学 位 論 文

Doctor's Thesis

A study on roles of phospholipids in gastric mucosal protection

胃粘膜防御因子としてのリン脂質の役割に関する研究

松田　ミミアン　グラシエラ

Matsuda Mimian Graciela

指導教官

岡部　紘明　教授

熊本大学医学部医学研究科臨床検査医学

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著 者 名 ： 松田 ミミアン グラシエラ
Matsuda Mimian Graciela

指 導 教 官 ： 臨床検査医学教授 岡部 紘明

審査委員名 ： 分子病理学担当教授 山本 哲郎
病理学第二担当教授 竹屋 元裕
微生物学担当教授 前田 浩
生化学第二担当教授 堀内 正公

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CONTENTS

要　旨

【目的】Helicobacter pylori（Hp）感染スナネズミ（第1部）および胃疾患患者（第2部）を対象として胃粘膜内のリン脂質を測定し、胃粘膜防御機構におけるリン脂質の役割について検討した。

第1部【対象および方法】Hp感染後3、6、9、12、18、24、36ヵ月群と、同期の月齢群をコントロールとして実験モデルを作成した。屠殺後、胃各部位の粘膜の一部を採取し、リン脂質をクロロホルム・メタノール法で抽出し、高速液体クロマトグラフィー（HPLC）を用いて各組織中のリン脂質を測定した。同時に、各組織片からHematoxylin-Eosin染色を行って胃粘膜の形態的変化を観察した。

【成績および考案】1. 対照動物では、総リン脂質量は部位別にみると、前胃で最も少なく、正常胃底腺、萎縮胃底腺、幽門腺、十二指腸の順に有意に（$P<0.001$）多かった。このことは、前胃と腺胃との境界にある弁状のバリアーが塩酸の逆流を防いでいると推察され、また十二指腸では胆汁によるリン脂質の加水分解（浸蝕）から防御する必要があるので、腺胃に比べてリン脂質の量が多いものと考えられた。これらの所見はHp感染動物でも同様の傾向（$P<0.001$）がみられた。2. 非感染群の胃粘膜は経過（加齢）とともに正常胃底腺領域の減少（粘膜萎縮）がみられた（$P<0.01$）が、その変化に伴って総リン脂質量も漸次減少した（$P<0.001$）。また、Hp感染群でも同様に感染後の経過とともに著明な萎縮性変化が認められた（$P<0.01$）が、総リン脂質量は萎縮の変化の度合いに平衡して有意に（$P<0.001$）減少していた。3. リン脂質の分画はphosphatidylinositol（PI）、

phosphatidylserine (PS)、phosphatidylethanolamine (PE)、phosphatidylcholine (PC)、 lysophosphatidylcholine (LPC)、sphingomyelin (SPH) の６種類であり、このうち、PS, PE, PC が他の脂質に比べて著しく多く含まれていた。この中でも PC は正常胃底腺領域、つまり塩酸分泌領域に最も多く認められたこと、および PC 以外のリン脂質分画ではこのような特異的分布はみられなかったことから、PC は塩酸に対する特異的防御因子と考えられた。

【結論】腺胃粘膜におけるリン脂質は adaptive cytoprotection として、すなわち①粘膜表面においては疎水性（物理学的）バリアーとして、②胃粘膜組織内では prostaglandin に分解されて（化学的に）粘液生成の促進や血流の増加などの作用を有し、両面から胃粘膜の防御機構に関与していることが示唆された。リン脂質から見る限り粘膜防御に対する Hp 感染による直接的影響はないと考えられた。

第２部【対象および方法】外来患者 105 人（表層性胃炎 20、十二指腸潰瘍 30、胃潰瘍 50、胃癌 5）を対象として、幽門輪から 2cm の大彎側の幽門部および胃角上 3cm の対側大彎の胃体部から内視鏡的生検を行い、リン脂質をクロロホルム・メタノール法で抽出し、HPLC を用いて各粘膜組織中のリン脂質を測定した。

【成績および考案】いずれの胃疾患でも、総リン脂質量は幽門粘膜よりも胃体部粘膜に高い傾向があったが、特に胃潰瘍では幽門粘膜と胃体部粘膜との間に有意差（$P<0.05$）がみられた。総リン脂質量は、表層性胃炎や十二指腸潰瘍の方が胃潰瘍や胃癌に比べて高かった。なお、測定したリン脂質分画、PI、PS、PE、PC、LPC、SPH のうち、PS、PE、PC が他に比べ著しく多く含まれていた。

【結論】リン脂質は塩酸からの胃粘膜防御機構を反映しており、いわゆる自己防御機構を示唆していると考えられた。リン脂質の中でも、PC、PE および PS が胃粘膜防御機構において重要な働きを有するものと考えられた。

SUMMARY

Part I

Background: The gastric mucosa exhibits its protection against various exogenous and endogenous irritants, including gastric acid and Helicobacter pylori (H. pylori). Surface-active phospholipids are reported to protect the gastric mucosa by their hydrophobicity. The present study focused on changes in mucosal phospholipid-related protection in Mongolian gerbils (M. gerbils) caused by H. pylori infection.

Materials and methods: Thirty-nine 4-week-old male M. gerbils were given standard strain of H. pylori orally, and divided into seven groups: 3-, 6-, 9-, 12-, 18-, 24-, 36- month-infection. As age-adjusted paired controls, 40 healthy M. gerbils were divided into seven groups: 4-, 7-, 13-, 19-, 25- and 37-month-old. Specimens were taken for phospholipid analysis from the following sites: the proximal fore-stomach (E-area), the fundic gland area (F-, f1, and f2-areas), and the pyloric area (P-area) of the stomach and from the duodenum (D-area). The contents of the following phospholipid subclasses were measured: phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), lysophosphatidylcholine (LPC), and sphingomyelin (SPH). The fundic gland mucosa was divided into three areas: F, f1 and f2, and their sizes are measured. Atrophic changes in the gastric mucosa of the M. gerbil were classified into four grades according to the percentage of

the F-area and the presence or absence of the f2-area: non-atrophic, mild atrophic, moderate atrophic, and severe atrophic changes.

Results: In controls, total phospholipid content was the highest in the D-area, followed by the F-, f1- and P-areas (similar levels), and lowest in the fore-stomach ($P<0.001$). The content decreased significantly with aging ($P<0.001$), in F-area of the gastric mucosa, but not in the other areas. The percentage of F-area decreased significantly with aging ($P<0.0001$). The total phospholipid content in the gastric mucosa decreased significantly with aging ($P<0.001$). The PS level was high in all five areas, except in the F-area. The PC level was high in all five areas except in the E-area. In the D-area, PE and PS were also in high levels. The H. pylori infected gerbils showed very similar results to those by the controls, regarding the total phospholipid contents, and the six-phospholipid subclasses, except for the result described below. In the H. pylori-infected gerbils, the age-related total phospholipid contents were similar to those of the control gerbils in spite of severe atrophic changes.

Conclusions: The present study suggests that phospholipid-related protection is the strongest in the duodenal mucosa, followed by gastric mucosa, and weak in the fore-stomach mucosa of M. gerbils. The phospholipid-related protection is almost equal in the gastric mucosa. PC is the major phospholipid to protect the gastric mucosa against gastric acid, probably by chemical protection. PS is also important for mucosal protection as the physical barrier in the fore-stomach and gastric mucosae, probably by its hydrophobicity. In the duodenal mucosa,

phospholipids are enriched to protect the mucosa from hydrolytic action of bile juice. H. pylori infection seems to induce adaptive cytoprotection in the gastric mucosa. However, the infection does not cause remarkable changes in phospholipid-related mucosa protection.

Part II.

Background: Phospholipids play an important role in gastric mucosal protection. The purpose of the present study was to investigate changes in various phospholipids in the fundic and pyloric gland mucosae of patients with gastric mucosal disease.

Methods: One hundred and five patients with superficial gastritis, duodenal ulcer, gastric ulcer or gastric cancer were studied. Patients underwent endoscopy to obtain biopsy specimens from both the fundic and pyloric gland mucosae. The phospholipid contents were measured by high performance liquid chromatography.

Results: Total phospholipid level was significantly greater in the fundic gland mucosa than in the pyloric gland mucosa ($P<0.05$), and the level in the fundic gland mucosa was high in all four gastric diseases studied. The difference was significant in patients with gastric ulcers ($P<0.05$). Total phospholipid levels were the highest in superficial gastritis, followed by duodenal ulcer, gastric ulcer and gastric cancer. In all four gastric diseases, phosphatidylserine (PS), phosphatidylethanolamine (PE), and phosphatidylcholine (PC) levels were high,

while phosphatidylinositol, lysophosphatidylcholine, and sphingomyelin levels were low. The PE and PC levels were higher in the fundic gland mucosa than in the pyloric gland mucosa, whereas the PS level was higher in the pyloric gland mucosa than in the fundic gland mucosa.

Conclusions: The fundic gland mucosa has stronger phospholipid-related protection than the pyloric gland mucosa, based on the levels of mucosal phospholipids. The main phospholipids for gastric mucosal protection are PC and PE (in the fundic gland mucosa) and PS (in the pyloric gland mucosa). Phospholipid-related protection is strong in superficial gastritis and duodenal ulcer, but is reduced in the pyloric gland mucosa in patients with gastric ulcers, and in both gastric gland mucosae in patients with gastric cancer.

LIST OF PUBLICATIONS

1. Mimian Graciela Matsuda, Atsunobu Misumi, Akitoshi Murakami, Chizuko Nakamura, Umeko Semba, Yoko Shibuya, Sebastião Mitsuji Miyazaki and Hiroaki Okabe. Changes in mucosal phospholipid-related protection in some gastric diseases. Digestive Endoscopy 2003; 15:37-44.

2. Sebastião Mitsuji Miyazaki, Mimian Graciela Matsuda, Atsunobu Misumi, Ubehiko Honmyo, Akitoshi Murakami, Hiroshi Murata, Katsuro Sagara, Ryouichi Kurano and Hiroaki Okabe. Blood flow, acidity and atrophic changes of the gastric mucosa in Mongolian gerbils infected with Helicobacter pylori. Digestive Endoscopy 2001; 13:195 201.

3. 宮崎光二セバスチャン、松田ミミアン、三隅厚信、鶴田潤二、岡部紘明、村田博司、蔵野良一、相良勝郎。Helicobacter pylori 感染のスナネズミの胃粘膜における血流量、胃内 pH および胃粘膜組織学的の変化。Therapeutic Research 2001; 22 suppl. 1: S98-S103.

4. 宮崎光二セバスチャン、松田ミミアン、岡部紘明、三隅厚信、鶴田潤二、村田博司、蔵野良一、相良勝郎。長期観察による Helicobacter pylori 感染スナネズミの胃粘膜の血流量および織学的変化。Therapeutic Research 2000. 21 suppl. 1: S159-S167

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ABBREVIATIONS

H. pylori	Helicobacter pylori
M. gerbil	Mongolian gerbil
HPLC	High Performance Liquid Chromatography
PI	Phosphatidylinositol
PS	Phosphatidylserine
PE	Phosphatidylethanolamine
PC	Phosphatidylcholine
LPC	Lysophosphatidylcholine
SPH	Sphingomyelin

Part I

INTRODUCTION

A fundamental property of the gastric epithelium is its ability to protect itself against various noxious factors such as acid, digestive enzymes, mechanical damage, and bacterial attacks (1). The concept of a "gastric mucosal barrier" arose from work by Code, Davenport, and colleagues (2-5), who studied the passive permeability of gastric mucosa to ions and recognized the very low permeability of this tissue to electrolytes. Under physiological circumstances, diffusion of acid from the lumen of the stomach into the mucosa was minimal; however, perturbations leading to mucosal damage and hemorrhage were associated with substantial loss of acid that was attributed to H^+ back diffusion. Indeed, the pathological significance of gastric acid in peptic ulcer disease had been recognized much earlier by Schwartz (6) as expressed in his dictum "without acid, no peptic ulcer" in 1910.

A lipid component of the gastric mucosal barrier seems to play an important role as a protective factor in the gastric mucosa. (7-11). An important property of the lipid component of mucosal barrier is its hydrophobicity, which protects the epithelium by repelling luminal fluids (12-17). Hydrophobicity can be measured by contact angle analysis and has been determined in a number of mammalian species (14-17) as well as in human (18). The presence of a phospholipid layer on the gastric epithelium (Fig.1), identified from both animal

and human gastric mucosae was considered responsible for the hydrophobic qualities (7, 13, 19-25).

Helicobacter pylori (H. pylori), a gram-negative bacillus that was first identified from a patient with chronic gastritis by Warren and Marshall in 1983 (26) has been implicated in various gastric diseases, including atrophic gastritis (27,28), intestinal metaplasia (28-30), gastric ulcer (28,29,31), mucosa-associated lymphatic tissue lymphoma (32), and gastric cancer (32-34). However the mechanisms by which H. pylori induce such gastric disorders remain unclear.

Previous reports have shown that H. pylori infection reduced the surface hydrophobicity of gastric mucosa as well as the phospholipid content (35-38). Wakabayashi et al. (39) have reported that H. pylori infection causes changes in the gastric mucosal phospholipid content and their fatty acid composition, which in turn, cause weakening of the gastric mucosal barrier.

On the other hand, many researchers have used Mongolian gerbils (*Meriones unguiculatus*) as animal models to investigate changes in the gastric mucosa developed by H. pylori infection (40-41).

The present study was carried out to clarify the roles of phospholipids in gastric mucosal protection in Mongolian gerbils (M. gerbils) infected with H. pylori.

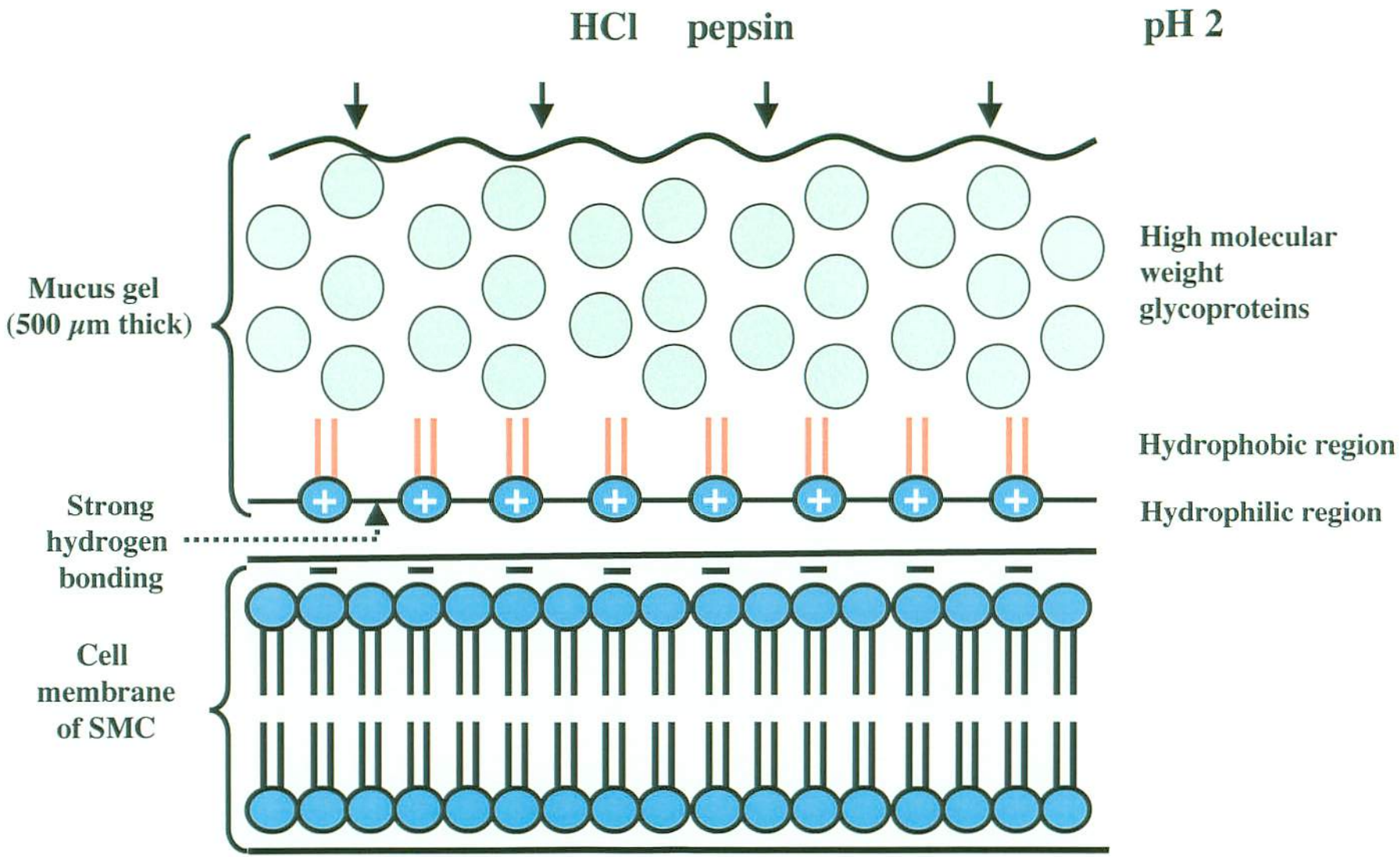


Figure 1. Schematic representation of the presence of a layer of surface-active phospholipids on the surface mucous cell (SMC). Under strongly acid conditions of stomach, ionization of phosphate is suppressed by and surfactant becomes becomes effectively cationic, resulting in much stronger adsorption. Un-ionized phopshate groups can now form hydrogen bonds giving strongly adsorbed layer strong cohesion.

MATERIALS AND METHODS

Animals

Thirty-nine 4-week-old male Mongolian gerbils (M. gerbils) (MGS/Sea SPF, Seiwa Experimental Animals, Fukuoka, Japan) were used in this study. Standard strain of Helicobacter pylori (ATCC 43504; American Type Culture Collection, Rocville, MD, USA) was administered orally to 39 M. gerbils using a feeding tube after 24 h fast according to Hirayama's method (41). Thereafter, animals were housed in plastic cages and were fed with pellet food (CE-2, Clea Japan, Tokyo, Japan) and water *ad libitum.*. Infection with H. pylori was confirmed immunologically with the anti-H. pylori IgG antibody method (Pyloriset Dry; Daiichi Pure Chemicals, Tokyo, Japan) three months after inoculation. Another forty healthy 4-week-old M. gerbils were used as controls.

The 39 infected animals were divided into seven consecutive groups according to the duration of infection in: 3-month infection (3MI, n=4), 6-month infection (6MI, n=6), 9-month infection (9MI, n=6), 12-month infection (12MI, n=5), 18-month infection (18MI, n=6), 24-month infection (24MI, n=6), and 36-month infection (36MI, n=6). As age-matched paired controls, 40 healthy M. gerbils were divided into seven consecutive control groups: 4-month-old control (3MC, n=4), 7-month-old (6MC, n=6), 10-month-old (9MC, n=6), 13-month-old (12MC, n=6), 19-month-old (18MC, n=6), 25-month-old (24MC, n=6), and 37-month-old (36MC, n=6).

Infection was reconfirmed before animals were killed by ether anesthesia. The stomach was removed, opened along the greater curvature, and specimens were taken for phospholipid analysis from the following sites: the proximal fore-stomach (E-area), both the greater and the lesser curvatures of the funded gland area, and the pyloric area (P-area) of the stomach and from the duodenum (D-area) (Fig.2). Thereafter, the stomach was fixed in 10 % formal in for histological examination.

Phospholipid analysis

Phospholipids were extracted according to the method of Bligh and Dyer (42). To each specimen was added 1.2 ml of the following mixture solution: methanol, 10 mol/L hydrochloric acid (HCl), and fluorescein (0.2 mg/ml in methanol) at a ratio of 200: 2: 1 (v/v), and then added 0.6ml of chloroform. Thereafter, it was homogenized at room temperature and vigorously stirred for 5 sec. Then, 0.6 ml each of chloroform and double-distilled water were added to the homogenate to stir gently for 30 sec. After standing for 5 min, the homogenate was again stirred gently for another 5 sec and centrifuged at 1,700g for 10 minute to separate the aqueous and organic phases. The organic phase was then dried in a centrifugal evaporator and stored at –80℃. Furthermore, the protein content in the aqueous phase was quantified according to Bradford's method (43).

Phospholipid subclasses were separated by high performance liquid

chromatography (HPLC) according to the method of Chen and Kou (44) using a liquid chromatography system (LC-9A solvent delivery system, type 7125 injector, SPD-6 A variable wavelength detector, Chromatopac CR-AD integrating recorder; Shimadzu, Kyoto, Japan). The chromatographic 03.1.19column used was a 15 x 4.6mm stainless steel column filled with a silica gel of particles 5 μm in size (Shim-pack CLC-SIL; Shimadzu, Kyoto, Japan).

The homogenate specimens stored at –80℃ were dissolved in a 50 μL of chloroform, and 3 μL of the sample was applied to HPLC after 15 sec of vigorous stirring. Phospholipids of the specimen were eluted with the mobile phase of acetonitrile-methanol: 85% phosphoric acid solvent (130: 4:1.5, v/v) at the flow rate of 1 ml/min and a pressure of 37kgf/cm^2, and were detected at 203 nm.

Phospholipid subclasses were identified by comparison with pure standards of phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), lysophosphatidylcholine (LPC), and sphingomyelin (SPH). Among these pure standards, PI, PE and PC were obtained from bovine liver, and the others from bovine brain (Sigma Chemical Co., St. Louis, USA). The levels of each of the six phospholipids subclasses were evaluated by integrated counts of respective peaks with internal standard of fluorescein. Calibration curves for authentic subclasses were linear, enabling a quantitative analysis. The phospholipid content of gastric mucosa was expressed as μg phospholipid (PL)/mg protein.

Division of the fundic area of the stomach

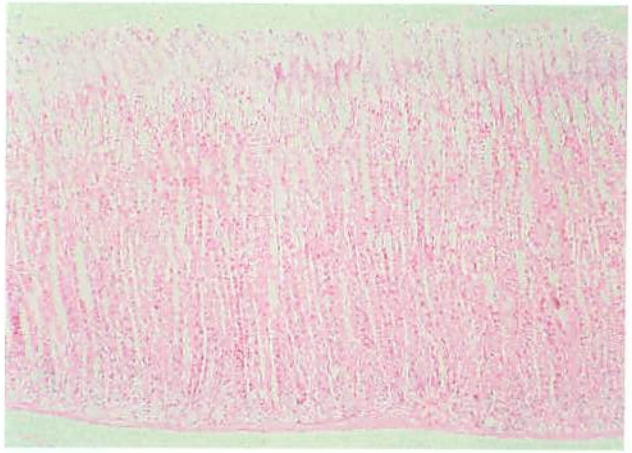
The fundic gland area was divided into three areas: F, f1 and f2. In the F-area, chief cells were observed continuously and a high density of pepsinogen granules was observed, visible as a dark blue area following hematoxylin-eosin (HE) staining. In the f1-area, the number of chief cells decreased and was visible as a light blue stain following HE. In the f2-area, parietal cells were scattered and chief cells were not seen (Fig.2).

Assessment of atrophic change of the stomach

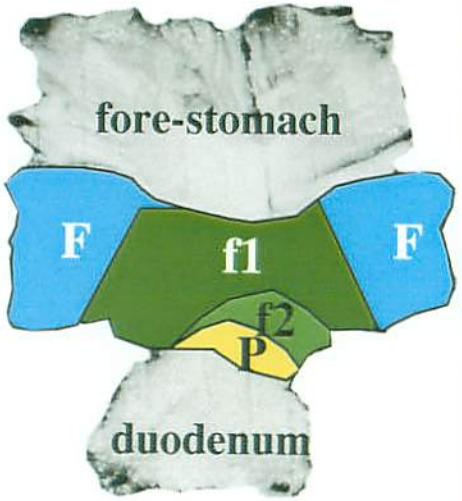
The location of the F-, f1-, f2- and P-areas was investigated in each histological section to delineate and measure the size of each area of the stomach, using a computer program (NHI 1.62 for Macintosh; National Institute of Mental Health). Atrophic changes in the gastric mucosa of the M. gerbil were classified into four grades according to the percentage of the F-area: non-atrophic, F-area ≥50%; mild atrophic changes, F-area 40<50%; moderate atrophic changes, F-area 20-<40%; and severe atrophic changes, F-area < 20%.

Statistical analysis

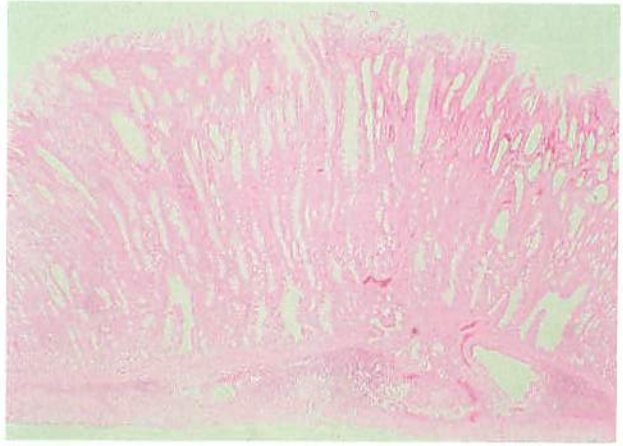
Data are expressed as mean ± SD. The Mann–Whitney *U*-test and Kruskal-Wallis were used for statistical analysis, and values of $P < 0.05$ were considered to be significant.



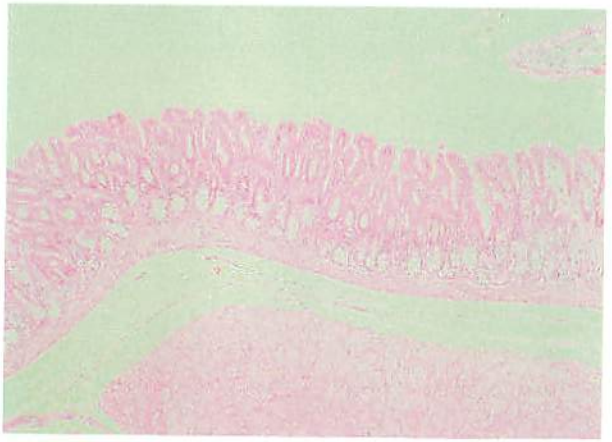
F Chief cells were observed continuously and densely (magnification x 25).



f1 Chief cells were observed discontinuously and not so densely (magnification x 25).



f2 Chief cells were not observable and parietal cells were observed dispersely (magnification x 10).



P Pyloric gland area (magnification x 25).

Figure 2. Parts of the stomach of M. gerbil. Specimens were taken from the fore-stomach, fundic area, pyloric area (P) and from the duodenum. The fundic gland area was subdivided into F, f1 and f2 areas.

RESULTS

Phospholipid content

Six subclasses of phospholipids were identified by the HPLC method in the order of PI, PS, PE, PC, LPC and SPH (Fig.3).

Total phospholipid content in the control gerbils

The total phospholipid content was deemed to be the sum of the six subclasses of phospholipids. In controls gerbils, total phospholipid content was 11.5 ± 5.0 in the E-area, 18.3 ± 10.1 in the F-area, 20.5 ± 6.3 in the f1-area, and 20.8 ± 9.4 in the P-area, and 37.0±11.2 μg PL/mg protein in the D-area. The total phospholipid content in the E-area was significantly lower than those of the other areas, and that of the D-area was significantly higher than those of the other areas ($P<0.001$). Meanwhile, no significant differences were found in the total phospholipid content among the F-, f1- and P-areas (Fig.4).

Total phospholipid content in the gastric mucosa of the control gerbils and aging

The data from the 3MC group, as well as the 3MI group were excluded from further statistical because gerbils of this group were at growing age (4 months old).

The mean of total phospholipid content in the F-, f1-, and P-areas was

expressed as the mean total phospholipid content in the gastric mucosa. The mean total phospholipid contents in the gastric mucosa were 23.5 ± 4.1, 16.3 ± 4.0, 18.8 ± 10.0, 16.3 ± 4.3, 18.3 ± 6.7, and 18.3 ± 8.4μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MC groups, respectively. The mean total phospholipid content in the gastric mucosa decreased significantly with aging ($P<0.001$; Fig.5).

Total phospholipid content in the F-area and aging in the control gerbils

The total phospholipid contents in the F-area were 24.0 ± 4.8, 16.6 ± 4.7, 12.6 ± 4.7, 14.6 ± 3.2, 16.4 ± 5.7, and 9.7 ± 1.6 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MC groups, respectively. The total phospholipid content decreased significantly with aging ($P<0.001$; Fig. 6).

Total phospholipid content in f1-area and aging in the control gerbils

The total phospholipid contents in the f1-area were 23.9 ± 3.0, 17.7 ± 2.8, 19.7 ± 5.8, 18.8 ± 4.9, 20.2 ± 10.0, and 23.1 ± 10.1 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MC groups, respectively. The total phospholipid content was not significantly different among the groups (Fig. 7).

Total phospholipid content in the P-area and aging in the control gerbils

In the control gerbils, the total phospholipid contents in the P-area were 22.1 ± 3.6, 13.2 ± 3.0, 25.1 ± 14.1, 16.01 ± 4.4, 19.7 ± 4.3, and 22.0 ± 2.7 μg

PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MC groups, respectively. The total phospholipid content was not significantly different among the groups (Fig.8).

Total phospholipid content in the duodenal mucosa and aging in the control gerbils

In the control gerbils, the total phospholipid contents in the duodenal mucosa were 37.4 ± 7.6, 39.8 ± 18.2, 45.3 ± 9.3, 32.9 ± 3.8, 36.2 ± 8.6, and 30.3 ± 4.6 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MC groups, respectively. The total phospholipid content was not significantly different among the groups (Fig.9).

Age-related correlation of atrophic change and total phospholipid content in the F-area of the control gerbils

In the control gerbils, the mean percentage F areas were 52.7±3.9, 52.8± 6.0, 44.1±5.0, 46.6±3.5, 44.2±3.5 and 33.3±7.7% in the 6-, 9-, 12-, 18-, 24- and 36MC groups, respectively. The percentage F area decreased significantly with aging ($P<0.01$, Fig 10). Furthermore, the total phospholipid content in the F-area decreased significantly with aging ($P<0.001$, Fig 10).

Contents of six phospholipid subclasses in control gerbils

Figure 11-a shows the contents of the six phospholipids subclasses in the

E-area of the control animals. The PS level was the highest out of the six subclasses (6.7 ± 2.8 μg PL/mg protein). The levels of PC (2.8 ± 1.7 μg PL/mg protein), PE (1.6 ± 0.7 μg PL/mg protein), LPC (0.9 ± 0.8 μg PL/mg protein), SPH (0.6 ± 0.3 μg PL/mg protein), and PI (0.5 ± 1.1 μg PL/mg protein) were much lower.

Figure 11-b shows the contents of the six phospholipids subclasses in the F-area of the control animals. The PC level was the highest out of the six subclasses (9.1 ± 4.9 μg PL/mg protein). The levels of LPC (2.9 ± 2.5 μg PL/mg protein), PE (2.7 ± 2.2 μg PL/mg protein), PS (2.2 ± 2.5μg PL/mg protein), SPH (1.8 ± 2.5 μg PL/mg protein), PI (0.5 ± 0.3 μg PL/mg protein) were much lower.

Figure 11-c shows the contents of the six phospholipids subclasses in the f1-area from the control animals. The PC level was the highest out of the six subclasses (8.5 ± 3.2 μg PL/mg protein), followed by PS (7.1 ± 2.4μg PL/mg protein). The levels of PE (3.1 ± 1.3μg PL/mg protein), LPC (1.8 ± 2.0μg PL/mg protein), SPH (0.9 ± 0.3μg PL/mg protein) and PI (0.5 ± 0.4μg PL/mg protein) were much lower.

Figure 11-d shows the contents of the six phospholipids subclasses in the P-area of the control animals. The PC and PS levels were the highest out of the six subclasses (8.5 ± 4.2 and 7.8 ± 3.9μg PL/mg protein), followed by PE (3.1 ± 1.4μg PL/mg protein). The levels of LPC (1.3 ± 0.8 μg PL/mg protein), SPH (0.8 ± 1.7μg PL/mg protein) and PI (0.5± 0.3μg PL/mg protein) were much lower.

Figure 11-e shows the contents of the six phospholipids subclasses in the

D-area of the control animals. The PC level was the highest out of the six subclasses (15.4 ± 5.3 PL/mg protein), followed by PE (11.6 ± 4.2 μg PL/mg protein) and PS (7.4 ± 4.1μg PL/mg protein). The levels of LPC (2.1 ± 1.8μg PL/mg protein), SPH (0.6 ± 0.8μg PL/mg protein) and PI (0.9 ± 0.5μg PL/mg protein) were much lower.

Total phospholipid content in the H. pylori infected gerbils

The f2-area (atrophic area) was excluded from statistical analysis considering that this area was not appropriate to evaluate the phospholipid-related mucosal protection due to the occurrence of various gastric diseases.

In the H. pylori infected gerbils, total phospholipid contents were 12.6± 6.7 in the E-area, 18.1± 11.4 in the F-area, 21.4 ± 8.4 in the f1-area, 23.5 ± 9.4 in the P-area, and 30.1± 11.9 μg PL/mg protein in the D-area, respectively (Fig. 12). As in the controls, total phospholipid content of the E- area was significantly lower than those of the other areas, and that of the D-area was significantly greater than those of the other areas ($P<0.001$).). In comparison between H. pylori and control gerbils, no significant differences were found in the E-, F-, f1- and P-areas.

Total phospholipid content in the gastric mucosa of the H. pylori infected gerbils and aging

In the H. pylori infected gerbils, the mean total phospholipid contents in

the gastric mucosa were 30.5 ± 13.7, 20.4 ± 8.1, 20.9 ± 12.3, 17.0 ± 6.0, 21.0 ± 6.9, and 19.0 ± 11.9 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MI groups, respectively (Fig.13). Total phospholipid content was significantly decreased in the older and longer infection groups ($P<0.05$) compared with the 6MI group. On the other hand, the contents did not show significant differences from those of age-matched control groups, suggesting that H. pylori infection does not affect phospholipid-related mucosal protection in the gastric mucosa (Fig.13).

Total phospholipid content in the F-area and aging in the H. pylori infected gerbils

In the H. pylori infected gerbils, the mean total phospholipid contents in the F-area were 34.6 ± 19.0, 15.5 ± 4.0, 22.0 ± 10.0, 12.6 ± 4.7, 16.7 ± 6.4, and 11.4 ±4.6 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MI groups, respectively (Fig. 14). The total phospholipid content was significantly lower in the 9- to 36MI groups, compared with the 6MI group ($P<0.01$). On the other hand, no significant differences were found between the age-matched control groups.

Total phospholipid content in fl-area and aging in the H. pylori infected gerbils

In the H. pylori infected gerbils, the mean total phospholipid contents in

the f1-area were 21.1 ±5.4, 23.8 ± 0.0, 24.5 ± 24.50, 15.4 ± 1.3 and 26.3 ± 5.8 μg PL/mg protein, in the 6-, 9-, 12-, 18- and 24MI groups, respectively (Fig. 15). The total phospholipid content was not significantly different among the groups. On the other hand, no significant differences were found between the age-matched control groups.

Total phospholipid content in the P-area and aging in the H. pylori infected gerbils

In the H. pylori infected gerbils, the mean total phospholipid contents in the P-area were 30.0 ± 7.2, 27.5 ± 9.6, 17.4 ± 13.9, 22.1 ±5.9, 23.5 ± 5.3, and 26.7±12.3 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MI groups, respectively (Fig.16). The total phospholipid content was not significantly different among the groups, similar to the result in the control gerbils. On the other hand, no significant differences were found between the age-matched control groups.

Total phospholipid content in the duodenal mucosa and aging in the H. pylori infected gerbils

In the H. pylori infected gerbils, the mean total phospholipid content in the duodenal mucosa were 35.5 ± 11.0, 35.8 ± 17.3, 31.1 ± 11.0, 33.1 ± 7.5, 31.0 ± 8.6, and 24.7 ± 9.4 μg PL/mg protein, in the 6-, 9-, 12-, 18-, 24-, and 36MI groups, respectively (Fig. 17). The total phospholipid content was not

significantly different among the groups, similar to the result in the control gerbils. On the other hand, total phopsholipid content was significantly lower in the 12MI group than in the 12MC group ($P<0.05$), whereas no significant differences were found between the others age-matched control-infected groups.

Age-related correlation of atrophic change and total phospholipid in the gastric mucosa of the H. pylori infected gerbils

In the H. pylori infected gerbils, the mean percentage F areas were 27.2± 2.3, 23.5±5.0, 28.0±8.1, 25.8±9.9, 14.5±10.1 and 7.1±7.6% in the 6, 9, 12, 18, 24 and 36 MI groups, respectively (Fig 18). The percentage F area decreased significantly with longer duration of infection with H. pylori ($P<0.01$). On the other hand the total phospholipid in the F-area decreased significantly in groups of longer infection ($P<0.001$). These results suggest that total phospholipid in the F area decreases with atrophic change that advances with the lapse of H. pylori infection in gerbils. However, interestingly, the age-related total phospholipid contents of the H. pylori infected gerbils were similar to those of the control gerbils in spite of severe atrophic changes with the infection.

Contents of the six phospholipids subclasses in the H. pylori infected gerbils.

Figure 19-a shows the contents of the six phospholipids subclasses in the E-area of the infected animals. The PS level was the highest out of the six subclasses (7.2 ± 3.7 μg PL/mg protein). The levels of PC (2.9 ± 1.8 μg PL/mg

protein), PE (1.8 ± 1.0 μg PL/mg protein), LPC (0.6 ± 0.5 μg PL/mg protein), SPH (0.6 ± 0.3 μg PL/mg protein), and PI (0.3 ± 0.4 μg PL/mg protein), were much lower. The contents of the six subclasses in the infected animals did not show significant differences from those in the control animals.

Figure 19-b shows the contents of the six phospholipids subclasses in the F-area of the infected animals. The PC level was the highest out of the six subclasses (9.4 ± 6.1 μg PL/mg protein). The levels of LPC (2.2 ± 1.3 μg PL/mg protein), PE (3.1 ± 2.7 μg PL/mg protein), PS (1.9 ± 1.4μg PL/mg protein), SPH (1.4 ± 0.9 μg PL/mg protein), and PI (0.8 ± 0.7 μg PL/mg protein) were much lower. The contents of the six subclasses in the infected animals showed no significant difference from those in the control animals.

Figure 19-c shows the contents of the six phospholipids subclasses in the f1-area of infected animals. The PC and PS levels were the highest out of the six subclasses (7.7 ± 3.3 and 7.6 ± 2.8 μg PL/mg protein, respectively), followed by PE (3.4 ± 1.9μg PL/mg protein). The levels of LPC (1.3 ± 0.8 and PL/mg protein), SPH (1.1 ± 0.4μg PL/mg protein) and PI (1.1 ± 2.0 μg PL/mg protein) were much lower. The contents of the six phospholipids subclasses in the infected animals did not show significant differences from those of the control animals.

Figure 19-d shows the contents of the six phospholipids subclasses in the P-area of the infected animals. The PC and PS levels were the highest out of the six subclasses (8.0 ± 3.3 and 9.0 ± 3.8 μg PL/mg protein), followed by PE (4.0 ±

1.5 μg PL/mg protein). The levels of LPC (1.4 ± 0.6 μg PL/mg protein), SPH (1.7 ± 1.1μg PL/mg protein) and PI (0.8 ± 0.9μg PL/mg protein) were much lower, showing a very similar pattern to that of the control gerbils.

Figure 19-e shows the contents of the six phospholipids subclasses in the D-area of the infected animals. The PC level was the highest out of the six subclasses (12.5 ± 5.5 μg PL/mg protein), followed by PE (8.7 ± 3.8 μg PL/mg protein) and PS (6.6 ± 4.2 μg PL/mg protein). The levels of LPC (1.5 ± 0.9μg PL/mg protein), SPH (SPH, 0.5 ± 0.3μg PL/mg protein) and PI (0.9 ± 0.5 μg PL/mg protein) were much lower, showing a very similar pattern to that of the control gerbils.

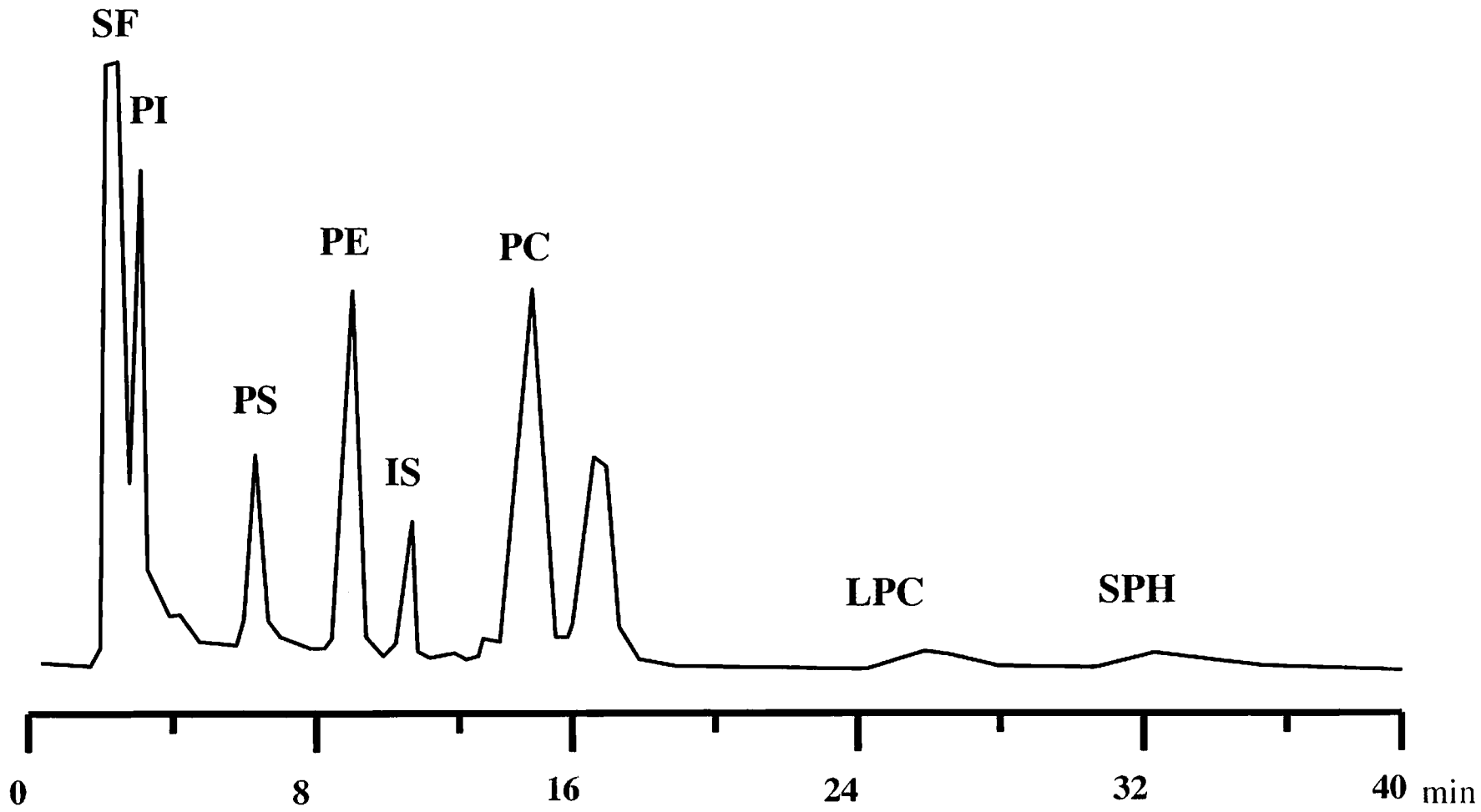


Figure 3. High performance liquid chromatography pattern of phospholipid extract from a biopsy specimen of gastric mucosa. Solvent front (SF), phosphatidylinositol (PI9, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SPH).

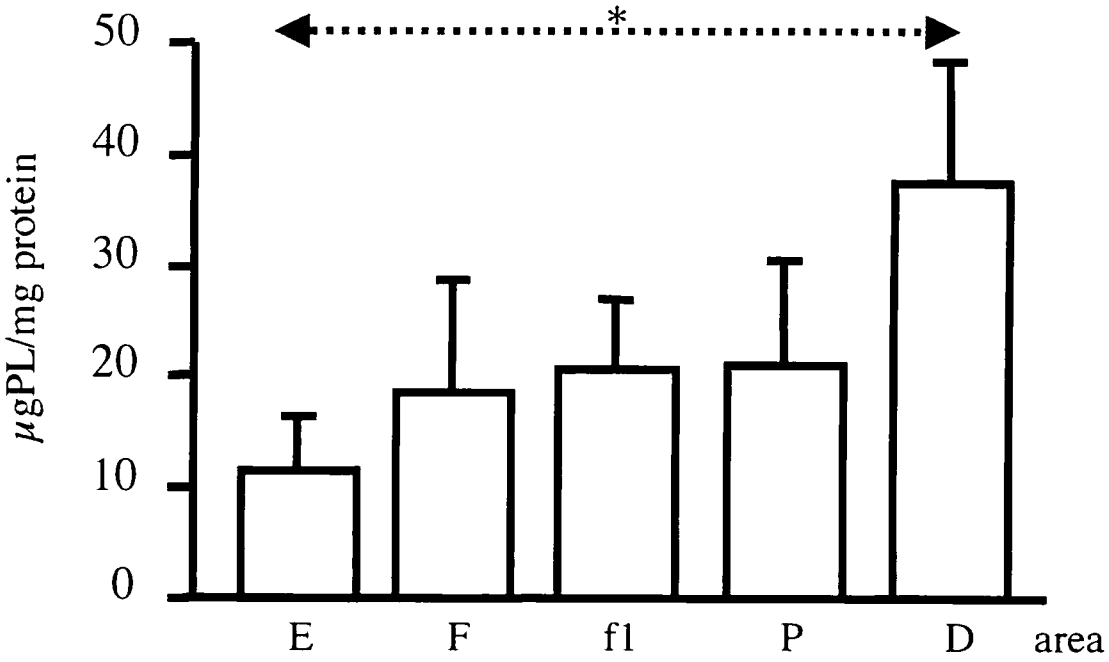


Figure 4. Total phospholipid contents in each area of the stomach and in the duodenum (D)from the control gerbils. The contents in the E-area was significantly lower than those in the F, f1, P and D areas, respectively (*P<0.001). The content in the D-area was significantly greater than those in the E-, F-, f1- and P-areas respectively (*P<0.001).

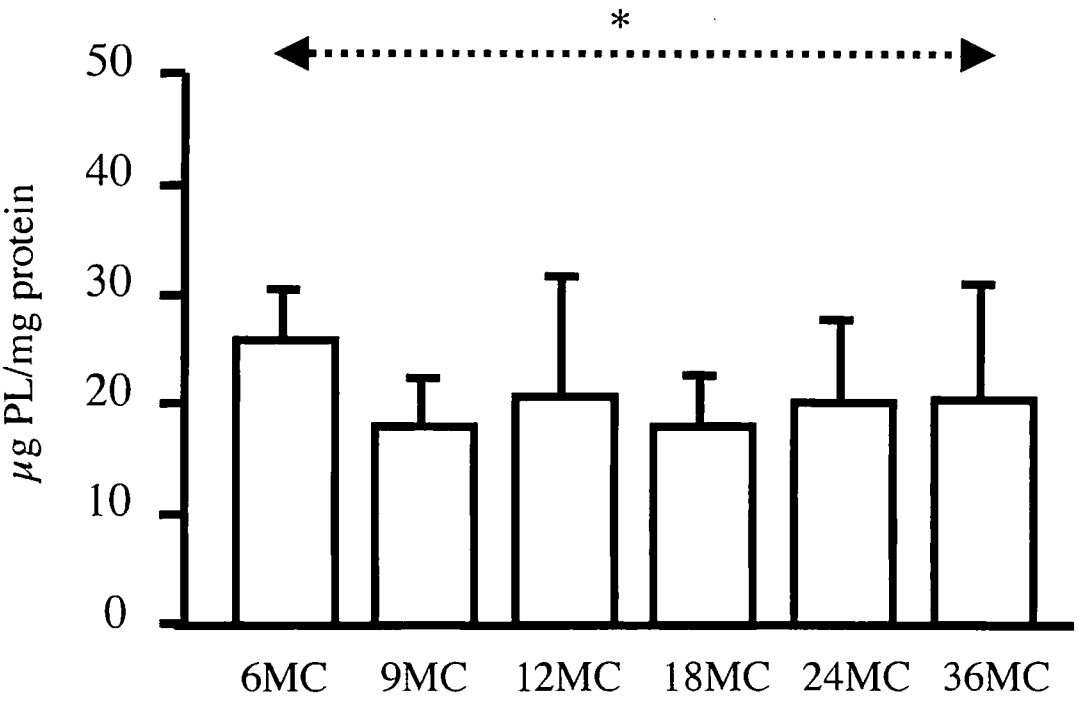


Figure 5. Mean total phospholipid content in the gastric mucosa of the control gerbils in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old)and 36MC (37-month-old), respectively.The total phospholipid content decreases significantly with aging (*P<0.001).

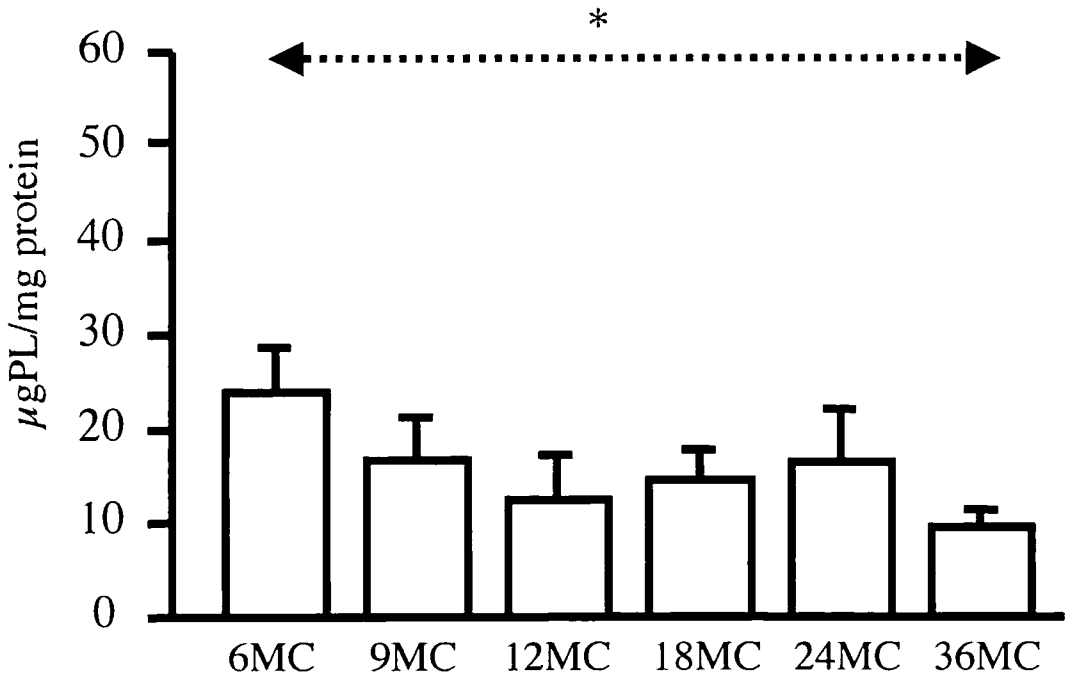


Figure 6. Total phospholipid contents in the F-area of control gerbils in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old)and 36MC (37-month-old), respectively. The content of total phospholipid decreases significantly with aging (*P<0.001).

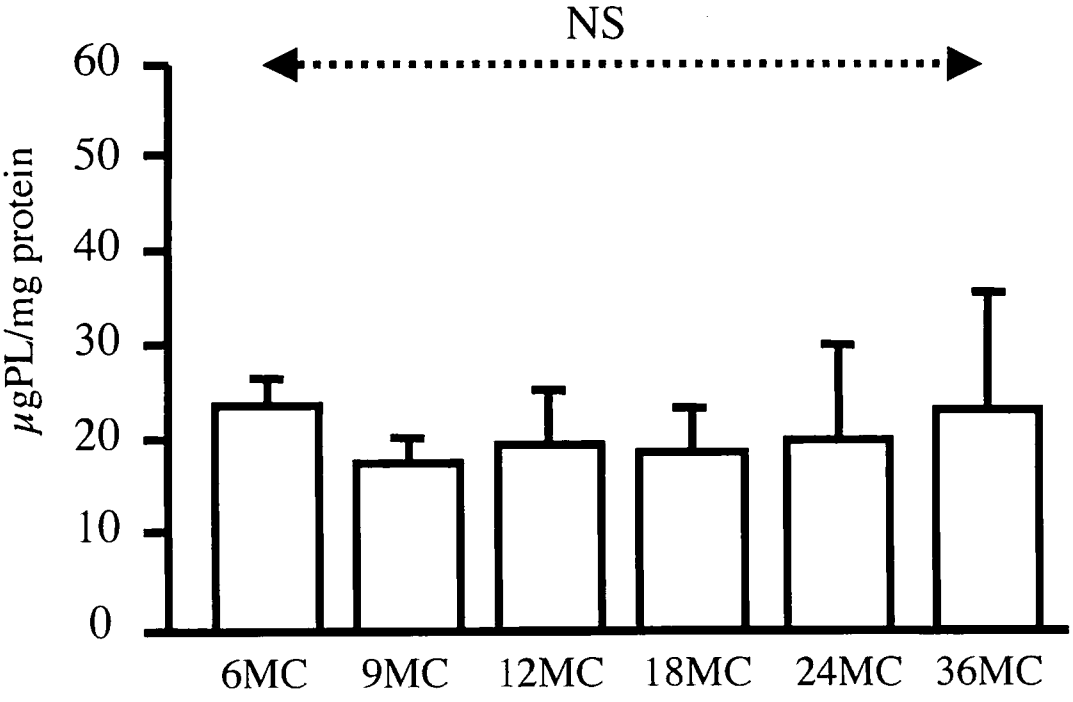


Figure 7. Total phospholipid contents in the f1-area of control gerbils in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old)and 36MC (37-month-old) groups, respectively.
Total phospholipid content was not significantly changed with aging.

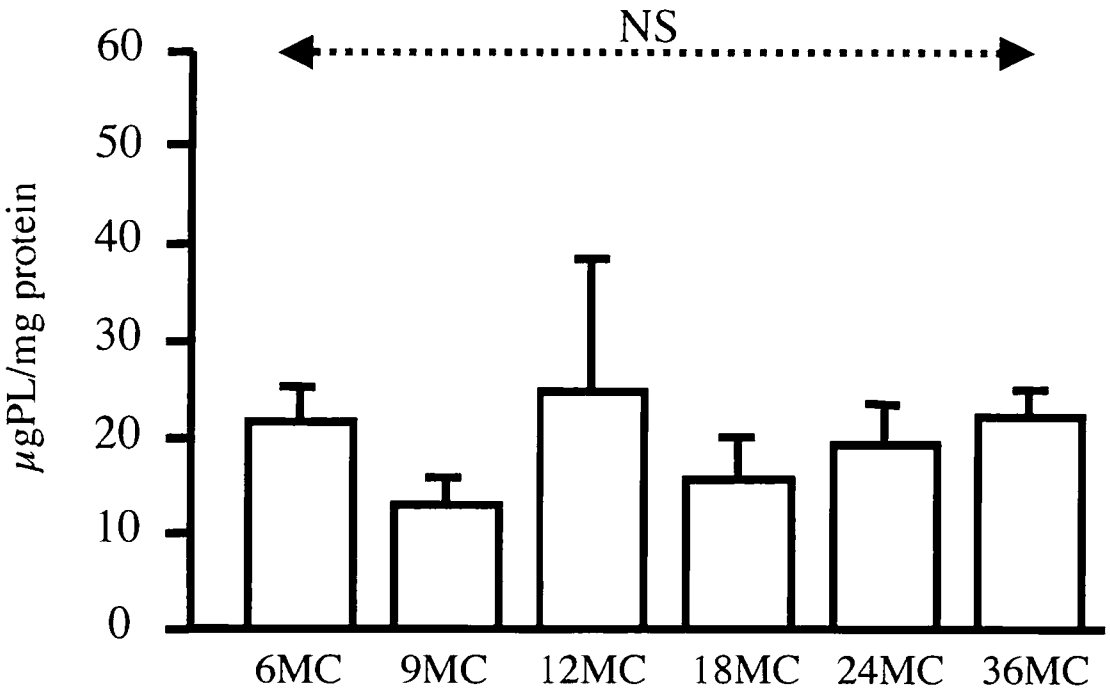


Figure 8. Total phospholipid contents in the P-area of control gerbils in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old)and 36MC (37-month-old groups, respectively. Total phospholipid content was not significantly changed with aging.

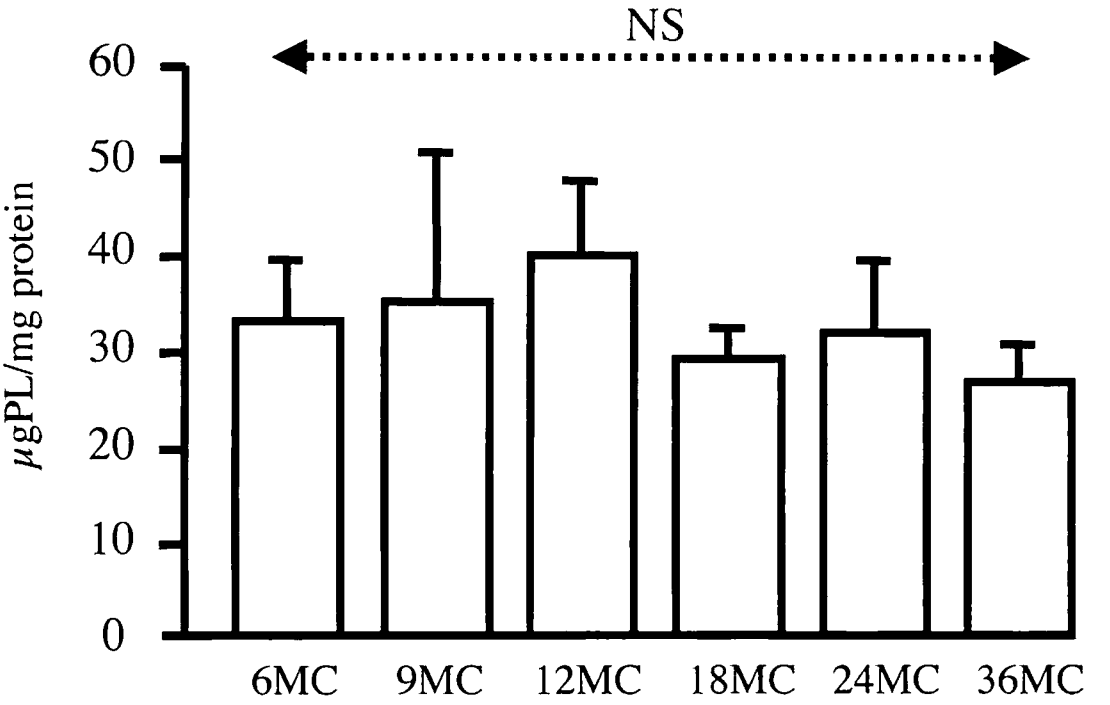


Figure 9. Total phospholipid contents in the D-area of control gerbils in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old)and 36MC (37-month-old) groups, respectively.Total phospholipid content was not significantly changed with aging.

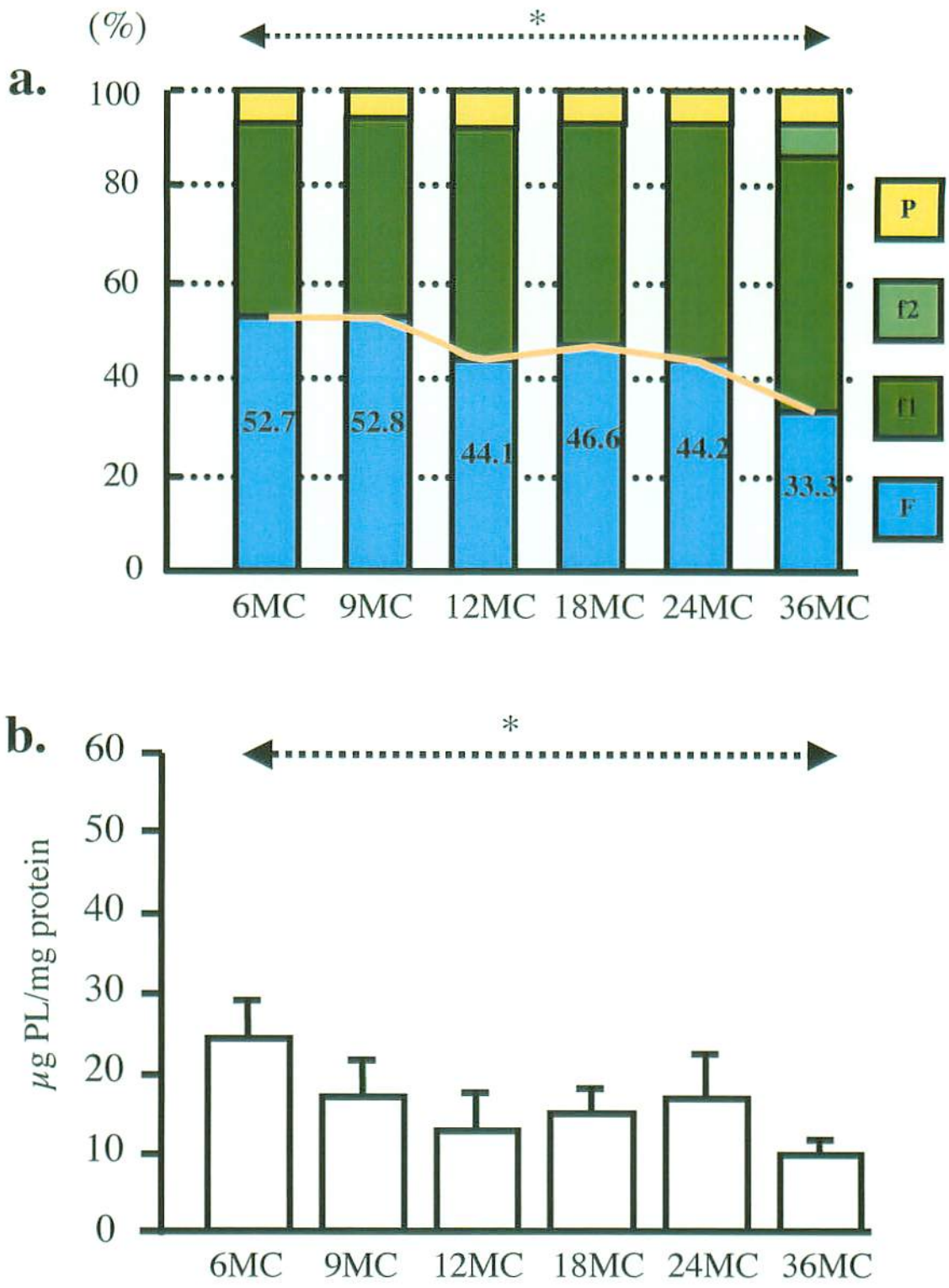


Figure10. Comparison between the grade of atrophic change and phospholipid content in the F-area in the 6MC (7-month-old), 9MC (10-month-old), 12MC (13-month-old), 18MC (19-month-old), 24MC (25-month-old) and 36MC (37-month-old) control groups, respectively. **a.**The percentage of F area decreases significantly with aging (*P<0.01). **b.** The content of total phospholipid in F-area also decreases significantly with aging (**P<0.001).

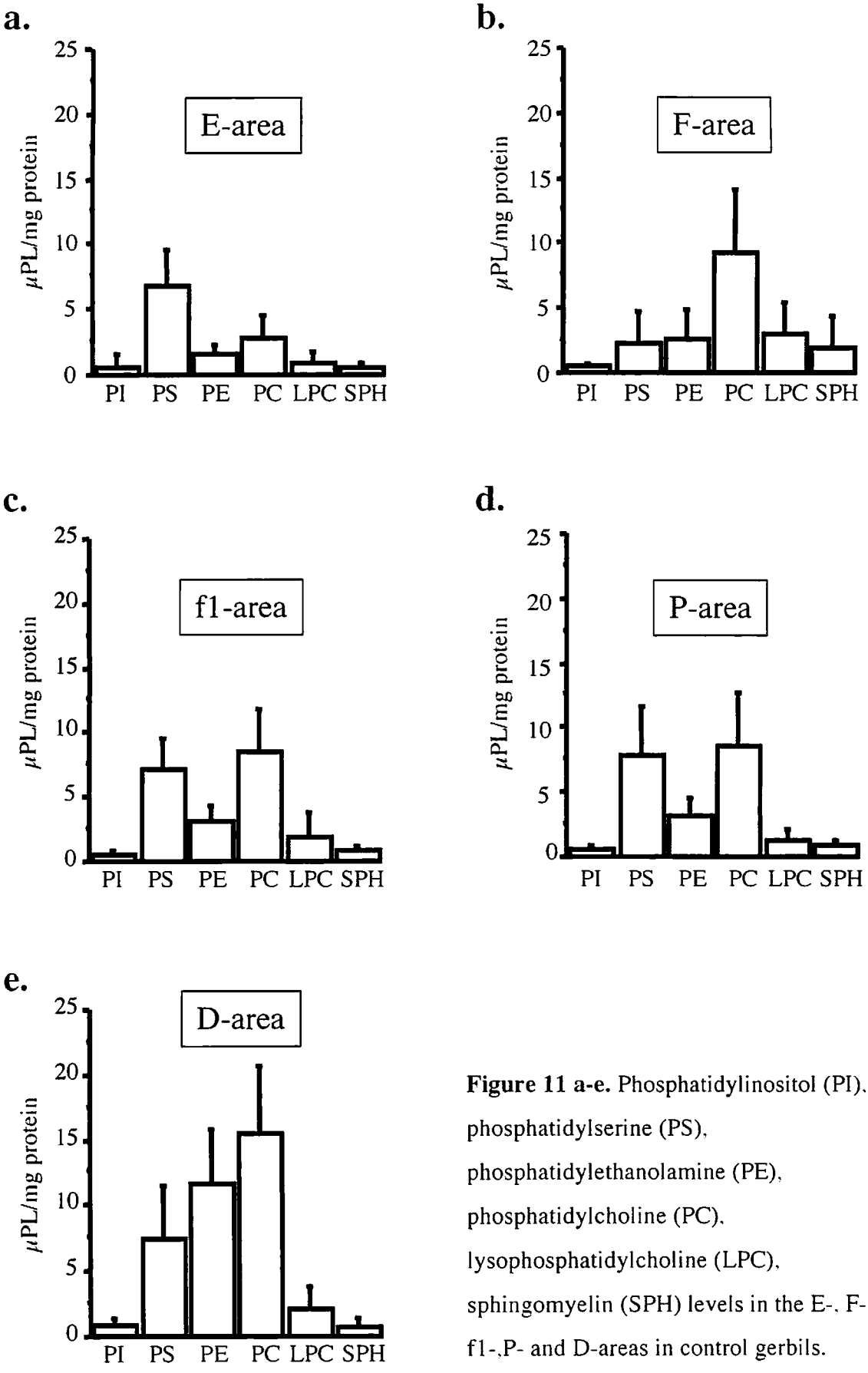


Figure 11 a-e. Phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SPH) levels in the E-, F-, f1-,P- and D-areas in control gerbils.

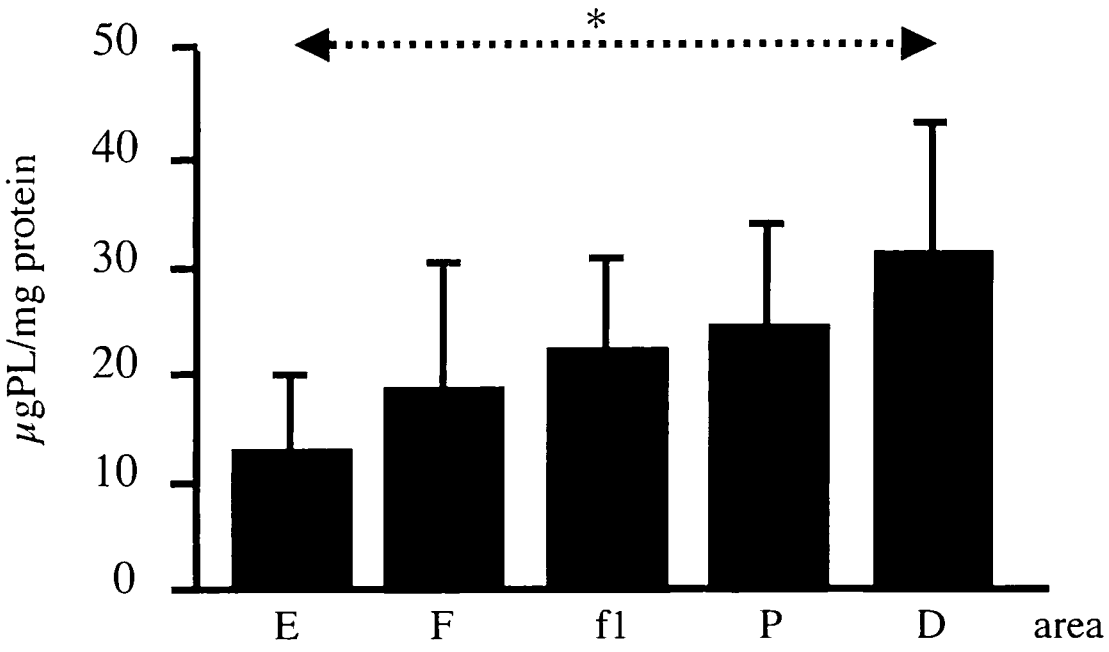


Figure 12. Total phospholipid contents in each area of the stomach and in the duodenum (D) from H pylori infected animals. The contents in the E-area was significantly lower than those in the F, f1, P and D areas respectively. (*P<0.001). The content in the D-area was significantly greater than those in the E-, F-, f1-, f2- and P-areas respectively (*P<0.001).

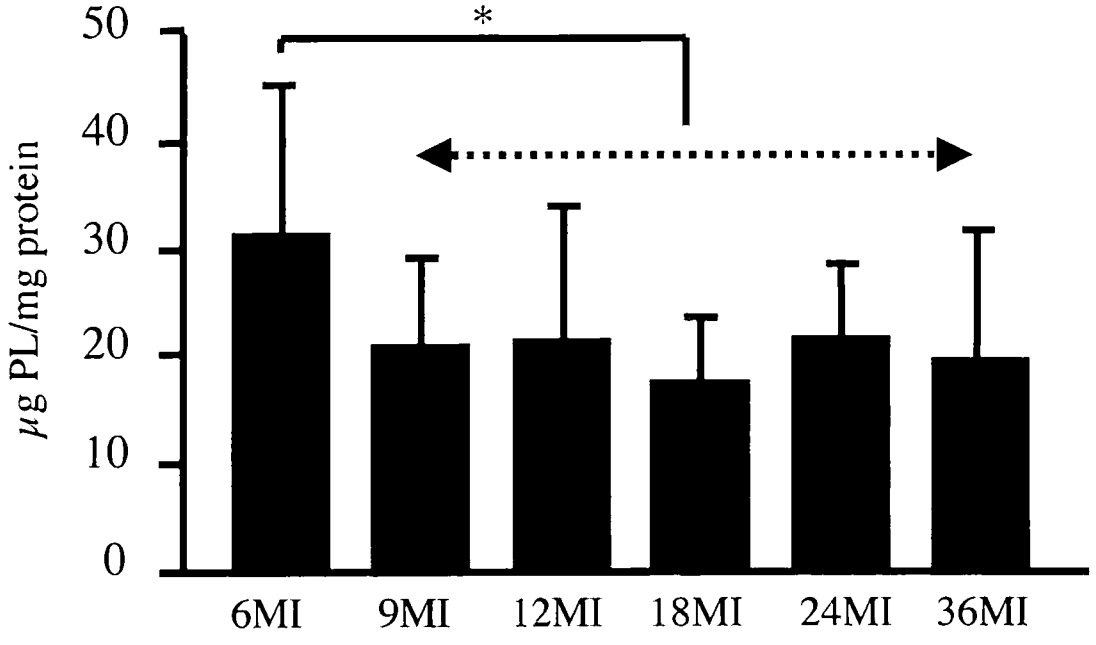


Figure 13. Mean total phospholipid content in the gastric mucosa of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. The total phospholipid content decreases significantly in the older and longer infection groups compared with the 6MI group (*P<0.05).

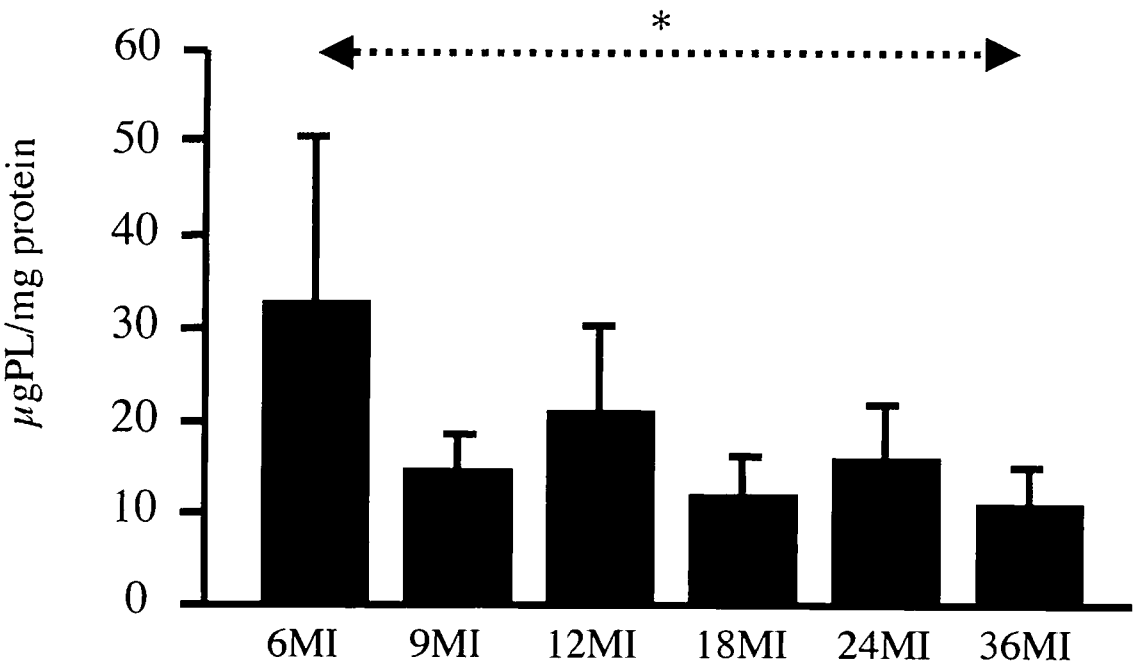


Figure 14. Total phospholipid content in the F-area of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. Total phospholipid content decreases significantly with aging and with the lapse of infection (*P<0.01).

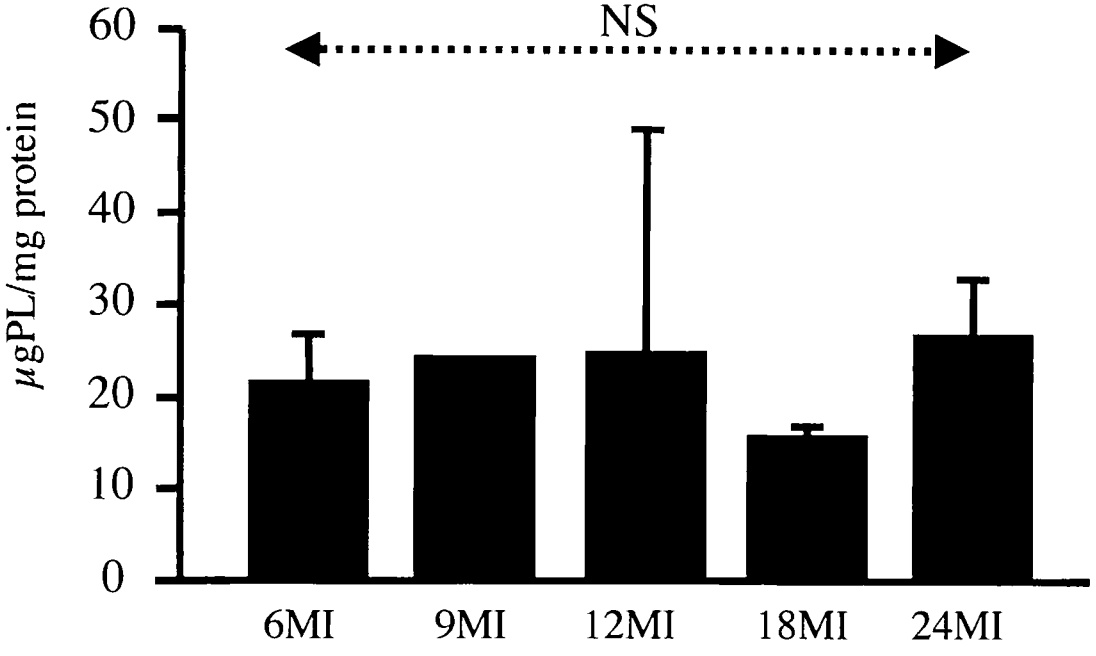


Figure 15. Total phospholipid content in the f-1area of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. Total phospholipid was not significantly changed with aging and with the lapse of infection .

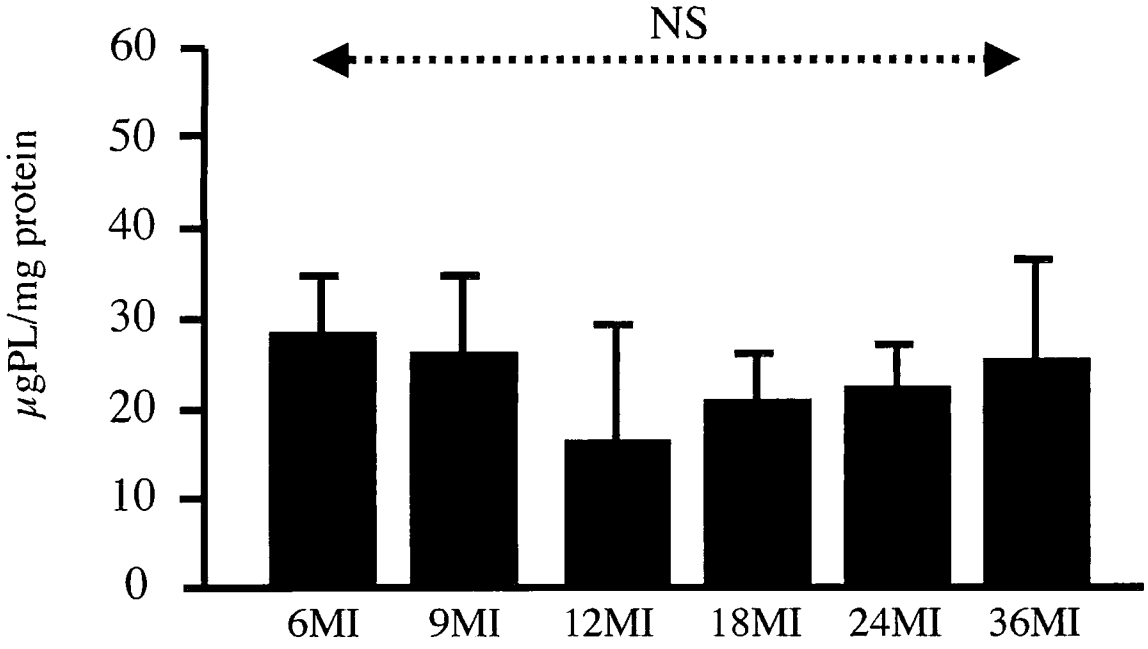


Figure 16. Total phospholipid content in the P-area of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. Total phospholipid was not significantly changed with aging and with thelapse of infection .

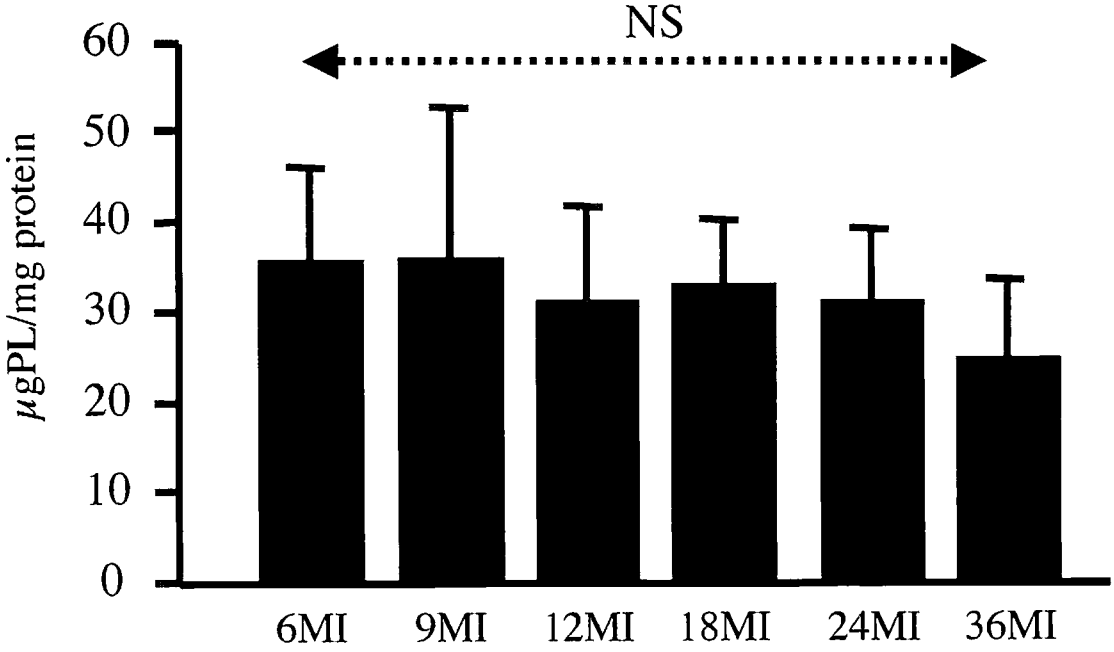


Figure 17. Total phospholipid content in the duodenal mucosa (D) of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. Total phospholipid was not significantly changed with aging and with the lapse of infection .

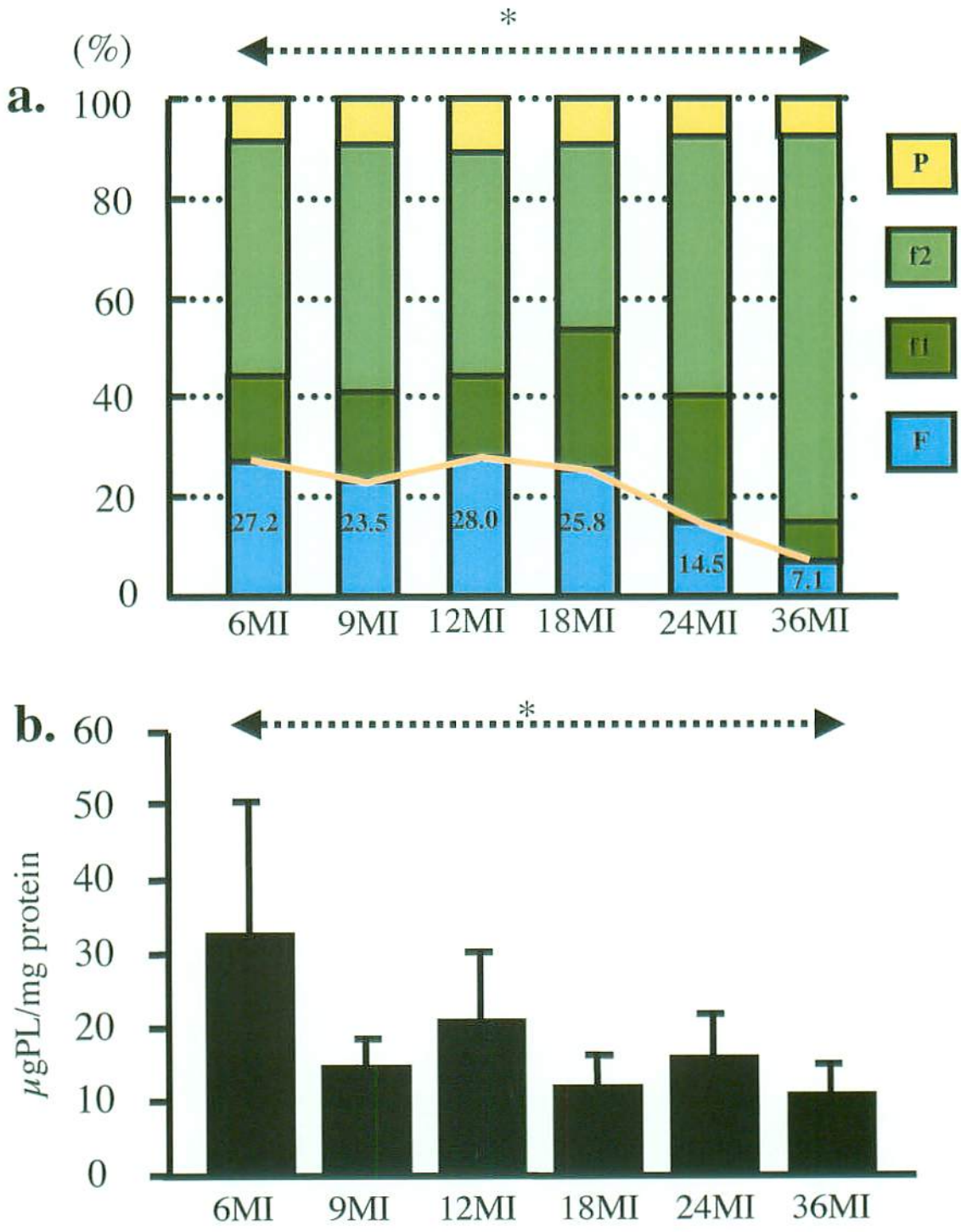


Figure 18. Comparison between the grade of atrophic change and phospholipid content in the F-area of H. pylori infected gerbils in the 6MI (6-month infection), 9MI (9-month infection), 12MI (12-month infection), 18MI (18-month infection), 24MI (24-month infection) and 36MI (36-month infection) groups, respectively. **a.** The percentage of F area decreases significantly with both aging and the lapse of infection (*P<0.01). **b.** The content of total phospholipid in the F-area also decreases significantly with both aging and the lapse of infection (**P<0.001).

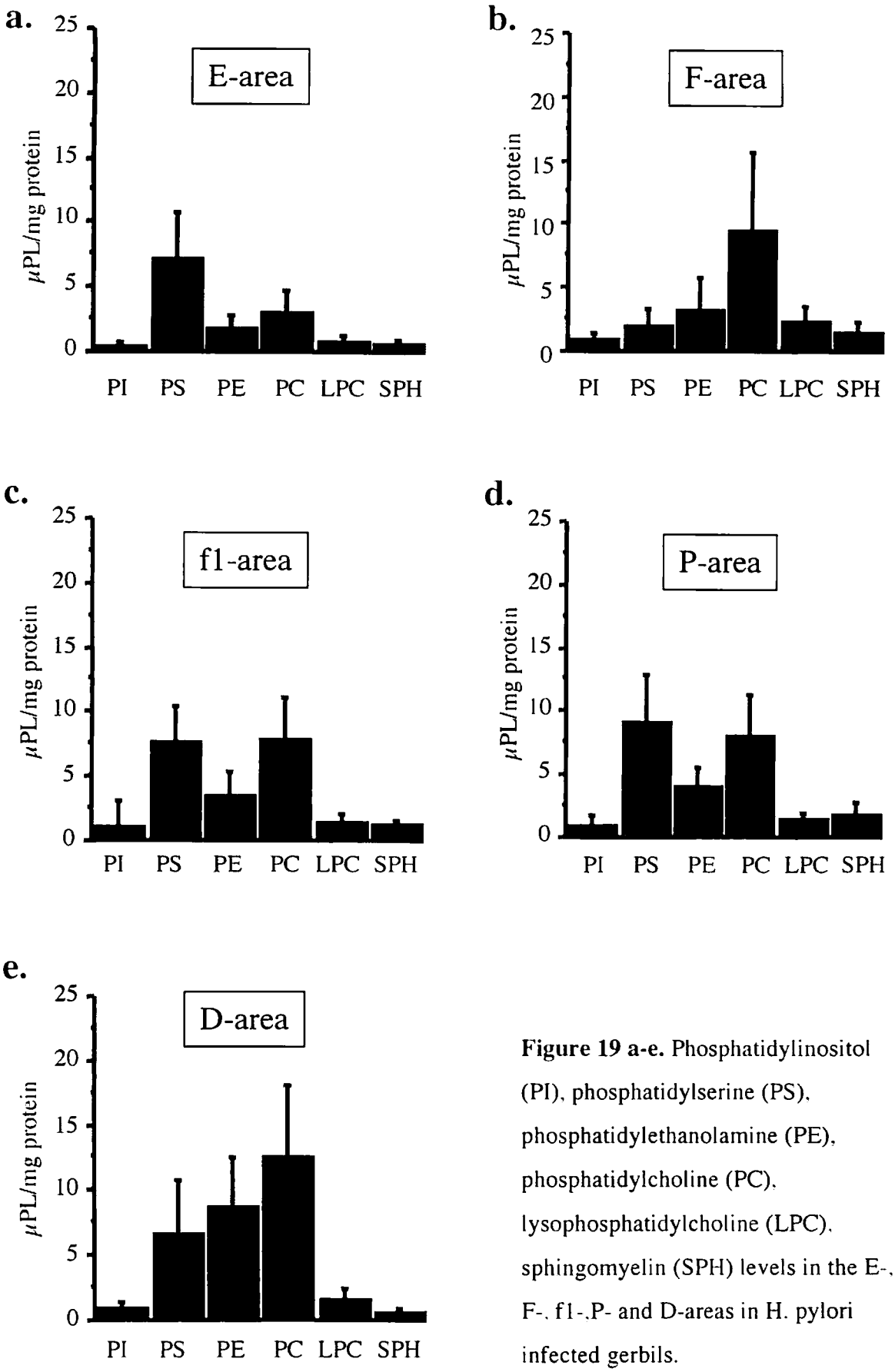


Figure 19 a-e. Phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC), sphingomyelin (SPH) levels in the E-, F-, f1-,P- and D-areas in H. pylori infected gerbils.

DISCUSSION

Various studies have shown that gastric mucosal phospholipids play an important role in the protective barrier of the gastric epithelium by their hydrophobic properties (7-25). H. pylori infection has attracted great attention since the bacillus was first identified from a patient with chronic gastritis by Warren and Marshall in 1983 (26). Many studies have clarified that H. pylori causes various gastric diseases (27-34). However, the mechanisms by which H. pylori induce such gastric disorders remain unclear.

Mongolian gerbils have been used as animal model to study the development of H. pylori-induced gastritis, gastric ulcer and to investigated the relationship between H. pylori infection and malignancy (40,41). Life span of M. gerbil is about 36 months, and puberty age is 8 weeks (2 months old) (45). Therefore, in this study, the 3MC and 3MI groups seem to be at growing age, and these groups were excluded from age-related statistical analysis. The 6- and 9MC, and the 6- and 9MI groups were young adults, the 12- to 24MC and the 12- to 24MI groups were adult and middle-aged animals, and the 36MC and 36MI groups were elderly animals.

Atrophic change has attracted much attention as age-related alteration of the gastric mucosa (46-49). In this study, the fundic area of the stomach of M. gerbil was divided into three areas: F, f1 and f2, to investigate changes in phospholipid-related protection in relation to mucosal atrophic changes

(background mucosa). In the F-area, chief cells were observed continuously and a high density of pepsinogen granules was observed, visible as a dark blue area following hematoxylin-eosin (HE) staining. In the f1-area, the number of chief cells decreased and was visible as a light blue stain following HE. In the f2-area, parietal cells were scattered and chief cells were not seen.

Unlike humans, M. gerbils have the fore-stomach, in addition to the fundic, pyloric, and duodenal mucosae. Consequently, specimens for mucosal phospholipid analysis were taken from the fore-stomach mucosa, F-, f1- and f2-areas of the fundic mucosa, pyloric mucosa, and duodenal mucosa.

This study shows that, in control M. gerbils, the total phospholipid content in the fore-stomach (E-area) was lower compared with those of the other areas of the stomach. The fore-stomach is lined with a layer of stratified squamous cells, and therefore it is non-glandular mucosa and mainly functions as a reservoir. Anatomically, there exists a circularly plicated structure around the border between the fore-stomach and glandular stomach. Actually, when the removed stomach of M. gerbil was opened, the fore-stomach was always filled with swallowed food. When had been fasted, M. gerbils swallowed their own hair to fill the fore-stomach. Considering these, the mucosa of the fore-stomach seems to be protected from reflux of gastric acid. This agrees with that content of total phospholipid is much lower in the fore-stomach mucosa than those of the other mucosae of control M. gerbils. Furthermore, among the six phospholipids subclasses, PS was the major phospholipid in this mucosa

The present study shows that the content of the total phospholipid is almost the same among the F-, f1- and P-areas in control M. gerbils, although the F-area is the main secreting site of gastric acid, and the others are less or non secreting sites. This result suggests that the phospholipid-related protection is similar in all areas of the gastric mucosa.

Lichtenberger et al. reported that the exogenous administration of phospholipids protects the rat gastric mucosa against hemorrhagic damage by strong acid (9). Furthermore, the same research group found that aspirin-induced damage to gastric mucosa was reduced by chemically associating aspirin with PC (50,51). Their research also suggested that PC and PE are of great importance, as part of a prostaglandin-induced cytoprotection of the gastric mucosa, which enhances hydrophobicity (9). Many others studies have reported the predominance of PC and PE for gastric mucosal protection (14,23-25). In the present study, among the six phospholipids subclasses, PC was measured at high levels in the glandular gastric mucosa, particularly in the F-area.

In the present study, PS was also measured at high levels in the f1- and P-areas. Moreover, the level of PS was similar in the fore-stomach, f1-, and P-areas, but its content was low in the F-area. In contrast, none of the previous researches has suggested the role of PS in gastric mucosal protection. Gutknecht and Walter reported that the HCl permeability of a PS bilayer membrane was approximately 50% lower than the HCl permeability of a PC bilayer membrane

(52). Graham and Lea also described that PS exhibited much lower permeability or greater hydrophobicity than PC (53). They also described that PS reduced its hydrophobicity at a higher pH (lower acidity), whereas PC did not change its hydrophobicity so much regardless pH. Therefore, it is highly like that PS is important in terms of repelling the diffusion of acid through the gastric mucosa.

It is widely accepted that prostaglandins (PGs) are important for protection of the gastric mucosa against various noxious agents (54-55), and increase the content of gastric mucosal phospholipid (9, 56-58). PGs are synthesized through cyclooygenase (COX) that catalyses the first two steps in the biosynthesis of the PGs from the substrate arachidonic acid (59). Arachidonic acid is released from the membrane phospholipids (particularly PC and PE) by the action of phospholipases (60). The findings described above suggest that PC seems to play its protective role as part of a prostaglandin-induced cytoprotection of the gastric mucosa rather than its hydrophobic property. This could explain that F-area does not need a higher content of total phospholipid compared with the other gastric areas to perform self-protection against gastric acid. Meanwhile, PS seems to exert mucosal protection by its hydrophobic property in the mucosae with less (the f1-area) or non-secreting (the fore-gastric and pyloric mucosae) gastric acid. This agrees with that in the research for humans, presented as part II in this thesis.

In this study, the total phospholipid content becomes low with atrophic change caused by aging, in the F-area, but not in the f1- or P-area. Therefore,

PC-supported cytoprotection in the oxyntic mucosa may be weakened with aging. It should be clarified in the future whether the weakening is due to reduced cell activity to produce phospholipid or due to reduced demand to response, namely reduction of acid output.

In the duodenal mucosa, the total phospholipid content was the highest. PC, PE and PS were the major phospholipids, and their levels were higher compared with the other areas. Anatomically and physiologically, the duodenum is the exposed site of bile juice that can damage the phospholipid coat by hydrolytic action. This may account for higher contents of phospholipids in the duodenum for mucosal protection.

Previous researchers have reported that H. pylori infection reduced the surface hydrophobicity of the gastric mucosa (35-38) and the phospholipid content (37) as a result of the action of H. pylori lipases and phospholipases A_2 in particular (61-65).Huhtinen et al. (66), however, did not find any correlation between the H. pylori status and the phospholipase A_2 catalytic activity concentration in gastric juice, and concluded that the main sources of phospholipase A_2 in gastric juice was probably other than H. pylori. Wakabayashi et al. (39) have reported that H. pylori infection reduce the content of PC in the gastric corpus, decreased linoleic acid composition and increased gastric mucosal arachidonic acid composition that may consequently cause the gastric mucosal barrier to be and weakened. Various studies have shown that mild irritant induced adaptive cytoprotection in the gastric mucosa (55, 67-68).

As for H. pylori infection, Brzozowski *et* al. stated that ammonia at the concentration produced by the bacilli was a mild irritant to induce adaptive cytoprotection in the rat stomach (69). Furthermore, recent studies reported that PG derived from both COX-1 and COX-2 was involved in adaptive cytoprotection developed in response to mild stressors (70-72).

In this study, the data from the H. pylori infection were compared with those from healthy controls. The results disclosed that H. pylori infection does not cause changes in phospholipid-related mucosal protection in the fore-stomach, gastric and duodenal mucosae. The results also disclosed that H. pylori infection does not affect the roles of PC in the glandular gastric mucosa, PS in the fore-stomach, glandular stomach, and PC, PE and PS in the duodenum. Therefore, H. pylori infection itself does not directly affect phospholipid-related mucosal protection. On the other hand, in relation to atrophic change of the gastric mucosa, total phospholipid content in the F-area decreased with atrophic change that advanced with the lapse of H. pylori infection. When compared with healthy controls, the H. pylori infected gerbils did not show a significant difference from healthy controls, in spite of severe atrophic changes. The findings of the present study suggest that the phospholipid-related mucosal protection in the gastric mucosa is strengthened in response to H. pylori infection as adaptive cytoprotection.

CONCLUSION

In conclusion, the present study suggests that the phospholipid-related protection in M. gerbils is the strongest in the duodenal mucosa, followed by gastric mucosa, and weak in the fore-stomach mucosa. The phospholipid-related protection is almost equal in the gastric mucosa. PC is the major phospholipid to protect the mucosa against gastric acid, probably by chemical protection in the mucosa. PS is also important for mucosal protection as a physical barrier in the fore-stomach and gastric mucosae, probably by its hydrophobicity. In the duodenal mucosa, phospholipids are enriched to protect the mucosa from hydrolytic action of bile juice. H. pylori infection seems to induce adaptive cytoprotection in the gastric mucosa. However, the infection does not cause remarkable changes in phospholipid-related mucosa protection.

Part II

INTRODUCTION

Gastric mucosa has a hydrophobic property (14,18) that plays an important role in gastric mucosal protection by repelling hydrogen ions (12-17). This property has been attributed to the presence of a phospholipid layer on the gastric epithelium identified from both animal and human gastric mucosae (7,13,19-25). However, little evidence has been presented about comparison of phospholipid contents between the fundic (oxyntic) and pyloric gland (non-oxyntic) mucosae.

The present study was carried out to compare the types and amounts of phospholipids in both the fundic and pyloric gland mucosae from patients with superficial gastritis, duodenal ulcer, gastric ulcer, or gastric cancer in order to clarified differences in mucosal phospholipid-related protection in each disease.

MATERIALS AND METHODS

Patients

One hundred and five patients were subjected to this study, consisting of 73 men and 32 women, with their age being 23-87 years old (median age 60.3± 16.3 years). Endoscopically, 20 of the patients had superficial gastritis; 30, duodenal ulcer; 50, gastric ulcer; and the remaining 5, gastric cancer (1 case was in gastric type and the others 4 cases were in intestinal type). The ulcer stage was evaluated using the classification of Sakita and Miyake et al in active (A), healing (H), and scar (S) stages (73,74) as follow: A-stage in 10, H-stage in 7, and S-stage in 13 patients for duodenal ulcer; and A-stage in 25, H-stage in 16, and S-stage in 9 for gastric ulcer patients. All patients gave their informed consents.

Tissue sampling

The patient underwent endoscopy to obtain four biopsy specimens. Two of them were taken from the greater curvature of the corpus corresponding to a site 3 cm proximal from the gastric angle on the lesser curvature (Fig.20). The other two were taken from a site 2 cm proximal to the pylorus on either the greater or lesser curvature of the antrum (Fig.20). Of these four specimens, two were used

for histological examination and the remaining two were stored at–80℃ for phospholipid analysis.

Phospholipid analysis

Phospholipids were extracted according to the method describe previously in the part I of this thesis.

Statistical analysis

Data are expressed as means ± SD Mann-Whitney's U test was used for statistical analysis, and values of $P < 0.05$ were considered to be significant.

RESULTS

Six subclasses of phospholipid were identified by HPLC method in the order of PI, PS, PE, PC, LPC and SPH.

Total phospholipid content

The total phospholipid was deemed to be the sum of the six subclasses of phospholipids. Total phospholipid content was significantly greater in the fundic gland mucosa than in the pyloric gland mucosa (19.4 ± 7.6 vs. 17.9 ± 6.4 μgPL/mg protein, $P<0.05$) in total cases (Fig.21).

The content was the highest in patients superficial gastritis (20.4 ± 8.5 for fundic gland mucosa; 20.1 ± 7.1 μgPL/mg protein for pyloric gland mucosa), followed by duodenal ulcer (18.7 ± 6.4 for fundic gland mucosa; 18.0 ± 6.1 μgPL/mg protein for pyloric gland mucosa). In these diseases, the content was not significantly different between the fundic and pyloric gland mucosae, although the content was less in the pyloric gland mucosa (Fig.21).

However, in patients with gastric ulcers, the content in the pyloric gland mucosa was significantly lower than that in the fundic gland mucosa (17.6 ± 6.5 vs. 19.4 ± 6.8 μgPL/mg protein , $P<0.05$) (Fig.21). There were no differences in total phospholipid content between the stages of ulcer in both gastric ulcer and duodenal ulcer patients.

In patients with gastric cancer, the content was the lowest and there was not a significant difference between the fundic and pyloric gland mucosae (17.4 ± 3.0 for fundic gland mucosa; 15.8 ± 4.8 μgPL/mg protein for pyloric gland mucosa) (Fig.21).

Contents of six phospholipid subclasses

The PC level was the highest out of the six phospholipid subclasses, followed by PS and PE and the levels of LPC, SPH, and PI contents were much lower in both gastric gland mucosae. This pattern of distribution did not differ among the four gastric diseases (Figs. 22-25).

Figure 22 shows the levels of the phospholipid subclasses in the fundic and pyloric gland mucosae from patients with superficial gastritis. The PS level was significantly greater in the pyloric gland mucosa than in the fundic gland mucosa (5.6 ± 1.9 vs. 4.7 ± 2.8 μgPL/mg protein, $P<0.05$). In contrast, the PE level was significantly greater in the fundic gland mucosa than in the pyloric gland mucosa (3.8 ± 1.52 vs. 2.7 ± 0.9 μgPL/mg protein, respectively; $P<0.01$). Similarly, the PC level was high in the fundic gland mucosa, compared with the pyloric gland mucosa (8.1 ± 3.4 vs. 7.6 ± 2.9 μgPL/mg protein, respectively), but the difference was not significant.

Figure 23 shows the levels of the phospholipid subclasses in the fundic and

pyloric gland mucosae from patients with duodenal ulcer. The PS and LPC levels were significantly greater in the pyloric gland mucosa than in the fundic gland mucosa (5.1 ± 1.6 vs. 4.3 ± 1.8 μgPL/mg protein, respectively; $P<0.01$ for PS ; 1.7 ± 0.9 vs. 1.5 ± 1.2 μgPL/mg protein, respectively; $P<0.05$ for LPC). In contrast, The PE level was significantly greater in the fundic gland mucosa than in the pyloric gland mucosa (4.0 ± 1.2 vs. 2.4 ± 0.7 μgPL/mg protein, respectively ; $P<0.0001$). PC content was also high in the fundic gland mucosa compared with the pyloric gland mucosa (7.3 ± 2.4 vs. 6.8 ± 1.9 μgPL/mg protein, respectively).

Figure 24 shows the levels of the phospholipid subclasses in the fundic and pyloric gland mucosae from patients with gastric ulcer. The PS level in the pyloric gland mucosa was not as high as that in the patient with superficial gastritis and duodenal ulcer (without significant difference between the pyloric and fundic gland mucosae : 5.0 ± 1.9 vs. 4.6 ± 2 μgPL/mg protein, respectively). In contrast, the levels of PE and PC were significantly higher in the fundic gland mucosa than in the pyloric gland mucosa (3.6 ± 1.1 vs. 2.4 ± 0.8 μgPL/mg protein, respectively ; $P<0.0001$ for PE; 7.6 ± 2.7 vs. 6.7 ± 2.5 μgPL/mg protein, respectively; $P<0.05$ for PC).

Figure 25 shows the levels of the phospholipid subclasses in the fundic and pyloric gland mucosae from patients with gastric cancer. The PS level was not

significantly different between the pyloric and fundic gland mucosae (4.8 ± 1.4 vs. 4.6 ± 1 μgPL/mg protein, respectively), similar to the case for gastric ulcer. In contrast, the PE level was significantly higher in the fundic gland mucosa than in the pyloric gland mucosa (3.2 ± 0.4 vs. 2.4 ± 0.7 μgPL/mg protein, respectively; $P<0.05$). The PC level was not significantly different between the fundic and pyloric gland mucosae (6.8 ± 1.3 vs. 6.0 ± 1.9 μgPL/mg protein, respectively).

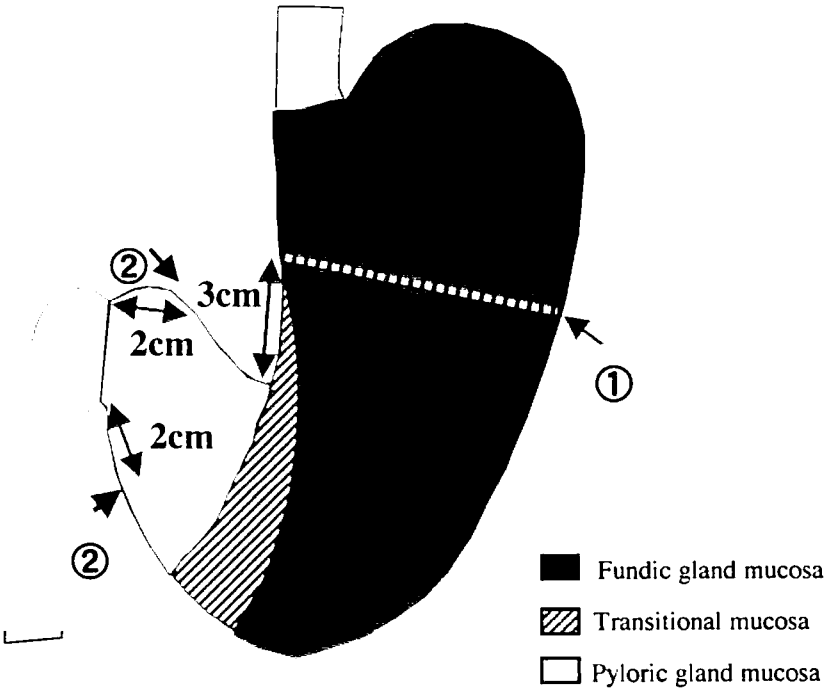


Figure 20. Sites were biopsy specimens were taken under endoscopy. ① Fundicgland mucosa on the greater curvature; ② Pyloric gland mucosa either on the greater or lesser curvature

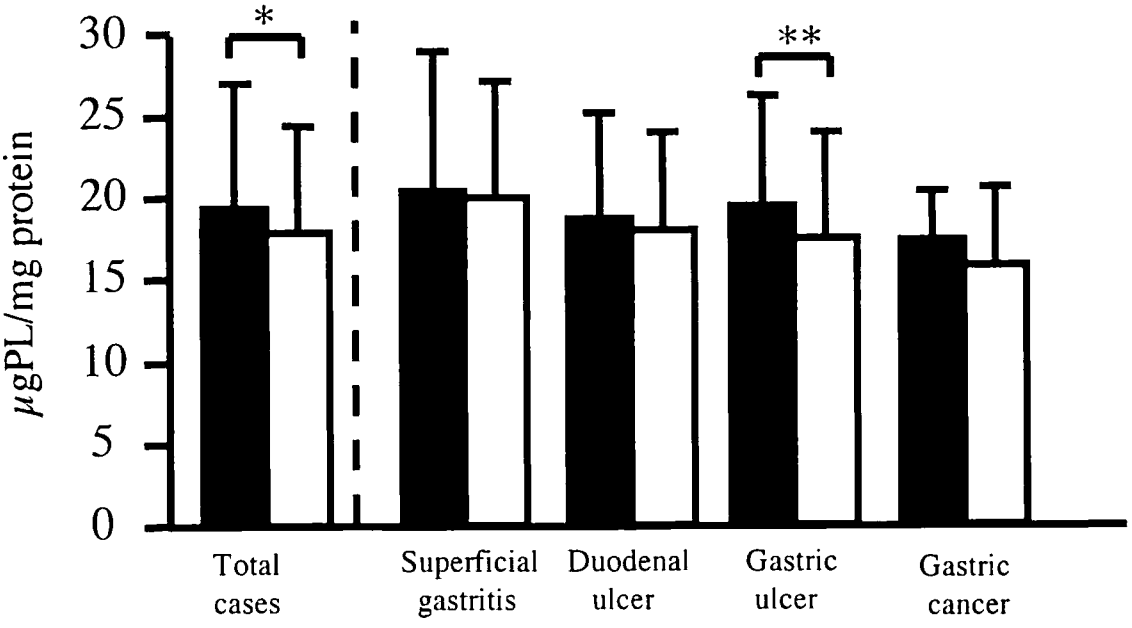


Figure 21. Total phospholipid contents in the fundic and pyloric gland mucosae in total cases, patients with superficial gastritis, those with duodenal ulcer, those with gastric ulcer and those with gastric cancer. The difference was significant between the fundic and pyloric gland mucosae in total cases (*P<0.05) and patients with gastric ulcer (**P<0.05).

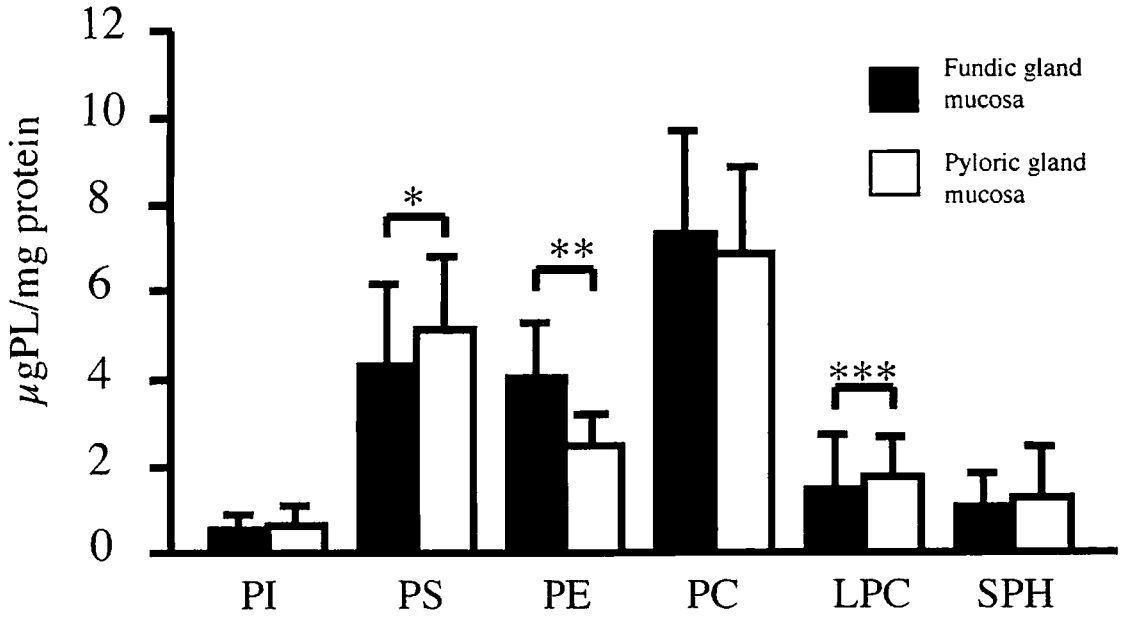


Figure 22. Phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC).lysophosphatidylcholine (LPC) and sphingomyelin (SPH) contents in the fundic and pyloric gland mucosae of patients with superficial gastritis.The difference was significant between the fundic and pyloric gland mucosae in PS (*P<0.05) and PE (**P<0.05) contents.

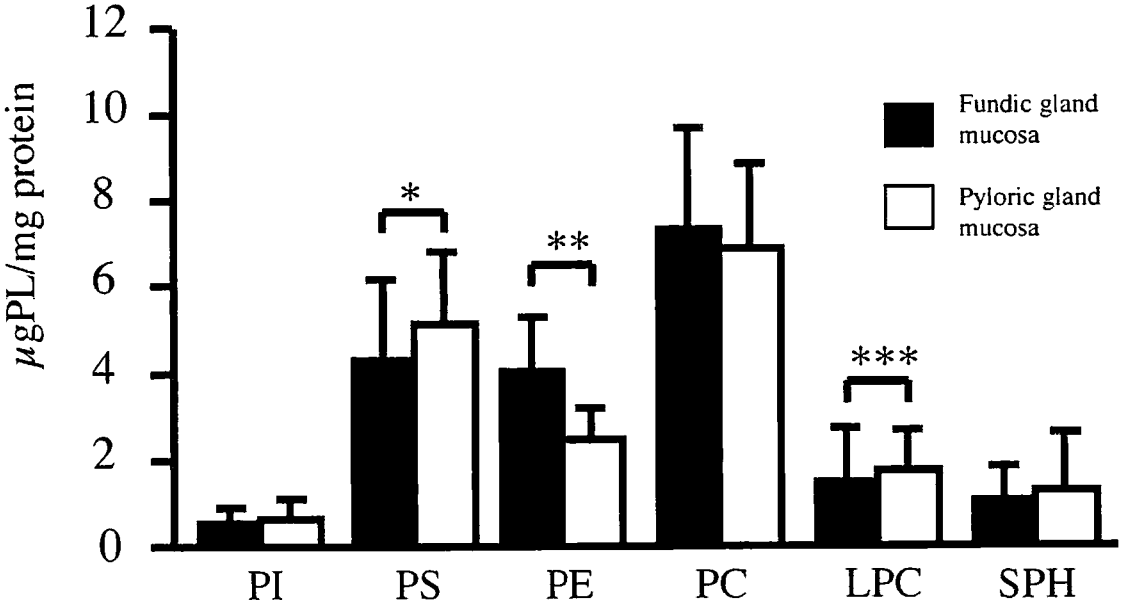


Figure 23. Phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), lysophosphatidylcholine (LPC) and sphingomyelin (SPH) contents in the fundic and pyloric gland mucosae of patients with duodenal ulcer.The difference was significant between the fundic and pyloric gland mucosae in PS (*P<0.05), PE (**P<0.0001)and LPC (***P<0.05) contents.

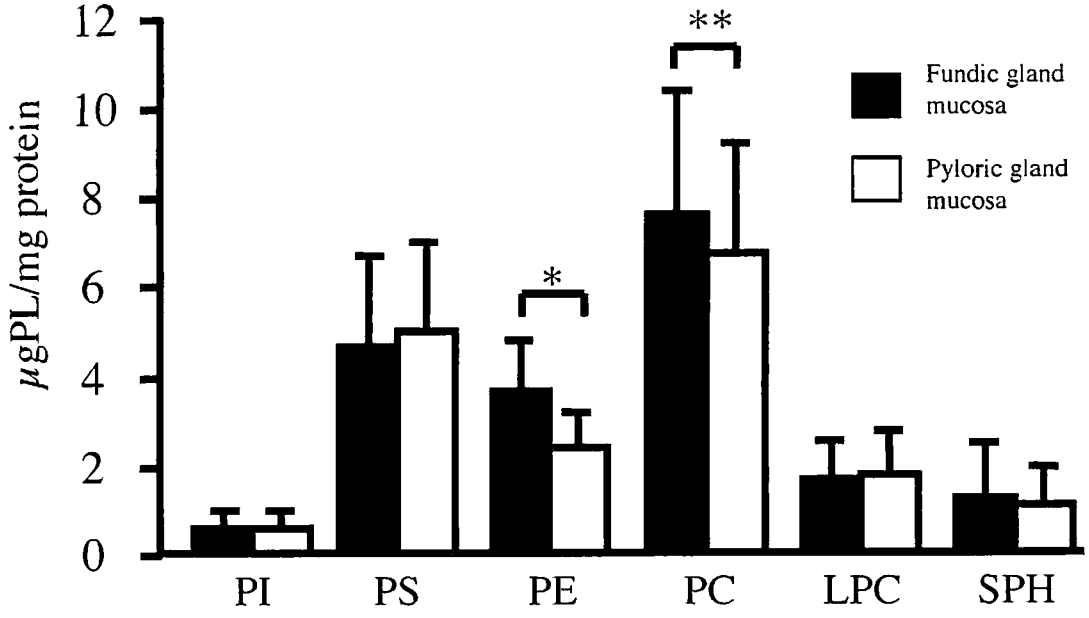


Figure 24. Phosphatidylinositol (PI), phosphatidylserine (PS),phosphatidylethanolamine (PE), phosphatidylcholine (PC),lysophosphatidylcholine (LPC), sphingomyelin (SPH) contents in the fundic and pyloric gland mucosae of patients with gastric ulcer.
The difference was significant between the fundic and pyloric gland mucosae in PE (*P<0.0001) and PC (**P<0.05) contents.

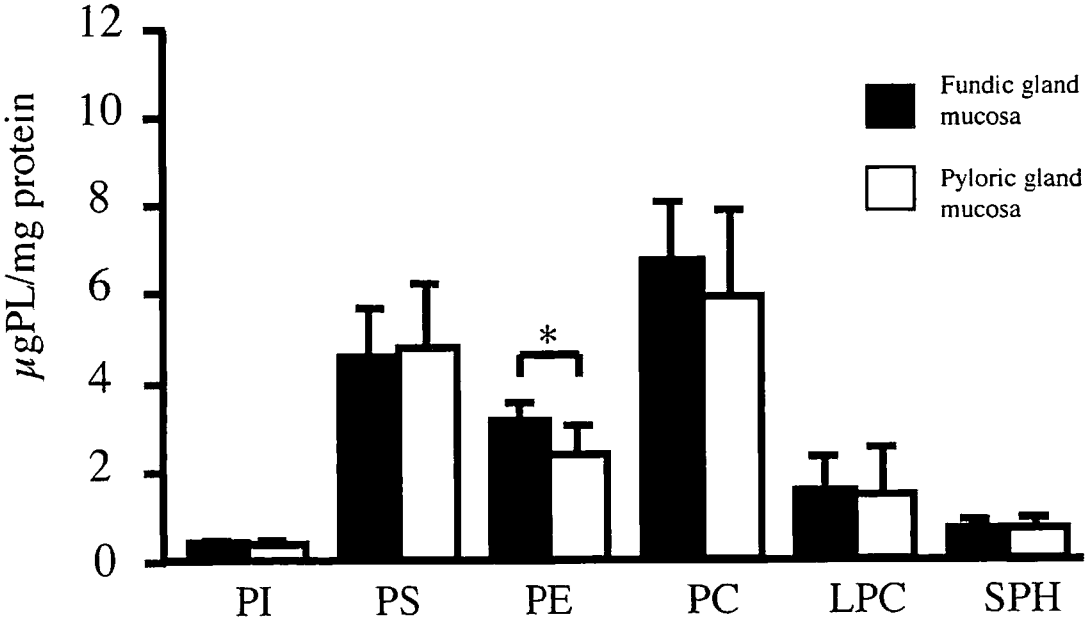


Figure 25. Phosphatidylinositol (PI), phosphatidylserine (PS),phosphatidylethanolamine (PE), phosphatidylcholine (PC),lysophosphatidylcholine (LPC) and sphingomyelin (SPH) contents in the fundic and pyloric gland mucosae of patients with gastric cancer.
The difference was significant between the fundic and pyloric gland mucosae in PE (*P<0.05) content.

DISCUSSION

Various studies have shown that gastric mucosal phospholipids play an important role in the protective barrier of the gastric epihelium by their hydrophobic properties (7-25). However, there have been very few reports on comparison between the fundic and pyloric gland mucosae.in terms of the hydrophobic barrier against gastric acid. In the present study, biopsy specimens were obtained from the two different sites: one from the fundic gland mucosa, and the other from the pyloric gland mucosa. In a previous study by Fujishima et al., it was found that the site of fundic gland mucosa is the area of maximum density of parietal cell (75), that is, the site of acid secretion. Therefore, we chose this site to investigate the role of phospholipid in the oxyntic gastric mucosa. We also chose the other site on the pyloric gland mucosa , in order to investigate the role of phospholipid in the non-oxyntic gastric mucosa.

In the present study, total phospholipid content was significantly greater in the fundic gland mucosa than in the pyloric gland mucosa in all four disease investigated, agreeing with the previous report by Maeda et al. (24). This result shows that the fundic gland mucosa possesses a stronger hydrophobic barrier compared with the pyloric gland mucosa. Consequently, it is highly likely that the fundic gland mucosa exerts a stronger protective barrier for self-protection against gastric acid.

Previous studies have suggested the importance of phospholipids in gastric

mucosal protection. Lichtenberger et al. reported that the exogenous administration of phospholipids protects the rat gastric mucosa against hemorrhagic damage by strong acid (9). Furthermore, the same research group found that aspirin-induced damage to gastric mucosa was reduced by chemically associating aspirin with PC (50,51). Their researches also suggested that PC and PE are of great importance, as part of prostaglandin-induced cytoprotection of the gastric mucosa, which enhances the hydrophobicity (9).

Many other studies have reported the predominance of PC and PE (14,23-25) for gastric mucosal protection. In the present study, PC (the highest) and PE (the third highest) were measured in high levels in all four gastric diseases investigated. In contrast, none of the previous researchers suggested that PS is also an important phospholipid, but we measured PS at the second highest level in all four gastric diseases investigated. Gutknecht et al. reported that the HCl permeability of PS bilayers membrane was approximately 50% lower than the permeability of a PC bilayers membrane (52). This indicates that PS is important in terms of repelling the diffusion of acid through the gastric mucosa.

Several reports have suggested that the surface-active phospholipid seems to be produced in surface mucus cells (21, 76,77), parietal cells (78-80) and chief cells (80). However, it is not clear which of these cells produce which specific subclass of phospholipid.

We found that PE level was significantly higher in the fundic gland mucosa than in the pyloric gland mucosa. Similarly, the PC level was higher in the fundic

gland mucosa compared with the pyloric gland mucosa in all four gastric diseases investigated, in agreement with a previous study by Maeda et al. (24). The findings of the present study suggest that the fundic gland mucosa requires greater levels of phospholipids, principally PE and PC, in order to bring about self-protection. In contrast, the PS level was higher in the pyloric gland mucosa than in the fundic gland mucosa in all patients, in agreement with Maeda et al. (24). This result suggests that PS is more important in the pyloric gland mucosa than in the fundic gland mucosa, in terms of the role of these phopsholipids in hydrophobic protection against gastric acid. However, further studies are needed to examine this hypothesis.

It is known that superficial gastritis and duodenal ulcer are associated with higher levels of gastric acid secretion, (81-83) but not gastric ulcer. In this study, the highest total phospholipid content was found in patients with superficial gastritis. Moreover, total phospholipid content did not differ significantly between the fundic and pyloric gland mucosae in patients with superficial gastritis or duodenal ulcer (Fig.6). Consequently, both the fundic and pyloric gland mucosae appear to possess strong enough defense mechanisms. This agrees with the fact that no ulcers arise in the stomach of patients with superficial gastritis or duodenal ulcer, as the diagnostic terminology indicates. However, the PS content was significantly higher in the pyloric gland mucosa than in the fundic gland mucosa in both superficial gastritis and duodenal ulcer. Therefore, it is highly likely that PS contributes to mucosal protection more in the pyloric gland

mucosa than in the fundic gland mucosa; as ptotection against the high levels of gastric acid secretion occurring in both superficial gastritis and duodenal ulcer.

In the present study, in gastric ulcer patients, the PE and PC levels were significantly lower in the pyloric gland mucosa than in the fundic gland mucosa. PS content was not as high in the pyloric gland mucosa, in contrast to cases of superficial gastritis and duodenal ulcer. We suggested that phospholipid-related protection is strong in the fundic gland mucosa, but is impaired in the pyloric gland mucosa. The findings of the present study agree with the fact that gastric ulcer almost never develop in the fundic gland mucosa (84).

Patients with gastric cancer showed the lowest total phospholipid content, and the lowest levels of PE and PC, in both gastric gland mucosae. Furthermore, the PS level was not as high in the pyloric gland mucosa, as it is in patients with gastric ulcer. Lower levels of phospholipids may reflect an impaired protective barrier of the gastric mucosa.

We found that the levels of PI, LPC, and SPH were much lower than the levels of PS, PE, and PC, in all patients. This suggests that PI, LPC, and SPH play a less important role in a protective hydrophobic barrier in the gastric mucosa.

CONCLUSION

In conclusion, we found that the fundic gland mucosa has a stronger phospholipid-related protection than the pyloric gland mucosa, based on the levels of mucosal phospholipids. The main phospholipids for gastric mucosal protection are PC, PE and PS; PC and PE are important in the fundic gland mucosa, and PS is important in the pyloric gland mucosa. Phospholipid-related protection appears to remain strong enough in patients with superficial gastritis or duodenal ulcer, but phospholipid-related protection is impaired in the pyloric gland mucosa of patients with gastric ulcer, and in both gastric gland mucosae in patients with gastric cancer.

REFERENCES

1. Slomiany B L, Sarosiek J, Slomiany A. Gastric mucus and the mucosal barrier.Dig.Dis. Sci. 1987; 5:125-145.
2. Code C F, Higgins J A, Moll J C, Orvis A L, Scholer J F. The influence of acid on the gastric absorption of water, sodium and potassium. J. Physiol. Lond. 1963; 166:110-119.
3. Code C F, Scholer J F,Orvis A L, and Higgins J A. Barrier offered by gastric mucosa to absorption of sodium. Am. J. Physiol. 1955; 183: 604.
4. Davenport H W. Gastric mucosal injury by fatty and acetylsalicylic acids. Gastroenterology 1965; 46: 245-253.
5. Davenport H W, Warner H A, and Code C F. Functional significance of gastric mucosal barrier to sodium. Gastroenterology 1964; 47: 142-152.
6. Schwartz K. Über penetrierende Magen und Jejunalgeschwüre. Beitr. Klin. Chir. 1910; 67: 96-128.
7. Slomiany A, Yano S, Slomiany B L, Glass G B J. Lipid composition of the gastric mucous barrier in the rat. J Biol chem. 1978; 253:3785-3791.
8. Slomiany A, Galicki N I, Kojima K, Banas-Gruszka Z, Slomiany B L. Glyceroglucolipids of the mucous barrier of dog stomach. Biochim Biophys Acta 1981; 665:88-91.
9. Lichtenberger L M, Graziani L A, Dial E J, Butler B D, Hills B A. Role of surface-active phospholipids in gastric cytoprotection. Science 1983;

219:1327-1329.

10. Murty V L N, Sarosiek J, Slomiany A, Slomiany B L. Effect of lipids and proteins on the viscosity of gastric mucus glycoproteins. Biochem Biophys Res Commun 1984; 121:521-529.
11. Sarosiek J, Slomiany A, Takagi A, Slomiany B L. Hydrogen ion diffusion in dog gastric mucus glycoprotein: effect of associated lipids and covalenty bound fatty acids. Biochem Biophys Res Commun 1984; 118:523-531.
12. Hills B A. Water repellency induced by pulmonary surfactants. J Physiol (Lond) 1982; 325:175-186.
13. Butler B D, Lichtenberger L M, Hills B A. Distributions of surfactants in the canine gastrointestinal tract and their ability to lubricate. Am J Physiol 1983; 244:G645-G651.
14. Hills B A, Butler B D, Lichtenberger L M. Gastric mucosal barrier: hydrophobic lining to the lumen of the stomach. Am J Physiol 1983; 244:G562-G568.
15. Hills B A. Gastric mucosal barrier: stabilization of hydrophobic lining to the stomach by mucus. Am J Physiol 1985; 249:G342-G349.
16. Goddard P J, Kao Y-CJ, Lichtenberger L M. Luminal surface hydrophobocity of canine gastric mucosa is dependent on a surface mucous gel. Gastroenterology 1990; 98:361-370.
17. Hills BA, Kirwood CA. Gastric mucosal barrier to hydrogen ions imparted by gastric surfactant in vitro. Gut 1992; 33: 1039-1041.

18. Spychal R T, Marrero J M, Saverymuttu S H, Nothfield T C. Measurement of surface hydrophobicity of human gastrointestinal mucosa. Gastroenterology 1989; 97:104-111.

19. Slomiany A, Slomiany BL, Horowitz MI. Studies on changes in lipid profiles of the rat gastric mucosa with stress ulcer. Clinica Chimica Acta 1975; 59: 215-226.

20. Wassef MK, Lin YN, Horowitz MI. Phospholipid-deacylating enzymes of rat stomach mucosa. Biochim. Biophys. Acta 1978; 528:318-330.

21. Wassef MK, Lin YN, Horowitz MI. Molecular species of phosphatidylcholine from rat gastric mucosa. Biochem. Biophys. Acta 1979; 573: 222-226.

22. Kao Y-C J, Lichtenberger L M. Localization of phospholipids-rich zones in rat gastric mucosa: possible origin of a protective hydrophobic luminal lining. J Histochem Cytochem 1987; 35:1285-1298.

23. Schmitz MGJ, Renooij W. Phospholipids from rat, human and canine gastric mucosa. Composition and metabolism of molecular classes of phosphatidylcholine. Gastroenterology 1990; 99:1292-1296.

24. Maeda M, Kiyohara H, Murakami A, Harada K, Misumi A, Ogawa M. Intramucosal phospholipids in human stomach: Distribution and relationship with mucosal atrophy. Kumamoto Med. J .1990; 43: 99-111.

25. Nardone G, Laccetti P, Civiletti C, Budillon G. Phospholipid composition of human gastric mucosa: a study of endoscopy biopsy specimens. Gut 1993; 34: 456-460.

26. Warren JR, Marshall BJ. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 1983; 1: 1273-1275.

27. Fujioka T, Kodama R, Honda S, Guei-Hua G, Nishizono A, Nasu M. Long-term sequelae of experimental gastritis with Helicobacter pylori: A 5-years follow-up study.

28. Honda S, Fujioka T, Tokieda M, Gotoh T, Nishizono A, Nasu M. Gastric ulcer, atrophic gastritis, and intestinal metaplasia caused by Helicobacter pylori infection in Mongolian gerbils. Scand J Gastroenterol 1998; 31: 454-460.

29. Hirayama F, Takagi S, Kusuhara H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in Mongolian gerbils infected with Helicobacter pylori. J Gastroenterol. 1996; 313: 755-757.

30. Genta RM, Gürer IE, Graham DY, et al. Adherence of Helicobacter pyori to area of incomplete intestinal metaplasia in the gastric mucosa. Gastroenterol. 1996; 111: 1206-1211.

31. Matsumoto S, Washizuka Y, Matsumoto Y, Tawara S, Yokota Y, and Karita M. Induction of ulceration and severe gastritis in Mongolian gerbil by Helicobacter pylori. J Med.Microbil. 1997; 46: 391-397.

32. Morgner A, Bayerdörffer E, Neubauer A, Stolte M. Gastric MALT lymphoma and its relationship to Helicobacter pylori infection: management and pathogenesis of the disease. Microsc Res tech 2000; 48:349-356.

33. Parsonnet J, Friedman GD, Vanderteen DP, Chang Y, Volgelman J, Orentreich

N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. N Engl J Med 1991; 325: 1127-1131.

34. The Eurogastric Study Group. An international association between Helicobacter pylori infection and gastric cancer. Lancet 1993; 341: 1359-62

35. Spychal RT, Goggin PM, Marrero JM, Saverymuttu SH, Yu CW, Corbishley CM, Maxwell JD, Northfield TC. Surface hydrophobicity of gastric mucosa in peptic ulcer disease. Relationship to gastritis and Campylobacter pylori infection. Gastroenterol. 1990; 98: 1250-1254.

36. Asante M, Ahmed H, Patel P, Davis T, Finlayson C, Mendall M, and Northfield T. Gastric mucosal hydrophobicity in duodenal ulceration: role of Helicobacter pylori infection density and mucus lipids. Gastroenterology 1997; 113: 449-454.

37. Nardone G, D'Armiento F, Corso G, Coscione O, Esposito M, Budillon G. Lipids of human gastric mucosa: effect of Helicobacter pylori infection and nonalcoholic cirrhosis. Gastroenterol. 1994; 107: 362-368.

38. Lichtenberger LM, Dial E, Ottlecz A, Romero JJ, Lechago JJ, Fox JG. Attenuation of hydrophobic phospholipid barrier is an early event in Helicobacter felis-induced gastritis in mice. Dig Dis Sci. 44(1): 108-115, 1999.

39. Wakabayashi H, Orihara T, Nakaya A, Miyamoto A, Watanabe A. Effect of Helicobacter pylori infection on gastric mucosal phospholipid contents and their fatty acid composition. J Gastroenterol Hepatol 1998; 13, 566-571.

40. Yokota K, Kurebayashi Y, Takayama Y, hayashi S, Isogai H, Isogai E, Imai K,

Yabana T, Yachi A, and Oguma K. Colonization of Helicobacter pylori in gastric mucosa of Mongolian gerbils. Microbiol. Immunol. Vol. 35(6), 475-480, 1991.

41. Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y. Establishment of gastric Helicobacter pylori infection in Mongolian gerbils. J Gastroenterol 31 (suppl IX): 24-28, 1996.
42. Bligh GH, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem. Physiol. 1959; 37: 911-917.
43. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72: 248-254.
44. Chen SS, Kou AY. Improved procedure for the separation of phospholipids by high-performance liquid chromatography. J. Chromat. 1982; 227: 25-31.
45. Troup GM, Smith GS, Walford RL. Life span, chronologic disease patterns, and age-related changes in relative spleen weights for the mongolian gerbils (Meriones unguiculatus). Exp Gerontol 1969; 4: 139-43.
46. Krasinski SD, Russell RM, Samloff IM, Jacob RA, Dallal GE, McGandy RB, Hartz SC. Fundic atrophic gastritis ion an elderly population. Effect on hemoglobin and several serum nutritional indicators. J Am Geriatr Soc 1986; 34: 800-806.
47. Kohli Y, Kato T, Suzuki K, Tada T, Fujiki N. Incidence of atrophic gastritis with age I Japan and Canada. Jpn J Med 1987; 26: 158-161.

48. Kasano T, Yoshida Y, Kihira K, Kimura K. Relationship between morphological and functional changes in the stomach with aging. Nippon Ronen Igakkai Zasshi 1991; 28: 606-610.

49. Miyazaki MS, Matsuda MG, Misumi A, Honmyo U, Murakami A, Murata H, Sagara K, Kurano R, Okabe H. Bloof flow, acidity and atrophic changes of the gastric mucosa in Mongolian gerbils infected with helicobacter pylori.

50. Kurinets A, Lichtenberger LM. Phosphatidylcholine-assocciated aspirin accelerates healing of gastric ulcers in rats. Dig. Dis. Science 1998; 43: 786-790.

51. Anand BS, Romero JJ, Sanduja SK, Lichtenberger LM. Phospholipid association reduces the gastric mucosal toxicity of aspirin in human subjects. Am. J. Gastroenterol. 1999; 94: 1818-1822.

52. Gutknecht J, Walter A. Transport of protons and hydrochloric acid through lipid bilayer membranes. Biochim. Biophys. Acta 1981; 641: 183-188.

53. Graham DE, Lea EJ. The effect of surface charge on the water permeability of phospholipid bilayers.Biochim Biophys Acta. 1972; 274: 286-93.

54. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, Hypertonic NaCl, and thermal injury. Gastroenterol 77: 433-443, 1979.

55. Robert A, Nezamis JE, Lancaster C, Davis JP, Field SO, Hanchar AJ. Mild irritants prevent gastric necrosis through "adaptive cytoprotection" mediated

by prostaglandins. Am J Physiol 8: G113-G121, 1983.

56. Lichtenberger LM, Richards JE, Hills BA. Effect of 16,16-dimethyl prostaglandin E_2 on the surface hydrophobocity of aspirin treated canine gastric mucosa. Gastroenterologgy 1985; 88:308-14.

57. Nishizawa Y, Moriga M. Effects of 15(S)-15-Methyl prostaglandin E_2 methyl ester on phospholipid metabolism in rat gastric mucosa. Biochem. Pharmacol. 1989; 38: 955-960,.

58. Scheiman J M, Kraus E R, Bonnville L A, Weinhold P A, and Boland C R. Synthesis and prostaglandin E_2 –induced secretion of surfactant phospholipid by isolated gastric mucous cells. Gastroenterology 1991; 100: 1232-1240.

59. Vane JR, Bakhle YS, Botting RM. Cyclooygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998; 38: 97-120.

60. Edward A. Phospholipase A_2 Mechanism: inhibition and role in arachidonic acid release. Drug Development research 1987; 10: 205-220.

61. Raedsch R, Stiehl A, Pohl S, Plachky J. Quantification of phospholipipase A_2 activity of Campylobacter pylori. Gastroenterol. 95:A404, 1988.

62. Lichtenberger LM, Hazzell SL, Romero JJ, Graham DY. Helicobacter pylori (Hp) hydrolysis of artificial phospholipid (PL) monolayers: insight into a potential mechanism of mucosal injury. Gastroenterol. 98:A78, 1990;

63. Langton SR, Cesareo SD. Helicobacter pylori associated phospholipase A_2 activity: a factor in peptic ulcer production?. J Clin Pathol 45:221-224, 1992.

64. Ottlecz A, Romero JJ, Hazell SL, Graham DY, Lichtenberger LM.

Phospholipase activity of Helicobacter pylori and its inhibition by bismuth salts. Biochemical and biophysical studies. Dig Dis Sci 38: 2071-80, 1993.

65. Berstad K, Berstad A Jr, Sjödahl R, Weberg R, Berstad A. Eosinophil cationic protein and phospholipases A_2 activity in human gastric juice. Sand J Gastroenterol 27: 1011-1017, 1992.

66. Huhtinen HT, Grönroos JM, Haapamäki MM, Nevalainen TJ. Phospholipase A_2 in gastric juice of Helicobacter pylori-positive and negative individuals. Clin Chem Lab Med 1998; 37(1): 61-64.

67. Brzozowski T, Konturek PC, Konturek SJ, Ernst H, Sliwowski K, Hahn EG. Mucosal irritation, adaptive cytoprotection, and adaptation to topical ammonia in the rat stomach. Scand J Gastroenterol 1996; 31: 837-846.

68. Konturek PC. Physiological, immunohistochemical and molecular aspects of gastric adaptation to stress, aspirin ant to H. pylori-derived gastrotoxins. J Physiol Pharmacol 1997; 48: 3-42.

69. Brzozowski T, Konturek PC, Konturek SJ, Drozdowicz D, Pajdo R, Pawlik M, Brzozowska I, Hahn EG. Expression of cyclooxigenase (COX)-1 and COX-2 in adaptive cytoprotection induced by mild stress. J Physiol Paris 2000; 94: 83-91.

70. Jackson LM, Wu KC,Mahida YR, Jenkins D, Hawkey CJ. Cyclooygenase (COX) 1 and 2 in normal, inflamed, and ulcerated human gastric mucosa. Gut 2000; 47: 762-770.

71. Takahashi S, Fujita T, Yamamoto A. Role of cyclooygenase-2 in

Helicobacter pylori-induce gastritis in Mongolian gerbils. Am J Physiol Gastrointest Liver Physiol. 2000; .279: G791-G798.

72. Takahashi M, Katayama Y, Takada H, Kuwayama H, Terano A. The effect of NSAIDs and a COX-2 specific inhibitor on Helicobacter pylori- induced PGE_2 and HGF in human gastric fibroblast. Aliment Pharmacol Ther 2000; 14 (1): 44-49.

73. Sakita T. Endoscopy in the diagnosis of early ulcer cancer. Clinics in Gastroenterology 1973; 2: 345-360.

74. Miyake T, Suzaki T, Oishi M. Correlation of gastric ulcer healing features by endoscopy, stereoscopic microscopy, and histology, and a reclassification of the epithelial regenerstive process. Dig Dis Sci 1980; 25: 8-14.)

75. Fujishima K, Misumi A, Akagi M. Histopathologic study on development and extension of atrophic change in the gastric mucosa. Gastroentrol. Japonica 1984; 19: 9-17.

76. Kao Y-C J, and Lichtenberger L M. Phopsholipid-and neutral lipid-containing organelles of rat gastroduodenal mucous cells. Possible origin of the hydrophobic mucosal lining. Gastroenterology 1991; 101: 7-21.

77. Scheiman JM, Kraus ER, Bonnville LA, Weinhold PA, Boland CR. Synthesis and prostaglandin E_2 –induced secretion of surfactant phospholipid by isolet gastric mucous cells. Gastroenterology 1991; 100: 1232-1240.

78. Itoh K, Watanabe T, Imatake K, Nagata T, Katoh K, Itoh E, Iwasaki A, Matsuo Y. Biological significance of phospholipids in the rat stomach from

the viewpoint of electron microscopy. Scand J Gastroenterol. 1989; 24: 23-26.

79. Hills B A. A physical identity for the gastric mucosal barrier. Med. J. Aust. 1990; 153: 76-81.

80. Ueda S, Kawamura K, Ishii N, Matsumoto S, Hayashi O, Okayasu M. Morphological studies on surface lining layer of the lung . Part VI. Surfactant-like substance in other organs (pleural cavity, vascular lumen and gastric lumen) than lungs. J. Med. Soc. Biol. Interface 1986; 17:132-156.

81. Cherry Dennis J, Fung WP, Matz LR. Gastric acid secretion in chronic atrophic gastritis and chronic (superficial) gastritis. Med J Aust. 1977; 1:813-814.

82. Christiansen PM. Gastric acid secretion in relation to duodenal and gastric ulcer. In: The physiology of gastric secretion. Edited by LS Semb, Myren J. Oslo, Universitetsforlaget, 1968; 569-576.

83. Krentz K. Gastric secretion in relation to gastric cancer and precancerous states. In: The physiology of gastric secretion. Edited by LS Semb, Myren J. Oslo, Universitetsforlaget, 1968; 595-604.

84. Oi M, Ito Y, Kumagai F, Yoshida K, Tanaka Y, Yoshikawa K, Miho O, Kijima M. A possible dual control mechanism in the origin of peptic ulcer. Gastroenterol. 1969; 57: 280-93.