Novel Ultrastructural Findings in a Helical Bacterium Found in the Baboon (*Papio anubis*) Stomach

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A helical bacterium, morphologically unrelated to *Campylobacter pylori*, has been observed in the stomach of the baboon, *Papio anubis*. This organism is found in both mucus and the canaliculi of oxyntic cells. Even though this organism is flagellated, electron micrographs show that it can both contract in length and bend. A novel system of tubules has been observed in the bacterial cytoplasm. These tubules may mediate both the bending and contraction observed.

INTRODUCTION

With the current interest in the association of *Campylobacter pylori* with human gastritis and peptic ulceration, several animal species have been examined as potential models of this infection (Tompkins *et al.*, 1988; Baskerville & Newell, 1987; Cave *et al.*, 1986). Some have been found naturally to harbour this organism, or a closely related strain (Baskerville & Newell, 1988; Jones & Eldridge, 1987; Reed & Berridge, 1988; Bronsdon & Schoenknecht, 1987) and attempts have been made to infect rhesus monkeys with strains of human origin (Newell & Baskerville, 1987).

In this study we have examined baboon stomachs and have found not only naturally occurring *C. pylori*, but a helical organism which shows some novel ultrastructural features. This paper describes the ultrastructure of this organism and its location in the baboon stomach. A preliminary description of this organism has already been published (Curry *et al.*, 1987).

METHODS

Baboons caught in the wild in East Africa were purchased from a non-human primate supply agency (Shamrock Farms (Great Britain), Victoria House, Small Dole, Henfield, Sussex BN5 9XE, UK). They were mainly olive baboons (*Papio anubis*; Napier & Napier, 1967), with an occasional hybrid of *P. anubis* and *P. cynocephalus*.

Upon arrival animals were commonly within a weight range of 3 to 4 kg. Age was estimated by dental examination to be 19 to 24 months (Siegal & Sciully, 1973). Animals were maintained in a carefully controlled environment at 20 °C ± 2 °C and at a relative humidity of 65 to 75%. Each animal was fed 250 g of a proprietary laboratory primate diet daily (Mazuri Primate Diet, SQC, Special Diet Services, Witham, Essex, UK) together with half an apple. Vitamin C was given orally as ascorbic acid tablets at 50 mg three times weekly, and tap water was freely available from individual automatic watering points.

The animals were derived from a separate drug safety evaluation investigation during which treated baboons received one of various test materials, while control baboons received either no treatment or inert vehicle (e.g. physiological saline or placebo). Material from control animals only was examined in this study.

At the end of each drug safety study all baboons were exsanguinated under deep intravenous barbiturate anaesthesia. During necropsy procedures a comprehensive selection of tissues were sampled, and careful attention was paid to their gross appearance but no observable abnormalities were seen in the stomach tissues used in this study.

Light microscopy. Samples of cardia, fundus and pylorus were fixed in Bouin's fluid (saturated aqueous picric acid 15 vols, 40% v/v, formaldehyde 5 vols and glacial acetic acid 1 vol.) with additional spare samples fixed in

Abbreviations: GALT, gut-associated lymphoid tissue.

neutral 10% phosphate-buffered formaldehyde (Carson *et al.*, 1973). Processed blocks were embedded in paraffin wax, sectioned at 5 µm and routinely stained with haematoxylin and eosin for light microscopy.

Culture technique. Duplicate samples of gastric mucosa were placed into nutrient broth for transport and cultivated on to a variety of media within 2 h of collection. Various conventional liquid and solid media were used at different times; *C. pylori* was isolated on blood agar and Skirrow's medium (Skirrow, 1977) from one animal. The organisms described here failed to grow on any of the more common laboratory media in an aerobic, anaerobic or microaerobic atmosphere.

Electron microscopy. Additional samples from the pyloric antrum, fundus and cardiac portion of the stomach were fixed at room temperature for a minimum of 4 h for transmission electron microscopy in either 3% (v/v) glutaraldehyde in 0.1 M-cacodylate buffer, pH 7.4, or 10% phosphate-buffered formaldehyde (Carson *et al.*, 1973). These were postfixed for 1 h at room temperature in 1% (w/v) osmium tetroxide in 0.1 M-cacodylate buffer, dehydrated in a graded series of alcohols and embedded in Agar 100 resin. Semi-thin sections stained with toluidine blue were examined to identify mucosal surface, crypts or presence of bacteria prior to ultrathin sectioning. Ultrathin sections were collected on to uncoated 200 mesh copper grids, stained with uranyl acetate and lead citrate and examined in an AEI EM 801 electron microscope.

RESULTS

Twelve animals (9 male and 3 female) were examined between October 1985 and November 1986. Four animals showed moderate or trace gut-associated lymphoid tissue (GALT), two of these also having moderate diffuse lymphocytic gastritis. An additional animal showing GALT had a minimal diffuse neutrophil infiltrate in the lamina propria. No ultrastructural differences between glutaraldehyde-fixed and formaldehyde-fixed tissue were discernible.

A non-*C. pylori*-like helical organism was found by electron microscopy in the stomach of eight baboons (Fig. 1), two showing GALT and one with both GALT and moderate diffuse lymphocytic gastritis. In the remaining four baboons, *C. pylori* was grown from one and a *C. pylori*-like (Fig. 2) organism seen by electron microscopy in two others. Material for electron microscopy from the remaining animal did not contain stomach mucosa.

The non-*C. pylori*-like helical organism was found in all regions of the baboon stomach sampled, but more commonly in the fundic and pyloric regions. In these various samples, the organisms were found in the mucus layer on the surface of the mucosa (Fig. 1) and also within the crypts (Fig. 3). In the fundic region they were also commonly found in the canaliculi of parietal (oxyntic) cells (Fig. 3). Often several organism were found in the same canaliculus. The membranous lining of the canaliculi has a microvillous appearance and these channels form an extensive network within the cells which communicate with the crypt lumen. Mixed populations with the *C. pylori*-like organism have not been observed at the electron microscopic level.

The helical organism had a bunch of sheathed flagellar filaments at both ends (Fig. 4). The exact number of filaments was difficult to ascertain in thin sectioned material but 10 to 12 seemed to be an average number at each end. The cell wall had a Gram-negative type of structure and the body was 4–8 µm long and 0.4–0.5 µm wide. However, because of its helical form, the overall width was greater than this. The slimmest organisms had an overall diameter of 0.8 µm and accomplished one complete helical turn every 0.44 µm of body length (Fig. 5). The fattest organisms had an overall diameter of 1.1 µm and completed one turn every 0.63 µm of body length (Fig. 5). All possible intermediate forms were found. Some organisms had a wide girth at the middle of the body and tapered slightly towards the ends, giving rise to a cigar-shaped profile. This was particularly apparent in organisms where the outer membrane-like component of the cell wall no longer followed the undulations of the body closely, giving rise to an almost smooth cigar-shaped form. In some fields taken in the lumina of crypts, from 24 to 100% of the organisms showed evidence of this membrane detachment. The membrane always remained attached to the helical peaks at the periphery of the organism and involved the whole length of the organism, i.e. no partial detachment has been observed. Such organisms did not show any apparent degenerative changes. Some micrographs showed organisms bent through angles of nearly 90° (Fig. 6), whereas others commonly showed a more gently curved form.

Fig. 2. *C. pylori*-like organism in mucus-filled crypt. Note absence of helical bacterium. Primary fixation in glutaraldehyde. Bar, 1.5 µm.

Fig. 1. Non-*C. pylori*-like helical bacterium in mucus. Primary fixation in formaldehyde. Bar, 5 μ m.

Fig. 3. Low-power micrograph of crypt showing helical organisms in both the lumen and the canaliculi of the oxyntic cells. Primary fixation in formaldehyde. Bar, 5 μ m.

Fig. 5. Section through a number of helical organisms. Note that one organism appears more tightly coiled than the others. Primary fixation in formaldehyde. Note obliquely cut cytoplasmic tubules (T) within the cytoplasmic matrix of the organisms. Bar, 1 μ m.

Fig. 6. Longitudinal section through a helical organism bent at an angle of almost 90°. Note obliquely cut cytoplasmic tubules (T) in the two organisms to the bottom left of this micrograph. Primary fixation in formaldehyde. Bar, 0.5 μ m.

Fig. 4. Longitudinal section of helical organism showing bunch of sheathed flagellar filaments arising from both ends. Primary fixation in formaldehyde. Bar, 1 μ m.

Fig. 7. Longitudinal section through the tip of an organism showing electron-translucent zone and electron-dense layer closely adherent to tip plasma membrane. Note longitudinal profiles of cytoplasmic tubules (T). Primary fixation in formaldehyde. Bar, 250 nm.

Fig. 8. Longitudinal section of organism in a canaliculus. Note cross-sections of bundles of tubules (T) in the bacterial cytoplasm. Bar, 0.5 μ m. Insets show two bundles of tubules magnified (bars, 500 nm). Primary fixation in glutaraldehyde.

Fig. 9. Slightly different orientation to Fig. 8, showing tubules (T) cut almost longitudinally. Primary fixation in formaldehyde. Bar, 0.2 μ m.

Internally the organism was filled with a dense cytoplasm comprising a central filamentous nucleoid area surrounded by a granular area containing the ribosomes. The terminal regions showed specializations which comprised an electron-translucent zone at the tip and electron-dense decorations on the cytoplasmic face of the plasma membrane surrounding this region (Fig. 7). The electron-dense layer was about 20 nm wide and extended, like the electron-translucent region, for a distance of 500 nm from the tip.

The most striking feature found internally was bundles of tubules, each between 10 and 12 nm in diameter, seen at regular intervals, running through the cytoplasm. The bundles were square, rectangular or rhomboid in cross-section, with from 20 to 100 tubules in each bundle when seen in perfect cross-section (Fig. 8). In the thin sections used in the electron microscope, longitudinally cut sections of these tubules were more difficult to pick out against the ribosomal matrix (Figs 7 and 9). The most commonly seen profiles of these tubules were cut obliquely (Fig. 6). These tubules insert near to, or in the terminal region of the organism.

DISCUSSION

The helical organism described here has not as yet been cultured, which makes definitive classification difficult. However, it is superficially similar to a new organism from the human stomach described by Dent *et al.* (1987). Both organisms are morphologically distinct both from true *Campylobacters* such as *C. jejuni* and from the gastric organism '*Campylobacter pylori*' (Jones *et al.*, 1985). The new human-derived helical organism appears to lack the internal bundles of tubules seen in the baboon bacterium (McNulty *et al.*, 1989) and, in addition, has not been observed to show any variation in number of complete body turns per unit length. As yet, there has been no opportunity to examine human oxyntic cells at the electron microscopic level, so that any association between the organism and this gastric cell remains to be determined.

Such differences may reflect the fact that the two organisms are not closely related or that the baboon bacteria have evolved specialized structures to exploit the environment found in the baboon stomach. The association between the baboon bacterium and oxyntic cells may be of fundamental significance. These cells are thought to secrete acid and have an extensive canalicular system opening into the crypt lumina (Lillibridge, 1964; Rohrer *et al.*, 1965). The canalicular system is not composed of single straight canaliculi, but of a highly branched network. To exploit the secretions of the oxyntic cells fully, flexing or bending would be advantageous to penetrate the depths of the canalicular system. Organisms bent up to 90° have been observed and the number of complete body turns per unit body length is variable. This strongly suggests that some contractile mechanism capable of shortening or bending the organism is incorporated in the body or wall of this bacterium. There are no ridges around this organism like those seen by Lee & Hazel (1988) and Lee *et al.* (1988) in the organism from the cat stomach, nor are there any periplasmic fibres like those seen in a flagellated microaerophilic bacterium associated with human gastroenteritis seen by Archer *et al.* (1988). The cytoplasmically located tubular system would seem to fit this purpose perfectly.

The internal tubules found in the baboon bacterium are most clearly seen in median longitudinal sections. They are *not* located in or near the periplasmic space, but are within the cytoplasmic matrix. The overall form of the bundles of cytoplasmic tubules is most easily interpreted as being a single bundle which either undulates or is wound helically within the body cytoplasm of the organism although it does not necessarily correspond with the body helix.

True spirochaetes characteristically possess filaments inside the body wall, but these lie between the plasma membrane and the outer flexible wall component and they do not occur in the protoplasm. In addition, spirochaetes do not possess free flagellar filaments as well as the internal filaments. Indeed, the axial filaments of spirochaetes are of a similar structure to normal bacterial flagella but they move within the flexible outer wall of the organism. Thus the baboon bacterium, which possesses both external sheathed flagellar filaments and a cytoplasmic tubular system, would not appear to be derived from, or related closely to, true spirochaetes. Spirochaetes have been reported in the stomachs of both baboons (McConnell *et al.*, 1974) and macacus rhesus monkeys (Doenges, 1939). As both studies used light microscopy, it is possible that the organisms were misidentified.

Naked bacterial flagellar filaments are solubilized in acid conditions (Ibrahim *et al.*, 1985). The baboon bacterium, with its predilection for acid-secreting oxyntic cells, must have acid-stable filaments. Such resistance to acid probably resides in the sheath covering the filament, rather than the filament itself. The ability to exist in the canaliculi of oxyntic cells may give a clue to the cultural conditions needed to grow this organism successfully. Acid or some other metabolite secreted by oxyntic cells may be essential for growth.

The electron-dense decoration seen on the inner face of the plasma membrane in the terminal region of this organism (Fig. 7) has been seen in several bacterial species and has been postulated as being responsible for mechanical support of flagellated poles of such organisms (Curry *et al.*, 1984).

A helical body form appears to be an essential feature of organisms living in mucus (Ferrero & Lee, 1988). Organisms morphologically similar to *C. pylori* have never been observed in association with the helical organism in any sample examined here at the electron microscopic level. This finding may show a mutually exclusive existence but could also reflect the enormous sampling errors associated with electron microscopy. There are reports of *C. pylori* (Chen *et al.*, 1985) and other helical bacteria (Weber *et al.*, 1958; Rollason *et al.*, 1984) in the canaliculi of human oxyntic cells but this association does not appear to be common. Moreover, these associations are based on microscopical appearance of the organism, the identity of which was not proven.

The baboon bacterium described here is most probably a commensal in the stomach as only a single animal showed evidence of gastritis. This is in contrast to the new human-derived helical organism (Dent *et al.*, 1987; McNulty *et al.*, 1989), which has only been found in patients with chronic active type B gastritis.

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