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Recovery of Injured *Campylobacter jejuni* Cells after Animal Passage

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Sixteen freeze-thaw-injured nonculturable stocks of *Campylobacter jejuni* were passed through rat gut, and seven were reisolated. These reisolated strains were converted to toxin producers, as they were before preservation, following consecutive passages through rat gut. This observation indicated the existence of an injured, viable, but nonresuscitated form of *C. jejuni* which can be resuscitated to a culturable and fully virulent form by passaging the organism through a susceptible host.

Campylobacter jejuni has the characteristic feature of degenerating into a coccoid form after a few days in culture. Generally, these coccoid forms lose their motility and the ability to grow in subculture (1, 2, 5). However, a microcosm study revealed the viability of this form of *C. jejuni* (6). The early degeneration of *C. jejuni* and thus the difficulties with culture and preservation of this organism have, over the years, hindered work on it (13). Here, we report the resuscitation of freeze-thaw-injured *C. jejuni* strains to fully virulent form when the organism is passed through the guts of susceptible rats.

C. jejuni strains from our laboratory which had been preserved in George's medium (approximately 2×10^9 CFU/ml) for 8 months at −70°C (4) were exposed to room temperature (>37°C) because of a major power breakdown in the institute. Electricity was restored after 4 days, but the strains failed to grow in subculture on *Campylobacter* blood agar plates (Campy-BAP) (0.2 ml was inoculated). On Gram staining, the organisms stained weakly gram negative and were found to be in coccoid form. These freeze-thaw-injured nonculturable stocks of the organism were inoculated into rat ileal loops by the method described earlier (10, 11). The animals were sacrificed after 18 h, and a culture was made from each loop on Campy-BAP (1). The plates were incubated in a candle extinction jar for 48 h at 42°C with one thickly inoculated *Escherichia coli* plate to reduce the oxygen tension further (9).

Seven of 16 freeze-thaw-injured strains were successfully resuscitated by passaging them through rat gut and were reisolated on Campy-BAP. The strains were confirmed by their known biotype and antibiotic susceptibility patterns. No *C. jejuni* was isolated from negative control loops. Although the strains were toxigenic prior to preservation and caused 0.28 to 0.70 ml of fluid accumulation per cm of rat gut (10, 11), they caused little or no fluid accumulation at the time of regeneration. However, in subsequent experiments, after two to three consecutive passages, fluid accumulation caused by the organisms increased to the before-preservation level (Table 1).

Resuscitation of freeze-thaw-injured *C. jejuni* strains from stock culture which failed to yield any colony on Campy-BAP confirms the existence of a viable but nonresuscitated stage of *C. jejuni*. A similar phase of bacterial cells, in which they remain intact and alive, has been observed with organisms such as *Salmonella enteritidis*, *Vibrio cholerae*, *E. coli*, and *Aeromonas hydrophila* (7, 12).

The existence of this viable but nonresuscitated stage of *C. jejuni* is most probably due to the fact that the bacterial cells are injured by their exposure to high temperature and the culture media and methods used are unable to create conditions which are conducive to the growth of these cells and which occur naturally in the environment or in a susceptible host (8). The complex microenvironment in rat gut facilitated the resuscitation of injured *C. jejuni* to culturable form and their multiplication. Very little or no fluid accumulation caused by these known toxigenic strains indicated apparent loss of the ability to cause such accumulation in this "pseudosenescent" state, but the ability to cause fluid accumulation could be restored by passage in animals.

The detection of an injured but viable form of *C. jejuni* strains and their resuscitation to culturable state is of para-

TABLE 1. Regeneration of *C. jejuni* strains by animal passage

Strain no.	Source	Accumulation of fluid (ml/cm) before preservation	Growth on subculture from RIL[a]	Accumulation of fluid (ml/cm)	
				Before passage	After passage
1	Chicken	0.30–0.50	+	0	0.30–0.45
2	Chicken	0.44–0.55	−	0	
4	Chicken	0.35–0.45	−	0	
5	Chicken	0.50–0.70	+	0.10	0.50–0.70
7	Chicken	0.30–0.45	−	0	
8	Chicken	0.30–0.50	+	0	0.30–0.45
9	Chicken	0.50–0.70	+	0.15	0.55–0.70
10	Chicken	0.45–0.55	−	0	
11	Chicken	0.30–0.35	+	0	0.30
13	Chicken	0.40–0.45	−	0	
15	Chicken	0.30–0.35	−	0	
16	Chicken	0.30–0.40	+	0	0.30–0.40
35	Human	0.40–0.65	−	0	
60	Human	0.30–0.50	+	0.15	0.45–0.50
411	Human	0.35–0.50	−	0	
430	Human	0.32–0.45	−	0	

[a] RIL, rat ileal loop.

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mount epidemiological significance in different waterborne outbreaks and may explain the failure to isolate the organism from suspect local water sources in affected areas despite extensive search (3, 14).

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