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Culture Supernatants of *Campylobacter jejuni* Induce a Secretory Response in Jejunal Segments of Adult Rats

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Culture supernatants of four *Campylobacter jejuni* strains induced a net sodium secretory flux (plasma-lumen) and an impaired glucose transport in perfused jejunal segments of adult rats in vivo.

Campylobacter jejuni is a microaerophilic, gram-negative rod associated with human gastroenteritis in all continents (1–3). Although the techniques and media for the isolation of this bacterium are well established (3, 7, 12), most aspects of the pathogenesis of *Campylobacter* infection remain obscure. Currently it is accepted that invasion is the principal pathogenic mechanism involved in *Campylobacter* gastroenteritis (3, 11). The purpose of this note is to report that the perfusion of culture supernatants of *C. jejuni* induces a secretory response in rat intestines.

Perfusion experiments were carried out with three control solutions and four culture supernatants of *C. jejuni*. The control solutions were as follows. (i) Glucose-saline solution, containing (per liter) 7.2 g of NaCl, 0.382 g of KCl, 0.5 g of $NaHCO_3$, 0.2 g of $CaCl_2$, and 3.6 g of glucose. (ii) Tryptic soy broth (TSB; Oxoid Ltd.) with 5% defibrinated sheep blood. This solution was incubated at 37°C for 72 h under a reduced atmosphere obtained with the GasPak system (BBL Microbiology Systems) without the catalyst (3) and used after centrifugation under refrigeration at 6,000 rpm for 15 min. (iii) Culture supernatant of *Escherichia coli* K-12. This solution was prepared by growing *E. coli* K-12 strain J53 in TSB at 37°C for 24 h without aeration and centrifuging under the same conditions used for the TSB–5% blood solution.

The culture supernatants of *C. jejuni* were prepared with four strains isolated from stool specimens of diarrheic children. Strains were isolated by smearing the stool specimens in Butzler medium and incubating the plates at 42°C for 48 h in a reduced atmosphere. Organisms were identified as *C. jejuni* when they were motile, curved or S-shaped, gram-negative bacilli, nonfermenters, oxidase and catalase positive; if they could grow at 37 and 42°C but not at 25°C and could not grow in 3.5% sodium chloride but could in 1% glycine; and if they were susceptible to nalidixic acid (1). Strains were stored frozen at −20°C in brucella broth (BBL) containing 15% glycerol, as described by Luechtefeld et al. (7). All the strains tested were freshly isolated, with no more than three passages in brucella agar (BBL) with 7% defibrinated sheep blood.

Supernatants were prepared by individually culturing the strains of *C. jejuni* in TSB supplemented with 5% defibrinated sheep blood at 37°C under a reduced atmosphere obtained with the GasPak system without the catalyst (BBL). After 72 h of incubation, the cultures were centrifuged under refrigeration at 6,000 rpm for 15 min. The concentrations of sodium, potassium, and glucose as well as the pH and osmolarity of the supernatants were determined. The supernatant pH was next adjusted to 7.0, and the sodium and glucose concentrations were adjusted to 130 meq/liter and 130 mg/100 ml, respectively. The osmolarity remained at 300 mosM/liter. Polyethylene glycol was used as a nonabsorbable marker at a concentration of 0.6 g/liter in supernatants and in perfusion solutions.

Seeding of *Campylobacter* culture supernatants in Butzler medium (42°C, reduced atmosphere) and of *E. coli* K-12 culture supernatants on blood agar plates (37°C, aerobic) showed no growth of these bacteria.

The perfusion experiments were carried out as follows. Male Wistar rats, about 150 to 200 g, were made to fast overnight but were allowed to drink water. After anesthesia with intraperitoneal injection of pentobarbital (40 mg/kg), the peritoneal cavity was opened by a midline ventral incision, and the jejunum was isolated. A polyethylene tube was inserted through a small incision made in the jejunum and was fixed in place with a ligature. A jejunum segment of 30 to 40 cm was utilized, and its distal section was then also cannulated.

TABLE 1. Effect of *C. jejuni* culture supernatants on sodium and glucose transport in rat jejunum in vivo

Solution	No. of perfusates	Transport (mean ± SEM)[a]	
		Sodium (μeq/min per cm)	Glucose (μg/100 ml per min per cm)
Control			
Glucose-saline	28	+612.4 ± 133.0	+4,304.4 ± 793.6
TSB–5% blood	22	+299.8 ± 74.4	+271.2 ± 117.5
E. coli K-12	21	+185.0 ± 35.9	+1,352.6 ± 140.4
C. jejuni			
14 FAISA	44	−1,402.9 ± 227.1	−901.7 ± 217.1
60892	30	−2,801.6 ± 692.2	−2,296.1 ± 779.1
23 HSP	23	−507.3 ± 85.2	+333.7 ± 130.9
10 HSP	28	−200.1 ± 126.8	+74.22 ± 73.8

[a] +, Absorption; −, secretion.

The intestinal contents were washed slowly with warm isotonic saline, taking care to avoid distending the experimental segment. The proximal cannula was connected to a Harvard peristaltic pump (model 1210), and the solutions were perfused at a rate of 0.57 to 1.98 ml/min. The perfusion lasted from 60 to 120 min. The first 30 min of the perfusion period was allowed for equilibration conditions to be reached. The second period was used to collect perfusate specimens, one sample each 10 min. The animal was killed at the end of the experiment by incising the heart. The jejunum was then removed, and its length was measured, always by the same person. Perfusate samples were stored at −20°C before being analyzed. At least four rats were perfused with each solution, producing 21 to 44 perfusate samples.

Net transport was calculated from changes in polyethylene glycol and individual solute concentrations by the usual water-marker technique equations (10). A transport value for each sample was calculated, and the mean transport rates ± standard errors of the mean were determined for each test group. The significance of differences in means was determined by the Student's *t* test for independent means.

A net sodium secretory flux (plasma-lumen) was observed in all the animals perfused with the supernatants of the four *C. jejuni* cultures (Table 1). This secretory flux was highly significant when compared with the effect of the control perfusing solutions, which induced absorption (lumen-plasma) ($P < 0.001$). Glucose transport was significantly affected ($P < 0.001$) by the supernatants of the four *C. jejuni* strains as compared with the glucose-saline solution and the *E. coli* K-12 supernatant. However, when compared with the TSB–5% blood solution, only two *C. jejuni* strains (14 FAISA and 60892) yielded significant results. Guerrant et al. (4) did not find toxigenic activity in *Campylobacter* supernatants assayed in rabbit loops, infant mouse cells, and CHO cells. Butzler and Skirrow (3) found that 16 of 100 *Campylobacter* culture supernatants were positive in infant mouse tests for thermostable enterotoxin. Madge (8) observed that young mice inoculated with standard single oral doses of *Campylobacter* cultures had significantly lower D-glucose and D-galactose absorption. In earlier studies with intestinal perfusion techniques, it was demonstrated that enterotoxic substances isolated from *Staphylococcus aureus* (13), *Clostridium perfringens* (9), *Klebsiella pneumoniae* (5), and *E. coli* (6) induce a net secretory process of water and electrolytes.

The results obtained in our experiments suggest that *C. jejuni* produces one or more substances that induce a secretory process in jejunal segments of adult rats that may be related to the diarrhea phase of *Campylobacter* enteritis. Since the *E. coli* K-12 supernatant and the other control solutions did not induce secretion, the possibility of a nonspecific secretory process seems to be ruled out. Further studies on the mechanism of this secretory process as well as on the nature of the substances involved are in course in our laboratory.

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