Title: Why does gastric acid damage the oesophagus? The absence of a biochemical protective barrier preventing oesophageal epithelial damage in gastro-oesophageal reflux

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Abstract

The distribution of pepsinogen 11 and agmatine has been studied in human oesophageal and gastric biopsies in normal patients and patients with a hiatus hernia having gastro-oesophageal reflux. Pepsinogen 11 has been found in the chief cells of the stomach, gastric gland lumen, gastric lumen and the parietal cell canaliculi but minimal amounts have been found in the oesophageal biopsies.

There is considerable agmatine in the gastric mucosa particularly at the site of the gastric mucus. This agmatine would provide an acid-protective mechanism. There is no equivalent layer of agmatine in the oesophagus which leaves the oesophageal mucosa vulnerable to damage when exposed to gastric acid. The damaged oesophageal epithelium does have some agmatine at the intercellular junctions and in the cytoplasm of some oesophageal epithelial cells in the prickle cell layer. The oesophageal agmatine in the damaged epithelium is minimal when compared to the amount in the normal gastric mucosa. The role of agmatine in tissue protection with reference to gastro-oesophageal reflux is discussed.

Keywords: Oesophagus, gastric reflux, agmatine, pepsinogen.

Introduction

The oesophageal epithelium is not normally exposed to gastric acid. However, if gastric acid refluxes through the gastro-oesophageal junction the oesophageal epithelium which is exposed to the gastric acid may be damaged. This has to be contrasted with the normal gastric epithelium which is not adversely affected by exposure to the gastric acid. Why does this difference exist?

In addressing this question it has been decided to examine the oesophageal epithelium and the gastric epithelium with particular attention being given to the biochemical and the cytological mechanisms of tissue protection. Evidence has

previously been presented that the decarboxylation of arginine is involved in gastric acid secretion (Steer 2005; Steer 2007). Arginine is the most basic amino acid (isoelectric point 11·15) and is significantly basic at physiological pH (7·4). The decarboxylation product of arginine, namely agmatine, is also extremely basic. It has been proposed that the presence of agmatine is one of the biochemical mechanisms for the protection of the gastric epithelium (Steer 2005). The present study has been undertaken to determine whether pepsinogen (an arginine-rich compound) and agmatine have any role in the oesophagus.

Material and methods

Biopsies have been taken at upper gastrointestinal endoscopy from the distal oesophagus and fundus of the stomach in fifteen patients (ten normal patients and five patients with a hiatus hernia). None of the normal patients have *Helicobacter pylori* infection but two of those patients with a hiatus hernia have *Helicobacter pylori* infection of the stomach. Biopsies have been resin embedded for immunohistochemical analyses using the method of Britten, Howarth and Roche (1993).

The endoscopic biopsies are placed in ice acetone containing 2mM phenyl methyl sulphonyl fluoride and 20mM iodoacetamide. The biopsies are fixed overnight at -20°C and then the fixative is replaced with acetone at room temperature for 15 minutes followed by methyl benzoate at room temperature for 15 minutes. The tissues are infiltrated with processing solution (5% methyl benzoate in glycol methacrylate solution – GMA solution A) at 4°C. There are three changes of processing solution with two hours in each change before embedding in freshly prepared embedding solution (GMA solution A / benzoyl peroxide). The capsules containing resin embedded biopsies are polymerized at 4°C for 48 hours and stored in airtight boxes at -20°C. Sections 2µm thick are cut from the resin embedded specimens and stained with polyclonal antibodies (Steer 2005). This study has involved using a rabbit polyclonal antibody to agmatine (1-amino-4-guanidobutane) (Chemicon International, Chemicon Europe, Chandlers Ford, Hampshire, U.K.), a rabbit polyclonal antibody to *Helicobacter pylori* (DakoCytomation, Ely, Cambridgeshire, U.K.) and a sheep polyclonal antibody to human pepsinogen 11 (Abcam, Cambridge, U.K.).

The immunohistochemical studies have involved using the following antibodies:

Antibody	**Clone**	**Source**
Agmatine	rabbit polyclonal	Chemicon International
Helicobacter pylori	rabbit polyclonal	DakoCytomation
Pepsinogen 11	sheep polyclonal	Abcam

Ethical approval for the study has been obtained. Permission to obtain the endoscopic biopsies as well as perform the cytochemical analyses have been obtained from the patients. The patients have been undergoing endoscopic examinations as part of the investigation of their presenting symptoms.

Results

Pepsinogen 11

The stratified squamous epithelium of the normal oesophagus and the oesophagus of patients with gastro-oesophageal reflux does not contain any cytoplasmic pepsinogen 11. However, in some patients, particularly those with gastro-oesophageal reflux, pepsinogen 11 positive material is present on the luminal surface of the oesophageal epithelium (figure 1).

When examining biopsies from the fundus of the stomach, pepsinogen 11 positive material is found in the chief cells and may take the form of dense cytoplasmic granules or cytoplasmic vacuoles (figure 2). Pepsinogen 11 positive material is also found in the lumen of the gastric glands, the gastric lumen and in the canaliculi of some parietal cells.

Agmatine

The stratified squamous epithelium of the normal oesophagus has no agmatine staining or minimal agmatine staining (figures 3 and 4). When agmatine is related to the normal oesophageal epithelium it is found on the luminal surface of the oesophageal epithelium (figure 5) and is patchily distributed at this site. It takes on a granular appearance (figure 6).

In those patients with gastro-oesophageal reflux agmatine may be found at two other sites in the oesophageal epithelium. Agmatine can be found at the intercellular junctions of the superficial layer of the oesophageal epithelium (figures 7 and 8). This agmatine staining is patchy and weak. Agmatine can also be found as a weak perinuclear cytoplasmic staining in the cells of the prickle cell layer of the oesophageal epithelium (figures 7 and 9).

When the gastric epithelium is examined for the presence of agmatine, there is intense staining (figure 10) in the mucus containing cytoplasmic areas of the gastric epithelium at the luminal surface of the stomach and in the pit region of the gastric glands. In these gastric biopsies agmatine is found in the chief cells and in the canaliculi of some parietal cells (Steer 2005; Steer 2007).

The amount of agmatine, the intensity of staining for agmatine and the amount of mucus in the mucus secreting gastric epithelial cells of patients infected with *Helicobacter pylori* is decreased when compared with the biopsies from patients lacking this infection (Steer 2005).

Discussion

Agmatine was first isolated from herring sperm (Kossel 1910). For many years agmatine was thought to be absent from mammalian tissues but this misconception was corrected in 1994 (Li et al 1994). Subsequent tissue analyses have shown that the tissue with the greatest concentration of agmatine is the stomach (Raasch et al 1995).

Acid is produced by the parietal cells of the gastric mucosa (Bradford and Davies 1950). The pH of this acid may reach a value of pH 1.2 which would be extremely damaging to the tissues of the body. Why is the normal oesophageal mucosa susceptible to damage by gastric acid whereas the normal gastric mucosa is not susceptible to such damage? When considering the protection of tissues against a very strong acid, the two

principles to be considered are the avoidance of exposure to the acid and the biochemical neutralization of the strong acid.

In the stomach, exposure to gastric acid is inevitable but how are the tissues protected? The most vulnerable site would be at the level of the epithelial mucus. Heatley (1959) proposed a pH gradient within the gastric mucus. The *in vivo* verification of this pH gradient at the epithelial level was made by Williams and Turnberg (1979). This change in pH has been attributed to active biocarbonate secretion (Garner and Flemstrom 1978). Are any other biochemical molecules involved in this pH gradient? In examining this possibility agmatine has been identified at this site (Steer 2005; present study). Agmatine is significantly basic. The agmatine can be taken up by the stomach (Molderings et al 2002) and the agmatine in the mucus is ideally sited to protect the gastric mucosa.

The oesophageal mucosa lacks a mucus layer equivalent to that found in the gastric mucosa. There is no oesophageal epithelial agmatine layer equivalent to that found in the gastric epithelium. Hence, if the oesophageal epithelium is exposed to any strong acid, this acid will have a deleterious effect on the oesophageal epithelium. This is seen when acid refluxes into the oesophagus *in vivo* or when the oesophageal epithelium is exposed to strong acid iatrogenically as in the Bernstein and Baker test for oesophageal reflux (Bernstein and Baker 1958).

Are there any biochemical mechanisms associated with normal anatomy which would help to prevent gastric acid reflux? The chief cells at the cardia of the human stomach produce group 1 and group 11 pepsinogens. Pepsinogens (proenzymes) are converted by acid into the active enzymes pepsins with the release of activation segment. Activation segment has a high concentration of basic amino acids particularly arginine and lysine (Kageyama and Takahashi 1980). Fifteen out of forty seven amino acids in the activation segment molecule from human pepsinogen 1 are basic amino acids. Arginine and its decarboxylation product, agmatine, are extremely basic at physiological pH. The high concentration of these chemicals at the cardia of the stomach assists in limiting any adverse events due to the strong acid. It is not surprising that a small amount of pepsinogen 11 and agmatine have been found in the distal normal oesophagus related to the luminal surface of the epithelium. It is interesting to note that in the frog (Hirschowitz 1984) there are specific glands in the lower oesophagus containing chief (peptic) cells. Have these oesophageal glands developed in the frog as a protective mechanism in addition to being a source of pepsinogen? In addition, the group 1 pepsinogens which are limited to being produced by the fundic mucosa are more negatively charged molecules than group 11 pepsinogens (Samloff 1971) and would therefore be biochemically more acid-protective than group 11 pepsinogens. Human pepsinogen 11 is a group 1 pepsinogen.

The presence of the specialised gastric glands at the cardia of the stomach and the secretory products of these gastric glands can be considered functionally as the biochemical component of the gastro-oesophageal anti-reflux mechanism. The diagrammatic representation of agmatine production and gastro-oesophageal protection is shown in figure 11.

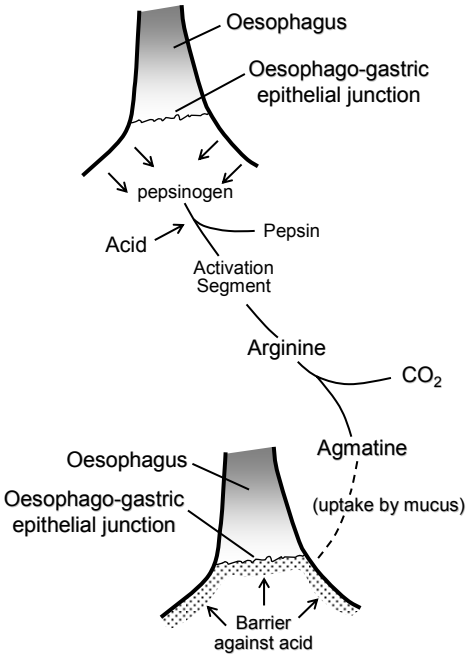

Figure 11. Diagrammatic representation of the production of agmatine and the defence of gastro-oesophageal tissues

If gastric acid refluxes into the oesophagus in the presence of a hiatus hernia the oesophageal defence mechanism breaks down (figure 12).

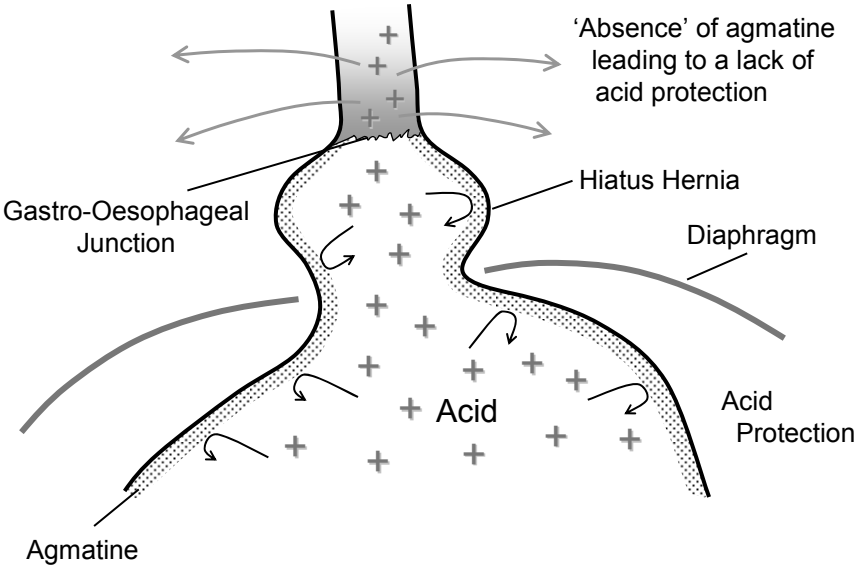

Figure 12. Gastro-oesophageal reflux and the lack of acid protection in the oesophagus

Acknowledgements

Grateful acknowledgement is made of the help received from Dr. Susan Wilson, Linda Jackson, Helen Rigden and Jon Ward of the Histochemistry Research Unit, University of Southampton School of Medicine, Anton Page of the Biomedical Imaging Unit, University of Southampton School of Medicine / Southampton University Hospital NHS Trust and Adie Falcinelli of the Learning Media, Southampton Hospital NHS Trust.

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Figure 1.

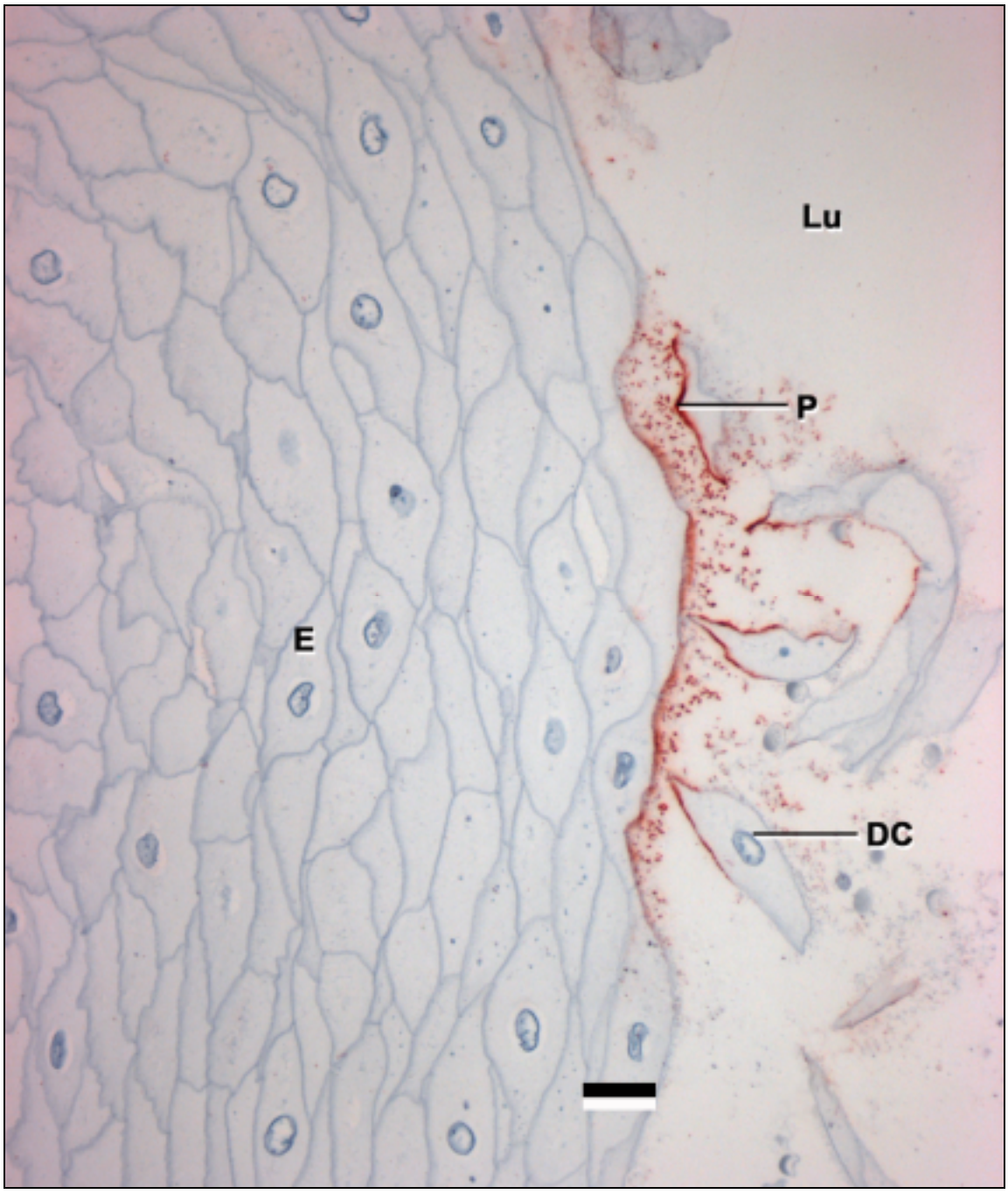

Figure 1. Pepsinogen 11. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a patient with gastro-oesophageal reflux and antral gastritis. The stratified squamous epithelium (E) and desquamating cells (DC) being shed into the oesophageal lumen (Lu) are shown. Pepsinogen 11 (P) is seen at the luminal surface of the oesophageal epithelium and surrounding the desquamating cells. Scale bar is 20µm.

Figure 2.

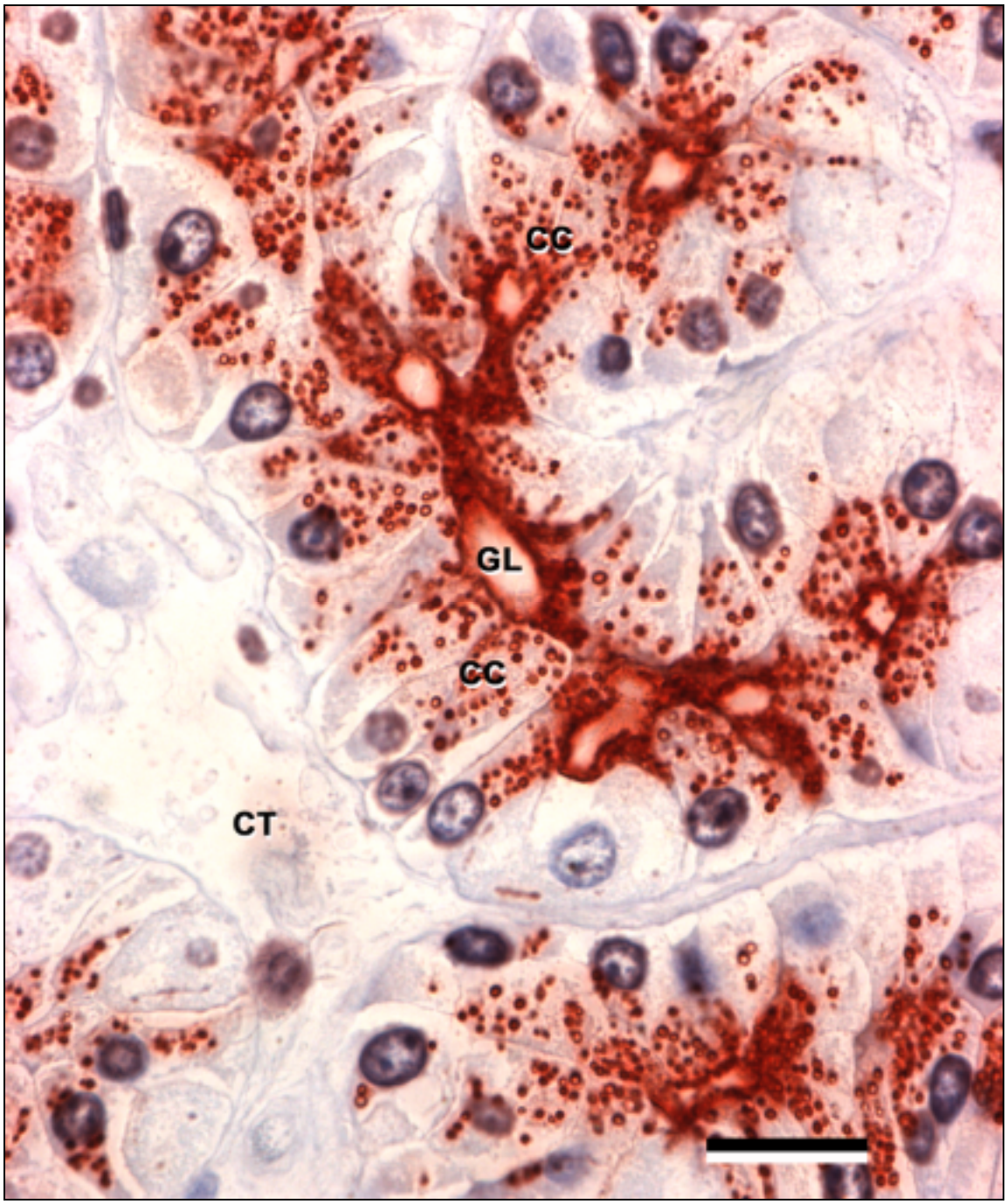

Figure 2. Pepsinogen 11. (Chromogen substrate).

Mucosal biopsy from high on the greater curve of the normal stomach approximately three centimetres distal to the gastro-oesophageal junction. Numerous chief cells (CC) contain vacuolated and non-vacuolated secretory granules. Pepsinogen 11 is seen in these secretory granules. The gastric gland lumen (GL) and subepithelial connective tissue (CT) are shown. Scale bar is 20µm.

Figure 3.

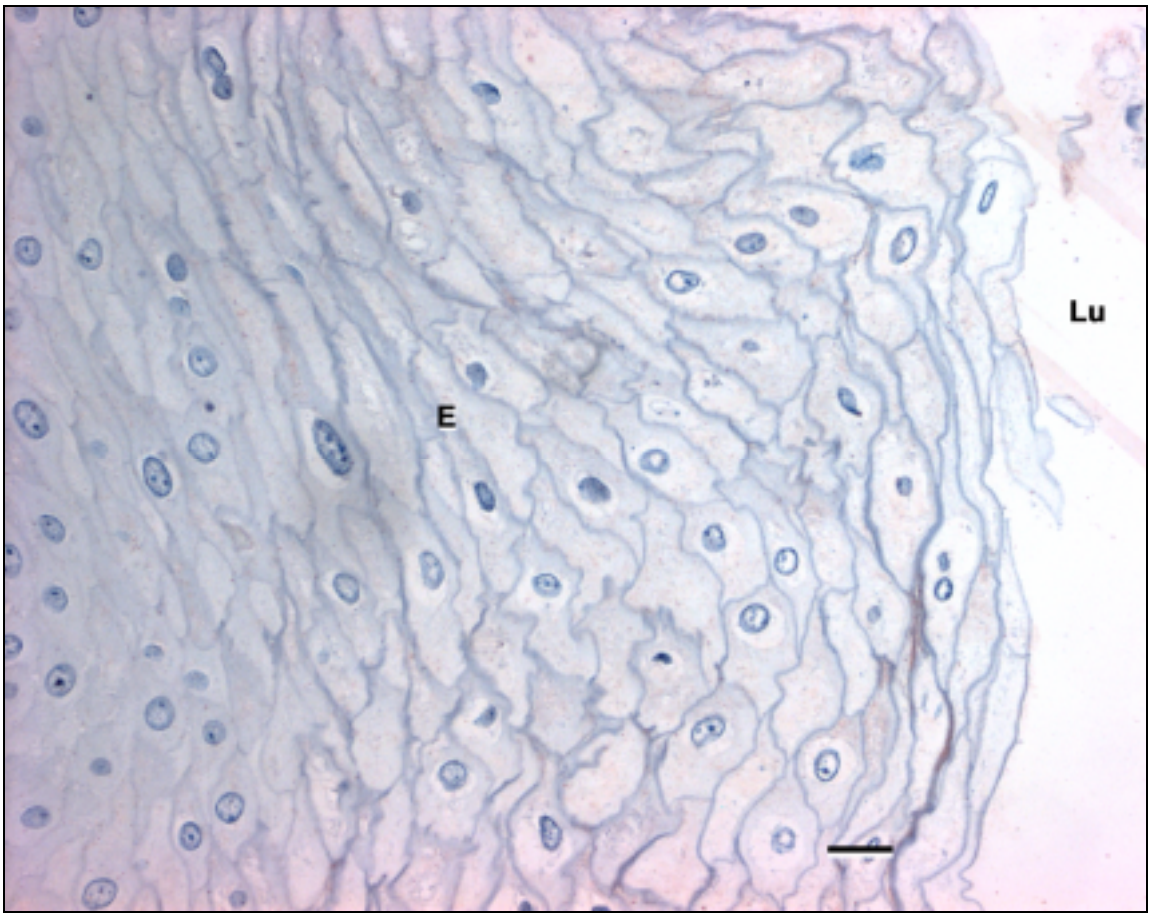

Figure 3. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a normal patient. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. There is no significant staining for agmatine. Scale bar is 20µm.

Figure 4.

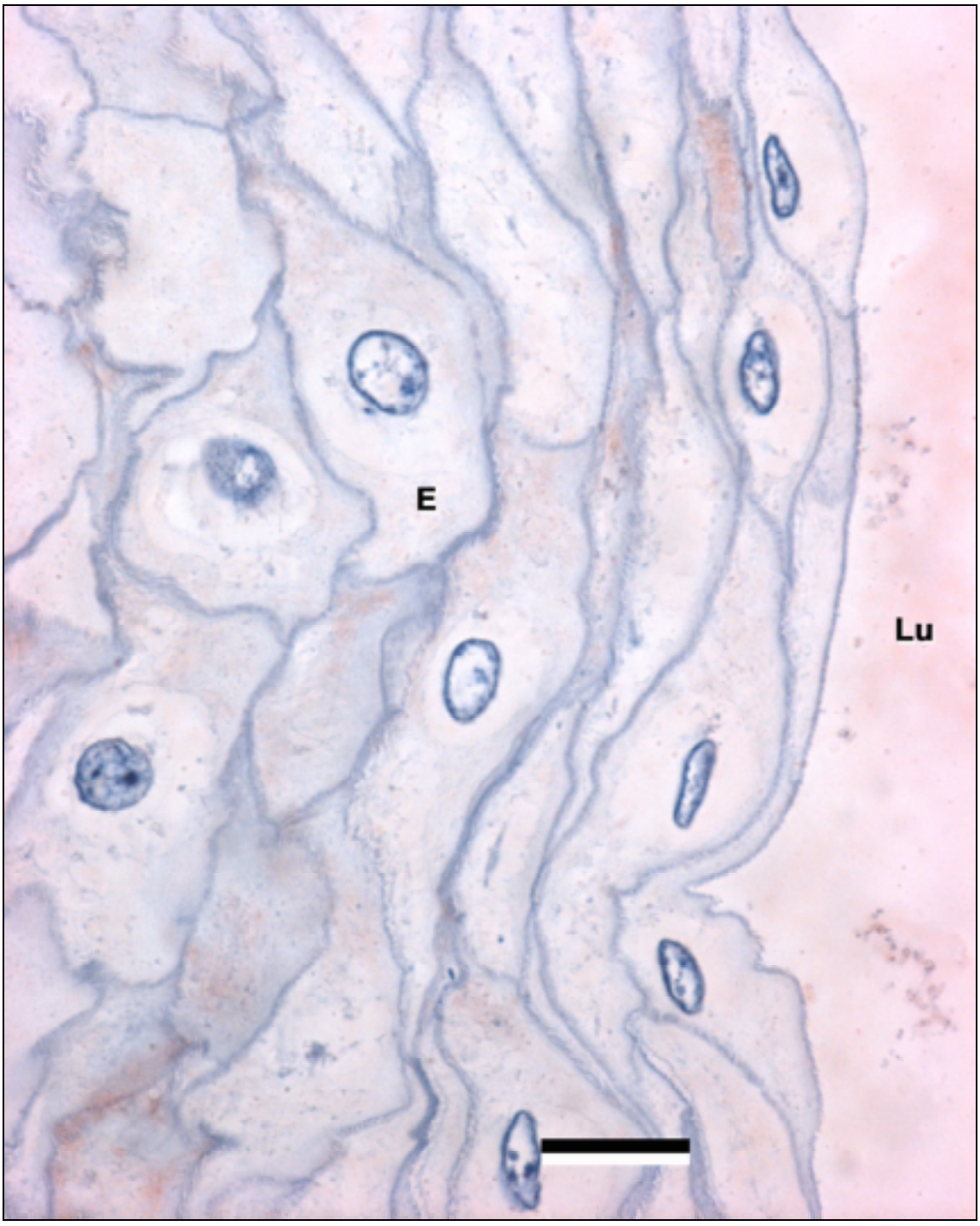

Figure 4. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a normal patient. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. There is no significant staining for agmatine. Scale bar is 20µm.

Figure 5.

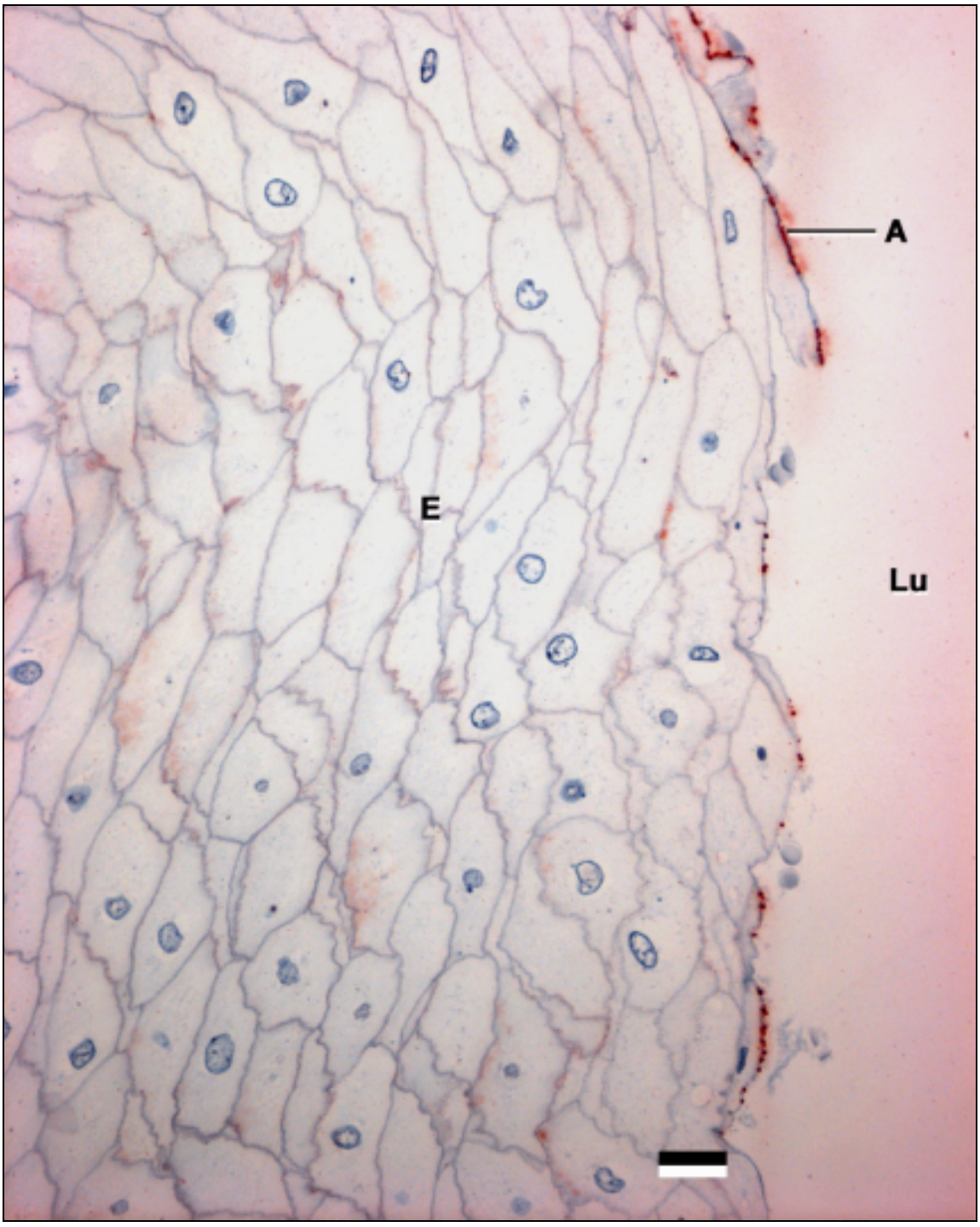

Figure 5. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a normal patient. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. Agmatine (A) is found on the luminal surface of the oesophageal epithelium. Scale bar is 20µm.

Figure 6.

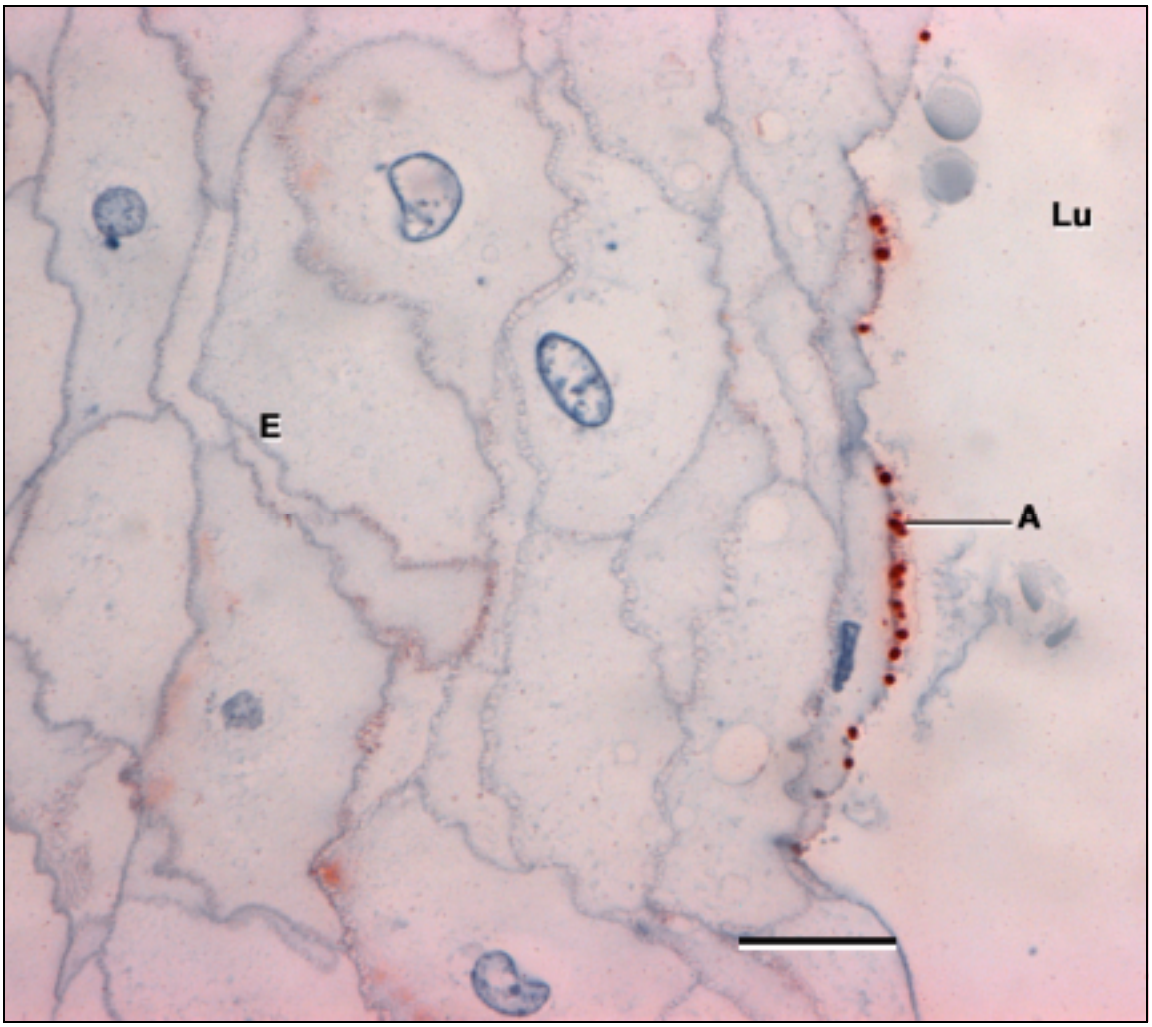

Figure 6. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a normal patient. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. Agmatine (A) is shown as a granular deposit on the luminal surface of the epithelial cells. Scale bar is 20µm.

Figure 7.

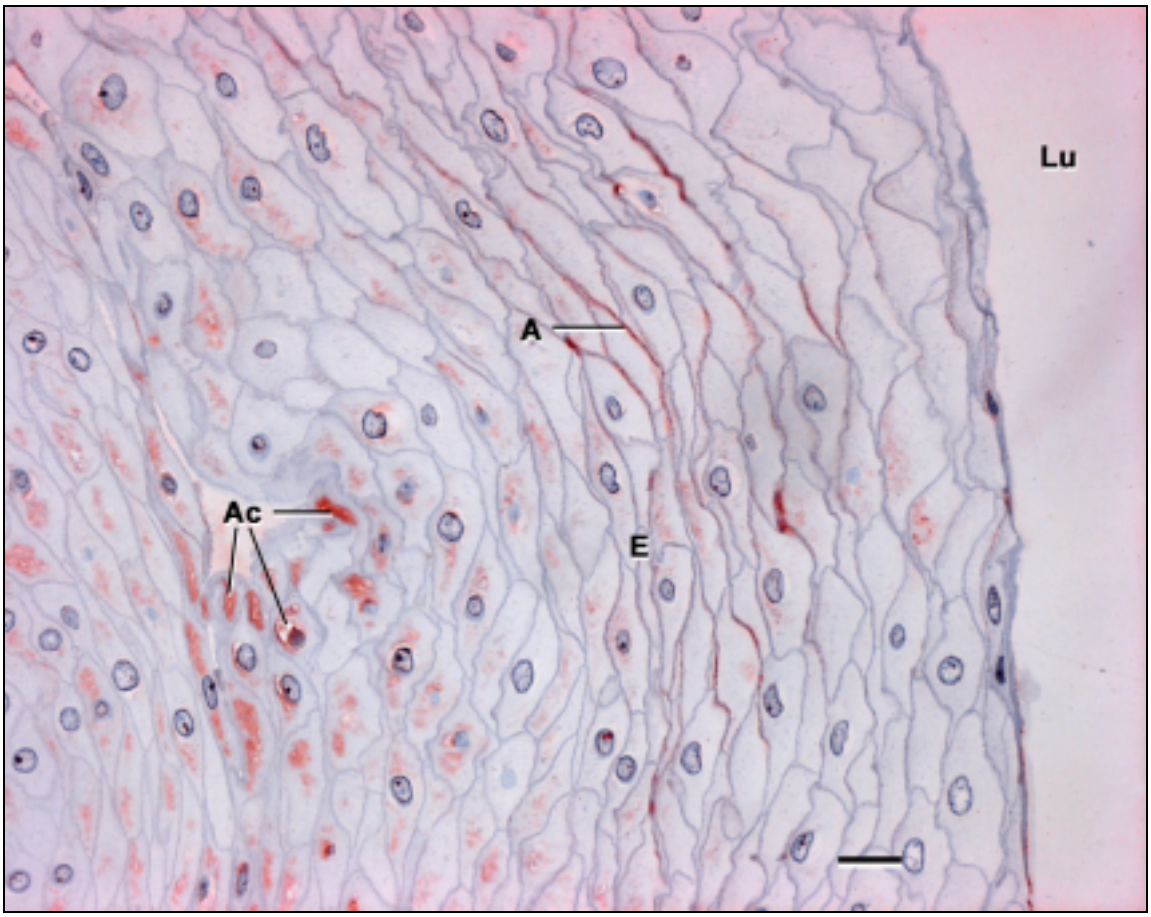

Figure 7. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a patient with a hiatus hernia and gastro-oesophageal reflux. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. Agmatine (A) is found at the intercellular junctions and there is also cytoplasmic agmatine (Ac). Scale bar is 20µm.

Figure 8.

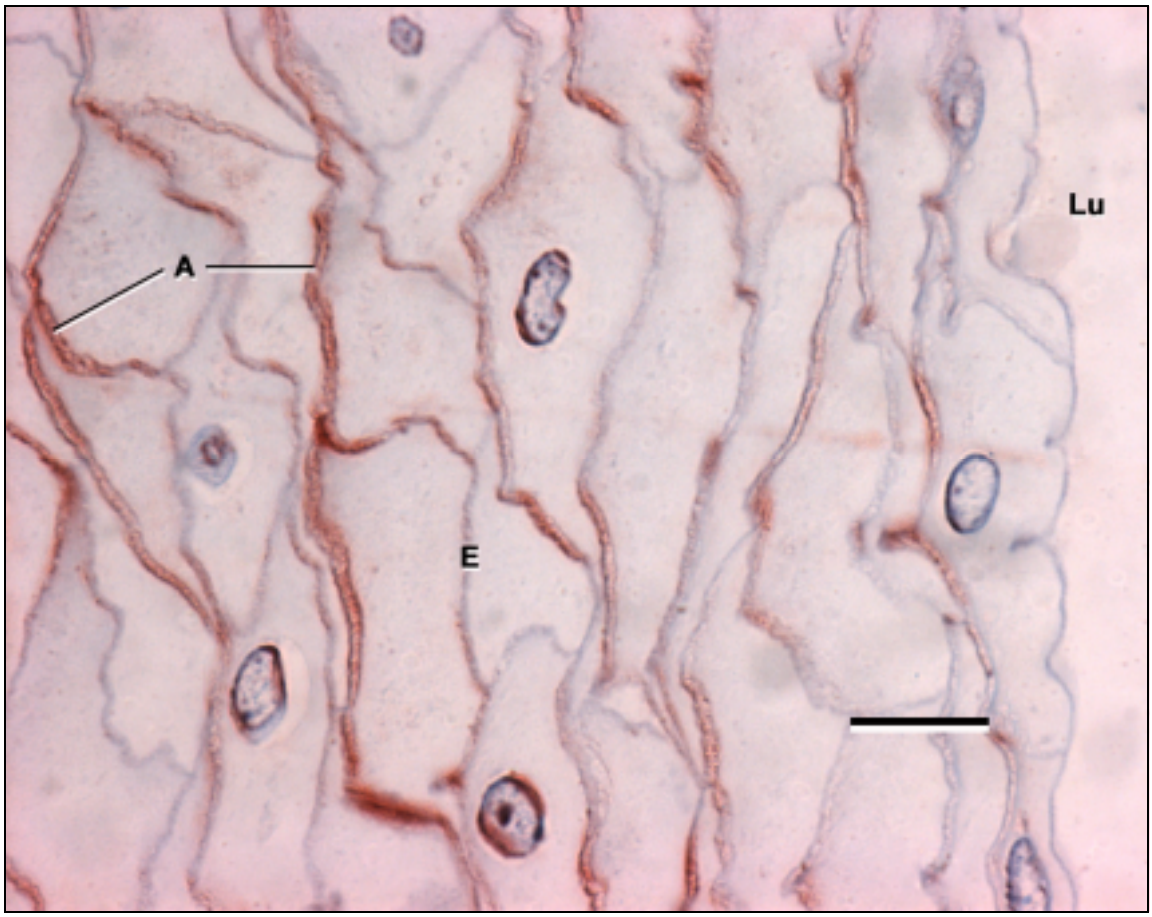

Figure 8. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a patient with gastro-oesophageal reflux secondary to a hiatus hernia. The oesophageal epithelium (E) and oesophageal lumen (Lu) are shown. Agmatine (A) is present at the intercellular junctions of some of the epithelial cells. Scale bar is 20µm.

Figure 9.

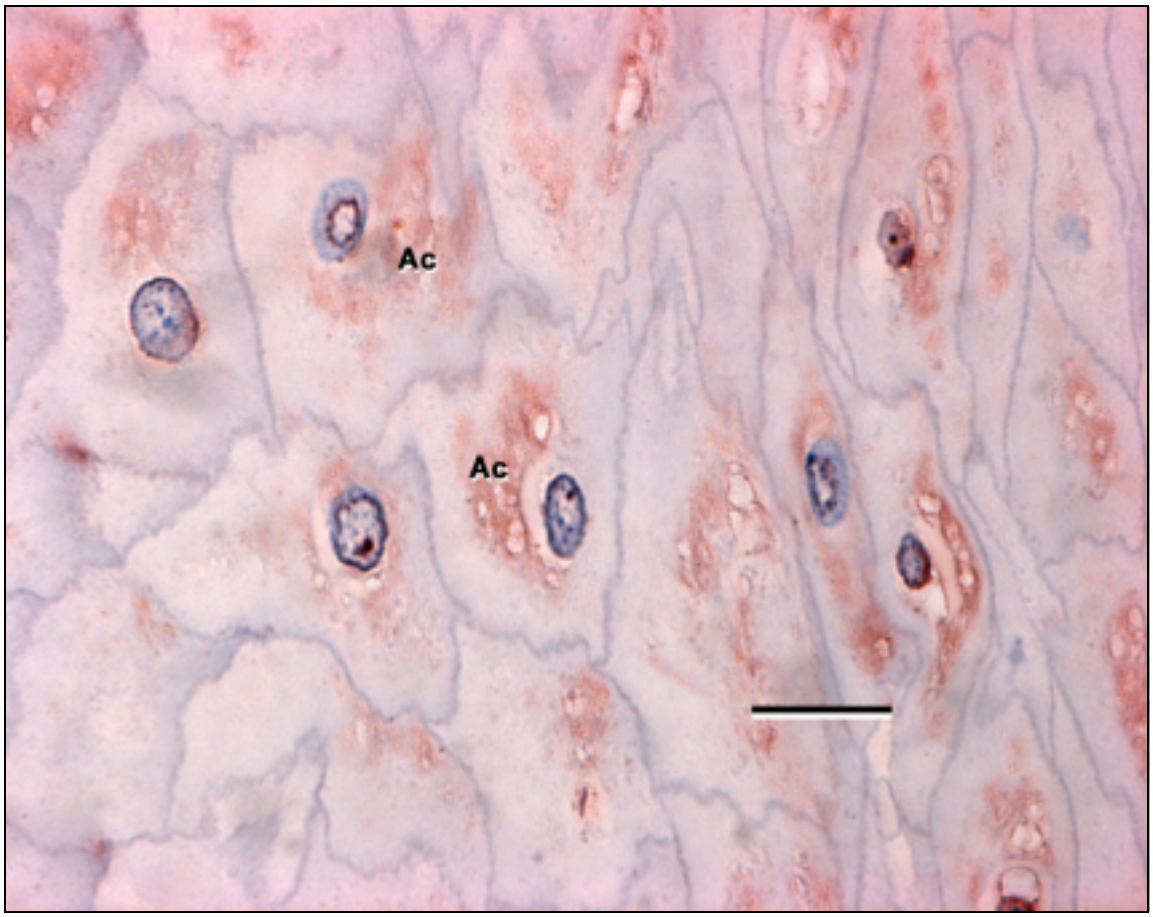

Figure 9. Agmatine. (Chromogen substrate).

Mucosal biopsy from the distal oesophagus of a patient with gastro-oesophageal reflux secondary to a hiatus hernia. Agmatine (Ac) is present in the cytoplasm of numerous epithelial cells. Scale bar is 20µm.

Figure 10.

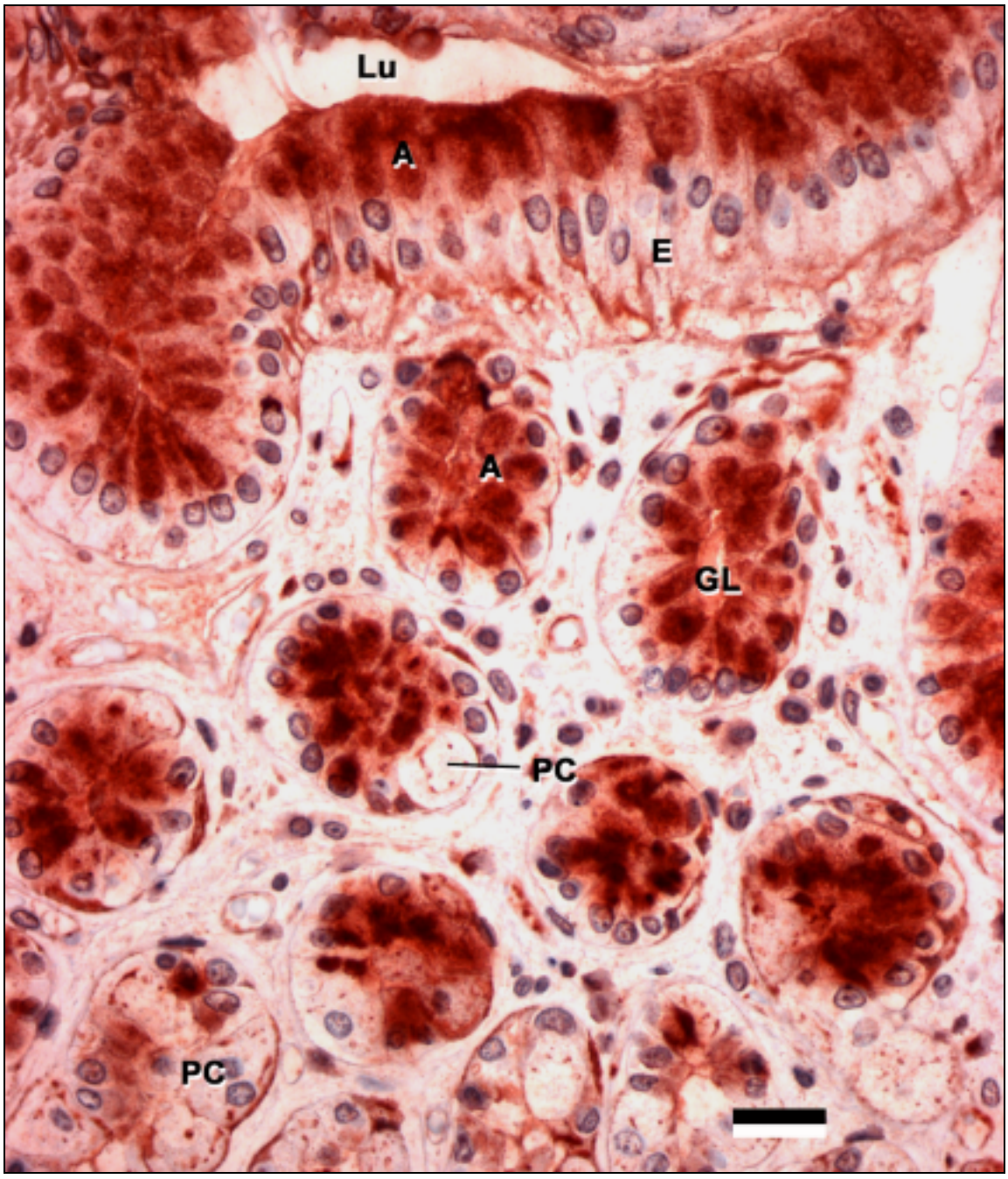

Figure 10. Agmatine. (Chromogen substrate).

Mucosal biopsy from high on the greater curve of the stomach three centimetres distal to the gastro-oesophageal junction. The gastric lumen (Lu), superficial gastric epithelium (E) and the lumen of a gastric gland (GL) are shown. Agmatine is present in the superficial epithelial cells (A) and in the canaliculi of parietal cells (PC). Scale bar is 20µm.