Association of *Treponema hyodysenteriae* with Porcine Intestinal Mucosa

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The association of *Treponema hyodysenteriae* with porcine caecal and colonic mucosal surfaces was studied by electron microscopy after orogastric inoculation of pigs with pure cultures. Examination of caecal and colonic mucosa from infected and control animals revealed that large numbers of the spirochaete were associated only with intestinal mucosal surfaces of infected animals. Further examination of the intestinal mucosa from infected pigs showed that *T. hyodysenteriae* colonized two sites preferentially: the mucus-filled crypts of Lieberkuhn and the mucus gel covering the epithelium. Furthermore, no evidence of either specific or nonspecific adhesion to the epithelium proper was found, suggesting that penetration of, or trapping in the mucus gel may be the predominant mechanism of mucosal association by *T. hyodysenteriae*. Moreover, *T. hyodysenteriae* was also observed to be highly motile in intestinal mucus, moving faster than any other organism present, and this 'high speed' motility appeared to facilitate penetration into the mucosa. The pattern of motility observed was also highly suggestive of chemotaxis, and this was subsequently confirmed using an *in vitro* assay to porcine mucus material. It is suggested, therefore, that motility and chemotaxis are important factors/mechanisms in the association and colonization of porcine intestinal mucosa by *T. hyodysenteriae*.

INTRODUCTION

Swine dysentery (SD), a mucohaemorrhagic diarrhoeal disease of pigs, has received a great deal of attention in recent years because of its economic importance to the swine industry. However, most of the research devoted to this subject has been restricted to the identification of the causative organism(s) or ways to treat or prevent SD, with much less attention being paid to the pathogenesis or pathophysiology of the disease. Although the aetiology and pathogenesis has not been clearly defined, a large, anaerobic spirochaete, *Treponema hyodysenteriae*, is considered to be the aetiological agent (Harris & Glock, 1986). Furthermore, other indigenous (e.g. fusobacteria and *Bacteroides* spp.) and endogenous (e.g. *Acetivibrio ethanoligignens*) anaerobic bacteria may also contribute to colonic lesion production in SD, and may be required for *T. hyodysenteriae* to express pathogenicity (Meyer *et al*., 1974, 1975; Whipp *et al*., 1977; Harris *et al*., 1978; Robinson *et al*., 1984).

Given that the first stage of the disease process in SD is likely to be association with the mucosal surface, it is not surprising that *T. hyodysenteriae* has been found in large numbers in the colonic mucosa of dysenteric pigs (Kennedy *et al*., 1973; Joens *et al*., 1981). By electron microscopy, for instance, *T. hyodysenteriae* has been observed within the crypts of the Lieberkühn, necrotic epithelial cells and the lamina propria (Glock & Harris, 1972; Kennedy & Strafuss, 1976; Teige *et al*., 1981), but there was no evidence that invasion was essential for lesion production or the initiation of disease (Glock *et al*., 1974; Albassam *et al*., 1985). Theoretically, these spirochaetes could colonize the mucosa by at least three different mechanisms: (i) specific or nonspecific adhesion to the epithelium or mucus (Knoop *et al*., 1979; Wilcock & Olander, 1979a, b); (ii) coadhesion to adherent micro-organisms (Kennedy, 1987); or

*Abbreviations*: SD, swine dysentery; HGM, hog gastric mucin.
(iii) chemotactic and/or motility regulated mucus association (Kennedy, 1987). Because of the likely importance some or all of these properties may play in the pathogenesis of SD, and the lack of information on the mechanism(s) of mucosal association by T. hyodysenteriae, the present studies were initiated to characterize the association of T. hyodysenteriae with porcine intestinal mucosa. In addition, the cell surface hydrophobicity, which has been suggested to be an important nonspecific adhesion mechanism in mucosal association of other bacteria (Magnusson et al., 1980), was also studied.

METHODS

Bacterial strains. The test organisms used were originally isolated from the faeces or colons of pigs with classic signs of swine dysentery. Strain B204 was obtained from the American Type Culture Collection (ATCC 31212), and strain 16-4 was provided by Richard Greening of this institution. Both strains had been given to dysentery free pigs and reisolated from the colon to insure that highly virulent organisms were used. From the date of re-isolation, the organisms were passed less than five times and were frozen at −70°C in growth medium containing 30% (v/v) glycerol.

Culture media, growth conditions and inocula preparation. T. hyodysenteriae strains were isolated from colons of pigs acutely infected with SD on trypticase soy agar (BBL) supplemented with 5% (v/v) sheep blood and spectinomycin (The Upjohn Company; 400 μg ml⁻¹), polymyxin B (Sigma; 25 μg ml⁻¹) and vancomycin (Sigma; 25 μg ml⁻¹) (TSA–SPV; Jenkinson & Wingar, 1981). Isolates were passed on TSA–SPV, grown in trypticase soy broth (BBL) supplemented with foetal calf serum (Gibco; 10%, v/v, final concentration) (TSB–FCS) for 48 h at 38°C in a Coy anaerobic chamber, and stored at −70°C in TSB–FCS broth containing glycerol (30%, v/v). A 1 ml portion of each frozen stock culture was used to inoculate 100 ml TSB–FCS and the culture was then incubated with shaking (100–110 r.p.m.) for 48–72 h at 38°C in a Coy anaerobic chamber that contained an atmosphere of 5% CO₂, 10% H₂ and 85% N₂ (by vol.). A 1% (v/v) inoculum of each culture was then transferred to fresh TSB–FCS, and these cultures were then incubated for 24 h as described above. The resulting cultures were used for inoculation of pigs. For some studies these steps were repeated except that brain heart infusion (BHI; Difco) broth supplemented with foetal calf serum (10%, v/v, final concentration) (BHI–FCS) was used as the growth medium. All manipulations of T. hyodysenteriae cultures were done in the anaerobic chamber. All media and equipment for T. hyodysenteriae growth were placed in the anaerobic chamber 24 h prior to use. The presence of T. hyodysenteriae in the faeces or intestines of experimental and control pigs was determined by selective plating on TSA–SPV and phase contrast microscopy.

Animals. Crossbred male and female pigs weighing approximately 6–10 kg were obtained from a closed herd at The Upjohn Company, and were used for all studies. Each experimental unit consisted of 8–10 pigs.

Animal preparation and inoculation. Pigs were inoculated by intubation on two consecutive days with 100 ml of broth culture that contained ~10⁸ viable cells ml⁻¹. Pigs were fasted for 48 h prior to inoculation and feed was returned 2 h after the last inoculation. Infected pigs used for the microscopic studies were killed, and necropsies were done 7 d after the first inoculation of the spirochaete. Samples were also obtained from uninoculated control pigs from the same herd at the same age.

Microscopic methods. For light microscopy, animals were killed and the caecum and colon were removed and opened. The intestinal contents were gently cleared from the surface with a rubber spatula, and a small piece of intestinal mucosa was placed on a glass slide 'mucosal side up'. A coverslip was then gently placed on the tissue, and the slide was examined immediately under phase-contrast microscopy (x 400) using a Leitz Laborlux D microscope. In some instances, it was necessary to press the cover slip down to observe the spirochaetes in the mucus gel. Intestinal scrapings were also observed for T. hyodysenteriae motility.

For scanning electron microscopy (SEM), intestinal tissues were fixed in 3.0% (v/v) glutaraldehyde in 0.1 M-sodium cacodylate buffer, pH 7.4, then washed in three changes of buffer, and post-fixed in 1% (w/v) OsO₄ in the same buffer for 1 h. The tissue then was washed in three changes of the cacodylate buffer, dehydrated by passage through an ethanol series, and critical-point dried with a Ladd (model #28000) critical-point dryer. Specimens were coated under vacuum with gold :palladium (60 : 40) in a Hummer X Sputter coater and examined in a JEOL JSM-T300 scanning electron microscope at 15–20 kV.

Two procedures were used for transmission electron microscopy (TEM). In the first procedure, pieces of intestinal tissue were fixed in 3% (v/v) glutaraldehyde in 0.1 M-cacodylate buffer pH 7.4 with 0.1% Ruthenium red for 1 h, then washed in three changes of buffer, and post-fixed in 1% (w/v) OsO₄ in the same buffer with 0.1% Ruthenium red. The tissue was then washed in three changes of buffer, and dehydrated in an ethanol series. Following this step, the tissue was placed in propylene oxide for 5 min, then immediately immersed in propylene oxide/Polybed 812 (1:1, v/v) and embedded in Polybed 812. The second procedure used was similar to that described above, except that the primary fixation was done in 3% glutaraldehyde in cacodylate buffer, followed by post-fixation in OsO₄ in cacodylate buffer, and tannic acid staining (0.5% tannic acid in water) for 1 h. Tissues
were sectioned using a Sorvall MT 5000 ultramicrotome and post-stained with 2% (w/v) aqueous uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). Sections were examined in a JEOL-1200 EX transmission electron microscope.

Chemotaxis assays. The techniques for measuring chemotaxis were based on a modification (Kennedy & Lawless, 1985) of the procedures described by Adler (1973). The bacterial suspensions were obtained by placing rectal swabs (containing faecal material) in 0.85% sterile saline, and mixing vigorously for 10 s. A 1 ml sample of the resulting suspension was placed into small test-tubes (10 × 74 mm). The capillary tubes (1–5 μl size Accupette Pipets; Dade Diagnostics), were filled with 1% (w/v) hog gastric mucin (HGM) (ICN Nutritional Biochemicals) dissolved in 0.85% saline and sealed at the upper end with stopcock grease. Control capillaries were filled with saline alone and used to assess background motility of the spirochaetes. By means of adhesive tape applied to the outside of the tubes, test and control capillaries were attached (one capillary per tube) so that the open ends of the capillaries were submerged 10 mm below the surface of the suspension. At this point the tubes were transferred to an anaerobic chamber and incubated for 1 h at 38 °C. After the incubation period the contents of the capillaries were expelled onto glass slides, coverslips were placed on them, and chemotaxis was assessed by observing the capillary contents under phase-contrast at 400 × magnification (Paster & Gibbons, 1986). Each chemotaxis assay was done in duplicate, 10–20 fields were counted, and the results were averaged. Chemotaxis was expressed as the ratio (Rmean) of the number of bacteria in attractant capillary to the number of cells in the control capillary (Kennedy & Lawless, 1985). Use of the Rmean value normalizes for experimental or day-to-day variation in data (Moulton & Montie, 1979). An Rmean value of 2.0 or greater was considered to be significant (Moulton & Montie, 1979).

Hydrophobicity studies. A modification (Kennedy et al., 1987) of the method of Rosenberg et al. (1980), with hexadecane (Sigma) as the hydrocarbon phase, was used to test for T. hyodysenteriae hydrophobicity. Briefly, the spirochaete was grown as described, washed twice in PUM buffer, which consisted of 22.2 g K2HPO4, 3H2O, 17.26 g KH2PO4, 1.8 g urea, 0.2 g MgSO4, 7H2O and distilled water to 1000 ml (Rosenberg et al., 1980), and resuspended to a final concentration of 1 × 10^9 bacteria ml^-1. For other experiments, faecal material was placed into PUM buffer or saline, and mixed vigorously for 30 s to prepare faecal suspensions of spirochaetes grown in vivo. To round-bottom tubes (16 mm diameter), containing 2.5 ml of bacterial suspensions, 0.5 ml of hexadecane was added. The resulting suspensions were incubated for 10 min at 37 °C, and mixed at 'high' speed for 2 min on a vortex mixer. After separation of the aqueous and organic phases, the aqueous phase from pure cultures of T. hyodysenteriae was measured at 400 nm on a Bausch & Lomb Spectronic 20 spectrophotometer. Control cells were treated identically without the addition of hydrocarbon to the system. The relative hydrophobicity was then determined by measuring the difference in optical density of test and control cell suspensions, or the difference in microscopic counts of spirochaetes from faecal suspensions, and determining the percentage of cells that entered the hydrocarbon phase.

RESULTS

Experimental dysentery in pigs

Inoculation of pigs with T. hyodysenteriae 16-4 and B204 resulted in mortality due to dysentery in approximately 15% and 75% of the animals, respectively. Both strains induced clinical signs of dysentery within 6–7 d post-inoculation, which consisted of tucked-in flanks, morbidity, and change in faecal consistency. The faecal consistency at this point was a soft to watery diarrhoea, which was followed by the appearance of mucus and blood by 6–10 d after the first inoculation. All pigs showing signs of dysentery were shedding T. hyodysenteriae in their faeces, whereas control pigs did not shed spirochaetes or develop any signs of SD.

Gross lesions of SD were not observed in the control pigs, but gross lesions were found in dead pigs or those killed at the time of necropsy in the infected groups. Lesions were limited to the colon for strain 16-4, whereas lesions were found in both the caecum and colon for strain B204. Various degrees of caecitis and colitis were observed, but were more severe for strain B204. Of pigs infected with strain B204 88% had pathological findings of SD, compared to only 43% of pigs infected with T. hyodysenteriae 16-4. Table 1 summarizes the data for the experimental and control pigs used for electron microscopy studies.

Association of T. hyodysenteriae with intestinal mucosa

By electron microscopy, numerous cells of the spirochaete were observed colonizing both the caecal and colonic mucosa of pigs infected with SD. SEM examination of faecal mucosa, for example, revealed abundant numbers of the spirochaete entangled in masses in mucous material and in the crypts of Lieberkühn (Fig. 1). The intestinal mucosa of control pigs also contained...
Treponema hyodysenteriae mucosal association

Fig. 1. Scanning electron micrograph of caecal mucosal surface from pig no. 4 (a); bar = 10 μm. Spirochaetes can be observed within the orifice of a crypt of Lieberkühn (b) or associated with mucus (c); (d) emphasizes that *T. hyodysenteriae* associates preferentially with mucus. Note the epithelium proper is relatively free of spirochaetes. Bar = 1 μm (for b–d).
Fig. 2. Transmission electron micrograph of colonic crypt from pig no. 24 showing a seemingly 'pure culture' of \textit{T. hyodysenteriae} in the mucus. Bar = 2 \mu m.

Table 1. \textit{Summary of data of experimental and control pigs}

<table>
<thead>
<tr>
<th>Pig no.</th>
<th>\textit{T. hyodysenteriae} strain</th>
<th>Pathological findings</th>
<th>Presence of \textit{T. hyodysenteriae}</th>
<th>Stool characteristics</th>
<th>Physical condition†</th>
<th>Average daily wt gain‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>16-4</td>
<td>Caecitis +</td>
<td>Selective +</td>
<td>Consistency Soft</td>
<td>Intermediate</td>
<td>0.01</td>
</tr>
<tr>
<td>24</td>
<td>B204</td>
<td>Colitis +</td>
<td>Selective plating +</td>
<td>Blood Intermediate</td>
<td>-</td>
<td>0.14</td>
</tr>
<tr>
<td>N</td>
<td>None</td>
<td>None -</td>
<td>None -</td>
<td>Consistency Normal</td>
<td>Intermediate</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* Microscopic examination revealed many, vigorously motile, large spirochaetes (+), or no spirochaetes (−).
† All the pigs were scored individually for their physical condition as (1) active (healthy), (2) intermediate and (3) inactive (lethargic).
‡ Average daily wt gain (kg) = \((\text{wt at day of sacrifice}) - (\text{wt at day of inoculation})/\text{no of days}\).

large numbers of bacteria that were seen in the mucus gel and in intestinal crypts. These bacteria were of several morphological forms, but fusiform-shaped bacilli appeared to dominate the mucosal flora of these pigs. Spirochaetes were not observed within the mucus gel, within intestinal crypts, or attached to the epithelium proper in control pigs not inoculated with \textit{T. hyodysenteriae}.

Further examination of the caecal and colonic mucosa from the pigs with SD revealed that the spirochaete appeared to prefer two sites for colonization: as can be seen (Fig. 1 a) these were the crypts of the Lieberkühn (Fig. 1 b) and the mucus gel covering the epithelium (Fig. 1 c). Due to the conventional methods used for fixation of tissues in these studies much of the surface mucus was lost. Nevertheless, in the areas of the section where small pieces of mucus were retained,
they were seen to be packed with the spirochaete (Fig. 1d). While an occasional spirochaete was observed apparently attached to the epithelium, direct association with the epithelium did not appear to be important for colonization or initiation of mucosal lesions. Likewise, *T. hyodysenteriae* was not observed to penetrate healthy epithelial cells. TEM of thin sections of intestinal mucosa confirmed the observations noted using SEM. Fig. 2, for example, shows *T. hyodysenteriae* colonizing a colonic crypt from pig no. 24. As can be seen, numerous cells of the spirochaete are located in the mucus in a crypt seemingly in 'pure' culture. Furthermore, Fig. 3 emphasizes that direct attachment to the epithelium by *T. hyodysenteriae* does not appear to be important for colonization. Some cells of the spirochaete are very close to, but do not appear to be in direct contact with, the epithelial surface, whereas other spirochaetes can be seen 'swimming' in a 'pool' of mucus. Association with the mucus (whether in the crypts or covering the epithelial surface) appeared to be the primary mechanism of association of *T. hyodysenteriae* with porcine intestinal mucosa. Fig. 4 shows *T. hyodysenteriae* penetrating a mucus-secreting goblet cell.

It was also noted that the spirochaete was often associated in mucus with two morphologically distinct bacteria, especially at the onset of the disease. Fig. 1(b) shows *T. hyodysenteriae* associated with a large spirillum in a crypt and Fig. 5 shows *T. hyodysenteriae* associated with 'corkscrew-shaped' bacilli in the mucus overlying the epithelium. It is not clear from these micrographs whether the spirochaete is 'attached' to or simply entangled with the other organisms.
Motility of *T. hyodysenteriae* in the intestinal mucosa

Scrapings and small pieces of intestinal mucosa from pigs with SD were examined by phase-contrast microscopy to observe the movement of *T. hyodysenteriae* in intestinal mucus. The spirochaete proved to be highly motile in intestinal mucus, moving faster than any other microorganisms observed in these preparations. Their motility was so efficient that they were easily able to push past other bacteria and appeared to be able to burrow through the mucus. Furthermore, movement of *T. hyodysenteriae* appeared to be directed toward the mucosal surface. This is consistent with observations made by others (Isadore M. Robinson, personal communication). It was also interesting to note that 'packs' of 2–8 spirochaetes would occasionally 'swim' together in parallel streams in the mucus gel or in channels in the mucus gel. Moreover, it was also observed that normal motility was, all at once, interrupted and all the spirochaetes in the field under observation would switch to a brief period of 'high frequency' motility. This frenzy of activity was presumably triggered by the release of material from the dying epithelium, and was suggestive that the spirochaetes could undergo chemotaxis. This was reinforced by the observation that motility was also interrupted by a switch in direction, where the spirochaete would move rapidly forward then backward repeatedly. No such spirochaetes or high speed motility was observed in control pigs.

Chemotaxis

*In vivo* grown *T. hyodysenteriae* B204 demonstrated a significant chemotactic response (*R_{che} = 11.7*) to HGM, since significantly more bacteria were observed to accumulate in test capillaries than in control capillaries. The mean number of cells of the spirochaete per field was 7·0 for
capillaries containing HGM compared to control capillaries, which showed only a mean of 0-6 spirochaetes per field. Attempts to study chemotaxis using pure cultures of T. hyodysenteriae grown in vitro were not possible as the spirochaete in 16–24 h cultures exhibited only a twitching-type motility.

Hydrophobicity studies

The relative hydrophobicity of T. hyodysenteriae was determined by the phase-partition test. The strains of T. hyodysenteriae examined proved to be strongly hydrophilic since $<1\%$ of the cells entered the hydrocarbon phase. This was true whether the cells were grown in different broth media (BHI–FCS, TSB–FCS) in vitro or when grown in vivo in pigs.

DISCUSSION

T. hyodysenteriae colonizes the intestinal mucosa of swine by association with intestinal mucus in both the mucus gel covering the epithelium and the mucus-filled crypts. Although T. hyodysenteriae attaches to epithelial cells in vitro (Knoop et al., 1979), no evidence of adhesion to the intestinal epithelium was found in this study. The finding that no specific orientation of the spirochaete to the epithelial surface occurred is consistent with this. Moreover, in terms of infected mucosa, it was clearly observed that the spirochaete was not ‘attached’ to the epithelial surface, but was instead colonizing the overlying mucus layer. Likewise, examination for a nonspecific adhesion mechanism (e.g. cell surface hydrophobicity) that might confer a random orientation to the epithelium was not observed in the present study. The association of T. hyodysenteriae with the intestinal mucosa, therefore, parallels very closely that recently reported

Fig. 5. Scanning electron micrograph of exudate overlying the mucosa from pig no. 4 showing the spirochaete associated with a 'corkscrew-shaped' organism and itself, and entangled in a fibrous-mucus matrix. Bar $= 1\mu m.$
for *Campylobacter jejuni* and certain spiral organisms of the indigenous microbiota that normally colonize the murine intestinal mucosa (Lee *et al.*, 1986). The present findings suggest that penetration of and/or trapping in the mucus gel may represent the predominant mechanism of mucosal association by *T. hyodysenteriae*. Similarly, it has previously been shown that other organisms may colonize the intestinal mucosa by nonadhesive mechanisms (Freter & O'Brien, 1981; Freter *et al.*, 1981a; Lee *et al.*, 1986; Kennedy, 1987, Kennedy *et al.*, 1987).

The observation that *T. hyodysenteriae* is highly motile *in vivo* also correlates well with the finding that motility may be an essential factor for mucosal colonization and pathogenesis by certain pathogens and indigenous intestinal bacteria (Allweiss *et al.*, 1977; Freter, 1978, 1980, 1982). Guentzel & Berry (1975) and Yancey *et al.* (1978), for instance, showed that nonmotile *Vibrio cholerae* were less virulent for orally challenged experimental animals than the wild-type strains, from which they were derived. Likewise, Newell *et al.* (1983) and Morooka *et al.* (1985) have reported similar findings for *C. jejuni*. The loss of virulence for all of the nonmotile strains described in the reports cited was apparently associated solely with a reduced capacity to associate with the intestinal mucosa, and not to a simple failure to multiply *in vivo* or loss of ability to produce toxin. Stanton & Savage (1983) reported that motile bacteria significantly outnumbered nonmotile bacteria in scrapings of mouse caecal mucosa. Subsequent comparison studies with one motile species (*Roseburia cececola*) isolated from the caecal mucosa of a mouse, and several nonmotile isogenic mutants, showed that motility was an essential factor to colonize the mouse caecum of conventional mice (Stanton & Savage, 1984). In support of this, *T. hyodysenteriae* was highly motile in the mucosa.

In addition, it has been noted that the ability to swim through viscous environments, such as mucus, depends on the possession of a specialized type of motility (Greenberg & Canale-Parola, 1977; Lee, 1985). Bacteria like *T. hyodysenteriae*, with a spiral morphology, are likely to have a selective advantage in the viscous mucus gel overlying the epithelium (Lee, 1985). Studies by Lee *et al.* (1986) have shown that indigenous intestinal spiral-shaped bacteria and *C. jejuni* were able to move more efficiently in solutions of high viscosity compared with motile rod-shaped intestinal bacteria. Likewise, in the present study *T. hyodysenteriae* moved faster than any other bacterium observed in preparations of intestinal mucus from pigs with SD. Interestingly, it appears that *T. hyodysenteriae* colonized the mucus gel and crypts in pigs in a manner analogous to *C. jejuni* and indigenous murine intestinal spirals in the mouse (Lee *et al.*, 1986).

The data presented in the present paper also provide strong evidence that chemotactic attraction constitutes a potentially important factor in the association of *T. hyodysenteriae* with mucus gel. *T. hyodysenteriae* was strongly attracted to HGM *in vitro* when the spirochaete was isolated from the pig gut. Furthermore, the motility pattern for *T. hyodysenteriae* observed in mucus scrapings was strongly suggestive that chemotaxis occurred *in vivo*. Conceivably, then, chemotaxis to sites containing mucus, in addition to the apparent superior motility, may allow *T. hyodysenteriae* to compete successfully for niches in the mucosa, thus facilitating the survival of this pathogen *in vivo*. Consistent with this idea were the findings that virtually all cells of the spirochaete noted in infected tissue were highly motile and that the spirochaetes appeared to move into the mucosa. Freter *et al.* (1981b) have reported that a chemotactic gradient extends throughout the mucus gel, with the strongest attraction occurring deep in the tissue. This correlates well with the observations of the present paper in that *T. hyodysenteriae* appeared to colonize the crypts (where mucus-secreting cells are located) preferentially. Moreover, *T. hyodysenteriae* penetrated, presumably by being chemotactically guided, mucus-secreting cells. It is not known, however, whether *T. hyodysenteriae* can utilize intestinal mucus, as can other intestinal bacteria (Cohen *et al.*, 1983), for carbon, nitrogen and energy sources. Nevertheless, the results of the present experiments indicate that motility and chemotaxis, singly and collectively with other survival mechanisms, may affect the ability of *T. hyodysenteriae* to succeed in colonizing the intestinal mucosa by allowing efficient penetration into the tissue. Moreover, these may be the only mechanisms, in the absence of either specific or nonspecific adhesion, that prevent *T. hyodysenteriae* from being eliminated from the gut by the natural flow of mucus. This is in agreement with several studies that have shown chemotaxis to play an important role in bacterial colonization of mucosal surfaces (Allweiss *et al.*, 1977; Freter
& O'Brien, 1981; Freter et al., 1981a; Uhlan & Jones, 1982; Paster & Gibbons, 1986). Consequently, the importance of motility and chemotaxis should not be underestimated since they may well supply that extra increment of fitness which is responsible for the colonization of intestinal mucosa by *T. hyodysenteriae*. Also, it was noted that *T. hyodysenteriae* was often associated with large spirilla, 'corkscrew-shaped' bacilli, or both at the onset of disease. A survey of faecal samples from nearly 100 pigs (from this herd) infected with SD, showed that these organisms (which are motile) were often present at early times after infection (M. J. Kennedy & R. J. Yancey, Jr, unpublished data). If association with these bacteria is necessary for colonization or pathogenesis by *T. hyodysenteriae*, this may help to explain its inability to produce SD in germ-free pigs fed pure cultures of *T. hyodysenteriae*.

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