● THE ATTACHMENT OF HELICOBACTER PYLORI TO HUMAN GASTRIC EPITHELIUM IN VITRO: A MODEL FOR THE STUDY OF THE PATHOMECHANISM OF COLONIZATION. J. Sarosiek, B.J. Marshall, S. Hoffman, L. Barrett, R. Guerrant, H. Anderson, J. Hamlin, R.W. McCallum. University of Virginia Health Sciences Center, Charlottesville, Virginia.

Adherence of Helicobacter pylori (HP) to an epithelial cell receptor seems to be a prerequisite of colonization. Herein, we describe a long-term culture of the human gastric epithelium as a model for the study of an intimate contact between HP and the epithelial cells in vitro, which may mimic an in vivo scenario.

Gastric mucosal cells were isolated from biopsy specimens obtained during a diagnostic endoscopy of 5 patients with HP-related gastroduodenal pathology. Epithelial cells were isolated in a calcium-free medium by mechanical dispersion or with collagenase. Gastric epithelial cells, placed in LabTech chambers (Nunc Inc.) coated with collagen or laminin, were cultured 2-6 weeks in F-12 Nutrient Mixture (Ham) medium supplemented with 10% FBS, EGF (2ng/ml), and 100μg/ml gentamicin. HP, isolated from patients with gastric pathology and cultured in Brucella broth were inoculated and incubated with gastric epithelial cells for 3h, washed and stained by Giemsa.

HP exhibited a similar attachment to the surface of mucosal cells isolated both by the collagenase-dependent and collagenase-free procedure. Three patterns of adherence of HP to the gastric epithelium were identified: predominantly diffuse type (40%), predominantly localized (40%) and mixed (20%).

Long-term human gastric epithelial cell culture appears to be a useful model for the study of adherence-related cell pathology. The pattern of adhesion (diffuse vs localized) may provide important clues regarding the pathogenesis of HP infection.

● ESOPHAGEAL MUCOSAL BARRIER: A NEW METHOD FOR THE STUDY OF THE DIFFUSION OF HYDROGEN ION WITH TRITIATED [^{3}H]HCL. J. Sarosiek, R.W. McCallum, T. Feng. University of Virginia Health Sciences Center, Charlottesville, Virginia.

Studies of the rate of diffusion of hydrogen ion into the esophageal mucosa were hampered by the lack of appropriate methodology to differente between losses of hydrogen ion from the acidic perfusate due to a real diffusion into the mucosa form the loss due to neutralization by simultaneously stimulated bicarbonate secretion. We present a new method of the measurement of diffusion of hydrogen ion into the mucosa based on the use of hydrochloric acid equilibrated with tritiated [^{3}H]H_2O.

Esophageal perfusion was conducted in 5 cats with a specially designed two-balloons tubing sealing entire esophagus between the upper and lower esophageal sphincters. Radioactivity of tritium was monitored in scintillation counter.

Cat esophageal mucosa has an ability to withstand two 10-min periods of perfusion with 100mEq/l HCL with only 2-5% losses of radioactivity from a perfusate. Within the next two 10-min of continuous perfusion periods a profound diffusion of hydrogen ion occurs leading to the maximal loss of hydrogen ion 8.4mEq/10min/cm^2. Both pepsin and bile acids introduced into the HCL solution facilitated the changes evoked by hydrochloric acid alone. Indomethacin (25mg/kg i.v.) showed a biphasic effect on the rate of diffusion of hydrogen ion. Within the first 20 minutes it inhibited, but during the next three 10min periods profoundly potentiated the rate of hydrogen ion diffusion into the esophageal mucosa.

The study demonstrates a new and very reliable method for the measurement of the rate of diffusion of hydrogen ion into the esophageal mucosa not hampered by simultaneous secretion of bicarbonate by challenged mucosa.

● THE IMPACT OF HELICOBACTER PYLORI COLONIZATION ON THE THE THICKNESS OF THE GASTRODUODENAL MUCUS LAYER. J. Sarosiek, B.J. Marshall, D.A. Peura, S. Hoffman, T. Feng, R.W. McCallum. University of Virginia Health Sciences Center, Charlottesville, VA 22908.

The gastroduodenal mucus layer is considered as a primary mucosal protective barrier, especially important in the maintenance of a pH gradient despite the highly acidic gastric milieu. Thus the measurement of the mucus layer thickness in various diseases could advance our understanding of gastroduodenal pathophysiology. We present a novel method for measuring the mucus layer and compare its thickness in endoscopic biopsy material from Helicobacter pylori (HP) positive and negative patients.

Endoscopic biopsies were obtained from 17 patients with gastroduodenal mucosa harboring HP and from 15 patients without current HP colonization. The thickness of the mucus layer was measured in fresh specimens by phase-contrast dark-field microscopy technique (Carl Zeiss, Germany).

	Mucus thickness (μm) (mean ±SD)		
	Corpus	Antrum	Duodenum
HP +	0.105 ±0.032	0.085 ±0.027	0.093 ±0.033
HP -	0.161 ±0.064	0.175 ±0.067	0.162 ±0.045
p<	0.01	0.001	0.001

This study suggets that chronic colonization of the gastroduodenal mucosa by HP impairs the mucus layer covering the surface epithelium. This compromised mucus layer in HP patients may increase the susceptibility of the mucosal epithelium to hydrogen ion damage with subsequent development of inflammation and/or peptic ulcer.

● EFFECTS OF rhbFGF MUTEIN CS23 (TGP-580) ON THE HEALING OF GASTRIC ULCERS INDUCED BY ACETIC ACID IN RATS. H. Satoh, A. Shino, N. Inatomi, H. Nagaya, F. Sato, S. Szabo, J. Folkman, Research and Development Division, Takeda Chemical Ind., Osaka, Japan, Brigham & Women's Hosp., Children's Hosp., Harvard Med. Sch., Boston, MA.

We found that recombinant human basic fibroblast growth factor and its mutein CS23 (TGP-580) accelerated the healing of chronic duodenal and colonic ulcers in rats (Dig. Dis. Sci., 34, 1323, 1989; Gastroenterology, 98, A203, 1990). In the present study, we examined the effect of TGP-580 on the healing of chronic gastric ulcers in rats.

Male SD rats weighing ~220g were used. Gastric ulcers were induced by injection of 20 μl of 20% acetic acid into the subserosal layer at the junction of the corpus and antrum. A drug or the vehicle was given orally twice daily for 14 days starting 2 days after the operation. The ulcerated area (mm^2) and severity of the ulcer (0: completely healed, 1: scar or almost completely healed, 2: moderate ulcer, 3: deep ulcer or perforation) were measured under a dissecting microscope. An ulcer index was obtained from the product of the area and the severity. In the other experiments, effects on gastric acid secretion and gastric lesion formation were studied in rats.

1. Effect on ulcer healing

TGP-580 (1, 10 and 100 μg/kg) accelerated the healing of ulcers dose-dependently; % improvement was 38, 55 and 75 ($p<0.01$)%, respectively (n=19~25). In the histological examination, maximal diameter of the ulcer crater was significantly decreased, and regeneration of the mucosa was enhanced by TGP-580. Cimetidine (100 mg/kg) moderately (43%) and EGF (1~100 μg/kg) mildly (15~27%) accelerated the healing. Co-administration of TGP-580 (10 μg/kg) and EGF (10 μg/kg) promoted the ulcer healing (64%).

2. Effect on acid secretion in the 3-hr-pylorus-ligated rat

TGP-580 given orally 1 hr before pylorus ligation did not affect the acid secretion, whereas cimetidine (100 mg/kg) inhibited the secretion by 86% ($p<0.01$).

3. Effect on ethanol-induced gastric lesions in rats

TGP-580 given orally 30 min before the administration of 1 ml of absolute ethanol did not inhibit the formation of gastric lesions. PGE_2 (100 μg/kg) decreased the lesions by 76% ($p<0.05$).

Conclusions: bFGF mutein TGP-580 accelerated the healing of gastric ulcers. The potency seems to be superior to that of cimetidine or EGF. The additive effect of TGP-580 and EGF suggests important roles for both growth factors (bFGF and EGF) in the healing of gastric ulcers. Antisecretory and mucosal protective action may not be involved in the effect of TGP-580.