ESCHERICHIA COLI CULTURE FILTRATE INDUCES DIARRHEA RESEMBLING CHOLERA

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Culture filtrates of some *E. coli* strains have been reported to produce rapid onset short duration diarrhea in dog Thiry-Vella loops. The diarrhea stops when loops are rinsed free of filtrate¹. In contrast, the diarrhea caused by cholera filtrates develops 2-3 hours after exposure of loops to filtrates, is long acting, and does not stop after the loops are rinsed^{2, 3}. High volume diarrhea resembling cholera occurs in patients from whom no vibrios can be isolated^{4, 5}. Therefore we tested culture filtrates of *E. coli* strains from vibrio-negative diarrhea patients for a cholera-like toxin.

Cultures of patients' admission rectal swabs on MacConkey's, plain agar and SS media showed pure growth of *E. coli* and no *V. cholerae* or other known pathogens. Identification was confirmed from growth patterns on triple sugar, M. I. U., Kligler-Iron agar and citrate media. Cultures were maintained on tryptose slants at 5°C. The entire growth from an overnight slant subculture was used to inoculate a 500 ml. aliquot of phosphate-buffered medium consisting of 2% casamino acids, 1.5% yeast extract, and 0.25% glucose which was incubated at 35°C without shaking for 48 hours. Cultures were centrifuged and the supernatant fluids filter-sterilized. After the addition of ammonium sulfate to 90% saturation and centrifugation, precipitated protein was resuspended in normal

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saline, dialysed overnight, and stored at 5°C. All preparations were tested in ligated rabbit loops by De's method⁶. Six of nine caused fluid accumulation in the rabbit.

Filtrates were then tested for diarrheagenic activity in 16 ligated cannulated 20 cm. jejunal loops of 10 anesthetized adult dogs. Fluid losses were replaced with i.v. normal saline and temperatures were kept at 36-37.8°C. Net fluxes of Na⁺ and water before, during and after exposure of loops to heated or unheated culture filtrates were measured using a test solution with NaCl 150 mM./l. and PEG or PSP as previously described³. Loops were exposed to 1.5-5 ml. of unconcentrated or 50-60 fold concentrated filtrates for 10 minutes and were then rinsed. When loop fluid accumulation occurred samples of fluid were collected under oil and analysed by standard methods. Serum protein and Na⁺ did not change significantly during studies.

Net fluxes in the dog loops were always absorptive before exposure to filtrates and remained so in the case of filtrates which did not cause fluid accumulation in rabbit loops. All filtrates of strains which caused fluid accumulation in rabbit loops produced two separate effects. The first was an immediate decrease in absorption in the presence of filtrates which ended within 20 minutes of the removal of the filtrate and rinsing of the loops. The second was a delayed, long acting net secretion with profus diarrhea. This effect, which was not affected by rinsing, occurred after a 10 minutes exposure of loops to even unconcentrated filtrate. The toxin which caused this long-acting secretion was eliminated by boiling filtrates for 20 minutes. In contrast the toxin causing the immediate effect was heat stable and was detectable only in concentrated filtrates.

After the filtrates which caused the immediate effect were rinsed out of the loops at the end of the 10 minute exposure absorption always returned to normal control levels*. Two hours later absorption decreased and by the 3rd hour after exposure to filtrates net loop fluid accumulation began. Fluid accumulation rose to 0.21 ml./min./loop by the 4th-6th hour and persisted unabated up to 10 hours when animals were painlessly sacrificed. Mean loop diarrhea volumes aspirated during the 0-1st, 1st-2nd, 2nd-4th and 4th-6th hours after exposure were 0, 1.4, 6.6 and 9.2 ml./hour respectively. In a protracted study of two dogs diarrhea persisted 24 hours, and peaked at 9-10 hours with a gradual decline thereafter. Mean composition at all times of 38 diarrhea samples from 10 loops of 9 dogs was: Na⁺ 150, K⁺ 5.2, HCO₃⁻ 29.6 mEq/l. and protein 254 mg./100 ml. Diarrhea composition resembled that in cholera studies.²

* +0.12 ml H₂O/min./loop and +21 uEq. Na⁺/min/loop.

Detection of cholera-like enterotoxins requires an animal model in which changes in flux can be continuously monitored after filtrates are rinsed out of loops. The acute dog model can be so used and in over 100 studies of cholera enterotoxin has given no false positive responses. Previous dog studies did not reveal a cholera-like *E. coli* toxin, possibly because shaken cultures (containing little or no cholera-like toxin) were used. Short and long acting toxins cannot be distinguished merely by injecting filtrates into ligated loops and reading at 18 hours because accumulated fluid may then be due to either or both toxins.

The cholera-like *E. coli* toxin is probably responsible for severe human *E. coli* diarrhea, since it causes a sustained diarrhea in dogs which resembles the human disease. There is a possibility that this *E. coli* toxin is similar to or identical with cholera toxin and we have been able to show in preliminary experiments in 3 dogs that in the same animal the long acting effect of this toxin as well as the effect of Wyeth 002 cholera toxin is neutralized by convalescent serum from a cholera patient. Cross immunity induced by cholera toxin and cholera-like *E. coli* toxin would be of great epidemiologic significance; our observations suggest that the possibility of transfer of toxigenicity from one genus of enteric bacteria to another merits further study.⁷

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