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Dog Gastric Lipase: Stimulation of Its Secretion In Vivo and Cytolocalization in Mucous Pit Cells

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Dog gastric lipase (DGL) secretion is stimulated in vivo by urecholine, pentagastrin, histamine, 16,16-dimethyl prostaglandin E_2, and secretin. Under fasting conditions, DGL is irreversibly inactivated by gastric acid below pH 1.5; consequently, DGL output can be underestimated. This problem has been resolved by buffering the acid or by using an antisecretory drug such as omeprazole during stimulation. There is a clear parallelism between the secretion of DGL and of gastric mucus. This observation led to the present investigation of the cellular localization of DGL using immunofluorescence techniques. Results showed that DGL is cytolocalized in mucous pit cells of gastric glands. Pepsinogen is found in chief cells. To the authors' knowledge, this is the first description of an enzyme (gastric lipase) secreted by mucous-type gastric cells. In contrast to other species, gastric lipase of the dog is located in cardiac, fundic, and antral mucosae.

Although the exact physiological role of preduodenal lipases in mammals is not yet definitively established, the tissue and cellular localizations of these enzymes are now well characterized in several species.[1-8] Human, rat, and rabbit preduodenal lipases have been purified and localized in different tissues of these species.[9-11] The cytolocalization was performed using immunofluorescence techniques with polyclonal antibodies.[1,6,7] The role of gastrointestinal hormones or neurotransmitters in the secretory mechanism of preduodenal lipases has also been explored.[12-22] Their secretion has been studied in vivo and in vitro using dispersed gastric glands or von Ebner glands. However, an exhaustive study cannot be performed in humans, and it is uncertain that rabbit or rat are good models because of their different dietary behavior.

In our search for an animal model, we have recently studied preduodenal lipase in the dog. The biochemical properties of dog gastric lipase (DGL) are very similar to those of preduodenal lipases previously purified; it is a 49-kilodalton glycoprotein containing 13% carbohydrate and formed by a single polypeptide chain of 377-379 amino acid residues, acting on both long- and short-chain triglycerides.[8]

The use of dogs with chronic gastric fistulas expands the scope of physiological studies of gastric lipase. Thus, the effect of gastric secretagogues administered by IV infusion can be observed in the conscious dog by analysis of gastric secretion.

As far as we know, only one study of the response of DGL to IV infusion of secretagogues was performed in 1970 by Blum and Linscheer[12] in dogs with Heidenhain pouches. They found that both histamine and urecholine were secretagogues of DGL. The lipolytic activities found were very low compared with those we are now able to detect with the standard DGL assay. Moreover, the values in this study are insignificant compared with the lipolytic activity found in the entire dog gastric mucosa.[8] An interesting observation by Blum and Linscheer was a pH optimum of 4 with urecholine- or histamine-stimulated gastric juice as lipase source and a pH optimum of 7 with unstimulated gastric juice. To explain this difference, these investigators proposed a structural alteration of the lipase under stimulated acidic conditions or, alternatively, the presence of two lipases responding differently to urecholine or histamine stimulation. Clementi et al.,[23] who observed the presence of a lipase in the alkaline mucus collected from dog gastric mucosa, did not observe any lipolytic activity in acidic gastric juice collected

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from Pavlov pouches. This discrepancy led us to reinvestigate DGL secretion.

In this paper, we determined the effect of several gastric secretagogues on the secretion of DGL and acid. We observed for the first time an in vivo inactivation of DGL under unbuffered conditions below pH 1.5. Furthermore, our results suggested that DGL might be secreted by mucous-type cells; this was confirmed by immunocytolocalization studies.

Material and Methods

Dogs

Six 2-year old male Beagle dogs weighing 13.5–17.5 kg (mean, 14.5 ± 1.4 kg) were studied. At the age of 6 months, they had been prepared with chronic gastric and duodenal cannulae as described by Thomas.[24] The gastric cannula was inserted in the pyloric area on the greater curvature (see Figure 1). The duodenal cannula was inserted opposite the pancreatic papilla. Dogs showed no symptoms of functional gastric disorders or any endoscopically detectable abnormalities in the stomach.

Each experiment was preceded by an 18-hour fast with free access to water. During the experiments, dogs stood in a Pavlov harness and were given a continuous IV infusion of 0.15 mol/L sodium chloride solution at a rate of 100 mL/h. After a 1-hour basal period, secretagogues were added to the infusion for 1–2 hours. The gastric secretion was collected at 15-minute intervals, and the duodenal cannula was kept open to prevent duodenogastric reflux.

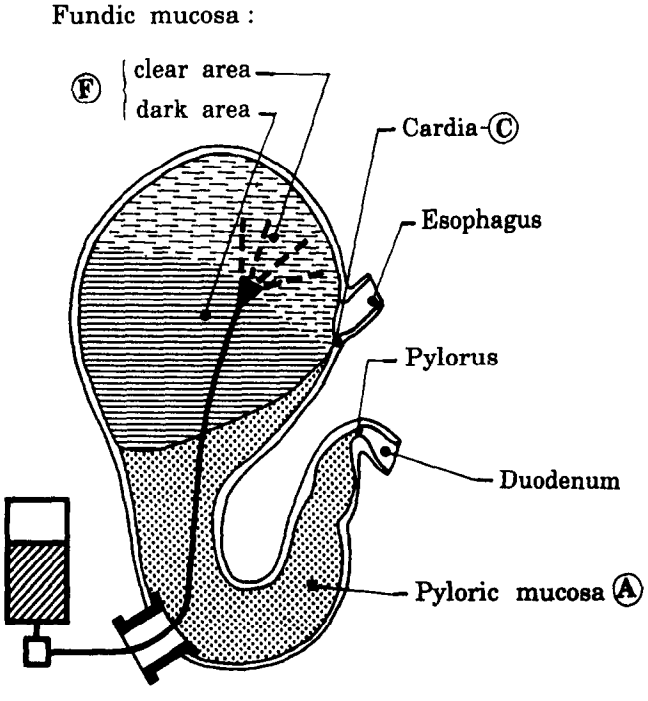


Figure 1. Scheme of dog stomach and sites where biopsy specimens were taken (C, cardiac mucosa; F, upper fundic mucosa; A, proximal pyloric mucosa) and scheme of the spray device for in vivo buffer administration.

Administration of Drugs

Pentagastrin (6 $\mu g \cdot kg^{-1} \cdot h^{-1}$, Peptavlon; ICI Pharma, Cergy, France), histamine (150 $\mu g \cdot kg^{-1} \cdot h^{-1}$; Prolabo, Paris, France), or urecholine (200 $\mu g \cdot kg^{-1} \cdot h^{-1}$, Bethanecol; Merck Sharp & Dohme, Paris, France) were infused IV for 1 hour after a 1-hour basal period; the number of experiments (n) was 9, 9, and 17, respectively.

Urecholine (200 $\mu g \cdot kg^{-1} \cdot h^{-1}$) was also used in association with omeprazole (Mopral; Astra laboratories, Nanterre, France), a proton pump inhibitor (n = 2). Omeprazole (10 mg), dissolved in 50 mL of a 12 mmol/L sodium bicarbonate solution, was administered 30 minutes after the urecholine stimulation started, through the duodenal cannula, which was closed for 30 minutes.

Synthetic secretin (1 $U \cdot kg^{-1} \cdot h^{-1}$, Sekretoline; Hoechst laboratories, Frankfurt, Germany) was infused IV for 2 hours after a 1-hour basal period (n = 5).

The effect of prostaglandins on DGL secretion was studied using Enprostil [16,16-dimethyl prostaglandin E_2 (PGE_2), Gardrine; Syntex laboratories, Puteaux, France]. After a 1-hour basal period, the gastric and the duodenal cannulas were closed and two capsules of Enprostil (35 μg/capsule) were given orally 15 minutes apart. Thirty minutes later, the two cannulas were opened, and gastric secretion was collected for 2 hours (n = 5). Control experiments (n = 3) were performed using a placebo in which Enprostil was removed from the capsules and replaced by the excipient (propyleneglycol) only.

In Vivo Neutralization of Gastric Acidity

To investigate the role of gastric acidity on the DGL stability, several of the experiments using pentagastrin, histamine, or urecholine as secretagogues (n = 4, 4, and 8, respectively) were performed while spraying the stomach with a glycine/HCl solution that buffers in the pH range 2–3. One molar glycine/HCl buffer, pH 6, was sprayed at a rate of 5 mL/5 min into the stomach during each 15-minute period of gastric juice collection as shown in Figure 1. Control experiments were performed without secretagogues. The spray volume was subtracted from the total collected volume every 15 minutes.

Enzyme Purifications

Pure DGL was obtained as described by Carrière et al.[8]

Dog pepsinogen was purified from gastric juice collected under urecholine stimulation. After adjustment to pH 7, gastric juice was incubated with gentle stirring for 30 minutes with a batch of Mono Q gel (anionic exchanger; Pharmacia, Uppsala, Sweden; equilibrated with 10 mmol/L Tris-HCl buffer, pH 7. All the pepsinogen was bound to the gel. The Mono Q gel was washed with 10 mmol/L Tris-HCl buffer and packed into a chromatographic column (24-mm ID; IBF, Villeneuve-la-Garenne, France). Using FPLC (Pharmacia), a linear NaCl concentration gradient was applied from 0 to 0.5 mol/L in 10 mmol/L Tris-HCl buffer, pH 7. The pepsinogen was eluted at a concentration of 0.3 mol/L NaCl. Pepsinogen fractions eluted from the Mono Q

column were concentrated by ultrafiltration on a YM 10 DiaFlo membrane (Amicron, Paris, France). The second step was a gel filtration chromatography performed on a Superose 12 HR 10/30 column (Pharmacia) using 10 mmol/L Tris-HCl buffer, pH 7, and 150 mmol/L NaCl. The protein elution profiles were recorded spectrophotometrically at 280 nm. Pure dog pepsinogen was identified by amino acid and N-terminal sequence analysis.[8]

Enzyme Activity Measurements

Gastric lipase activity was determined at pH 5.5 as described by Gargouri et al.[25] using a pHstat (Radiometer, Copenhagen, Denmark) with tributyrin as substrate. The lipase output in secretion experiments was expressed in international units per 15 minute-period (1 unit equals 1 micromole of butyric acid released per minute). Some secretion samples contained both soluble and insoluble mucus; it was ascertained that DGL had an homogeneous distribution and was not selectively concentrated in the mucus gel.

Pepsin activity was assayed using Azocoll (Calbiochem, La Jolla, CA)[26] as substrate and expressed as porcine pepsin equivalents.

Measurement of Secreted Volume and Acid Outputs

The volume and the pH of gastric juice secreted every 15 minutes were measured to the nearest 0.5 mL and 0.1 pH unit, respectively. Acidity was measured with a glass electrode by titration with 0.1N NaOH to pH 7 and checked according to the equation $[H^+] = 10^{-pH}$. The results were expressed in millimoles per 15-minute periods.

pH Stability of DGL in Gastric Juice

Dog gastric juice stimulated by pentagastrin and containing pepsin (1–2 mg/mL) was used. Because this juice was not collected with a buffer, its lipase was totally inactivated by acidity. Several samples of this juice were adjusted to various pHs from 0.5 to 6. The same amount of purified DGL (30 U/mL final concentration) was added to each sample and incubated for 4 hours at 37°C at a fixed pH. The residual lipase activity was then measured.

Antibodies

Polyclonal antibodies against DGL and dog pepsinogen were raised in guinea pig using the method described by Moreau et al.[6] with purified DGL and dog pepsinogen.

After sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) of dog gastric juice and an homogenate of dog gastric mucosa, the specificities of DGL and dog pepsinogen antisera were established by protein-blotting onto nitrocellulose membrane.[27]

Biopsy Specimen Sampling

Using an endoscope introduced through the gastric cannula, mucosal biopsy samples were taken from two dogs used for the secretory studies. Three paired biopsy specimens were sampled from each of the two dogs as shown in Figure 1. One of each pair of specimens was immediately fixed for microscopy studies. The other biopsy specimen (1–2 mg) was homogenized in 5–10 µL of 10 mmol/L phosphate buffer, 0.15 mol/L NaCl, pH 7.2, containing 0.5% (vol/vol) Triton X-100 (Merck, Nogent, France) and assayed for lipase activity. Triton X-100 had no effect on DGL activity at this concentration.

Immunofluorescence Studies

As described in previously published reports,[6,7] the biopsy specimens of gastric mucosa were fixed for 1 hour at 4°C in phosphate-buffered saline (PBS) containing 2% (wt/vol) paraformaldehyde and 2% (vol/vol) glutaraldehyde. Tissues were dehydrated in dimethylformamide (Fluka, Mulhouse, France) and embedded in Lowicryl K4M (Polaron Equipment Ltd., Waterford, England).[28] Semithin sections (250 nm) were cut for further immunofluorescence labeling.[29] Polyclonal antisera (guinea pig anti-DGL and guinea pig anti-dog pepsinogen) were used at a 1:200 dilution. Fluorescein-conjugated sheep anti-guinea pig immunoglobulin G (Cappel-Flobio; Cooper Biomedical, Malvern, England) was diluted 200 times in PBS. Serum of nonimmunized guinea pig was used as control, and no labeling was detected in any zone of biopsy collection.

Statistical Analysis

Values are given as mean ± SEM. n is always the number of experiments with different dogs. The Kruskal–Wallins test was used to analyze the differences between control data and data obtained under stimulation within a same time period. A probability value $P < 0.05$ was considered significant. Bonferroni test was used when comparing results after stimulation and control values over multiple time periods.

Results

Stimulation of DGL Secretion

Effect of IV infusion of pentagastrin. Figure 2 shows the volume of gastric secretion and H^+ and

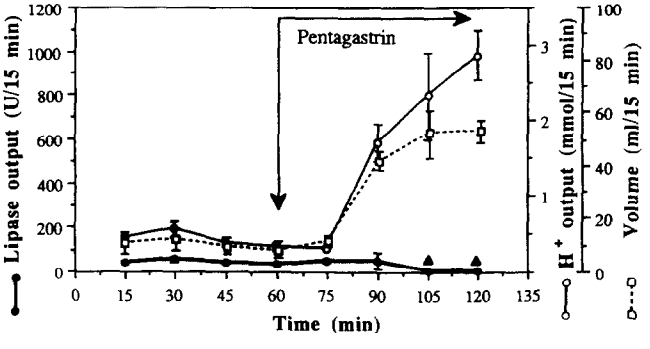


Figure 2. Effect of IV infusion of pentagastrin (6 µg · kg^{-1} · h^{-1}) after a 60-minute basal period (n = 5). *$P < 0.05$ by comparison with data obtained during the same period of control experiments in buffered conditions without stimulation.

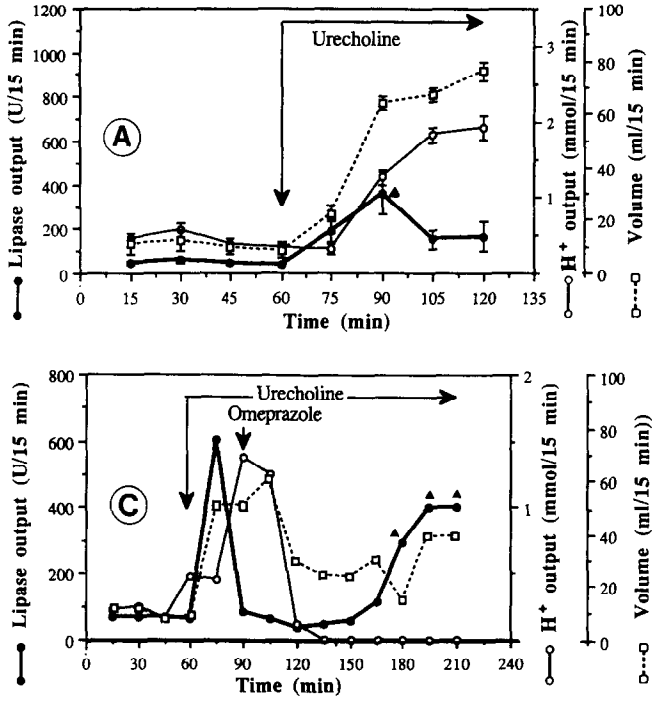


Figure 3. Effect of IV infusion of urecholine (200 $\mu g \cdot kg^{-1} \cdot h^{-1}$). (*A*) Infusion of urecholine alone after a 60-minute basal period (n = 9). (*B*) Infusion of urecholine associated with 1 mol/L glycine/HCl buffer, pH 6, sprayed regularly into the stomach after a 60-minute basal period (n = 8). (*C*) Infusion of urecholine associated with omeprazole (10 mg). The urecholine stimulation was started after a 60-minute basal period, and omeprazole was administrated as a bolus dose intraduodenally 30 minutes after the stimulation started (n = 2). $^{\blacktriangle}P < 0.05$ by comparison with data obtained during the same period of control experiments in buffered conditions without stimulation.

DGL outputs per 15 minutes after 6 $\mu g \cdot kg^{-1} \cdot h^{-1}$ of IV pentagastrin. In contrast to what was observed in humans,[15,20,22] DGL secretion was not stimulated by pentagastrin. Indeed, 45 minutes after the beginning of the infusion, measurements of DGL were markedly decreased. This unexpected result led us to investigate the effect of other gastric secretagogues.

Effect of IV infusion of urecholine. As shown in Figure 3*A*, while H^+ output increased continuously throughout urecholine infusion (200 $\mu g \cdot kg^{-1} \cdot h^{-1}$), after an initial increase in DGL output to a maximum value of 362 ± 97 U/15 min, there was a significant decrease to 166 ± 64 U/15 min 1 hour after the infusion started. This may represent inactivation of secreted DGL under acidic conditions; when the pH of gastric juice was near 1, DGL was usually undetectable.

The effect of pH on DGL stability in gastric juice is shown in Figure 4. Total DGL activity was preserved at pH values above 1.5 (residual DGL activity ≥90% after 4 hours at 37°C), but below this value, the enzyme was rapidly and irreversibly inactivated (Figure 4). Because each juice sample contained pepsin (1–2 mg/mL expressed in terms of porcine pepsin), the enzymatic activity of DGL was not apparently affected by pepsin (optimal pH for pepsin activity is around pH 3) and depended only on the level of acid. Thus, the DGL output measured during stimulation by pentagastrin or urecholine was probably underestimated because of concomitant stimulation of acid secretion.

To determine the exact amount of secreted DGL, gastric juice was pH-buffered in vivo by a regular spray of glycine/HCl solution into the stomach during stimulation with gastric secretagogues. A control experiment performed without stimulation showed that the DGL activity in basal secretion increased from 49 ± 9 U/15 min to 133 ± 50 U/15 min when the buffer was sprayed, reaching 189 ± 39 U/15 min after 45 minutes of spray. In total, DGL basal secretion was 606 ± 40 U/h compared with 190 ± 23 U/h

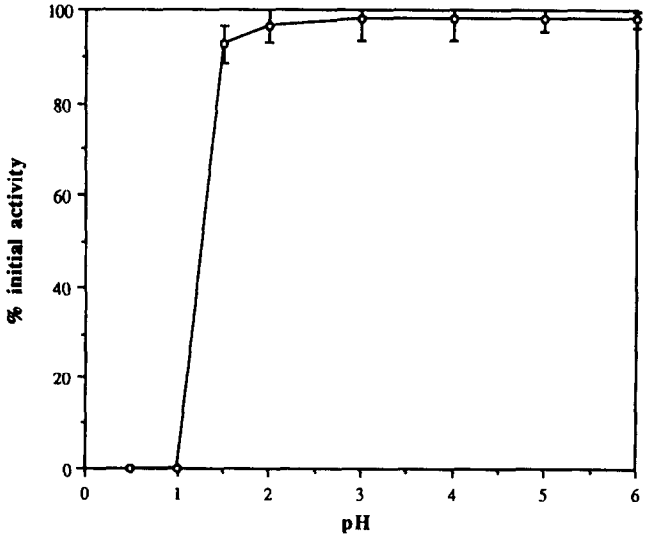


Figure 4. Inactivation of DGL at various pHs. Samples of gastric juice containing a known amount of DGL (30 U/mL) were adjusted at various pHs from 0.5 to 6.0 and incubated during 4 hours at 37°C (see Materials and Methods).

without buffering. When buffer spraying was used with the infusion of 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$ urecholine (Figure 3B), H^+ output increased at a reduced rate and DGL output increased up to a maximal value of 1001 ± 154 U/15 min after 30 minutes of stimulation. After this initial peak of secretion, lipase level then remained rather constant at a slightly lower value (753 ± 146 U/15 min, 60 minutes after the stimulation started). Thus, the total DGL secreted per hour under urecholine stimulation was 2836 ± 361 U/h compared with 865 ± 124 U/h without buffering the gastric secretion ($P < 0.001$). Compared with the buffered basal secretion, urecholine stimulated DGL output 4.7-fold (Figure 5). Both DGL output and volume vary in a dose-dependent manner up to 200 $\mu g \cdot kg^{-1} \cdot h^{-1}$ urecholine (Figure 6A).

Effect of pentagastrin or histamine. Like urecholine, pentagastrin (6 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and histamine (150 $\mu g \cdot kg^{-1} \cdot h^{-1}$) stimulated the volume of gastric secretion and H^+ output. The total DGL secreted per hour under pentagastrin stimulation, in association with glycine/HCl buffer, was 1228 ± 257 U/h compared with 119 ± 44 U/h without buffering. Compared with the buffered basal secretion, pentagastrin stimulated DGL output 2.0-fold. Similar to urecholine, pentagastrin stimulated DGL output in a dose-dependent manner up to the maximal dose used (6 $\mu g \cdot kg^{-1} \cdot h^{-1}$) (Figure 6B).

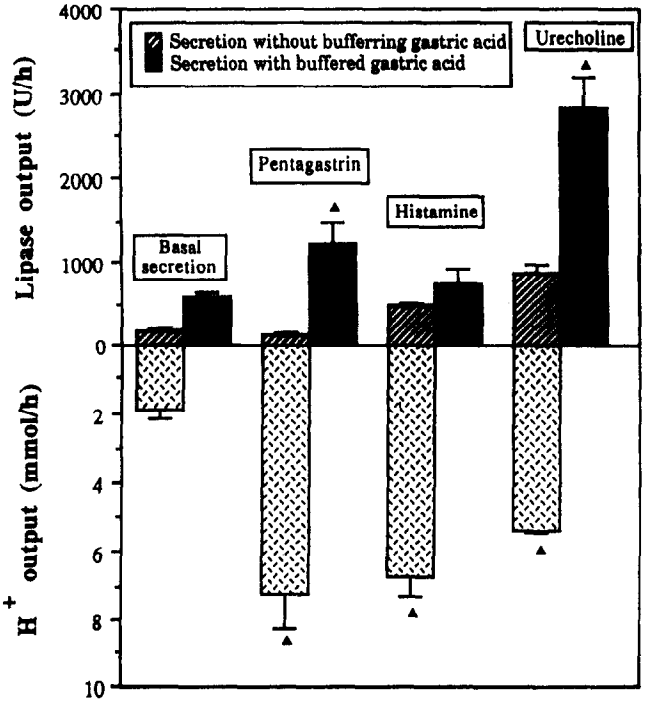


Figure 5. DGL and H^+ outputs per hour after stimulation by gastric secretagogues associated or not associated with 1 mol/L glycine/HCl buffer, pH 6, sprayed in vivo. $^{\blacktriangle}P < 0.05$ by comparison with data obtained during the same period of control experiments in buffered conditions without stimulation. (Buffer control, n = 4; pentagastrin, n = 5; pentagastrin + buffer, n = 4; histamine, n = 5; histamine + buffer, n = 4; urecholine, n = 9; urecholine + buffer, n = 8.)

The stimulation of DGL output was very low under histamine infusion (150 $\mu g \cdot kg^{-1} \cdot h^{-1}$) in association with glycine/HCl buffer, 736 ± 189 U/h compared with 606 ± 40 U/h in buffered basal secretion (1.2-fold). The difference between these two values was not significant. In contrast with the secretory response produced by pentagastrin or urecholine, DGL output did not increase in a dose-dependent manner with histamine but appeared biphasic. Up to 150 $\mu g \cdot kg^{-1} \cdot h^{-1}$, DGL output varied with histamine dose but above this value, it decreased to the level of basal secretion (Figure 6C).

Effect of gastric acid inhibitors. To confirm the role of gastric acidity on DGL inactivation, omeprazole, a powerful inhibitor of the proton pump,[30-36] was used in association with an infusion of urecholine (Figure 3C) without buffering of gastric secretion. As previously shown in Figure 3A, a peak of DGL output was observed, but apparent DGL secretion decreased promptly while H^+ output was rising. Although proton output was abolished within 30 minutes of omeprazole administration, DGL activity required about 100 minutes to recover a sustained output of 400 U/15 min. The total volume secreted decreased by half and was mostly mucus. This delay was not observed when gastric acid was buffered in vivo. Moreover, DGL output was lower (1600 ± 134 U/h) than the output observed with the same dose of urecholine in association with glycine/HCl buffer (2836 ± 361 U/h).

Another inhibitor of gastric acid secretion belonging to the PGE_2 family[33,35-37] was used with the same purpose as omeprazole, recognizing that the mechanism of inhibition of acid secretion is entirely different. Enprostil (16,16-dimethyl PGE_2) was administrated orally in a pharmacological dose used in humans after a 60-minute basal period. A control experiment was performed with a placebo. Studying the effect of Enprostil alone (Figure 7), it was confirmed that H^+ output was inhibited to a significant degree. Furthermore, Enprostil increased DGL output significantly during the second hour (778 ± 88 U/h compared with 152 ± 34 U/h with the placebo). The delay for DGL stimulation was longer using Enprostil than using gastric secretagogues such as urecholine, pentagastrin, or histamine.

Effect of IV infusion of secretin. In addition to their antisecretory effects, E_2 prostaglandins are also secretagogues of gastric mucus.[38-44] To check the possibility that DGL may be secreted in parallel with gastric mucus, secretin, another secretagogue of

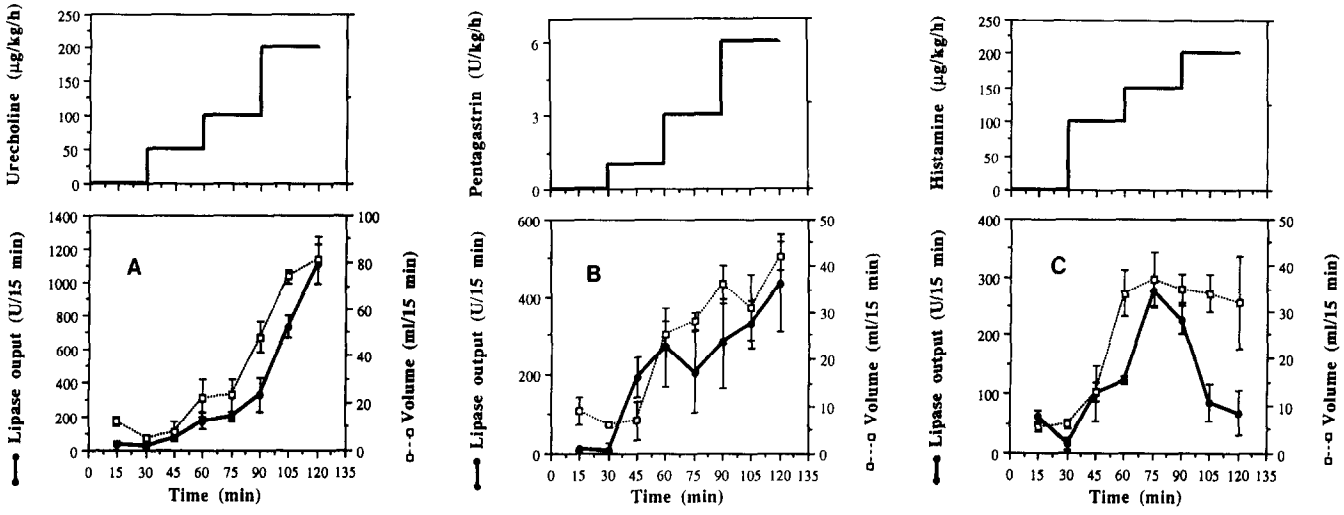


Figure 6. Dose-response curves of volume and DGL output under buffered conditions. (*A*) Urecholine infusion, (*B*) pentagastrin infusion, and (*C*) histamine infusion. The *upper panel* shows the stepwise increase in drug infusion. H^+ output is not represented, because these experiments were performed in buffered conditions.

mucus,[44-46] was tested (Figure 8) in a pharmacological dose (1 $U \cdot kg^{-1} \cdot h^{-1}$) currently used to stimulate pancreatic secretion in dogs. Proton output (0.4 mmol/15 min in basal secretion) decreased 30 minutes after the secretin infusion started, reaching a sustained level of 0.03 mmol/15 min. DGL output required 90 minutes after the secretin infusion, to reach a maximal level of 525 ± 250 U/15 min. Identical results were obtained with or without buffering the gastric secretion during the stimulation period (data not shown). Compared with the buffered basal secretion (606 ± 40 U/h), the stimulation factor was 3.

Cellular Localization of DGL and Pepsinogen

Immunoblotting controls. Supernatants of biopsy specimens and dog gastric juice were subjected to SDS-PAGE followed by immunoblotting using nitrocellulose membranes. Guinea pig anti-DGL reacted strongly with the DGL band (49 kilodaltons) in biopsy supernatant as well as in gastric juice (Figure 9). Guinea pig anti-pepsinogen revealed two bands corresponding to pepsinogen (around 40 kilodaltons) and to pepsin (around 38 kilodaltons) in biopsy specimens and gastric juice. A third unknown faint band was also revealed in biopsy specimens.

Fluorescent microscopy. Immunofluorescence was performed on samples from three different zones showing DGL and dog pepsin activities (see Figure 1). Biopsy specimens from site F showed DGL labeling in cells located into the pit of the fundic glands (Figure 10*A* and *D*), whereas pepsinogen labeling was located in cells of the base of fundic glands (Figure 10*C* and *D*). We detected no cell containing both DGL and pepsinogen. Cells containing DGL or pepsinogen were morphologically different. Pepsinogen secretory granules were located in chief cells, whereas DGL was located in foveolar mucous-type cells from the pit of gastric glands (Figure 10*B*). Labeling was also positive when antibodies specific to DGL or dog pepsinogen were used on biopsy specimens taken from zones *C* and *A*, and the same cellular distribution was observed.

Discussion

Effect of Gastric Acid Secretion on DGL Stability

In contrast to human gastric lipase which is stable at pH 1,[2] DGL is irreversibly inactivated below pH 1.5 (Figure 4). In basal gastric secretion or during hormonal stimulation in fasting conditions, secreted DGL is partially or totally inactivated when this pH threshold is reached. By contrast, during normal digestion, gastric acid is buffered by the components of the meal, and pH values remain above 2 for approximately 3 hours (unpublished observations). In this situation, DGL is stable and active towards alimentary triglycerides. This explains the paradox of the absence or low level of DGL activity in basal or stimulated gastric secretion despite the fact that gastric mucosa contains substantial amounts of lipolytic activity (approximately 50,000 U/stomach for 15 kg of body wt[8]).

The inactivation of DGL by gastric acid is clearly shown using urecholine as gastric secretagogue. DGL and H^+ secretions are both stimulated by urecholine. Measurable DGL output decreases rapidly after a se-

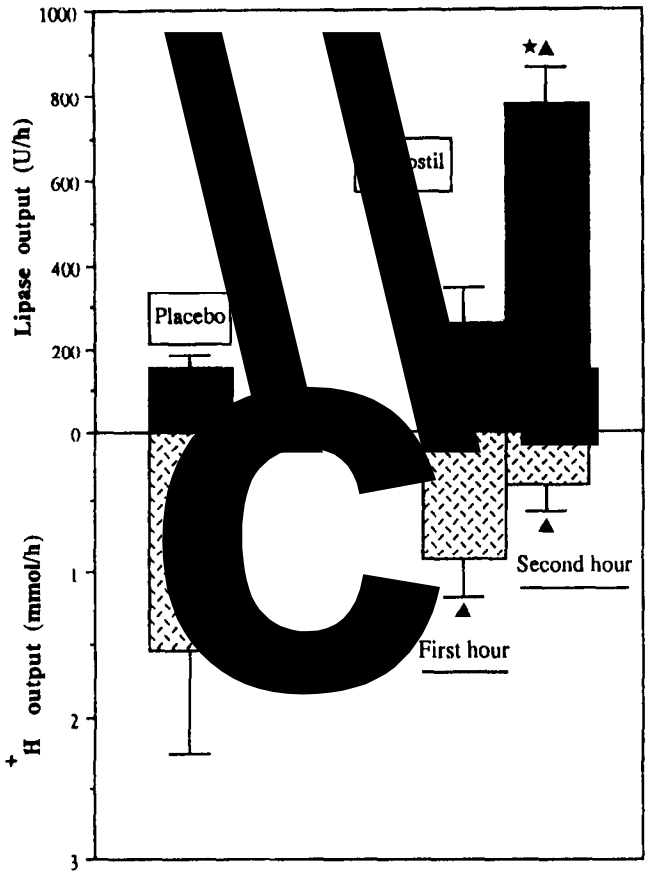
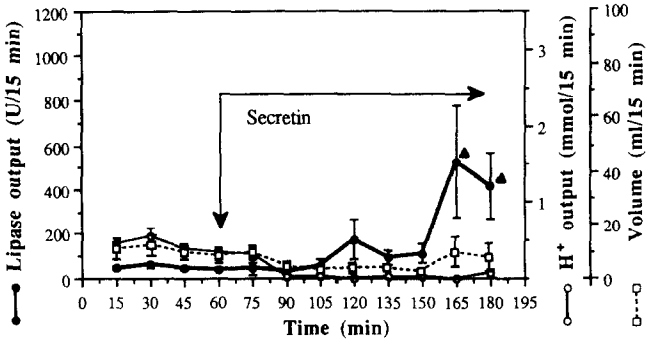


Figure 7. DGL and H^+ outputs per hour after treatment with 16,16-dimethyl PGE_2. Two capsules of Enprostil were administrated orally after a 60-minute basal period (n = 5). The control experiments were performed with a placebo (n = 3). $^{\blacktriangle}P < 0.05$ by comparison with data obtained during the same period of control experiments using a placebo (Kruskal–Wallins test). $^{*}P < 0.05$ by comparison with the data obtained during the first hour of stimulation; α-level = 0.006 using Bonferroni test.



8. Effe infusion of secretin (1 $U \cdot kg^{-1} \cdot h^{-1}$) after a nute bas d (n = 5). $^{\blacktriangle}P < 0.05$ by comparison with the btained the first hour of stimulation (Kruskal–Wallins test); α-level = 0.001 using Bonferroni test.

cretion peak, whereas H^+ output increases continuously and reaches a sustained level under stimulation (Figure 3A). If a buffer neutralizing gastric acid is sprayed in vivo during the stimulation period (Figure 3B), DGL output reaches a higher level and its secretion remains sustained. The sensitivity of DGL towards acid is overcome during urecholine stimulation by using omeprazole (Figure 3C), which inhibits gastric acid secretion in both humans[31,32] and dogs.[30,34]

Stimulation of DGL Secretion Using Gastric Secretagogues

Infused IV, pentagastrin, histamine, and urecholine (Figure 5) stimulate DGL secretion in the following order: urecholine > pentagastrin > histamine. The respective DGL outputs are 4.7-, 2.0-, and 1.2-fold the output in buffered basal secretion expressed in units of DGL secreted per hour. On the other hand, H^+ outputs are found in the same range for each of these three secretagogues. In contrast to our results, Blum and Linscheer[12] suggested that the

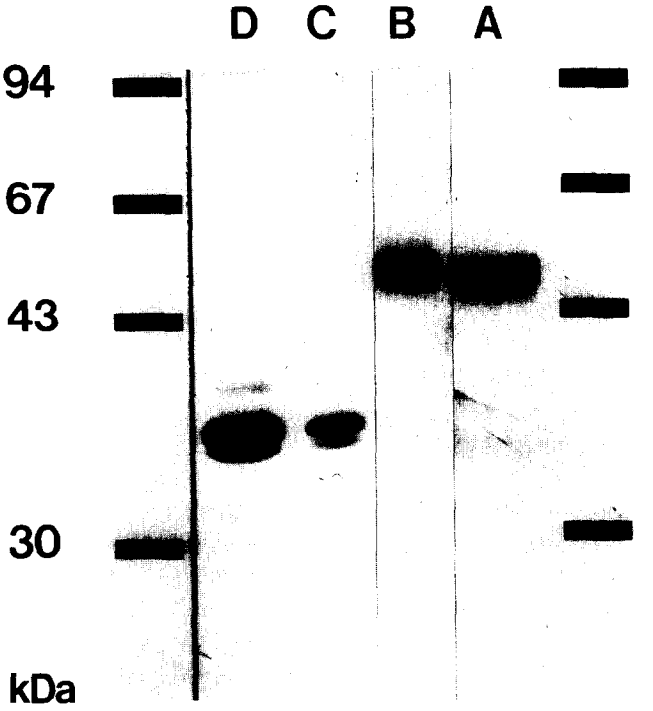


Figure 9. Immunoblotting of DGL and dog pepsinogen. Dog gastric juice and supernatant of fundic biopsy homogenate in PBS Triton X-100 were subjected to SDS-PAGE followed by immunoblotting. Guinea pig antibodies to DGL were found to react only with a relative molecular mass 49-kilodalton band corresponding to that of DGL in the fundic biopsy (*lane A*) and in gastric juice (*lane B*). Guinea pig antibodies to dog pepsinogen were found to react mainly with two bands with M_r around 39 and 38 kilodaltons corresponding to pepsinogen and pepsin, in gastric juice (*lane C*) and in the fundic biopsy (*lane D*). These antibodies reacted slightly with an unknown 41-kilodalton band in the fundic biopsy (*lane D*).

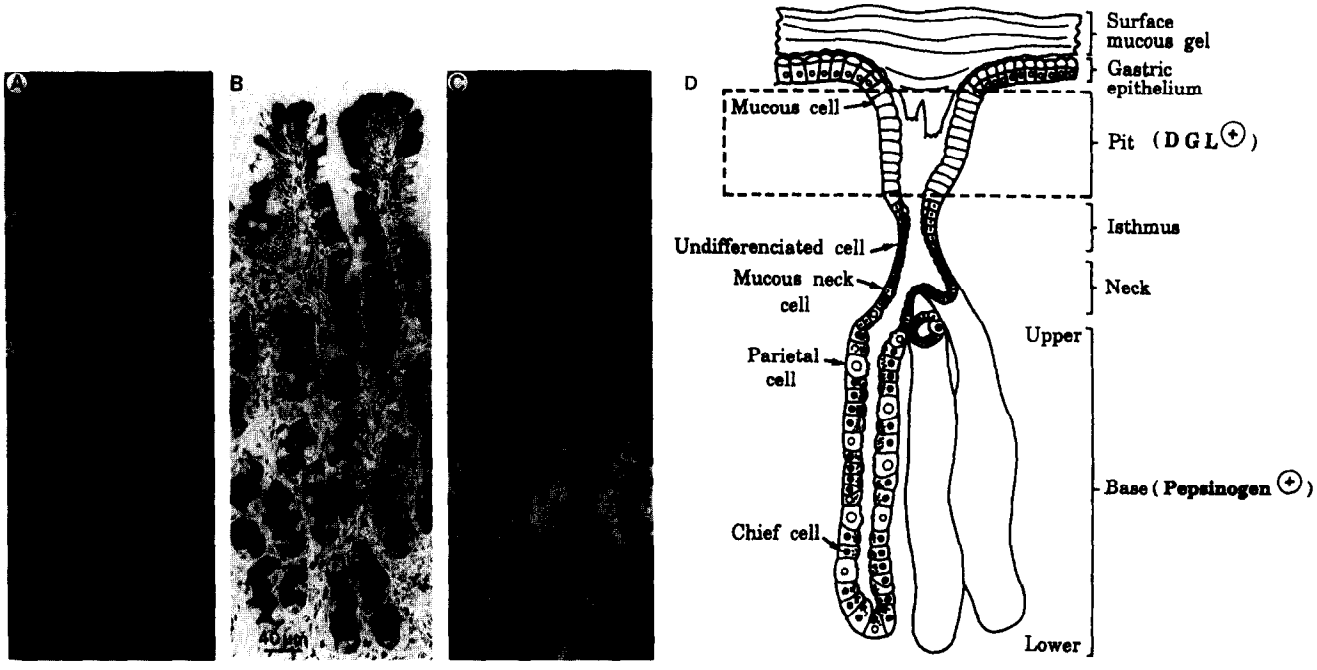


Figure 10. Dog fundic gland immunolabeling. Sections were incubated with guinea pig antibodies to DGL (*A*) and dog pepsinogen (*C*) and revealed by fluorescein-labeled immunoglobulins to guinea pig. Another section was stained with Azur II (*B*) for general identification of the cells in the fundic gland. (*D*) Scheme of fundic gastric gland with localization of the different cellular types.

effects of histamine and urecholine were similar. This is what we observed when gastric acidity was not buffered. The explanation of the discrepancy presumably relates to acid inactivation of DGL. Similarly, the fact that Clementi et al.[23] did not observe lipolytic activity in acidic dog gastric juice is probably due to the same inactivation.

We compared DGL and human gastric lipase outputs in basal and maximally stimulated secretions. In healthy humans,[22] the basal (3020 ± 496 U/h) and pentagastrin-stimulated secretion (12,598 ± 2036 U/h) represent 1%–3% and 4%–12%, respectively, of the total lipolytic activity found in gastric mucosa (100,000–300,000 U/stomach).[4] In dogs, the buffered basal (606 ± 40 U/h) and buffered urecholine-stimulated secretion (2836 ± 361 U/h) represent 1.2% and 5.7%, respectively, of the total lipolytic activity found in gastric mucosa.[9] These results are very similar in humans and dogs with stimulation factors of 4.2 and 4.7 for human gastric lipase and DGL, respectively. In both species, only a small amount of the total lipolytic activity found in gastric mucosa is secreted in response to maximal stimulation with known secretagogues.

Inhibitors of gastric acid secretion were investigated as alternatives to the spray device for buffer administration used in vivo. Omeprazole associated with urecholine stimulation results in an increase in active DGL output in gastric juice (see Figure 3C). However, for the same dose of urecholine, the measured total DGL output (units per hour) is higher with urecholine and buffer (see Figure 3B). Omeprazole administration may have a secondary effect on DGL secretion. PGE_2 and its analog 16,16-dimethyl PGE_2 (Enprostil) are known to be inhibitors of acid secretion in humans[36] and dogs.[33,35,37] Enprostil was found to be more potent than PGE_2 and to be active orally.[35] As expected, Enprostil inhibited gastric acid secretion in our experiments (Figure 7) but stimulated DGL secretion in absence of any other secretagogue.

PGE_2[33,38–44] and urecholine[44,47–51] are known to stimulate gastric mucus secretion. DGL secretion was investigated during stimulation by secretin, which is also a gastric mucus secretagogue.[44–46] Secretin infused IV in pharmacological doses stimulates DGL secretion with the same delay (around 90 minutes) observed with PGE_2 (Figure 8). The delay observed for DGL output with both PGE_2 and secretin may be related to mucus output. According to Bolton et al.,[39] it is possible that all freshly secreted mucus first appears as barrier mucus adherent to the gastric mucosa; as a result of acid and pepsin activity, it is then broken down and shed. We can imagine that DGL, secreted in parallel with mucus, could initially be retained in adherent barrier mucus. This could also explain the delay observed using omeprazole under urecholine stimulation of DGL (see Figure 3C). By contrast, when urecholine is used under buffered conditions without omeprazole (see Figure

3B), DGL output increases without delay, probably because the secreted enzyme is drained from the barrier mucus by acid secretion.

There is a marked analogy between gastric mucus and DGL secretion under pentagastrin, histamine, and urecholine stimulation. Our data taken with data in the literature concerning gastric mucus secretion seem to indicate that DGL could be produced by mucous-type cells (Table 1). Gerard et al.[50] studied the synthesis, distribution, and secretion of gastric mucus in dogs bearing total gastric fistulas. Stimulants used were the vagomimetic drug urecholine, food ingestion, histamine, and gastrin. Urecholine induced secretion of mucus by fundic pit cells. After this excretory phase, increased synthesis of mucus by pit cells and prepyloric antral glands was noted. The effects of food were similar to those of urecholine, perhaps, as postulated by Gerard et al., because food ingestion induces marked central vagal stimulation in the dog.[50] Administration of histamine and gastrin resulted in increased synthesis of mucus by fundic and antral pit cells but not in stimulation of mucus secretion. However, the increasing size of secretory granules may lead to exocytosis. This could explain the lower secretion of DGL observed during stimulations with pentagastrin or histamine.

Immunocytolocalization of DGL in Mucous Pit Cells of Gastric Glands

Whereas in humans and rabbits gastric lipase is located in chief cells,[6,7] DGL is located in mucous-type cells as suggested by the secretory data. Specific antibodies against DGL react strongly with mucous pit cells of gastric glands (Figure 10A). No labeling was detected in epithelial mucous cells or in other cells of gastric glands. By comparison, dog pepsinogen is located in chief cells (Figure 10C) as found in humans and rabbits. No colocalization of DGL and dog pepsinogen has been detected. It is noteworthy that in contrast to humans and rabbits in whom gastric lipase is only found in the proximal stomach, it has been shown that dogs have gastric lipase in the antral area as well as in more proximal areas of the stomach. Moreover, for the first time, to our knowledge, mucous-type cells have been shown to secrete an enzyme. It still has to be shown that these cells produce both DGL and gastric mucus.

Table 1. Comparison of Stimulation of Gastric Mucus and DGL in Dogs

Secretagogue	Stimulation of gastric mucus secretion[a]	Stimulation of DGL secretion[b]
Urecholine	++++	+++++
Pentagastrin	++	++
Histamine	+	+

[a]Data from Gerard et al.
[b]Present data.

General Conclusions

DGL secretion has probably been underestimated in earlier studies because of its inactivation by gastric acid under fasting conditions. This problem has been resolved by buffering the acid secretion in vivo or by using antisecretory drugs during stimulation of DGL secretion.

The parallelism observed between DGL and gastric mucus secretions can be explained by the cytolocalization of DGL in mucous pit cells of gastric glands. These cells respond to various stimuli: a cholinergic agonist (urecholine), gastrointestinal hormones (pentagastrin and secretin), and local transmitters (histamine and PGE_2).

Up to now in three different species studied, the cellular distributions of gastric lipase and pepsinogen are different, i.e., colocalization in human chief cells of fundic glands,[6] localization in different rabbit chief cells of cardiac mucosa,[7] and, finally, localization of DGL in mucous pit cells and dog pepsinogen in chief cells of cardiac, fundic, and proximal pyloric mucosae. Why is the capacity for gastric lipase secretion a property of different cell types according to species? The same question can be asked more generally for preduodenal lipases, which are localized in different tissues of the upper digestive tract according to the animal species, i.e., lingual in rat,[1-3] pharyngeal in calf,[2,52] and gastric in human,[4-6] dog,[2-8] and rabbit.[2,3] These problems deserve further investigation.

References

1. Roberts IM, Jaffe R. Lingual lipase: immunocytochemical localization in the rat von Ebner gland. Gastroenterology 1986;90:1170–1175.
2. Moreau H, Gargouri Y, Lecat D, Junien JL, Verger R. Screening of preduodenal lipases in several mammals. Biochim Biophys Acta 1988;959:247–252.
3. DeNigris SJ, Hamosh M, Kasbekar DK, Lee TC, Hamosh P. Lingual and gastric lipases: species differences in the origin of prepancreatic digestive lipases and in the localization of gastric lipase. Biochim Biophys Acta 1988;959:38–45.
4. Moreau H, Laugier R, Gargouri Y, Ferrato F, Verger R. Human preduodenal lipase is entirely of gastric fundic origin. Gastroenterology 1988;95:1221–1226.
5. Abrams CK, Hamosh M, Lee TC, Ansher AF, Collen MJ, Lewis JH, Benjamin SB, Hamosh P. Gastric lipase: localization in the human stomach. Gastroenterology 1988;95:1460–1464.
6. Moreau H, Bernadac A, Gargouri Y, Pieroni G, Verger R. Immunocytolocalisation of human gastric lipase in chief cells of the fundic mucosa. Histochemistry 1989;91:419–423.
7. Moreau H, Bernadac A, Tretout N, Gargouri Y, Ferrato F,

Verger R. Immunocychemical localization of rabbit gastric lipase and pepsinogen. Eur J Cell Biol 1990;51:165–172.
8. Carrière F, Moreau H, Raphel V, Laugier R, Bénicourt C, Junien JL, Verger R. Purification and biochemical characterization of dog gastric lipase. Eur J Biochem 1991;202:75–83.
9. Tirrupathi C, Balasubramanian KA. Purification and properties of an acid lipase from human gastric juice. Biochim Biophys Acta 1982;712:692–697.
10. Field RB, Scow RO. Purification and characterization of rat lingual lipase. J Biol Chem 1983;258:14563–14569.
11. Moreau H, Gargouri Y, Lecat D, Junien JL, Verger R. Purification and characterization of rabbit gastric lipase. Biochim Biophis Acta 1988;960:286–293.
12. Blum AL, Linscheer WG. Lipase in canine gastric juice. Proc Soc Exp Biol Med 1970;135:565–568.
13. Szafran H, Popiela T, Szafran Z. The effect of 2-deoxy-D-glucose and insulin stimulation on the secretion of gastric lipase. Scand J Gastroenterol 1971;6:55–58.
14. Popiela T, Szafran H, Szafran Z. Secretion of gastric juice in 50. Gerard A, Lev R, Jerzy Glass GB. Histochemical study of the mucosubstances in the canine stomach. II. The effect of histamine, gastrin, urecholine, and food. Lab Invest 1968;19:29–39.
15. Szafran Z, Szafran H, Popiela T, Trompeter G. Coupled secretion of gastric lipase and pepsin in man following pentagastrin stimulation. Digestion 1978;18:310–318.
16. DeNigris SJ, Hamosh M, Kasbekar DK, Fink CS, Lee TC, Hamosh P. Secretion of human gastric lipase from dispersed gastric glands. Biochim Biophys Acta 1985;836:67–72.
17. Finks CS, Hamosh M, Hamosh P, Denigris J, Kasbekar DK. Lipase secretion from dispersed rabbit gastric glands. Am J Physiol 1985;248:G68–G72.
18. Field RB, Dromy R, Hand AR. Regulation of secretion of enzymes from von Ebner's gland of rat tongue. J Dent Res 1987;66:586–587.
19. Field RB, Hand AR. Secretion of lingual lipase and amylase from rat lingual serous glands. Am J Physiol 1987;253:G217–G225.
20. Moreau H, Saunière JF, Gargouri Y, Pieroni G, Verger R, Sarles H. Human gastric lipase: variations induced by gastrointestinal hormones and by pathology. Scand J Gastroenterol 1988;23:1044–1048.
21. Ruellan C, Moreau J, Bouisson M, Ribet A. The Ebner glands: a pancreatic-like gland secreting an acid lipase. Secretory regulation in vitro. Int J Pancreatol 1988;3:293–300.
22. Moreau J, Bouisson M, Balas D, Ravaud A, Stupnik S, Buscail L, Vaysse N, Ribet A. Gastric lipase in alcoholic pancreatitis: comparison of secretive profiles following pentagastrin stimulation in normal adults and patients with pancreatic insufficiency. Gastroenterology 1990;99:175–180.
23. Clementi A, Urbano A, Cambria A. Sede della secrezione della gastrolipasi nel sistema ghiandolare della mucosa gastrica del cane. Boll Soc Ital Biol Sper 1969;44:802–804.
24. Thomas JE. An improved cannula for gastric and intestinal fistulas. Proc Soc Exp Biol Med 1941;46:260.
25. Gargouri Y, Pieroni G, Rivière C, Saunière JF, Lowe PA, Sarda L, Verger R. Kinetic assay of human gastric lipase on short- and long-chain triacylglycerol emulsions. Gastroenterology 1986;91:919–925.
26. Will PC, Allbee WE, Witt CG, Bertko RJ, Gaginella TS. Quantification of pepsin A activity in canine and rat gastric juice with the chromogenic substrate azocoll. Clin Chem 1984;30:107–711.
27. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 1979;76:503–509.
28. Altman LG, Schneider BG, Papermaster DS. Rapid embedding of tissues in Lowicryl K4M for immunoelectron microscopy. J Histochem Cytochem 1984;32:1217–1223.
29. Ferracci H, Bernadac A, Gorvel JP, Maroux S. Localization by immunofluorescence and histochemical labeling of aminopeptidase *N* in relation to its biosynthesis in rabbit and pig enterocytes. Gastroenterology 1982;82:317–324.
30. Larsson H, Carlsson E, Junggren U, Olbe L, Sjostrand SE, Sundell G. Animal studies with omeprazole, a potent inhibitor of gastric acid secretion. Scand J Gastroenterol 1982;17(Suppl 78):302.
31. Olbe L, Haglund U, Leth R, Lind T, Cederberg C, Ekenved G, Elander B, Fellenius E, Lundborg P, Wallmark B. Effects of substituted benzimidazole (H 149/94) on gastric acid secretion in humans. Gastroenterology 1982;83:193–198.
32. Lind T, Cederberg C, Ekenved G, Haglund U, Olbe L. Effect of omeprazole—a gastric proton pump inhibitor—on pentagastrin stimulated acid secretion in man. Gut 1983;24:270–276.
33. Soll AH. Mechanism of action of antisecretory drugs. Studies on isolated canine fundic mucosal cells. Scand J Gastroenterol 1986;21(Suppl 125):1–6.
34. Larsen KR, Ives MM, Jensen III NF, Carlsson E, Larsson H. Omeprazole and cimetidine versus pentagastrin in canine ex vivo gastric chamber. Am J Physiol 1989;256:G390–G395.
35. Robert A, Schultz JR, Nezamis JE, Lancaster C. Gastric antisecretory and antiulcer properties of PGE_2, 15-methyl PGE_2, and 16,16-dimethyl PGE_2. Gastroenterology 1976;70:359–370.
36. Robert A. Cytoprotection by prostaglandins. Gastroenterology 1979;77:761–767.
37. Kauffman Jr GL, Reeve JJ, Grossman MI. Gastric bicarbonate secretion: effect of topical and intravenous 16,16-dimethyl prostaglandin E_2. Am J Physiol 1980;239:G44–G48.
38. Bolton JP, Cohen MM. Stimulation of nonparietal cell secretion in canine Heidenhain pouches by 16,16-dimethyl prostaglandin E_2. Digestion 1978;17:291–299.
39. Bolton JP, Palmer D, Cohen MM. Stimulation of mucus and nonparietal cell secretion by the E_2 prostaglandins. Dig Dis Sci 1978;23:359–364.
40. Domschke W, Domschke S, Hornig D, Demling L. Prostaglandin-stimulated gastric mucus secretion in man. Acta Hepatogastroenterol 1978;25:292–294.
41. Johansson C, Kollberg B. Stimulation by intragastrically administered E_2 prostaglandins of human gastric mucus output. Eur J Clin Invest 1979;9:229–232.
42. Waterbury LD, Mahoney JM, Peak TM, Cohn RG, Garay GL. Stimulatory effect of enprostil, an anti-ulcer prostaglandin, on gastric mucus secretion. Am J Med 1986;81:30–33.
43. Seidler U, Knafla K, Kownatzki R, Sewing KF. Effects of endogenous and exogenous prostaglandins on glycoprotein synthesis and secretion in isolated rabbit gastric mucosa. Gastroenterology 1988;95:945–951.
44. Seidler U, Sewing KF. Ca^{2+}-dependent and -independent secretagogue action on gastric mucus secretion in rabbit mucosal explants. Am J Physiol 1989;256:G739–G746.
45. André C, Lambert R, Descos F. Stimulation of gastric mucous secretions in man by secretin. Digestion 1972;7:284–293.
46. Kowalesky K, Patchkowski T, Kolodej A. Effect of secretin on mucinous secretion by the isolated canine stomach perfused extracorporeally. Pharmacology 1978;16:78–82.
47. Janowitz HD, Hollander F, Jackson C. Stimulation of cell-free gastric mucus by the topical application of acetylcholine. Proc Soc Exp Biol Med 1951;76:578–580.

48. Horowitz MI, Hollander F. Evidence regarding the chemical complexity of acetylcholine-stimulated gastric mucus. Gastroenterology 1961;40:785–793.
49. Altamirano M. Alkaline secretion produced by intraarterial acetylcholine. Am J Physiol 1963;168:787–803.
50. Gerard A, Lev R, Jerzy Glass GB. Histochemical study of the mucosubstances in the canine stomach. II. The effect of histamine, gastrin, urecholine, and food. Lab Invest 1968;19:29–39.
51. Zalewsky CA, Moody FG, Allen M, Davis EK. Stimulation of canine gastric mucus secretion with intraarterial acetylcholine chloride. Gastroenterology 1983;85:1067–1075.
52. Bernback S, Hernell O, Blackberg L. Purification and molecular characterization of bovine pregastric lipase. Eur J Biochem 1985;148:233–238.

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