Mucosal expression and luminal release of epidermal and transforming growth factors in patients with duodenal ulcer before and after eradication of *Helicobacter pylori*

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Abstract

Background—Epidermal growth factor (EGF) and transforming growth factor- α (TGF α) are potent gastric acid inhibitors and stimuli of mucosal growth and protection but their involvement in *Helicobacter pylori* associated duodenal ulcer has been little examined.

Aim—To assess gastric acid secretion, plasma gastrin concentrations, mucosal content of EGF and TGF α , and mucosal expression of these peptides and their receptor (EGFr) as well as salivary and gastric luminal release of EGF under basal conditions and after pentagastrin stimulation in 10 healthy subjects and in 25 *H pylori* positive patients with duodenal ulcer before and after two weeks of triple anti-*H pylori* therapy and four weeks after the termination of this therapy.

Results—Pentagastrin stimulation caused a significant increase in salivary and gastric release of EGF both in healthy controls and patients with duodenal ulcers but in the patients, the eradication of *H pylori* resulted in several fold higher gastric luminal (but not salivary) EGF release than before the anti-*H pylori* therapy. Mucosal contents of immunoreactive EGF and TGF α and mucosal expression of EGF, TGF α , and EGFr in *H pylori* positive patients with duodenal ulcer were significantly higher than those in healthy *H pylori* negative controls and this increase persisted after eradication of *H pylori*. Basal plasma gastrin was significantly reduced after two weeks of triple therapy and four weeks after the *H pylori* eradication all ulcers were completely healed.

Conclusions—(1) *H pylori* infection in patients with duodenal ulcer was accompanied by enhanced plasma gastrin and increased mucosal content and expression of TGF α , EGF, and EGFr; (2) *H pylori* eradication resulted in ulcer healing, reduction in plasma gastrin, and enhancement of gastric (but not salivary) luminal release of EGF, particularly after pentagastrin stimulation; and (3) enhanced mucosal content and expression of TGF α , EGF, and EGFr and increased luminal release of EGF may contribute to ulcer healing after eradication of *H pylori*.

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Keywords: *Helicobacter pylori*, EGF, TGF α , EGF receptors, gastrin, duodenal ulcer.

Helicobacter pylori infection is the cause of initially acute and then chronic active gastritis (type B) and plays a major part in the pathogenesis of duodenal ulceration.¹⁻³ It is accompanied by an increment in plasma gastrin,⁴ possibly due to the impaired feedback control of the release of this hormone by luminal acid, probably due to the deficiency of its paracrine suppression by somatostatin.⁵

Epidermal growth factor (EGF)⁶ is secreted in humans mainly by salivary glands and is present in digestive secretions such as saliva, gastric, duodenal, and pancreatic juice, and in urine as urogastrone.⁷⁻¹⁰ Transforming growth factor- α (TGF α) is another growth factor produced predominantly in the gastric mucosa, especially when exposed to topical irritants.^{11 12} Similar to somatostatin, both EGF and TGF α are powerful inhibitors of gastric acid secretion^{9 10} and promoters of mucosal growth, repair, and ulcer healing.¹⁰⁻¹³ There is a pronounced increase in the EGF receptors and EGF producing cells around acetic acid induced gastric ulcers in rats^{14 15} and a novel cell lineage in human mucosal ulcerations secreting EGF adjacent to peptic ulcer¹⁶ suggesting that EGF plays an important part in ulcer healing. This notion has been supported by the findings that patients with peptic ulcer show reduced release of EGF into plasma, saliva, and gastric juice.¹⁷⁻¹⁹ Recent studies by Tunio and Hobsley²⁰ showed, however, that there is an increased rather than decreased release of histamine stimulated EGF into the gastric juice in patients with duodenal ulcer possibly due to increased expression of an EGF producing new cell lineage in the ulcer area¹⁶ but the *H pylori* status in these patients was not determined.

Although *H pylori* infection is considered to be a major pathogenic factor in the formation of peptic ulceration and accompanying gastric secretory disorders, little information is available as to whether the presence of *H pylori* in gastric mucosa affects the expression and the release of EGF and TGF α under basal conditions and after gastric secretory stimulation in duodenal ulcer and whether the eradication of *H pylori* affects the mucosal expression and luminal release of these peptides in the stomach.

This study, carried out on healthy controls and *H pylori* positive patients with duodenal

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ulcer, was designed to determine the influence of *H. pylori* eradication on plasma gastrin release and on luminal release (salivary and gastric) of EGF and mucosal expression of EGF, TGF α , and EGFr in gastric mucosa of these patients.

Methods

Twenty five *H. pylori* positive patients with duodenal ulcer and 10 *H. pylori* negative healthy controls were included in the study. All subjects gave informed consent and the project was reviewed and accepted by the ethical committee of the Jagiellonian University School of Medicine, Kraków, Poland.

Gastric contents were aspirated twice, firstly, during endoscopy using a special tube inserted through the endoscope, and secondly, in a separate secretory test, including continuous gastric juice collection during 30 minutes in basal conditions and 60 minutes of intravenous infusion of pentagastrin (2 μ g/kg/h) (Peptavlon, ICI, Alderley Park, UK) using a standard technique of gastric aspiration. Both collections of gastric juice were performed after about 12 hours of overnight fasting with smoking and ethanol forbidden for this period. Also for at least 48 hours before the collection the subjects were asked to stop any medication affecting gastric acid secretion, including H₂ receptor antagonists, (ranitidine or cimetidine) or antibiotics. During basal and pentagastrin induced stimulation, the saliva was constantly expectorated and collected into 15 minute aliquots. Gastric juice was constantly aspirated and collected into separate 15 minute aliquots and their volumes were measured. The samples of saliva and gastric contents were immediately adjusted to pH 7.0 by adding 100 mM NaOH and stored at -70°C until radioimmunoassay of EGF using commercially available reagents (Amersham, Buckinghamshire, UK) and following the protocol suggested by Amersham. The antiserum, raised in rabbits against human EGF, was used at a final dilution of 1:20 000.

Plasma gastrin was also determined under basal conditions (just before endoscopy) using radioimmunoassay with antiserum 4562 (kindly donated by Professor J E Rehfeld of Aarhus University, Aarhus, Denmark) at a final dilution of 1:140 000 as described.⁵

During endoscopy, at least six biopsy samples were obtained from the distal portion (antrum) of the stomach and fixed in buffered formalin and then used for the assessment of the severity of gastritis, the presence of *H. pylori*, and immunocytochemical detection of EGF, TGF α , and EGFr. Two additional biopsy samples were taken from the fundic mucosa for determination of immunoreactive EGF and TGF α and immediately stored at -70°C until analysis.

The presence of *H. pylori* was detected using haematoxylin and eosin staining and the quantity of *H. pylori* was graded according to Marshall and Warren²¹ on a four point scale from absent (grade 0) to abundant (grade 3).

The severity of gastritis in antral mucosa was estimated on a five point scale²² from normal (grade 0) to superficial gastritis (grade 1), and slight, moderate, and severe gastritis (grades 2, 3 and 4 respectively). The extent of intestinal metaplasia, the numbers of mononuclear inflammatory cells in the lamina propria, neutrophils, and eosinophils were estimated in grades showing a range of activity of gastritis from normal (grade 0) to slight (grade 1), moderate (grade 2), and heavy (grade 3) increase as described previously.²² These gradations closely resemble those of the Sydney system.²³

For immunocytochemistry, serial sections obtained from paraffin wax blocks were dewaxed, rehydrated, pretreated with citrate buffer (pH 6.0) in a microwave oven at 700 W for 10 minutes, and incubated with specific monoclonal antibody (12 hours; 4°C) for EGF (1/40; GF 0.1, Oncogene Science, New York, USA), for TGF α (1/20, G10, Oncogene Science, New York, USA), or for EGFr (1/40, Sigma Chemical Co, St Louis, MO, USA) followed by the avidin biotin complex (ABC) method (ABC kit, Oncogene Science, New York, USA). The cytoplasm staining reactions were graded in accordance with the intensity of staining by examining 100 consecutive cells in three regions of the gastric mucosa, the surface epithelium, the neck region (neck), and the basal portion of gastric glands (base) as described previously.^{15 24-26} Coded specimens were independently assessed by two observers. The intensity of staining was graded as follows: 0=no staining, 1+=weakly staining, 2+=moderately positive staining (cytoplasm positive but other cytoplasm compounds also visible), or 3+=densely staining, equal to the simultaneously stained positive control. The number of cells positive for EGFr was evaluated for 100 consecutive cells in three different regions of the mucosa. Negative control sections were processed immunohistochemically after replacing the primary antibody with irrelevant monoclonal antibody or phosphate buffered saline (PBS). Positive control sections were obtained from pancreatic carcinoma (TGF α), oesophageal squamous carcinoma (EGFr), and mandibular gland (EGF) showing maximal labelling with the appropriate antibody. We validated our counts for EGF and EGF α expression and for EGFr labelling. The same person performed a second independent count on sections of mucosa immunostained for EGF, TGF α , and EGFr without knowledge of the results of the first counts. Results of one person were also compared with those obtained from the second person. There was less than 5% variation from the initial counting.

The stored mucosal samples for radioimmunoassay of EGF and TGF α were weighed, thawed, and homogenised in 0.01 M PBS (pH 7.2; 5 ml/g wet tissue). The homogenates were centrifuged at 12 000 g for 20 minutes at 4°C until assay. The EGF radioimmunoassay in tissue homogenates was performed as in the saliva and gastric juice samples described earlier.^{5 9 10} The TGF α

radioimmunoassay in these homogenates were performed with species specific human TGF α antiserum (<0.5% cross reactivity with rat or mouse TGF α or human EGF) and recombinant human TGF α (Berlex Bioscience Inc, Alameda, CA, USA) used both as a standard and as 125 I-labelled tracer. Sheep anti-human TGF α was used at 1:40 000 in 1% normal sheep serum in radioimmunoassay buffer and incubated for 24 hours at 4°C. The assay was highly specific for human TGF α with no displacement of 125 I-labelled tracer found with rat, mouse, or human EGF up to 0.1 μ g/ml. The sensitivity of the assay was 5 pg TGF α per tube. The dose response curve of immunoreactive TGF α found in the gastric mucosa was parallel to standard (recombinant human TGF α) in the TGF α radioimmunoassay.

Gastric secretory studies, plasma gastrin determination, and endoscopy combined with biopsy and histology and immunocytochemistry were performed once in healthy *H. pylori* negative subjects and repeated in patients with duodenal ulcer after two weeks of triple therapy (20 mg omeprazole twice daily, 500 mg amoxycillin four times a day, and 500 mg metronidazole twice daily) and four weeks after the termination of this therapy.

STATISTICS

Data are presented as means (SEM). The Wilcoxon signed rank test was used with paired data and the Mann-Whitney U test with unpaired data. A p value <0.05 was considered significant.

Results

Figures 1 and 2 show the values of basal plasma gastrin concentrations, gastric luminal concentrations of EGF, and gastric acid

Figure 1: Plasma gastrin and gastric luminal EGF concentrations in 10 healthy young *H. pylori* (*Hp*) negative subjects and 25 patients with duodenal ulcer (DU) before and after two weeks of triple therapy and four weeks after termination of therapy. Values are means (SEM).

*Significant increase above the concentrations in healthy subjects. †Significant decrease below the value in patients with duodenal ulcer before the start of *H. pylori* eradication.

Figure 2: Gastric acid secretion under basal conditions and after intravenous infusion of pentagastrin (P-gastrin) in 10 healthy subjects and 25 patients with duodenal ulcer with *H. pylori* (*Hp*) infection before and after two weeks of triple therapy and four weeks after termination of this therapy. Values are means (SEM). *Significant increase above the value in healthy subjects. †Significant decrease below the value in patients with duodenal ulcer before the eradication of *H. pylori*.

outputs under basal conditions and after pentagastrin stimulation in 10 healthy subjects and in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after the termination of therapy.

Basal plasma gastrin concentration in *H. pylori* positive patients with duodenal ulcer before the start of therapy averaged 62 (8) pM/l and it was significantly higher than that recorded in healthy controls (Fig 1). After two weeks of triple therapy, when 15 out of 25 patients showed endoscopically healed ulcers, all patients showed a pronounced and significant reduction in plasma gastrin and this reduction was also seen four weeks after termination of the treatment in the patients with duodenal ulcer.

In patients with duodenal ulcer, basal gastric acid output (Fig 2) before and after the eradication of *H. pylori* was not significantly different from that in *H. pylori* negative healthy controls. The maximal acid response to pentagastrin in *H. pylori* positive patients with duodenal ulcer before the anti-*H. pylori* treatment and after two weeks of triple therapy was slightly but significantly higher than that in *H. pylori* negative healthy controls but four weeks after the anti-*H. pylori* therapy, this stimulated acid output was not significantly different from that in *H. pylori* negative healthy controls.

Concentrations of EGF in gastric content (collected at the time of endoscopy) in *H. pylori* positive patients with duodenal ulcer averaged 100.8 (11.2) pM/l and this was significantly higher (by about 45%) than that in *H. pylori* negative healthy controls (Fig 1). After two weeks of triple therapy and four weeks after stopping therapy the basal gastric EGF concentrations still remained significantly increased above the value recorded in healthy controls.

Figures 3 and 4 present the concentrations and outputs of EGF in the gastric juice aspirated continuously from the stomach (with

Figure 3: Gastric EGF concentrations and outputs under basal conditions and during pentagastrin (P-gastrin) infusion in 10 healthy subjects. Values are means (SEM). *Significant increase above the basal values recorded during a 15 minute period just before the start of pentagastrin infusion.

saliva expectorated throughout the experiment) during the basal 30 minute period and after intravenous infusion of pentagastrin at a dose of 2 µg/kg/h in healthy subjects and

patients with duodenal ulcer. There was no significant difference in basal gastric concentrations and outputs of EGF between healthy controls and *H pylori* positive patients

Figure 4: Gastric EGF concentrations and outputs under basal conditions and during pentagastrin (P-gastrin) infusion in 25 patients with duodenal ulcer before triple therapy and four weeks after the termination of this therapy. Values are means (SEM). *Significant increase above the values obtained in corresponding periods in the same patients with duodenal ulcer before the eradication of *H pylori*.

Figure 5: *H. pylori* (Hp) density by histological examination of biopsy samples of antral mucosa in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant decrease below the value in patients with duodenal ulcer before the start of triple therapy.

with duodenal ulcer. In these patients, four weeks after the termination of triple therapy, when all ulcers were endoscopically healed and *H. pylori* was detected histologically (Fig 5) in only two out of 25 patients (the rapid urease (CLO) test was negative in all 25 patients), the basal and pentagastrin stimulated EGF concentrations and outputs were considerably increased compared with the corresponding values recorded in these patients before the *H. pylori* eradication (Fig 4). For comparison, the basal salivary concentrations and outputs of EGF in normal *H. pylori* negative subjects were similar to those recorded in saliva of the patients with duodenal ulcer. After pentagastrin stimulation, the salivary EGF concentrations and outputs were significantly increased compared with basal values but they were lower than those recorded in gastric juice of these subjects (Table I).

TABLE I Basal and pentagastrin induced salivary EGF concentrations (pM/l) and outputs (nM/15 min) in *H. pylori* negative healthy controls (n=10) and in patients with duodenal ulcer (n=25) before and after eradication of *H. pylori*

	Time (min)	EGF	
		(pM/l)	(nM/15 min)
Healthy <i>H. pylori</i> subjects	15	40 (7)	0.64 (0.10)
	30	29 (9)	0.35 (0.08)
	45*	38 (5)	0.42 (0.06)†
	60*	75 (8)*	0.68 (0.10)†
	75*	70 (12)*	1.47 (0.22)*†
	90*	68 (14)*†	1.64 (0.34)*†
<i>H. pylori</i> + patients with duodenal ulcer (before <i>H. pylori</i> eradication)	15	37 (6)	0.49 (0.06)
	30	22 (5)	0.60 (0.05)
	45*	26 (9)	0.54 (0.07)
	60*	32 (12)	1.65 (0.08)*
	75*	58 (6)*	3.62 (0.01)*
	90*	64 (7)*	2.51 (0.02)*
<i>H. pylori</i> - patients with duodenal ulcer (after <i>H. pylori</i> eradication)	15	32 (5)†	0.38 (0.12)†
	30	28 (4)†	0.44 (0.10)†
	45*	75 (9)*	2.25 (0.08)*†
	60*	49 (10)†	2.88 (0.30)*†
	75*	58 (9)*†	2.94 (0.42)*†
	90*	62 (7)*†	2.17 (0.28)*†

Values are means (SEM).
*Significant increase v basal values recorded before the start of pentagastrin infusion.
†Significant change v the values of EGF recorded in the gastric juice of these subjects.
*Time during pentagastrin infusion.

Figure 6: Immunostaining for EGF and TGFα in biopsy samples of antral mucosa obtained from 10 healthy *H. pylori* (Hp) negative controls and 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant increase above the value obtained before the triple therapy.

In the gastric mucosa of healthy subjects there was only a negligible EGF immunoreactivity, localised mainly in the lumen of gastric glands (Fig 6). TGFα immunoreactivity was more prominent and confined to surface epithelial cells and parietal cells. *H. pylori* positive patients with duodenal ulcer before the *H. pylori* eradication showed about 65% higher EGF immunoreactivity and only about 20% higher TGFα immunoreactivity than *H. pylori* negative healthy controls. Also the EGFr expressed mainly in the superficial epithelial cells and in the upper two thirds of the gland was about 45% higher in *H. pylori* positive patients with duodenal ulcer than in healthy controls (Fig 7). This increased EGF, TGFα, and EGFr immunostaining in patients with duodenal ulcer remained significantly raised after two weeks of triple therapy and four weeks after termination of therapy.

Mucosal contents of TGFα both in healthy controls and in *H. pylori* positive patients with duodenal ulcer were about eight to 10 times

Figure 7: Immunostaining for EGFr containing cells in the biopsy samples obtained from 10 healthy *H. pylori* (Hp) negative controls and 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant increase above the value obtained in healthy controls.

TABLE II Immunoreactive EGF and TGF α contents (pM/g wet tissue) in biopsy samples of fundic mucosa of healthy subjects (n=10) and in patients with duodenal ulcer (DU) (n=25) before, after two weeks of triple therapy, and four weeks after termination of treatment

	EGF (pM/g)	TGF α (pM/g)
Normal controls	0.28 (0.09)	4.51 (0.42)†
DU patients before treatment	0.79 (0.11)*	6.82 (0.86)*‡
After 2 weeks of treatment	0.67 (0.21)*	5.04 (2.11)*‡
After 4 weeks of treatment	0.53 (0.10)*	4.72 (1.94)*‡

Values are means (SEM).

*p<0.05 between healthy control and patients with duodenal ulcer.

‡p<0.05 EGF and TGF α contents in healthy controls v patients with duodenal ulcer before or after triple therapy.

higher than that of EGF in the mucosal biopsy samples (Table II). Both EGF and TGF α contents in *H. pylori* positive patients with duodenal ulcer were significantly higher than those in healthy subjects and they showed only a small tendency to decline after the eradication of *H. pylori* in these patients.

The activity of gastritis had dramatically declined after two weeks of *H. pylori* therapy (Fig 8), whereas the severity of gastritis showed a more gradual decline after two weeks of therapy and four weeks after termination of therapy (Fig 9).

Discussion

This study shows that pentagastrin stimulated EGF release in the human stomach originates, at least in part, directly from the gastric mucosa

Figure 8: Activity of gastritis in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant decrease below the value obtained in *H. pylori* (Hp) positive patients with duodenal ulcer before the triple therapy.

Figure 9: Severity of gastritis in 25 patients with duodenal ulcer before and after two weeks of triple therapy and four weeks after termination of therapy. *Significant decrease below the value obtained in *H. pylori* (Hp) positive patients with duodenal ulcer before the triple therapy.

and that the eradication of *H. pylori* in patients with duodenal ulcer results in a pronounced increase in this EGF release suggesting that this growth factor might contribute to ulcer healing in the course of anti-*H. pylori* therapy.

In the basal state during endoscopy, EGF concentrations in the gastric juice showed significantly higher values in *H. pylori* positive patients with duodenal ulcer when compared with those in *H. pylori* negative healthy controls. This agrees with enhanced mucosal content of immunoreactive EGF and TGF α and immunohistochemical expression of these peptides in the gastric mucosa of patients with duodenal ulcer. However, when the saliva was expectorated, the basal EGF concentrations in saliva and gastric juice of *H. pylori* positive patients with duodenal ulcer were not significantly different from those in healthy controls. This disagrees with previous studies, claiming lower basal concentrations of EGF in patients with peptic ulcer than in healthy normal subjects¹⁷⁻¹⁹ but supports a recent report of Tunio and Hobsley,²⁰ who also found no significant difference in basal EGF release into the saliva and gastric juice between patients with duodenal ulcer and healthy controls.

In our study, when the saliva was constantly expectorated and the gastric content continuously aspirated, a fall in the gastric EGF content was found both in *H. pylori* negative healthy controls and *H. pylori* positive patients with duodenal ulcer suggesting that basal EGF in gastric contents originates, at least in part, from the saliva. However, the salivary concentrations and outputs of EGF after pentagastrin infusion in healthy subjects and patients with duodenal ulcer were significantly lower than those measured in the gastric juice of these subjects. This indicates that during pentagastrin stimulation gastric luminal EGF originates from the gastric mucosa rather than from the swallowed saliva. As in previous reports with histamine stimulation,¹⁹ we also found a large increase in EGF release into gastric juice after the stimulation with pentagastrin both in normal *H. pylori* negative subjects and in patients with duodenal ulcer. This increase in EGF concentrations and outputs was accompanied by a large increase in gastric acid secretion caused by pentagastrin. This indicates that pentagastrin, similar to histamine, stimulates the production of EGF mainly in the stomach and this occurs in a manner that seems to be linked with gastric acid stimulation. It is unclear whether pentagastrin stimulates gastric EGF release via histamine release and what the cellular source of the gastric EGF is.

The major finding of this report is that after eradication of *H. pylori* and ulcer healing in patients with duodenal ulcer, there was a large increase in basal EGF release and a several-fold rise in gastric EGF outputs after pentagastrin stimulation. It is of interest that the mucosal content and expression of EGF and TGF α , which was significantly higher in *H. pylori* infected patients with duodenal ulcer than in healthy controls, remained increased also after

H. pylori eradication. Thus, the increased EGF release coincided with greater expression of EGF and TGF α in the *H. pylori* eradicated gastric mucosa. It is unclear, however, why during the actual *H. pylori* infection, the content of EGF in the gastric juice was reduced despite the enhanced mucosal content of immunoreactive EGF and TGF α and mucosal expression of immunohistochemical content of these patients before the start of anti-*H. pylori* therapy. It is possible that proteases produced by *H. pylori* contribute to rapid degradation of the EGF released from the mucosal cells so that the measurable amounts of EGF in the gastric contents were much lower than those actually released from these cells. It is also possible that the adhesion of *H. pylori* to the gastric mucosa somehow restrains the release of EGF from the mucosal cells. Further studies are needed to elucidate the interrelation between the expression and release of EGF and the infection of the mucosa by *H. pylori*.

As EGF and TGF α are potent mitogens for epithelial cells both in vivo and in vitro,^{2,9-12} it is reasonable to assume that overexpression of these growth factors could contribute to the increase in the mucosal cell proliferation found in *H. pylori* infected stomach.²⁷⁻²⁹ This increased epithelial cell proliferation during long term *H. pylori* infection could serve as an indicator of risk for gastric cancer.³⁰ Additional factors that could explain the mucosal cell proliferation in the *H. pylori* infected stomach might be an increase in plasma gastrin possibly due to the reduced release of somatostatin.⁵ The overexpression of EGF receptors in the mucosa of patients with duodenal ulcer compared with healthy subjects could explain a possible link between growth factors and mucosal cell proliferation in these patients.²⁷⁻³⁰

- 1 Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; i: 1273-5.
- 2 Konturek PCh, Konturek SJ. Role of *Helicobacter pylori* infection in gastroduodenal secretion and in pathogenesis of peptic ulcer and gastritis. *J Pharmacol Physiol* 1994; 45: 333-50.
- 3 Hirschi AM. *Helicobacter pylori*. *Klin Wochensh* 1994; 106: 536-42.
- 4 Levi S, Haddad G, Ghosh P, Beardshall K, Playford R, Calam J. *Campylobacter pylori* and duodenal ulcer; the gastrin link. *Lancet* 1989; i: 1167-8.
- 5 Konturek JW, Gillessen A, Konturek SJ, Domschke W. Eradication of *Helicobacter pylori* restores the inhibitory effect of cholecystokinin on postprandial gastrin release in duodenal ulcer patients. *Gut* 1995; 37: 482-7.
- 6 Cohen S. Isolation of a mouse submaxillary protein acceleration incisor eruption and eye opening in newborn animal. *J Biol Chem* 1962; 237: 1555-62.
- 7 Elder JB, Williams G, Lacey E, Gregory H. Cellular localization of human urogastrone/epidermal growth factor. *Nature* 1978; 271: 466-7.
- 8 Gregory H. Isolation and structure of urogastrone and its relationship to epidermal growth factor. *Nature* 1975; 257: 325-7.
- 9 Konturek JW, Bielanski W, Konturek SJ, Bogdal J, Oleksy J. Distribution and release of epidermal growth factor in man. *Gut* 1989; 30: 1194-200.
- 10 Konturek JW, Brzozowski T, Konturek SJ. Epidermal growth factor in protection, repair, and healing of gastroduodenal mucosa. *J Clin Gastroenterol* 1991; 13 (suppl): S88-97.
- 11 Polk WH, Dempsey PJ, Russel WE, Brown PI, Beauchamp RD, Bernard JA, et al. Increased production of transforming growth factor alpha following acute gastric injury. *Gastroenterology* 1992; 102: 1467-74.
- 12 Barnard JA, Beauchamp RD, Russell WE, Dubois RN, Coffey RJ. Epidermal growth factor - related peptides and their relevance to gastrointestinal pathophysiology. *Gastroenterology* 1995; 108: 564-80.
- 13 Konturek SJ, Brzozowski T, Majka J, Dembinski A, Slomiany A, Slomiany BL. Transforming growth factor alpha and epidermal growth factor in protection and healing of gastric mucosal injury. *Scand J Gastroenterol* 1992; 27: 649-55.
- 14 Lee H, Hansson HA, Norstrom E, Helander HF. Immunoreactivities for epidermal growth factor (EGF) and for EGF receptors in rats with gastric ulcers. *Cell Tissue Res* 1991; 265: 211-8.
- 15 Konturek PCh, Brzozowski T, Konturek SJ, Ernst H, Drozdowicz D, Pajdo R, et al. Expression of epidermal growth factor and transforming growth factor alpha during ulcer healing. *Scand J Gastroenterol* 1997; 32: 6-15.
- 16 Wright NA, Pike C, Eila G. Induction of a novel epidermal growth factor-secreting cell linkage by mucosal ulceration in human gastrointestinal stem cells. *Nature* 1990; 343: 82-5.
- 17 Hansen G, Muller C, Sinha P, Bohnenkamp W, Kottgen E. Gastric ulcer is accompanied by a decrease of epidermal growth factor in gastric juice and saliva. *J Clin Chem Clin Biochem* 1989; 27: 539-45.
- 18 Ohmura E, Emoto N, Tsushima T, Watanabe S, Takeuchi T, Kawamura M, et al. Salivary immunoreactive human epidermal growth factor (IR-hEGF) in patients with peptic ulcer disease. *Hepatogastroenterology* 1987; 34: 160-3.
- 19 Calabro A, Orsini B, Brocchi A, Falchini M, Fedi P, Surrenti C. Gastric juice immunoreactivity epidermal growth factor levels in patients with peptic ulcer disease. *Am J Gastroenterol* 1990; 85: 404-7.
- 20 Tunio AM, Hobsley M. Epidermal growth factor in saliva and gastric juice the response to histamine. *Gut* 1995; 37: 335-9.
- 21 Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; i: 1311-4.
- 22 Karttunen T, Niemela S, Lehtola J. *Helicobacter pylori* in dyspeptic patients. Quantitative association with severity of gastritis, intragastric pH and serum gastrin concentration. *Scand J Gastroenterol* 1992; 26: 124-34.
- 23 Price AB. The Sydney system: histological division. *J Gastroenterol Hepatol* 1991; 6: 209-22.
- 24 Thomas DM, Nasim MM, Gullick WJ, Alison MR. Immunoreactivity of transforming growth factor alpha in the normal and adult gastrointestinal tract. *Gut* 1992; 33: 628-31.
- 25 Jankowski J, McMenamin R, Yu C, Hopwood D, Wormesley KG. Proliferating cell nuclear antigen in oesophageal diseases: correlation with transforming growth factor alpha expression. *Gut* 1992; 33: 587-91.
- 26 Brzozowski T, Konturek P Ch, Ernst H, Stachura J, Konturek SJ, Hahn EG. Gastric adaptation to injury by repeated doses of aspirin strengthens mucosal defence against subsequent exposure to various strong irritants in rats. *Gut* 1995; 37: 749-57.
- 27 Lynch DA, Mapstone NP, Clarke AM, Sobala GM, Jackson P, Morrison L, et al. Cell proliferation in *Helicobacter pylori* associated gastritis and the effect of eradication therapy. *Gut* 1995; 36: 351-3.
- 28 Caghill RJ, Xia H, Kilgallen C, Beattie S, Hamilton S, O'Marion C. Effect of eradication of *Helicobacter pylori* infection on gastric epithelial cell proliferation. *Dig Dis Sci* 1995; 40: 1627-31.
- 29 Thomsen LL, Gavin JB, Tasman-Jones C. Relation of *Helicobacter pylori* to human gastric mucosa in chronic gastritis of the antrum. *Gut* 1990; 31: 1230-6.
- 30 Caghill RJ, Sant S, Beattie S, Hamilton H, O'Marion C. *Helicobacter pylori* and increased epithelial cell proliferation: a risk factor for cancer. *Eur J Gastroenterol Hepatol* 1995; 6: 1123-7.