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# An electron microscopic study of *Helicobacter pylori* in the surface mucous gel layer

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**Summary.** This study describes the distribution of spiral and coccoid forms of *Helicobacter* (*H.* ) *pylori* within the surface mucous gel layer (SMGL) on human gastric mucosae. Gastric mucosae infected with *H. pylori* were obtained from surgically removed stomachs of 14 cases of gastric cancer. The glycocalyx of the spiral and coccoid forms of *H. pylori* was examined for specific lectin labeling. Coccoid forms were identified in 11 of these cases (78.6%). The SMGL, which was from 50 to 100 $\mu$m in thickness, contained *H. pylori* throughout its entire thickness. Variously-sized vacuole-like clear areas were present near *H. pylori*. The glycocalyx on both the spiral and coccoid forms was similar in its staining with the 8 types of lectins tested. However, the staining pattern of the lectins varied among different samples. The numerous fibrillae-like filaments radiated from the surface of the bacteria and appeared to link the bacteria to the surface mucous cells or the surrounding mucus. These fibrillae-like filaments were not specifically stained by the lectin reactions, suggesting absence of sugar molecules.

**Key words:** *Helicobacter pylori*, Glycocalyx, Fibrillae-like filaments, Coccoid form, Surface mucous gel layer

## Introduction

It has been well established that *Helicobacter (H. ) pylori* infection is closely associated with peptic ulcer, chronic gastritis and gastric cancer (Marshall and Warren, 1984; Hessey et al., 1990; Kazi et al.,1990; Asaka et al., 1994; Ogata, 1995, 1997; Ogata and Araki, 1996). The structural features of stomach and duodenum infected with *H. pylori* have been reported in a number of publications (Thomsen et al., 1990; Steer, 1992; Noach et al., 1994; Ogata, 1995, 1997; Ogata and Araki, 1996). However, these studies mainly concerned changes of the surface mucous cells induced by *H. pylori* infection, but the relationship between *H. pylori* and the surface mucous gel layer (SMGL) has not been examined in detail.

Previous transmission electron microscopy (TEM) studies revealed the fibrillae-like filaments between the outer surface of *H. pylori* and the surface mucous cells (Caselli et al., 1989; Jones and Curry, 1992; Noach et al., 1994; Taniguchi et al., 1994). In this study, similar filament-like structures were also observed between the outer surface of *H. pylori* and the surrounding mucus.

Bacterial glycocalyx generally possesses the barrier and protective functions that enable then to survive and persist in natural environments (Costerton et al., 1981). Previous cytochemical studies of the glycocalyx on *H. pylori* were with the specimens stained with ruthenium red (Goodwin et al., 1989; Hessey et al., 1990; Thomsen, et al., 1990; Jones and Curry, 1992; Drouet et al., 1993). The specific sugar components of this glycocalyx cannot be determined with ruthenium red to demonstrate surface polysaccharides which stains all anionic polymers. On the other hand, different lectins possess specific affinity for individual hexoses or oligosaccharide sequences, so they can partially elucidate carbohydrate components. In this study, the specimens were stained with 8 different lectin-horseradish peroxidase (HRP) conjugates.

The fact that spiral forms of *H. pylori* convert to coccoid forms in prolonged culture has been observed (Jones and Curry, 1990). Recently, the existence of coccoid forms of *H. pylori* in the human stomach have been observed by electron microscopy (Chan et al., 1994; Janas et al., 1995). However, the extracellular coats of the coccoid forms have not been reported. In this study, coccoid forms were observed in 11 of 14 cases infected with *H. pylori*. This cytochemical study indicates that the glycocalyx and fibrillae-like filaments of coccoid and spiral forms are similar.

## Materials and methods

### Tissues

Specimens from 14 cases of human gastric cancers were obtained at surgery (9 males, 5 females; age range:

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23-75 years) (Table 2). The diagnosis of *H. pylori* infection was previously confirmed by the CLO test (Delta West Pty. Ltd., Australia) on endoscopic specimens. The resected stomach was opened and the mucosa of macroscopically normal portions about 20 mm in diameter from the middle corpus of the greater curvature were dissected out. The specimens were pinned on a supporting board with the mucosal surface facing upwards without prior rinsing and immersed in half-strength Karnovsky's fixative containing 2% paraformaldehyde-2.5% glutaraldehyde in 0.1M cacodylate buffer containing calcium chloride, pH 7.4, and then irradiated in a domestic microwave oven (500 W, 2450 MHz) for 5 seconds for four times with intervals of several seconds (Mizuhira et al., 1990). After 15 min, the specimens were cut into small pieces of tissue (4x3 mm) under a dissection microscope, followed by 45-min postfixation in the same fixatives at 4 °C. The fixed tissues were washed in three changes of 0.1M phosphate buffer (PB) for 30 min and then in 3 changes of 0.01M phosphate-buffered saline (PBS) for 30 min at 4 °C. Subsequently, they were infiltrated in PBS containing 10%, 15% and 20% sucrose respectively for 4 hr, 12 hr and 24 hr at 4 °C. They were immersed in PBS containing both 20% sucrose and 5% glycerin for 2 hr at 4 °C. The specimens were embedded in O.C.T. compound (Miles Inc., USA), rapidly frozen in isopentane cooled with liquid nitrogen, and stored in liquid nitrogen until sectioning. Frozen sections, about 10 $\mu$m thick, were cut with a cryostat (Sakura Co., Japan) at -25 to -35 °C and mounted on albumin-coated glass slides. The slides were air dried for 1 hr and thawed in two changes of PBS containing 10% sucrose for 10 min at room temperature. The sections were incubated in 1% bovine serum albumin (BSA, Nacalai Tesque Inc., Kyoto, Japan)-PBS containing 10% sucrose in a moist chamber for 20 min at room temperature. And then they were washed with PBS containing 10% sucrose at 4 °C prior to lectin staining.

### *Lectin staining*

We used the following eight lectins, and their binding specificities to the sugar residues and hapten sugars are shown in Table 1. HRP conjugates of *Phaseolus vulgaris* (PHA $E_4$), *Ulex europaeus* agglutinin I (UEA I), *Canavalia ensiformis* (Con A), *Arachis hypogaea* (PNA), *Ricinus communis* agglutinin I (RCA 120) and *Dolichos biflorus* agglutinin (DBA) were purchased from Honen corporation (Tokyo, Japan). *Limax flavus* agglutinin (LFA) and *Sambucus nigra* agglutinin (SNA) conjugated to HRP were obtained from E-Y laboratories (San Mateo, CA). The respective inhibitors, i.e. N-acetyl-D-glucosamine, L-fucose, α-metyl-D-mannoside, D-galactose, Lactose, N-acetyl-D-galactosamine and N-acetylneuraminic acid were purchased from E-Y laboratories (San Mateo, CA).

**Table 1.** Lectin characteristics.

| LECTIN | MAIN CARBOHYDRATE BINDING AFFINITY | HAPTEN SUGAR |
|---|---|---|
| PHA $E_4$ | Bisecting GlcNAc | D-GlcNAc |
| UEA I | α-L-Fuc | L-Fuc |
| LFA | NANA | NANA |
| SNA | NANA-α(2-6)-GalNAc | Lactose |
| Con A | α-D-Man> α-D-Glc | α-MM |
| PNA | Gal-β (1-3)-GalNAc | D-Gal |
| RCA 120 | Gal-β (1-4)-GlcNAc | D-Gal, Lactose |
| DBA | α-D-GalNAc | D-GalNAc |

GlcNAc: N-acetylglucosamine; Fuc: fucose; NANA: N-acetylneuraminic acid; GalNAc: N-acetylgalactosamine; Man: mannose; Gal: galactose; Glc: glucose; α-MM: α-methyl-D-mannoside.

The lectin staining was performed with the direct peroxidase method. The cryostat sections were incubated with each of the above lectin-HRP conjugates (50-100 $\mu$g/ml) in PBS containing 10% sucrose and 0.1% BSA for 24-72 hr at 4 °C and then washed in six changes of PBS containing 10% sucrose for a total of 60 min at 4 °C. The sections were then fixed with 2% glutaraldehyde in PB for 20 min and washed in 3 changes of PB for 30 min at room temperature. To visualize the peroxidase activity, the specimens were treated with 3,3'-diaminobenzidine 4HCl (DAB; Wako Pure Chemicals, Tokyo, Japan) solution containing 0.2 mg/ml DAB and 10 mM sodium azide in 50 mM Tris-HCl buffer (pH 7.6) for 30 min. Subsequently they were reacted with a DAB solution containing 0.005% $H_2O_2$ for 5-10 min at room temperature (Graham and Karnovsky, 1966). In these steps, endogenous peroxidase activity was completely blocked by the addition of 10 mM sodium azide to the DAB solutions. The DAB reaction was terminated by washing with distilled water. The sections were post-fixed with 1% osmium tetroxide in PB for 20 min and washed in 2 changes of distilled water for 20 min. They were dehydrated in a graded ethanol series, and embedded in Epon 812. Ultrathin sections were cut, stained lightly with uranyl acetate and lead citrate, and examined with TEM, a JEM-100S (JEOL, Japan). The reactivity of each lectin-HRP conjugate with glycocalyx of *H. pylori* was classified into 4 levels of relative intensity, from 0 to +3 (Table 2).

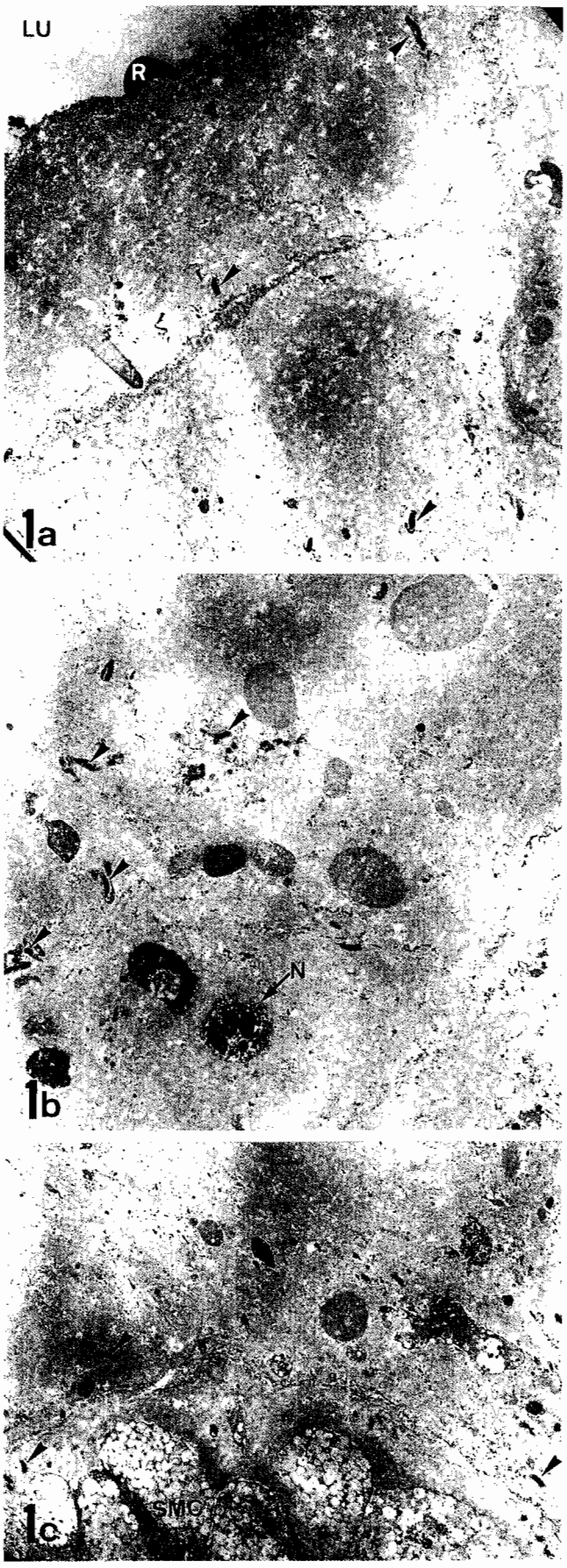
### *Controls*

To confirm the specificity of lectin binding, the frozen sections were preincubated with appropriate hapten sugars at a concentration of 0.1M for 30 min and then incubated with lectin-HRP conjugates in the presence of 0.1M hapten sugars. Non-specific binding of HRP was checked by incubation with HRP (E-Y laboratories, San Mateo, CA) alone (Ito et al., 1985).

## Results

### *1) Spiral forms of H. pylori in SMGL*

The SMGL appeared as a continuous layer of




uninterrupted mucus. *H. pylori* were scattered throughout the entire thickness of the SMGL, namely, near the luminal surface (Fig. 1a), in the mid layer (Fig. 1b) and adjacent to the surface mucous cells (Fig. 1c). Some bacteria were in parallel array (Fig. 2) or formed a small cluster (Fig. 4). Occasionally, *H. pylori* colonized around fragments of cell debris (Fig. 5). Dividing *H. pylori* was relatively frequent in the SMGL (Fig. 6).

At higher magnification, gastric mucus appeared to be preserved as fine granular and fibrillar substances. In the SMGL colonized with *H. pylori*, there were vacuole-like clear areas near or around the bacteria (Figs. 2-4). The sizes of clear areas ranged from 1 $\mu$m (Figs. 2, 4) up to 10 $\mu$m (Fig. 3).

*2) Extracellular coats of spiral form*

2-A) Glycocalyx

The glycocalyces of *H. pylori* were stained with some or all of the 8 types of lectin-HRP conjugates (Table 1). The carbohydrate components specifically recognized by these lectins were N-acetylglucosamine, α-L-fucose, N-acetylneuraminic acid, N-acetylneuraminyl-α(2-6)-N-acetylgalactosamine, α-D-mannose, galactose-ß(1-3)-N-acetylgalactosamine, galactose-ß(1-4)-N-acetylglucosamine and α-D-N-acetylgalactosamine. In the specimens stained with the lectin-HRP conjugates, the glycocalyx appeared as a high density granular layer about 40 nm in thickness around the cell wall membrane (Figs. 7-12). The glycocalyx was also observed around the flagellar sheath (Fig. 9a,b). The staining pattern of the lectins on the glycocalyx of *H. pylori* was variable in different specimens and the results are summarized in Table 2. There was also a range of staining intensities (Fig. 8). The reason for variable or absence of the lectin staining reaction in some specimens is not clear.

2-B) Fibrillae-like filaments

Some *H. pylori* were in close apposition to the surrounding mucus in SMGL. But others were separated from the uniformly granular SMGL by a clear or less granular zone. In these areas, the bacteria appeared to be anchored to the surrounding mucus by the numerous fibrillae-like filaments of about 2 nm in diameter. These structures were not uniformly present around the whole microorganism (Fig. 10). These filaments were similar to those seen between the bacterial surface and the surface mucous cells (Figs. 11, 12). These fibrillae-like filaments were not stained with any lectin tested (Figs.

**Fig. 1.** Low magnification view of 3 different layers of the same area of the SMGL infected with *H. pylori*. **a.** Upper one third layer of the SMGL. Isolated *H. pylori* (arrowheads) are sporadically seen. LU: gastric lumen; R: red blood cell. **b.** Middle layer. *H. pylori* (arrowheads) have a scattered distribution. N: neutrophil. **c.** Lower one third layer. *H. pylori* (arrowheads) colonize in the SMGL adjacent to the surface mucous cells (SMC). Stained with LFA-HRP. x 1,600

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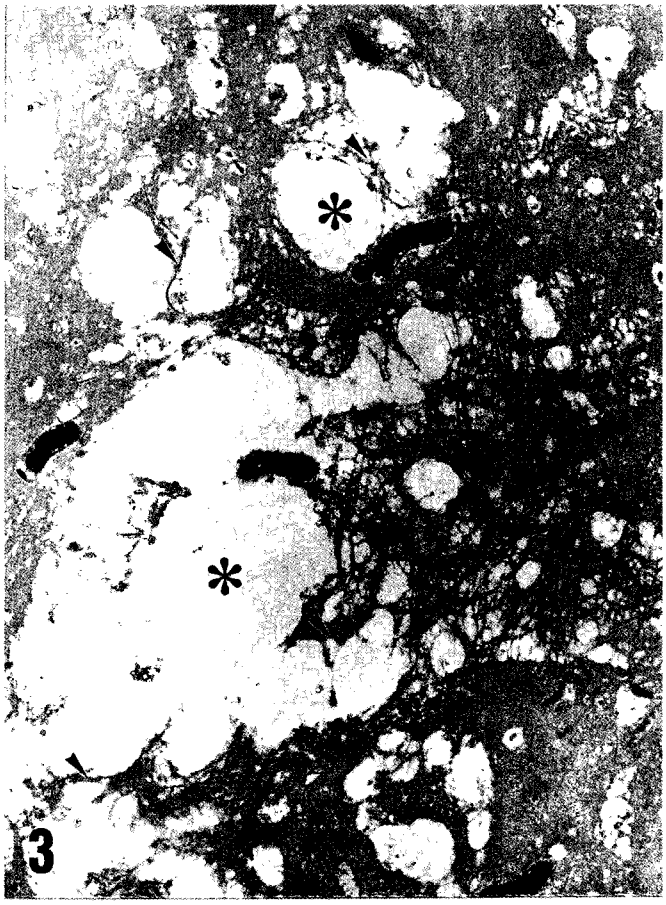

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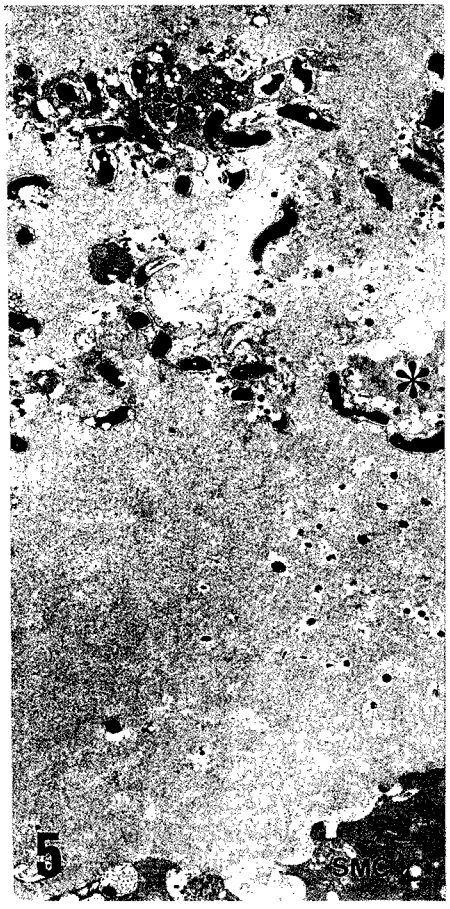


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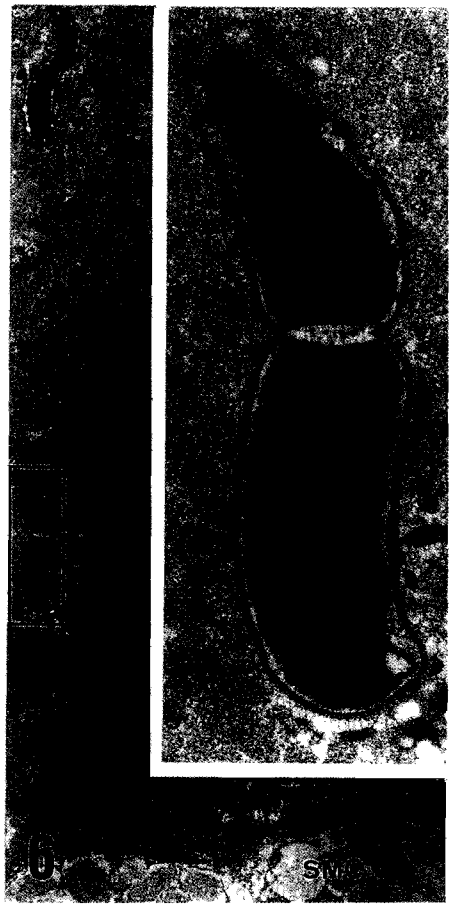


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**Fig. 2.** Spiral forms of *H. pylori* arrange in parallel to each other in the SMGL. Note the lack of mucus around *H. pylori* (arrows) and many clear areas (arrowheads) are seen in the SMGL. One coccoid form (C) is present. Stained with SNA-HRP. x 5,600

**Fig. 3.** Large vacuole-like clear areas (asterisks) are seen in the SMGL. Thin septa (arrowheads) between the clear areas suggest the apposed clear areas fuse to each other. Stained with SNA-HRP. x 5,400

**Fig. 4.** A small cluster of *H. pylori* seen in the middle layer of the SMGL. Small clear areas of the mucus (arrowheads) are seen around *H. pylori*. Stained with UEA I-HRP. x 4,100

**Fig. 5.** Numerous *H. pylori* adhere to the floating detached fragments of surface mucous cells (asterisks) in the SMGL. Stained with PHA $E_4$-HRP. SMC: surface mucous cells. x 4,600

**Fig. 6.** A specimen stained with SNA-HRP. The cell division of *H. pylori* is seen in the SMGL. SMC: surface mucous cells. x 6,000. Inset shows higher magnification of the area in the square shown in Fig. 6. The glycocalyx is not stained by the lectin. x 31,000

**Table 2.** Staining patterns of glycocalyx around *H. pylori* with lectins.

| CASES | AGE | SEX | PHA $E_4$ | UEA I | LFA | SNA | Con A | PNA | RCA 120 | DBA |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 46 | F | +3 | +2 | +1 | +1 | +3 | +2 | +1 | +1 |
| 2 | 39 | M | +3 | +2 | +1 | - | +2 | +2 | - | - |
| 3 | 65 | M | +3 | +3 | +1 | +2 | +1 | - | - | +1 |
| 4 | 23 | F | +2 | +2 | +2 | +2 | - | - | - | - |
| 5 | 61 | M | +2 | +1 | +2 | +2 | - | - | - | - |
| 6 | 64 | F | +2 | - | +1 | +1 | - | +2 | - | - |
| 7 | 64 | M | +2 | - | - | - | - | +1 | - | - |
| 8 | 70 | M | +1 | - | - | - | - | - | +2 | +1 |
| 9 | 51 | M | +1 | +2 | - | - | - | - | - | - |
| 10 | 58 | M | +1 | +2 | - | - | - | - | - | - |
| 11 | 72 | M | - | +2 | +1 | - | +2 | +2 | - | - |
| 12 | 58 | F | - | +2 | +1 | - | +2 | - | +1 | - |
| 13 | 55 | F | - | - | +1 | +2 | +3 | - | +2 | - |
| 14 | 75 | M | - | - | +1 | +2 | - | - | +3 | - |

+3:>90% cells; +2: 10-90% cells; +1:<10 cells; -: no cells

10-12).

### *3) Coccoid forms of H. pylori in SMGL*

In 11 (78.6%) of 14 cases, coccoid forms of *H. pylori* were recognized under TEM (Figs. 2, 13-21). In 5 cases (45.5%), coccoid forms were more numerous than spiral forms. They were seen in all layers of the SMGL, but they preferentially located in the middle and the luminal side of mucous layer (Figs. 13-21). Frequently, coccoid forms were seen in clusters (Fig. 13). Around these clusters, there were numerous clear areas in the SMGL (Fig. 13).

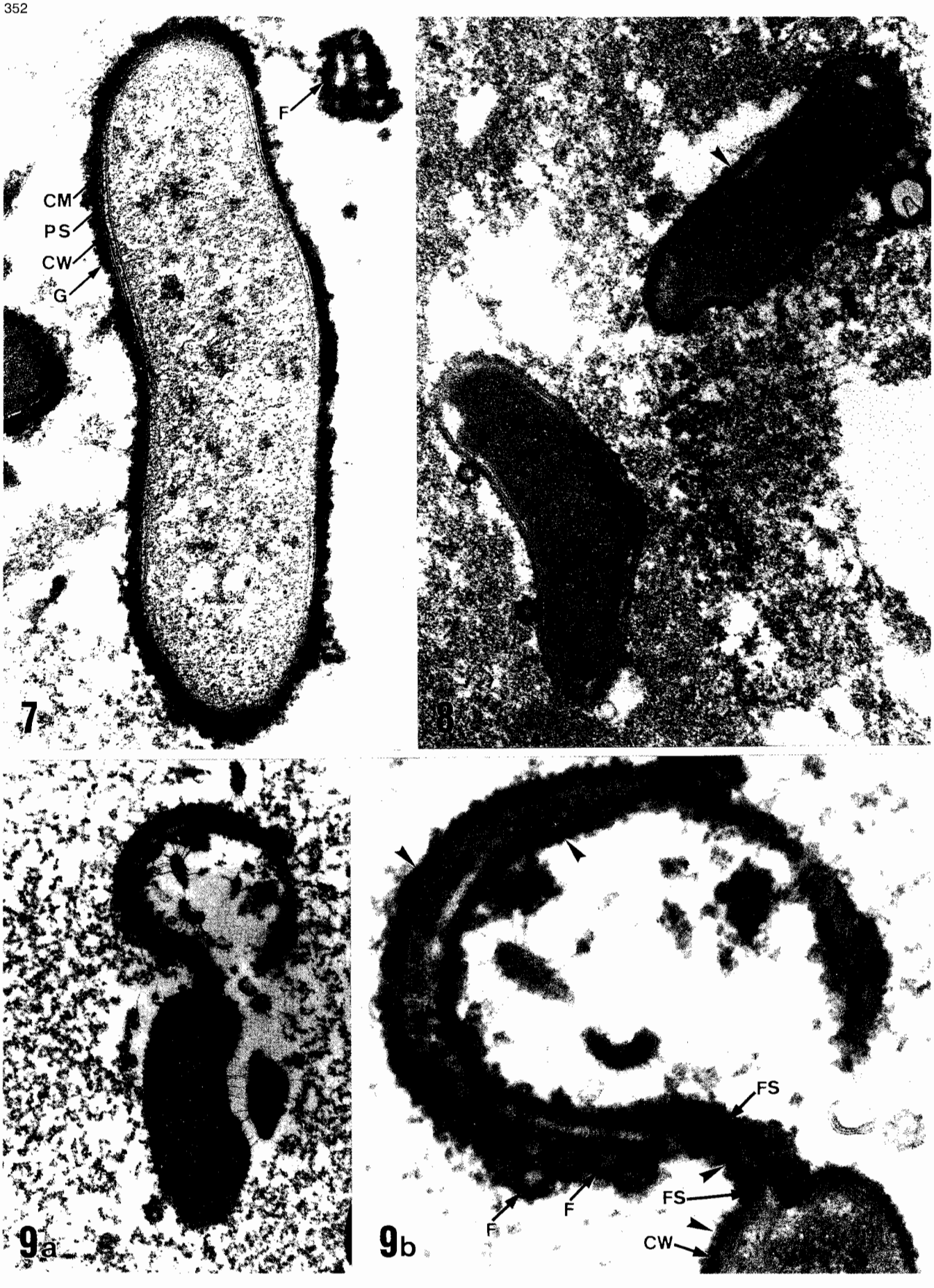
Full coccoid forms were spherical and enclosed with the characteristic cell wall and cytoplasmic membranes but lacked flagella (Figs. 14, 17, 19, 20). Their average diameter was about 1 $\mu$m greater than that of a spiral form (up to 0.7 $\mu$m). Some U-shaped forms, which may be a transitional form from a spiral form to a coccoid form were also observed (Figs. 13, 15, 16, 18, 21). The periplasmic space of these transitional forms was filled with granular dense materials. The diameter of U-shaped forms was about 1 $\mu$m.

The glycocalyces of the full coccoid, U-shaped and spiral forms were stained similarly with all examined lectins (Figs. 14-17, 21). Coccoid forms also appeared joined to the surrounding mucus by fibrillae-like filaments about 2 nm in diameter (Figs. 18, 19). Sometimes, coccoid forms were in close contact with the surface mucous cell (Fig. 21) and appeared to adhere by fibrillae-like filaments (Fig. 20) as in spiral forms (Fig.

**Fig. 7.** A spiral form of *H. pylori* stained with UEA I-HRP. The glycocalyx (G) of about 40 nm in thickness around the bacterial cell wall membrane (CW) is strongly stained with the lectin appearing as granular deposits. The glycocalyces around transected flagellar sheaths (F) are also stained. CM: cytoplasmic membrane; PS: periplasmic space. x 55,000

**Fig. 8.** The glycocalyx (arrowhead) around the upper right *H. pylori* is strongly stained with UEA I-HRP, but that of the lower left *H. pylori* is unstained. x 40,000

**Fig. 9.** A specimen stained with LFA- HRP. **a.** The glycocalyces around the bacterial body and flagella are strongly stained with the lectin. **b.** Higher magnification of a part of Fig. 9a. Note the glycocalyces (arrowheads) around the flagellar sheath (FS) are stained with the lectin and they are continued to the glycocalyx around the cell wall membrane. CW: cell wall membrane; F: transversely cut flagella. a, x 31,000; b x, 94,000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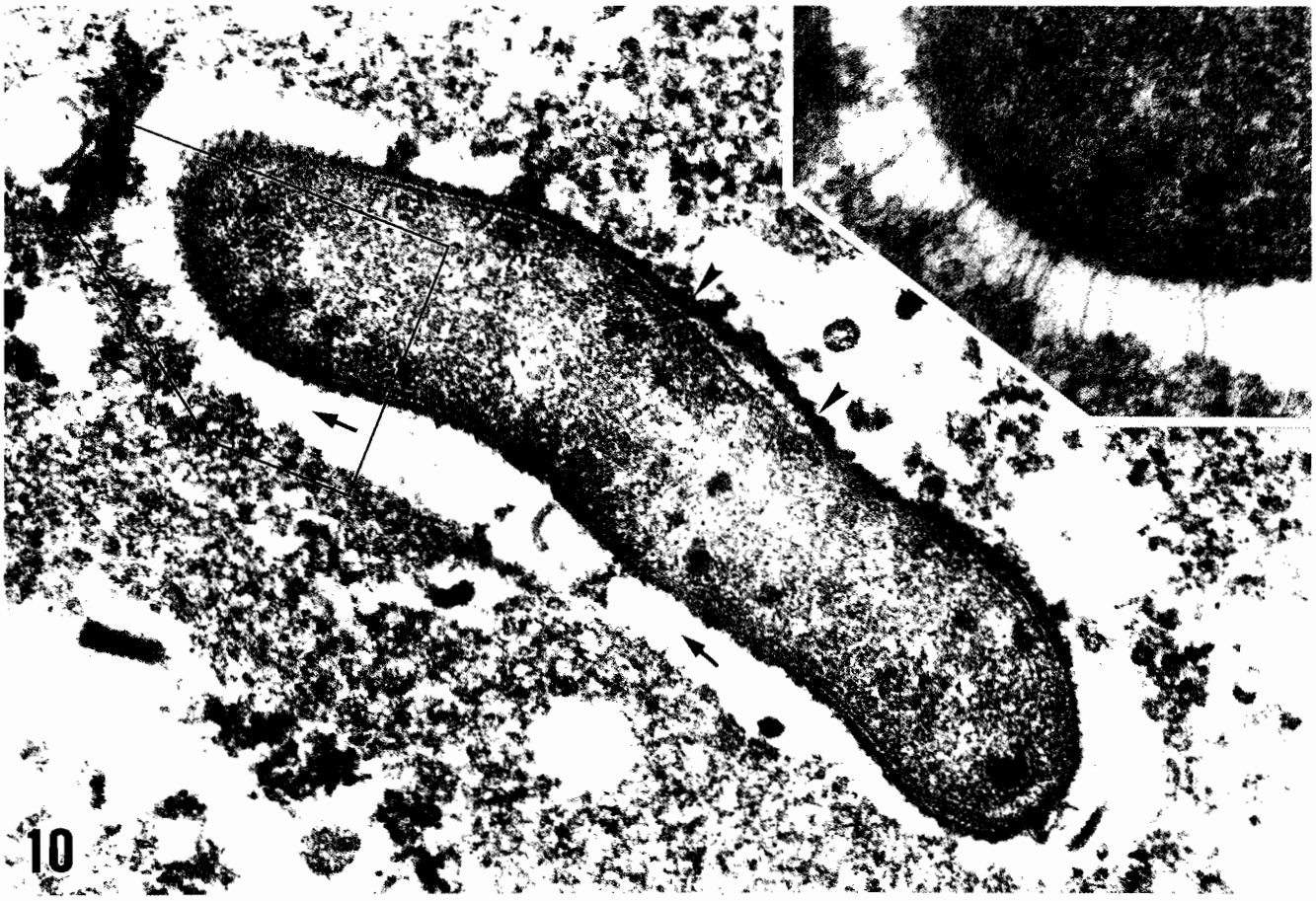

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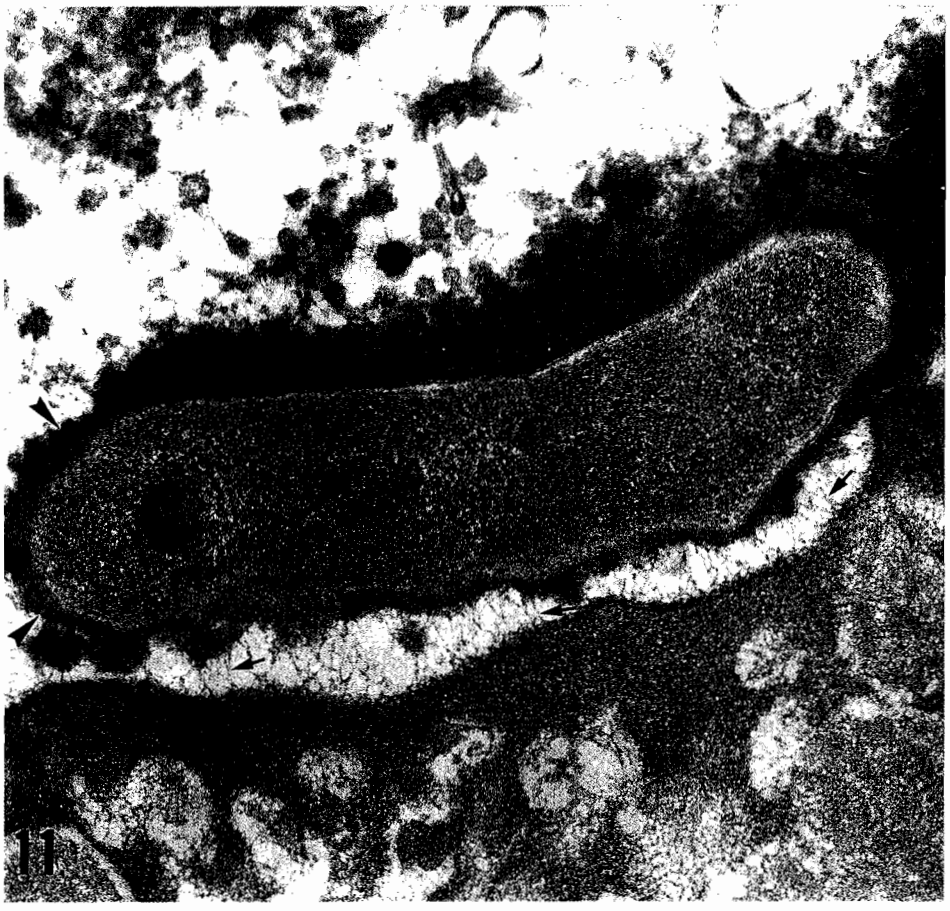

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**Fig. 10.** A spiral form of *H. pylori* stained with SNA-HRP. A less granular zone is seen between *H. pylori* and the surrounding mucus. Note that the fibrillae-like filaments (arrows) radiate from *H. pylori* anchoring to the adjacent mucus. The glycocalyx (arrowheads) is seen in the area without the filaments, while it is scarce or invisible in the region with the filaments. The surrounding mucus are also stained with SNA-HRP. x 48,000. Inset shows higher magnification of the area in the square shown in Fig. 10. The fibrillae-like filaments with ca. 2 nm in diameter are not stained with the lectin. x 78,000

**Fig. 11.** A specimen stained with SNA-HRP. *H. pylori* attaches to the surface mucous cell by numerous radiating fibrillae-like filaments. The fine filaments (arrows) radiate from the bacterial surface facing the surface mucous cell and connect to its apical membrane. These filaments are not stained with the lectin. The bacterial glycocalyx facing the lumen (arrowheads) is heavily stained with the lectin. x 58,000

**Fig. 12.** A specimen stained with Con A-HRP. *H. pylori* adheres to a detached fragment (asterisk) of the surface mucous cell by fibrillae-like filaments (arrows) in the SMGL. Its glycocalyx (arrowheads) is stained with the lectin, but the filaments are unstained. x 47,000

11). These fine filaments were not stained by the lectins (Figs. 18-20).

## Discussion

Using galactose oxidase-cold thionin Schiff-paradoxical Concanavalin A staining for gastric mucins and immunostaining for *H. pylori* in surgically removed stomachs, Akamatsu et al. (1995) reported that *H. pylori* were observed not only on the apical surface of the surface mucous cells but more abundantly in the SMGL. In addition, they described marked disintegration of


**Fig. 13.** A cluster composed of U-shaped forms (arrows) and full coccoid forms (arrowheads) is seen in the SMGL. Note many clear areas in SMGL. R: red blood cell. Stained with DBA-HRP. x 5,400

gastric mucous layer and large vacuolations of the SMGL infected with *H. pylori* by light microscopic observation. In this current study, spiral and coccoid forms of *H. pylori* were observed in whole layers of the SMGL. In addition, variously-sized vacuole-like clear areas were seen around and adjacent to *H. pylori*. These clear areas may be formed by the results of uptake of the mucus by *H. pylori* as their nutrients. Biochemically, the

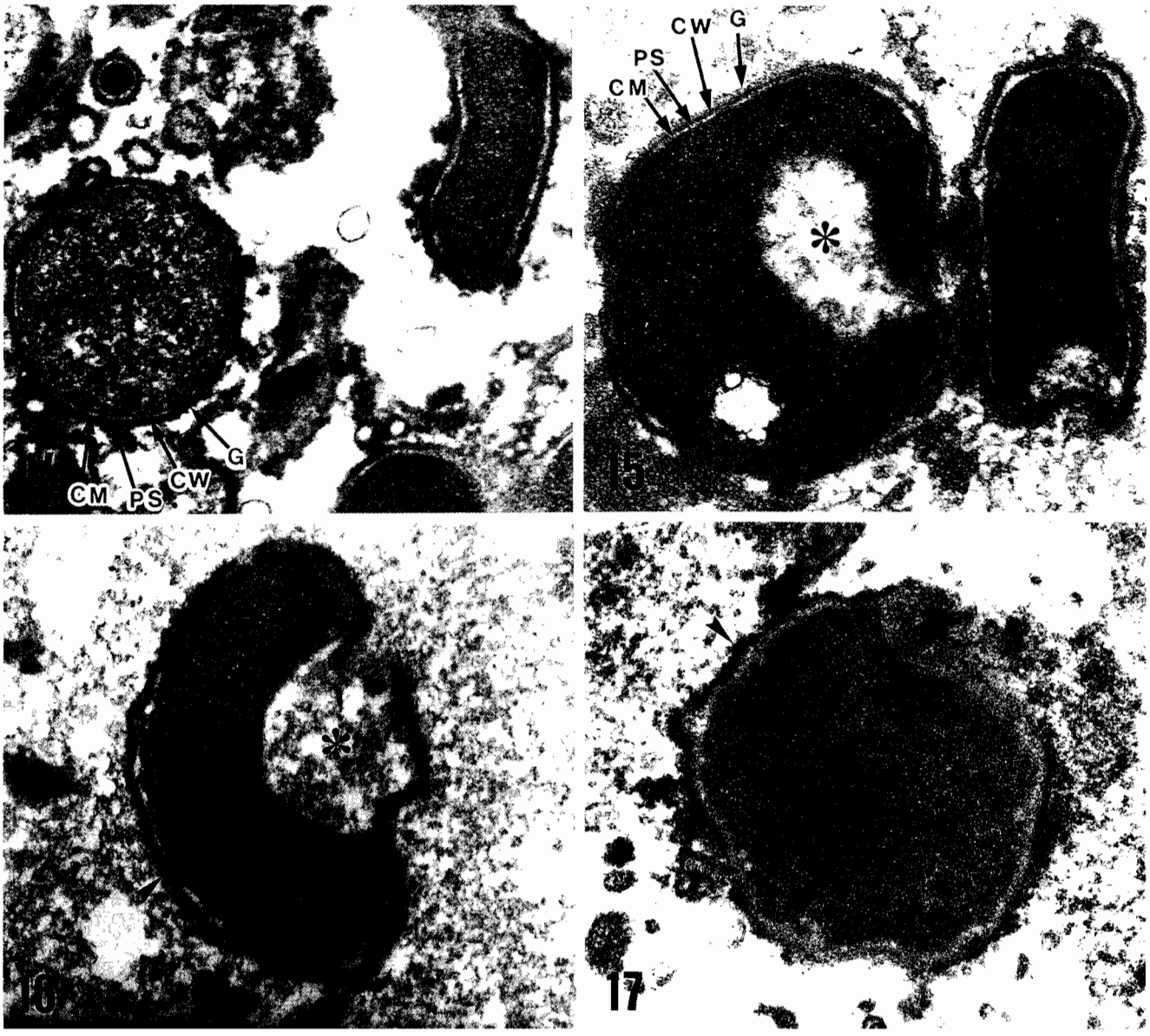


**Fig. 14.** A specimen stained with PHA $E_4$-HRP. A full coccoid form (left) and a spiral form (right) are seen in SMGL. A full coccoid form has cell wall membrane (CW), cytoplasmic membrane (CM), periplasmic space (PS) and glycocalyx (G). Note that the diameter of the full coccoid form is larger than that of the spiral form. The glycocalyces of both forms are similarly stained with the lectin. x 39,000

**Fig. 15.** A specimen stained with LFA-HRP. Both a U-shaped pre-coccoid form (left) and a spiral form (right) have the cell wall membrane (CW), cytoplasmic membrane (CM), periplasmic space (PS) and glycocalyx (G). The periplasmic space of the U-shaped form dilates (asterisk). The glycocalyces of both forms are stained with the lectin. x 45,000

**Fig. 16.** A specimen stained with Con A-HRP. A U-shaped form shows the bend of cytoplasm and the cell wall membrane stretched between the arms. Fine granular materials accumulate in the periplasmic space (asterisk). The bacterial glycocalyx (arrowhead) is stained with the lectin. x 44,000

**Fig. 17.** A specimen stained with SNA-HRP. The glycocalyx of a full coccoid form (arrowhead) is stained with the lectin. x 43,000

decrease of mucous layer thickness can be explained by protease and phospholipase A2 elaborated by *H. pylori* (Slomiany and Slomiany, 1992). Ogata and Araki (1996) observed detached fragments of apical cytoplasm of surface mucous cells in mucosae infected with *H. pylori*. The detached cell fragments are seen even in the middle layer of the SMGL heavily colonized with numerous *H. pylori*.

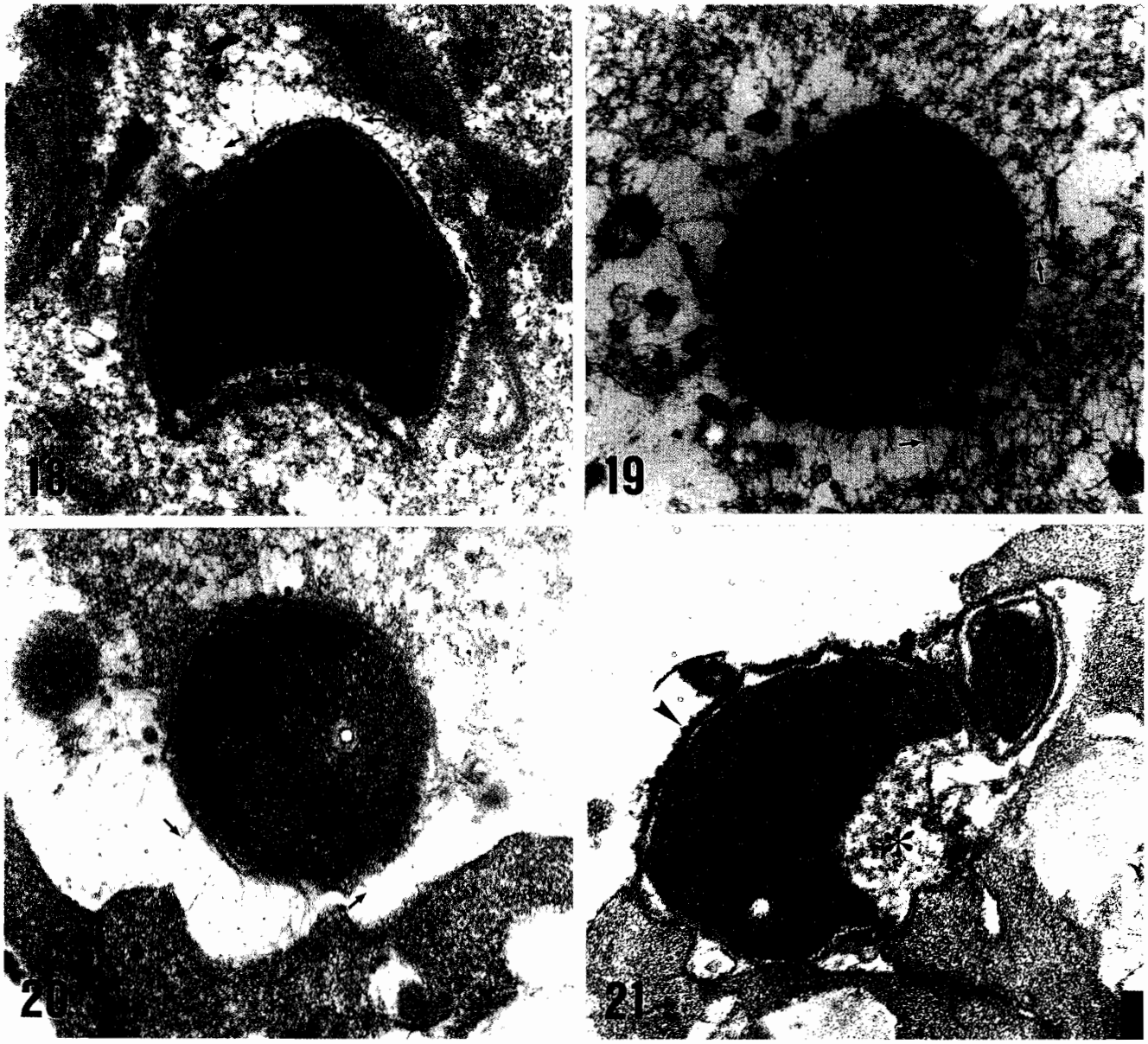

**Fig. 18.** A specimen stained with Con A-HRP. A pre-coccoid form adheres to the surrounding mucus by the radiated fibrillae-like filaments (arrows) unstained with the lectin. Its periplasmic space (asterisk) is filled with granular substances. x 42,000

**Fig. 19.** A specimen stained with Con A-HRP. The fibrillae-like filaments (arrows) radiate from the surface of a full coccoid form and connect to the surrounding mucus. Note these filaments are not stained by the lectin. x 48,000

**Fig. 20.** A specimen stained with RCA 120-HRP. A full coccoid form of *H. pylori* adheres to the surface mucous cell by the fibrillae-like filaments unstained with the lectin (arrows). x 40,000

**Fig. 21.** A specimen stained with DBA-HRP. An intermediate form of *H. pylori* between a spiral and a coccoid forms closely contacts with the apical membrane of a surface mucous cell. Fine granular materials accumulate in the periplasmic space (asterisk). The bacterial glycocalyx (arrowhead) facing the gastric lumen is stained with the lectin. x 40,000

Costerton et al. (1981) defined the glycocalyx of gram-negative bacteria as any polysaccharide, containing bacterial surface structure that is distal to the surface of the outer membrane or the cell wall. It is now believed that the bacterial glycocalyx provide a barrier and protection from the environment that contains bacteriophage, surfactants, antibodies, phagocytic cells and clearance mechanism (Costerton et al., 1981). The glycocalyx of *H. pylori* has been studied in specimens stained with ruthenium red (Goodwin et al., 1989; Hessey et al., 1990; Thomsen, et al., 1990; Jones and Curry, 1992; Drouet et al., 1993). The glycocalyx of *H. pylori* visualized by ruthenium red was about 40 nm thick (Goodwin et al., 1989). However, each carbohydrate component is not distinguished with ruthenium red.

In this study, 8 lectins were used to determine the carbohydrate components of the glycocalyx of *H. pylori*. There were vast variations in staining pattern of glycocalyx of *H. pylori* with each lectin (Table 2). But PHA $E_4$, UEA I, LFA and SNA reactions were positive in more than half the cases, while the DBA reaction was negative in most cases. These results suggest that the major components of the glycocalyx were α-L-fucose, bisecting N-acetylglucosamine and N-acetylneuraminic acid. The variations of the staining pattern of the bacterial glycocalyx with each lectin may be due to the interstrain variation, the glycocalyx-minus mutant, various phases of the bacterial cell cycle and/or possibly reflected pathogenic differences between *H. pylori* strains containing the different genes (Atherton et al., 1997). The glycocalyx of the flagella of *H. pylori* has not been previously reported. In the present study, it was shown that the flagella also had the glycocalyx and stained similarly to the glycocalyx of the cell body of *H. pylori*.

Electron microscopic studies have already described that there are two different modes of the attachment of *H. pylori* to surface mucous cells (Caselli et al., 1989; Jones and Curry, 1992; Noach et al., 1994; Taniguchi et al., 1994). In one mode, *H. pylori* is in close contact with the plasma membranes of the surface mucous cells, where the membranes form plateau-like extrusion-adhesion pedestals. In the other mode, *H. pylori* appears attached to the luminal surface of the surface mucous cells by fibrillae-like filaments of 2nm in diameter. Evans et al. (1988) reported that *H. pylori* possesses a surface-associated fibrillar hemagglutinin as a putative colonization factor antigen, which has an affinity for N-acetylneuraminyllactose. The fibrillar hemagglutinin had a closely packed, parallel, flexible, 2 nm-diameter structure resembling fibrillae by TEM observation. The fibrillae-like filaments observed between *H. pylori* and the surface mucous cell by Caselli et al. (1989), Jones and Curry (1992), Noach et al. (1994) and Taniguchi et al. (1994) may correspond to the fibrillar hemagglutinin reported by Evans et al. (1988), but further studies are needed to confirm this assumption. The fine filaments between the bacteria and the surface mucous cells observed in this study were not lectin stained. This result suggests that the fibrillae-like filaments are a kind of protein unconjugated with sugar molecules.

In this study, the adhesion of *H. pylori* to the surrounding mucus by fibrillae-like filaments was frequently observed and this finding has never been reported in the literature. Tzouvelekis et al. (1991) reported that some *H. pylori* strains bind to human gastric purified mucin in vitro. Patterson et al. (1975) showed that *Ruminococcus albus* attach to the nutritive substances by fibrillar extracellular coat material. Similarly, *H. pylori* adhere to the surrounding mucus obtaining their nutritive substances, i.e., the gastric mucus in a stable state.

Coccoid forms of *H. pylori* appeared in older bacteriological cultures and have been considered to be degenerative forms. However, this concept may have to be reconsidered in the light of recent evidence that the coccoid form is capable of regrowth (Steer, 1992). Jones and Curry (1992) suggest that the coccoid structure favors the survival of the organism by minimizing the bacterial surface during unfavourable external conditions.

In the human stomach, coccoid forms of *H. pylori* were also reported (Chan et al., 1994; Janas et al., 1995). Janas et al. (1995) described that coccoid forms of *H. pylori* were closely associated with damaged surface mucous cells of human stomach, while the spiral forms were localized in the proximity of unaffected surface mucous cells or surface mucous cells damaged in varying degrees. Chan et al. (1994) reported that the coccoid forms often adhered to the gastric epithelium, but they could also be found free in the mucus, usually occurring in clusters. In the present study, the full coccoid and transitional U-shaped forms were seen throughout the SMGL, frequently forming clusters. They were more often observed in the middle and upper layer of the SMGL than in the lower layer near the surface mucous cells.

Although several electron microscopic studies on coccoid forms of *H. pylori* have been published (Bode et al., 1988; Jones and Curry, 1992; Steer, 1992; Chan et al., 1994; Noach et al., 1994; Janas et al., 1995), there is no description of their extracellular glycocalyx and fibrillae-like filaments. This study revealed that both full coccoid and U-shaped forms having the glycocalyx around the cell wall were stained with lectins similarly to those of the spiral forms (Table 2). In addition, the fibrillae-like filaments radiated from the surface of coccoid forms to the surrounding mucus. These fibrillae-like filaments were not lectin stained either. These results suggest that the coccoid forms of *H. pylori* have the same glycocalyx as those of the spiral forms.

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