## NOTES

## Studies on a Canine Intestinal Spirochete: Scanning Electron Microscopy of Canine Colonic Mucosa

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A large homogeneous population of spirochetes was observed attached to the colonic mucosa of clinically normal dogs. Their association with the colonic mucosa and their mode of attachment resembled those of a spirochete reported in primates.

The colonization of various animal species by intestinal spirochetes has been known for many years (4, 5). In dogs such infestations apparently are common and generally occur without clinical signs (3, 6, 8). Indeed, it appears that, as in the case of some primates (7), spirochetes may comprise part of a dog's normal or autochthonous enteric flora (8).

After the isolation and cultivation of one such canine agent (8), scanning electron microscopy was used in an attempt to visualize the spirochete in its natural habitat and gain some insight into its possible association with the colonic mucosa.

The dogs used in this study were as previously reported (8). Segments of colon distal to the cecum were tied off and injected with cold phosphate-buffered 3% glutaraldehyde (pH 7.3). The segments were then removed and placed in cold fixative for 1 h, after which time the segments were opened and gently washed with phosphate buffer (pH 7.3). The washed segments were then placed in fresh, cold fixative for 24 h and postfixed with osmium tetroxide and thiocarbohydrazide by the methods of Kelly et al. (1). The tissues were then rinsed in phosphate buffer (pH 7.3), dehydrated with ethyl alcohol, criticalpoint dried, and sputter coated with gold.

Depending on the degree of colonization and on the particular area examined, spirochetes were observed on the surface of the mucosa both as isolated tufts or microcolonies (Fig. 1) and as dense, tangled masses that involved substantial areas of the mucosal surface (Fig. 2).

Although the canine agent reported here ap-

peared to be of a smaller diameter than the one observed in primates (approximately 0.20  $\mu$ m compared with 0.5  $\mu$ m), it bore marked similarities as to: (i) anatomical site within the host, (ii) mode of attachment, and (iii) perpendicular orientation to the mucosal surface.

One unusual phenomenon observed in several scanning electron microscope preparations was the apparent ability of adjacent spirochetes to be in phase relative to their movement, suggesting a possible organized or coordinated beating or wavelike action on the part of attached spirochetes (Fig. 3).

Although the canine agent reported here bears some superficial similarities to a spirochete isolated from cases of swine dysentery and referred to as *Treponema hyodysenteriae*, the canine isolate is distinct from the swine agent in that it: (i) is smaller, (ii) differs in ultrastructural detail, and (iii) is less hemolytic on media containing blood (8).

This study confirms that spirochete infestations of the colonic mucosa can occur in clinically normal dogs and that the microbe is not limited to the lumen of the colon but can enter into an intimate association with the mucosal surface. On the basis of what has been observed by scanning electron microscopy in primates (7), in swine (2), and now in dogs, similar situations will undoubtedly be observed in various other species known to harbor intestinal spirochetes.

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FIG. 1. Scanning electron micrograph of colonic mucosa revealing small isolated tuftlike microcolonies.
×5,800.
FIG. 2. Large matlike accumulation of spirochetes around an opening to a crypt of Lieberkühn. ×6,000.



FIG. 3. One of several areas where agent's movement appears to be in phase, suggesting some form of possible coordination.  $\times 6,800$ .

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