. The cytopathic effect increased with time, with maximum titers observed after four days. This was obviously a cytotoxin and we called it verotoxin (VT).

Of 136 *E. coli* strains tested, 10 induced a cytotoxic response in Vero cells. Eight of these strains were associated with diarrhea (seven in human infants and one in a weanling pig), and two were cheese isolates, not implicated in disease. The sero-types of VT-positive strains varied, but 4 of the 10 were serotype O26. Among the 10 VT-producing isolates, 3 distinct cyto-toxins were identified by isoelectric point, molecular weight, and neutralization studies.⁵⁶

The discovery of VTs and the establishment of the assay for their detection has assisted in the subsequent linkage of these toxins to hemorrhagic colitis (HC)⁷ and hemolytic uremic syndrome (HUS).⁸ All of the HC and HUS *E. coli* isolates produce high levels of one or more VTs. Especially important is serotype O157:H7, responsible for numerous illness outbreaks. Therefore, the characterization of VTs and the elucidation of their role in the pathogenesis of these diseases have been the subjects of numerous studies that followed the discovery of VT.

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Received November 12, 1991