Ultrastructural Aspects of Localized Membrane Damage
in Spirillum serpens VHL Early in Its Association
with Bdellovibrio bacteriovorus 109D

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Abstract. The freeze-fracture technique and electron microscopy have been used to demonstrate that localized damage is inflicted upon the cytoplasmic membrane of Spirillum serpens VHL within 20 to 30 min after the start of its association with Bdellovibrio bacteriovorus 109D. This damage is not observed in uninfected Spirillum cells, nor in infected cells within the first 10 min. This damage takes the form of a "blister" which, when viewed stereoscopically in electron micrographs, is seen to project toward the interior of the Spirillum cell. Shortly after its formation, the blister becomes elaborated into a series of ridges which may assume forms ranging from an elaborate spiral to a series of loops or knots. The formation of a blister is shown to involve both the inner and outer leaves of the membrane bilayer, and evidence is presented to indicate that the blister site corresponds to the site of attachment of the Bdellovibrio cell. The hypothesis is proposed that this ultrastructural damage is the cytological basis for the controlled and localized leakage through the cytoplasmic membrane into the periplasmic space of the Spirillum cell at locations adjacent to the Bdellovibrio cell. It is suggested that this localized membrane damage may be the ultrastructural basis for the high efficiency with which bdellovibrios are known to incorporate cytoplasmic materials from the other bacteria in whose periplasmic spaces they develop.

Key words: Bdellovibrio bacteriovorus—Spirillum serpens—Freeze Fracture—Electron Microscopy—Ultrastructure—Membrane Damage—Organismic Associations.

It has been clearly established that congenial bacteria associating with members of the bacterial genus Bdellovibrio suffer early and extensive disruption of their outer wall membranes and periplasmic spaces (Abram et al., 1974; Burnham et al., 1968; Scherff et al., 1966; Starr and Baigent, 1966; Varon and Shilo, 1969; for reviews see: Starr and Seidler, 1971; Starr and Huang, 1972; Stolp, 1973). Furthermore, biochemical evidence has been published to the effect that the cytoplasmic membranes of the attacked bacteria soon lose their selective permeability, at least with respect to small molecules (Crothers and Robinson, 1971; Rittenberg and Shilo, 1970; Varon et al., 1969). Rittenberg and Shilo (1970)
indicate that this event occurs (although not to as great an extent) even when penetration of the other bacteria by *Bdellovibrio* is blocked by the addition of streptomycin. This seems consistent with the recently published findings of Abram *et al.* (1974) which indicate that *Bdellovibrio* engages the cytoplasmic membrane of its associant prior to the act of penetration and that, in fact, such engagement is a necessary prerequisite to such penetration.

It has not previously been established whether the associant’s cytoplasmic membrane is disrupted simultaneously over its entire surface or whether the disruption is localized at the site of contact with the invading bdellovibrio. The present report presents ultrastructural evidence to the effect that *Bdellovibrio* inflicts localized damage upon the cell membranes of the entered bacteria, damage which is restricted to the vicinity of the inhabiting *Bdellovibrio*. Such localized damage may serve to produce the controlled leakage of nutrient cytoplasmic materials into the periplasmic space at the locus where the *Bdellovibrio* is developing.

**Materials and Methods**

*Spirillum serpens* strain VHL was used as the associant organism for *Bdellovibrio bacteriovorus* strain H-109D. *S. serpens* was maintained on peptone-yeast extract agar; it was also grown in YPSC broth supplemented with 2 mM Ca$^{2+}$ and 3 mM Mg$^{2+}$ (Huang and Starr, 1973), which was later inoculated with the *Bdellovibrio*. The *Bdellovibrio* was maintained in such lysate form at room temperature, with subculture into fresh YPSC cultures of *S. serpens* every 1 to 2 weeks.

A 50 ml overnight culture of *S. serpens* grown at 30°C in cation-supplemented YPSC broth was challenged at 30°C in a shaking waterbath with 5 ml of a *Bdellovibrio* lysate which had been grown together with *S. serpens* for 16–18 hrs in the same medium at 30°C. Periodically (0, 10, 20, 30, and 40 min) during the challenge period, the interaction between *Bdellovibrio* and *Spirillum* was stopped, either by chilling the culture in an ice-bath or by fixation in phosphate-buffered glutaraldehyde. Cultures which had been chilled in an iced-bath were occasionally checked microscopically, and the infection did not appear to proceed so long as the cultures were maintained at 0°C. While being held at 0°C, the culture was centrifuged and resuspended in 20% glycerol buffered with 0.025 M Tris buffer (pH 7.5) and augmented with 2 mM CaCl$_2$ and 3 mM MgSO$_4$. After about 1 to 1.5 hrs, the culture was again centrifuged and resuspended in 40% glycerol buffered and augmented as above. After an additional 1 to 1.5 hrs, the culture was again centrifuged and reusa-

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1 For reasons laid out in some detail elsewhere (Starr, 1975), the terms “parasite/host” and “predatory/prey” seem (at least to one of the present writers) to be inappropriate for designating the relationship between *Bdellovibrio* and the other bacteria with which it is associating. The relationship is best viewed as an antagonistic organismic association (an antagonistic symbiosis), and the organisms which are associating could—without coloring the relationship by applying ambiguous names to it—be called “associants” or “symbionts”. In this terminology (Starr, 1975), the organism which has been called the bdellovibrio’s “host” or “prey” might be termed the “entered (or inhabited) associant (or symbiont) of the bdellovibrio”, and the bdellovibrio which has been called the “parasite” or “predator” might be referred to as the “inhabiting (or entering) associant (or symbiont)".