Ultrastructural Organization of *Helicobacter pylori* under Natural Conditions and during *Ex Vivo* Culturing

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Ultrastructure of *Helicobacter pylori* were studied by transmission electron microscopy in freshly isolated and *ex vivo* cultured biopsy specimens of gastric antral mucosa from patients with duodenal ulcer.

Key Words: *Helicobacter pylori; ultrastructure*

Helicobacter pylori (HP) described in 1983 [6,14] plays an important role in the pathogenesis of chronic gastritis [7] and peptic ulcer [9] and is associated with some forms of stomach cancer [3].

Approaches to improving the reliability of negative results of bacteriological diagnosis of HP infection in patients with peptic ulcer are now discussed. Uncommon approaches to the search for criteria of reliability of these results seem to be promising, one of such approaches being ultrastructural analysis of biopsy specimens of the gastric mucosa, both freshly isolated and cultured as for isolation of HP (*ex vivo*).

We studied the ultrastructure of HP under natural conditions and after *ex vivo* culturing.

MATERIALS AND METHODS

Biopsy specimens of gastric antral mucosa from patients with duodenal ulcer collected during esophagogastroscopy [13] prior to anti-Helicobacter therapy and specimens from the same patients cultured *ex vivo* on solid nutrient medium as for routine isolation of HP culture [2] were examined under transmission electron microscope.

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The preparations were fixed [4], dehydrated in ascending alcohols, and embedded in LR White [8].

Ultrathin (15 nm) sections were made on an LKB-3 ultramicrotome, placed onto formvar-film covered grids, stained [10], and analyzed under a GEM-100B microscope.

RESULTS

Electron microscopy of biopsy specimens collected before anti-Helicobacter therapy showed pronounced colonization of the gastric mucosa with HP, the majority of bacterial cells were bud-shaped and only some bacteria looked like twisted rods. The bacteria were located in the mucus covering the mucosa and on the epitheliocyte surface (Fig. 1, *a*).

Cell wall had a well-developed inner layer without structural defects, outer membrane of the cell wall was somewhat thickened, and a microcapsule was clearly seen on its surface. Small vesicles abundantly pinching from the cell wall and closely contacting with epitheliocytes were also coated with a microcapsule. Electron transparent layer formed in the immediate vicinity of bacterial cells in surrounding mucus, the thickness of this layer was directly proportional to the number of small vesicles covering the bacterial cells. Bacterial cells divided by forming constrictions (Fig. 1, *b*).

Ultrastructure of cultured mucosa specimens from patients with duodenal ulcer was studied for the first

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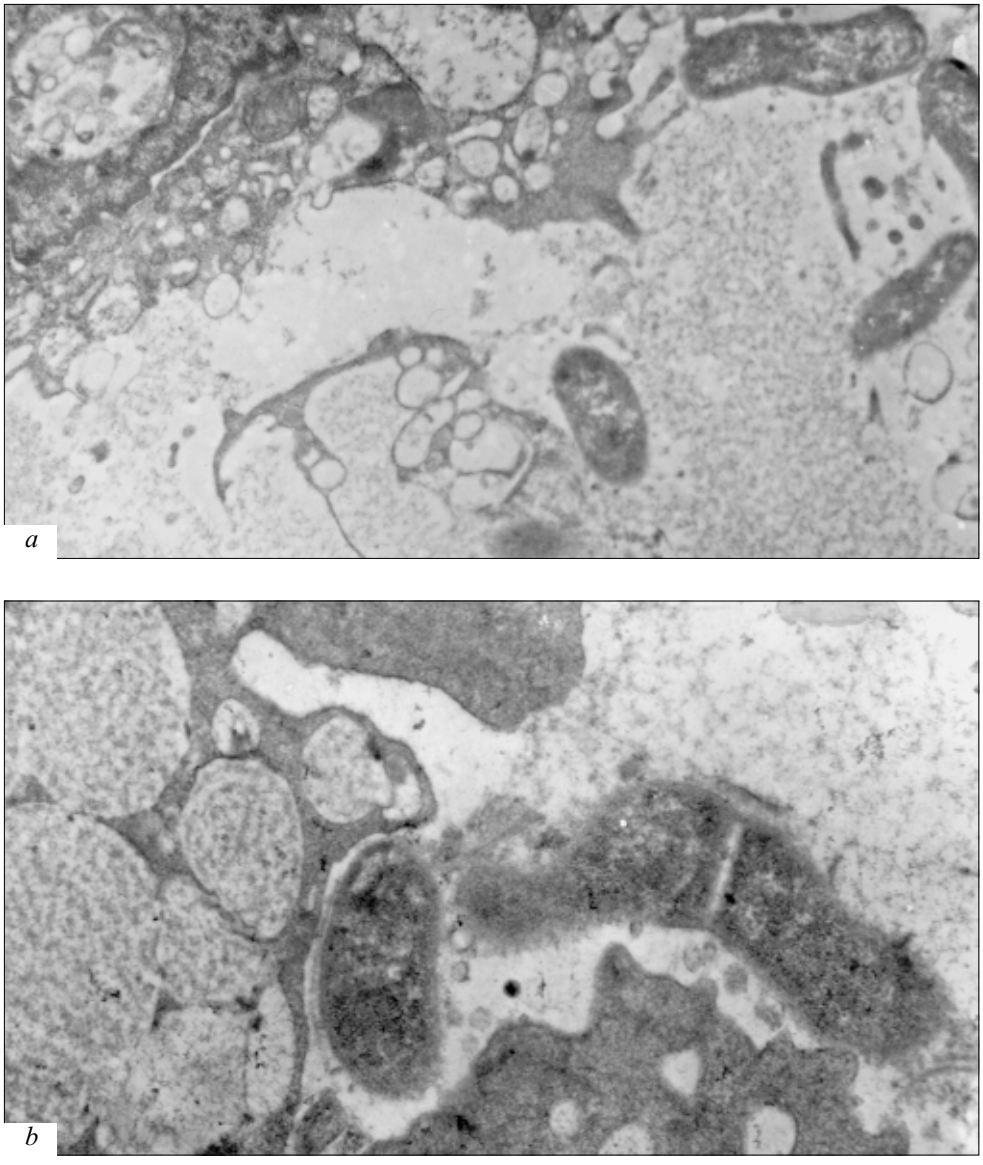

Fig. 1. Ultrastructure of *Helicobacter pylori* under natural conditions. *a*) typical bud-shaped and twisted rod-shaped cells, ×20,000; *b*) division of a rod-shaped cell, ×30,000.

time. It was demonstrated that HP grew in culture despite the fact that mucosa elements surrounding the microorganism actually represented cell detritus.

The presence of dividing bacterial cells characterized by pronounced polymorphism in the preparations confirmed viability of bacteria under these conditions. Polymorphism of HP cultures was directly associated with impaired structure of the rigid layer of the cell wall. This resulted in the formation of oval, pear-shaped, and irregularly shaped cells. Typical bud-shaped and twisted rod-shaped cells [10] were only occasionally seen. Numerous small vesicles separated from the cell wall (Fig. 2, *a*) and spheroplasts were seen in the immediate vicinity of bacterial cells (Fig. 2, *b*).

In morphological studies, special attention is usually paid to changes of HP shape [1]. It is generally accepted that coccoid forms of HP appear as a result of therapy and attest to the loss of some bacterial functions [5,11]. Therefore, we also paid special attention to the presence of coccoid HP cells. Our results indicate that round cell can represent a cross-section of bud-like or twisted rod-like bacteria. The true shape of HP can be evaluated by a complex analysis ultrathin sections and data of scanning electron microscopy.

Ultrastructure of HP in the gastric mucosa of untreated patients is similar to the structure of HP in *ex vivo* cultured biopsy specimens.

Morphological analysis of mucosa specimens cultured *ex vivo* on solid nutrient medium as for isolation

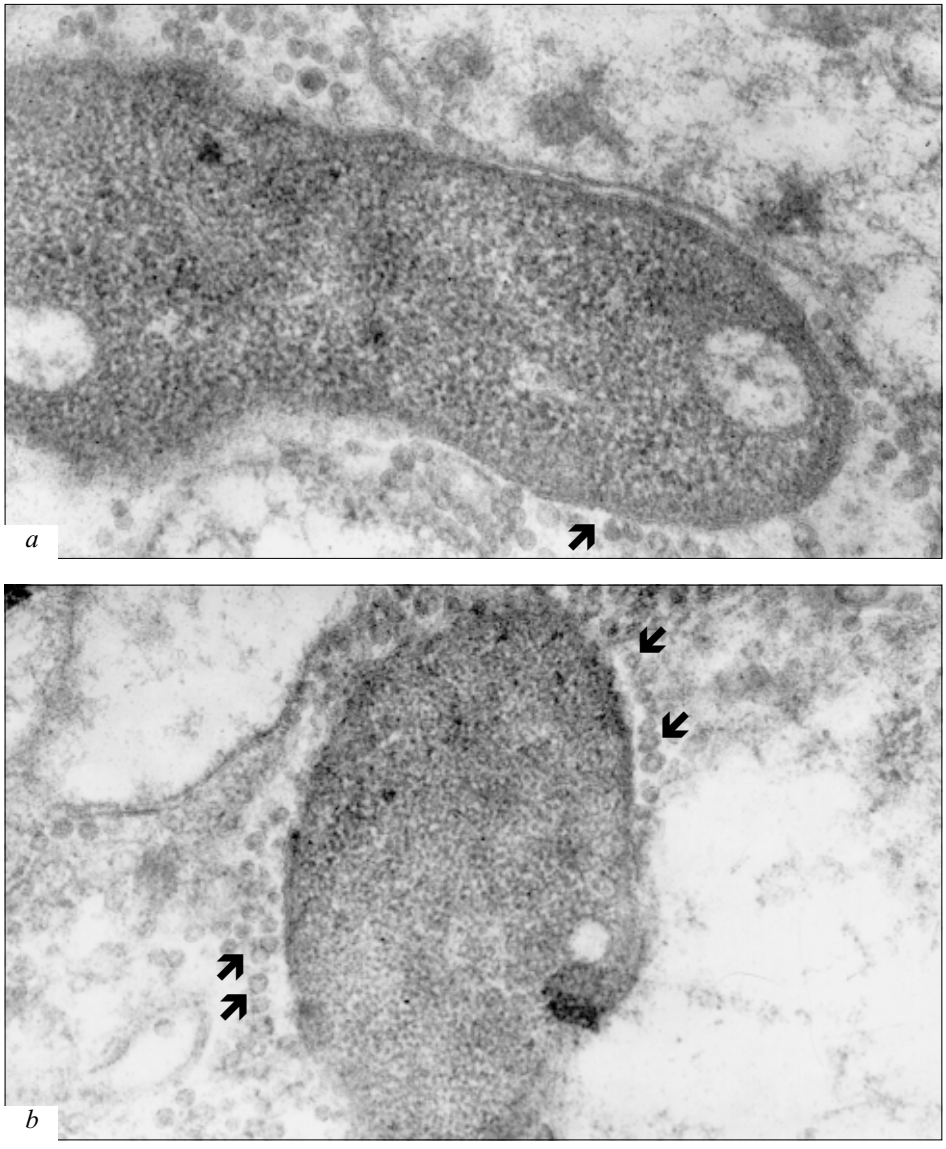

Fig. 2. Ultrastructure of *Helicobacter pylori ex vivo*. *a*) typical *H. pylori*, ×75,000; *b*) cell with defective cell wall, ×75,000. Arrows show vesicles.

of HP culture from clinical material within the framework of a diagnostic study will be very useful primarily in the analysis of negative results of routine bacteriological assay.

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