Histochemistry (1986) 86:5–17

Histochemistry

Histamine in endocrine cells in the stomach

A survey of several species using a panel of histamine antibodies

R. Håkanson[1]*, G. Böttcher[2], E. Ekblad[2], P. Panula[4], M. Simonsson[2], M. Dohlsten[3], T. Hallberg[3], and F. Sundler[2]

Departments of Pharmacology[1], Histology[2] and Microbiology[3], University of Lund, Sölvegatan 10, S-223 62 Lund, Sweden, and Department of Anatomy[4], University of Helsinki, Finland

Accepted June 12, 1986

Summary. Antibodies to histamine were used to examine the localization of the amine in cells of the stomach and upper small intestine of a great variety of species, including cartilaginous and bony fish, amphibia, reptiles (lizard), birds (chicken) and a large number of mammals. In all species gastric histamine was localized in endocrine cells (invariably found in the epithelium) and mast cells (usually with an extra-epithelial localization). The endocrine cells were identified as such by immunostaining with antibodies to chromogranin A and the mast cells were identified by toluidine blue staining. Histamine-immunoreactive endocrine cells were found almost exclusively in the acid-producing part of the stomach; only rarely were such cells observed in the pyloric gland area. They were fairly numerous in the gastric mucosa of the two subclasses of fish as well as in the amphibia and reptile species studied. Here, the majority of the histamine-immunoreactive endocrine cells seemed to have contact with the gastric lumen (open type cells) and were located in the surface epithelium (certain fish only) or together with mucous neck cells at the bottom of the pits. In the chicken, histamine-immunoreactive endocrine cells were numerous and located peripherally in the deep compound glands. They were without contact with the lumen (closed type) and had long basal extensions ("paracrine" appearance), running close to the base of the oxyntico-peptic cells. In mammals, the number of histamine-immunoreactive endocrine cells in the stomach varied greatly. They were particularly numerous in the rat and notably few in the dog, monkey and man. In all mammals, the histamine-immunoreactive endocrine cells were of the closed type and located basally in the oxyntic glands. They often had a "paracrine" appearance with long basal processes. Histamine-storing mast cells, finally, were few in both subclasses of fish as well as in the amphibian species and in the lizard. They were fairly numerous in chicken proventriculus (beneath the surface epithelium), few in the oxyntic mucosa of mouse, rat and hamster, moderate in number in hedge-hog, guinea-pig, rabbit, pig and monkey, and numerous in cat, dog and man. In the oxyntic mucosa of the latter three species mast cells sometimes seemed to have an intraepithelial localization which made their distinction from endocrine cells difficult. In newborn cats (1–3 days old) in human foetuses (17–24 weeks gestational age) mast cells were relatively few in the gastric mucosa and the histamine-containing endocrine cells were easier to demonstrate as a consequence. Patients with achlorhydria (and pernicious anemia) or suffering from hypergastrinemia due to gastrinoma had a greatly increased number of histamine-storing endocrine cells in the oxyntic mucosa compared with normal individuals.

* To whom offprint requests should be sent

Introduction

Histamine in the stomach has previously been found to occur in mast cells in all mammals and in endocrine cells in certain rodents, notably the mouse, rat, and Mastomys (*Praomys natalensis*), by means of the o-phthalaldehyde (OPT) histofluorescence method (Håkanson and Owman 1967, 1969; Aures et al. 1968; Håkanson et al. 1969, 1970, 1971, 1973; Håkanson 1970). However, this method does not detect neuronal histamine and is probably therefore of low sensitivity. The production of histamine antibodies has made it possible to apply immunocytochemical techniques to the cellular localization of histamine. Panula et al. (1984, 1985) recently demonstrated histamine by immunofluorescence in hypothalamic neurones and in endocrine-like cells in the rat and guinea-pig stomach.

In the present study we have used four histamine antibodies (three polyclonal and one monoclonal) to examine the localization of histamine in cells in the stomach of several species representing different phyla.

Material and methods

Antibodies. Four different histamine antibodies were used (Table 1):

1. Monoclonal histamine antibodies were raised by Pharmacia, Uppsala, Sweden.

2. Two antisera (code no. Hist II and Hist III) were raised in rabbits against histamine coupled to succinylated hemocyanin by the carbodiimide reaction (Panula et al. 1984, 1985).

3. One antiserum (code no. 8431, Milab, Malmö, Sweden) was raised in a rabbit against histamine coupled to serum albumin with carbodiimide. Histamine dihydrochloride was covalently bound to human serum albumin (HSA, crystallized human albumin, no. A-9511, Sigma) with 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide (ECDI, Sigma). Briefly, 100 mg histamine dihydrochloride, 10 mg HSA and 200 mg ECDI were dissolved in 1 ml water. The solution was incubated at room temperature for 2 h and dialyzed at 4° C against large volumes of 0.9% saline. After dialysis the volume was adjusted so that the protein concentration was 3 mg/ml. The amount of histamine bound to HSA was determined by the addition of ^{3}H-histamine (NEN Chemicals) to the incubation mixture. Approximately 30–35 molecules of histamine were bound per molecule of HSA.

Table 1. Working dilutions for the various histamine antibodies

Fixation and processing			Formalin-fixed cryostat sections		Formaldehyde vapour-fixed paraffin sections		DEPC-vapour-fixed paraffin or plastic sections	
Antibody	Code no.	Source	FITC	PAP	FITC	PAP	FITC	PAP
Monoclonal antibodies	–	Pharmacia, Uppsala, Sweden	1:40[a]	–	1:40[a]	1:80	–	–
Polyclonal antibodies	Hist II	Helsinki	1:400	–	1:400	–	1:600	1:6000
Polyclonal antibodies	Hist III	Helsinki	–	–	–	–	1:600	1:5000
Polyclonal antibodies	8431	Lund	1:160	–	1:160	–	1:1000	1:5000

[a] Diluted from stock solution

Table 2. Relative density of histamine-storing endocrine cells and mast cells in the gastric mucosa of some species of various subclasses (or phyla) of vertebrates

Fishes	Endocrine cells	Mast cells	*Amphibia*	Endocrine cells	Mast cells	*Mammals*	Endocrine cells	Mast cells
Cartilaginous fish			Frog (*Rana temporaria*)	+ +	+	Mouse	+ +	+
Spiny dogfish (*Squalus acanthias*)	+ +	+	Toad (*Bufo bufo*)	+ +	+	Hamster	+ +	+
Ray (*Raja clavata*)	+ +	+	*Reptilia*			Rat	+ + +	+
Bony fish			Lizard (*Lacerta vivipara*)	+ +	+	Guinea-pig	+ +	+
Daddy sculpin (*Cottus scorpius*)	+ +	+	*Birds*			Hedge-hog	+ +	+ +
Cod (*Gadus morhua*)	+ +	+	Chicken (*Gallus gallus*)	+ + +	+[a]	Rabbit	+ +	+ +
Redspotted plaice (*Pleuronectes platessa*)	+ +	+				Cat	+ +	+ + +
Stickleback (*Gasterosteus aculeatus*)	+ +	+				Dog	+	+ + +
						Pig	+ +	+ +
						Squirrel monkey	+	+ +
						Man	+	+ + +

Few cells +, moderate number of cells + +, many cells + + +

[a] In the proventriculus of the chicken, mast cells are few in the compound glands, but quite numerous beneath the surface epithelium

Immunocytochemistry. Tissue specimens were collected from the acid-producing and antropyloric parts of the stomach (or from analogous structures) and from the upper small intestine of the following phyla or subclasses thereof (at least three of each species): cartilaginous and bony fish, amphibia, reptiles, birds and mammals (cf. Table 2). Gastric mucosal biopsy specimens were collected from one patient with a pancreatic gastrinoma and a manifest Zollinger-Ellison syndrome including acid hypersecretion, two patients with long-standing achylia and pernicious anaemia and from four patients with normal acid secretion. In addition, specimens were taken from four human foetuses, 17–24 weeks of gestation. These specimens were collected at legal abortions. All tissue specimens were processed according to either of two alternative protocols: 1) They were placed overnight in an ice-cold solution of 4% formaldehyde in 0.1 *M* phosphate buffer, pH 7.2, and rinsed in Tyrode solution containing 10% sucrose for at least 24 h. The specimens were frozen on dry ice and sectioned on a cryostat microtome at −20° C. Cryostat sections (10 μm thick) were collected on chrome alun-subbed slides and air-dried. They were then hydrated in phosphate-buffered saline (PBS) containing 0.25% Triton X-100 (PBS-T) for 10 min. 2) Alternatively, specimens were frozen in a mixture of propane and propylene at the temperature of liquid nitrogen. After freeze-drying, these specimens were vapour-fixed in diethylpyrocarbonate (DEPC) (Pearse and Polak 1975) or formaldehyde (Björklund et al. 1972) and embedded in paraffin or plastic (Epon, Araldite or Durcopan). Paraffin sections were cut at 6–8 μm thickness, placed on slides, deparaffinized in xylene and rehydrated. Plastic sections were cut at 1–2 μm thickness, placed on slides and etched in 10% potassium hydroxide. For the demonstration of histamine the sections were subjected to the indirect immunofluorescence procedure (Coons et al. 1955) or to the peroxidase-antiperoxidase (PAP) procedure (Sternberger 1979). The working dilutions of the four histamine antibodies (and other details) are given in Table 1. The histamine antibodies were diluted in PBS-T and applied to the sections at 4° C over night. The slides were washed for 20 min in PBS-T and the sections were exposed to either fluorescein isothiocyanate (FITC)-labelled or unlabelled second antibody (sheep anti-rabbit IgG, diluted 1:80, Milab, Malmö, Sweden, or rabbit anti-rat IgG, diluted 1:40, Dakopatts, Copenhagen, Denmark, when the monoclonal antibody was used) in PBS-T for 2 h at room temperature. The sections were then washed in PBS for 20 min and mounted in PBS-glycerin for examination of immunofluorescence or incubated with PAP complex (dilution 1:80, Dakopatts, Copenhagen, Denmark) and stained for peroxidase. At times, plastic sections were counterstained with toluidine blue in order to improve the background. Control sections were incubated with histamine antibodies preabsorbed with histamine, L-histidine, tele-methylhistamine, β-alanyl-L-histidine, L-histidyl-L-leucine, thyrotropin-releasing hormone (pGlu-His-Pro-NH_2), 5-hydroxytryptamine, noradrenaline (Sigma,

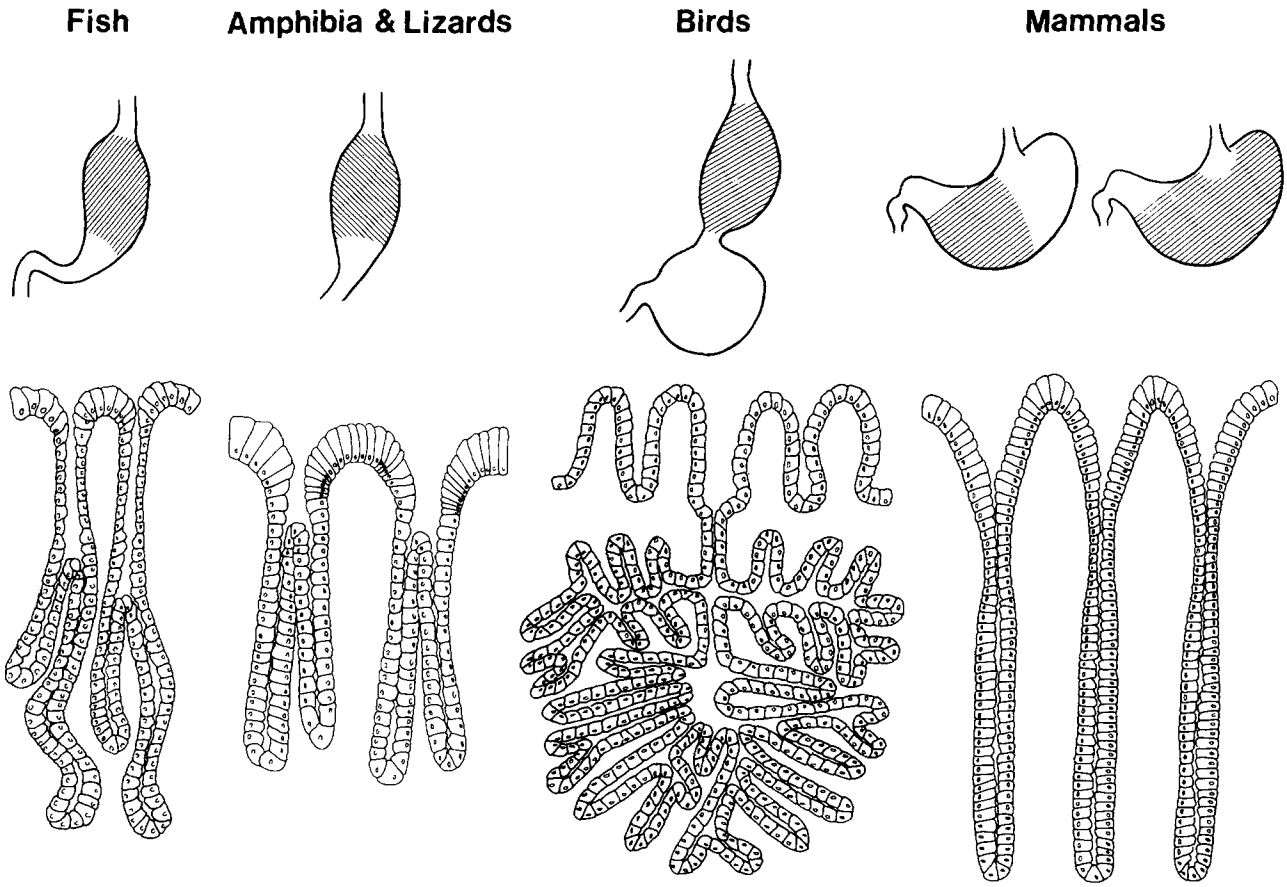


Fig. 1. Schematic illustration of gastric (fundic) glands in different phyla. The shapes of the stomachs in the different phyla are outlined to give an idea of the distribution of such glands (hatched area). In the case of mammals two alternatives are given: with or without rumen (forestomach). In fish the glands are usually branched and convoluted, in amphibia and lizards they are straight. In birds (proventriculus and gizzard shown) the glands are tubular and form spherical structures with a central cavity into which a large number of tubules open. The fundic glands of fish, amphibia, reptiles and birds contain combined oxyntico-peptic cells. The fundic (oxyntic) glands of mammals contain separate oxyntic (parietal) cells and peptic (chief) cells

St. Louis, Mo, USA) at 10, 100 or 1000 μ*M*. Histamine (5–50 μ*M*) abolished the immunostaining in mast cells and endocrine cells, whereas the other substances (except tele-methylhistamine) were ineffective even at the highest concentrations. Tele-methylhistamine (1 mM) reduced the intensity of the immunostaining with all four antibodies.

For identification of the histamine-immunoreactive cells, adjacent sections were stained according to one of the following alternative procedures: 1) Mast cells were identified by their metachromasia when stained with toluidine blue in ethanol solution (Romeis 1948). This staining could be performed on the formaldehyde-fixed material only. 2) Peptide hormone-producing endocrine cells were demonstrated collectively by immunostaining using an antiserum to chromogranin A (code nr. 137-1, diluted 1:640; a kind gift from Dr. D.T. O'Connor, V.A. Center, La Jolla, CA, USA). Chromogranin A antisera are known to stain peptide hormone-producing cells (with few exceptions) in many peripheral organs (O'Connor et al. 1983; Cohn et al. 1984; Wilson and Lloyd 1984; Nolan et al. 1985), including the endocrine cells in the oxyntic mucosa of mammals (Wilson and Lloyd 1984).

Results

Effect of fixation and tissue processing

Formalin-fixed cryostat sections gave satisfactory immunofluorescence with all four antisera. Formaldehyde-fixed paraffin sections gave less satisfactory immunostaining. DEPC-fixed paraffin sections produced excellent immunostaining with the three polyclonal antisera; the monoclonal antibody was inferior by comparison (Table 1).

The description of histamine-storing cells given below is based on studies using an optimal combination of fixation, tissue processing and immunocytochemical protocol. Histamine could be detected in sections from paraffin or plastic blocks stored for up to 15–20 years at room temperature.

Histamine in the stomach of different phyla

Fish

Both cartilaginous and bony fish species display variations in the design of the upper gastrointestinal tract. The majority of cartilaginous and bony fish species have a stomach, the distal portion of which is slightly bent upon itself to form the pyloric region. Within these anatomical divisions there is a histological differentiation characterized by the occurrence of fundic glands, extending over most of the mucosa of the body of the stomach (Fig. 1). The fundic glands possess but one type of exocrine cell. This gland cell is thought to produce both pepsin and hydrochloric acid (Andrew 1959; Kapoor et al. 1975; Giraud and Yeo-

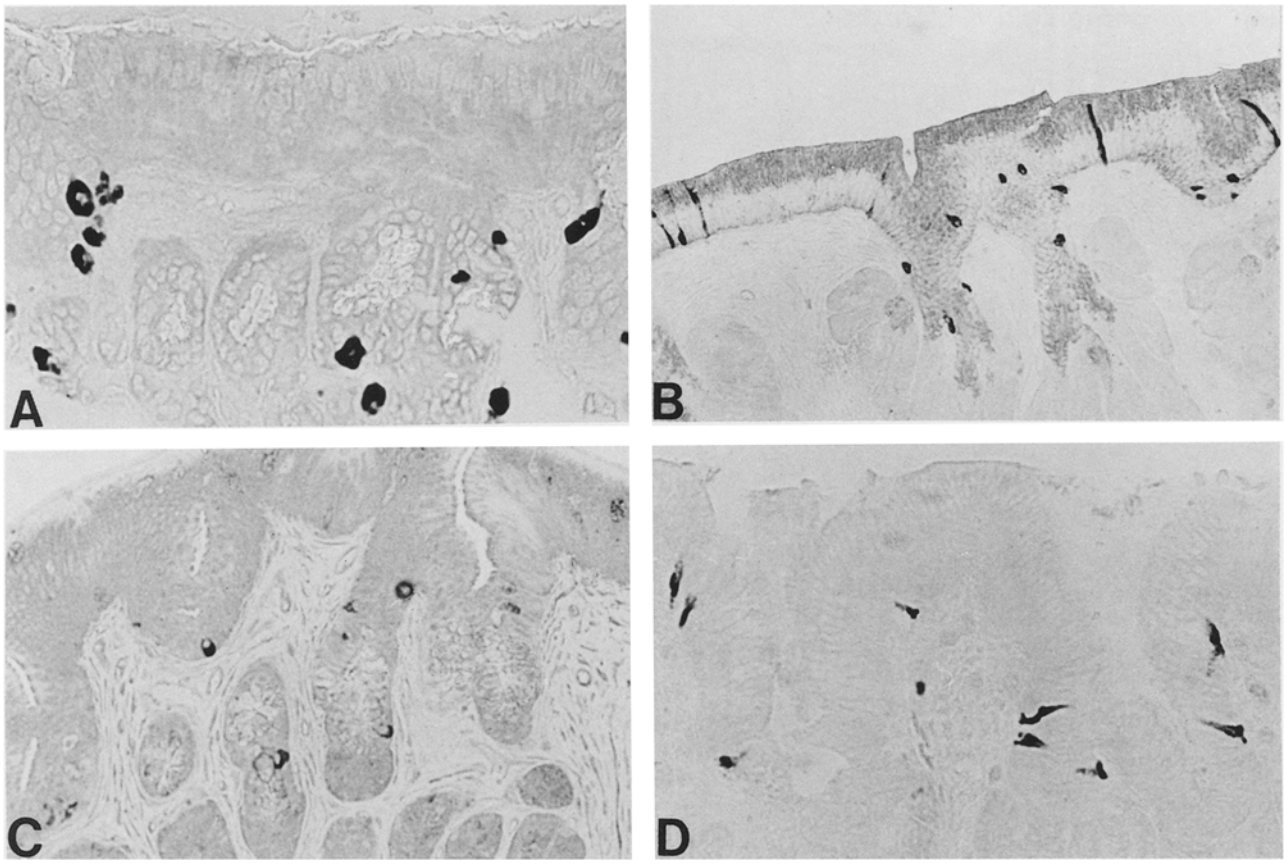


Fig. 2A–D. Histamine-immunoreactive endocrine cells in the fundic glands of the ray (**A**), daddy sculpin (**B**), redspotted plaice (**C**) and cod (**D**). Paraffin sections, DEPC fixation, antiserum HIST II, PAP staining. Many of the immunoreactive endocrine cells, particularly in the daddy sculpin, are of the open type, which means that they are in contact with the gastric lumen via an apical process. The immunoreactive endocrine cells in ray, plaice and cod are found basally in the pits, in daddy sculpin they occur both in the surface epithelium and in the pits. **A, C, D** ×200, **B** ×100

mans 1982). The epithelium of the surface and lining of the gastric pits consists of columnar mucus-secreting cells. Mucous "neck" cells, different from the surface cells and true gland cells, are present in some species of fish at the junction of the gland with the gastric pit.

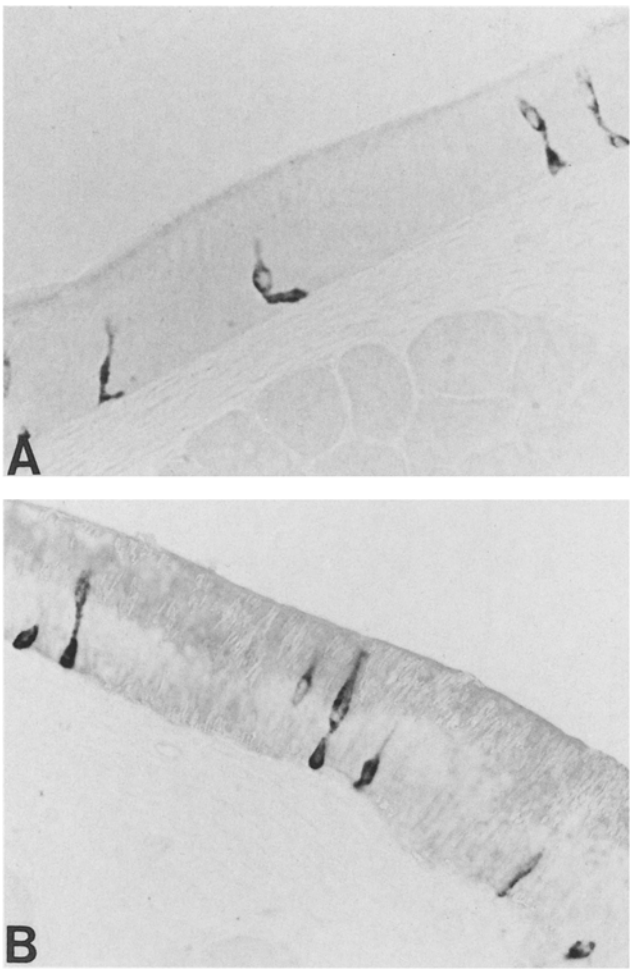
Spiny dog-fish (Squalus acanthias) and ray (Raja clavata). In these two species of cartilaginous fish, fundic glands are rather sparsely distributed in the mucosa. Histamine-immunoreactive endocrine cells (open type) were preferentially located at the base of the pits but they were seen also higher up (Fig. 2A). Such cells were absent from the distal (antropyloric) part of the stomach and the upper small intestine. Mast cells were few throughout the stomach.

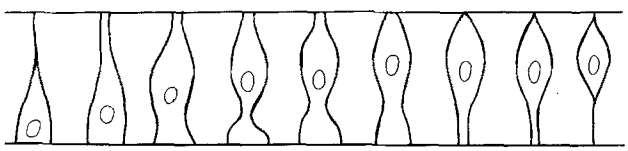
Daddy sculpin (Cottus scorpius). Randomly and rather sparsely distributed deep glandular structures open into the columnar surface epithelium. Histamine-immunoreactive endocrine cells of varying shapes (mostly flask-shaped with an apical and/or basal process) (Fig. 2B) were disseminated within the surface epithelium and in the ducts joining the gastric glands with the pits. The endocrine cells were invariably of the open type, i.e. they had luminal contact (Fig. 3). They were not seen outside the fundic part of the stomach. Mast cells were few.

Cod (Gadus morhua), redspotted plaice (Pleuronectes platessa), and stickleback (Gasterosteus aculeatus). These bony fish have a gastric mucosa with deep fundic glands so close together as to occupy most of the mucosa. Numerous, intensely immunoreactive endocrine cells, mostly of the open type, were accumulated at the junction between glands and gastric pits (Fig. 2C and D). Occasionally, immunoreactive endocrine cells occurred also in the surface epithelium. They usually had an irregular shape, sometimes with a thin descending process. There was no overt difference between cartilaginous (dog-fish and ray) and bony fish (cod, redspotted plaice and stickleback) with respect to number and shape of the histamine-storing endocrine cells in the proximal part of the stomach. Histamine-immunoreactive endocrine cells were absent from the antropyloric part and from the upper small intestine. Mast cells were few and scattered.

Amphibia

The stomach in anuran amphibia is recognized as a dilation of the alimentary tract. The surface epithelium, as in the fish, consists of columnar mucus-secreting cells. The greater part of the mucosa is occupied by fundic glands (Fig. 1). Mucous neck cells are common at the junction of gastric glands and pits. The fundic glands are made up of a single





C

Fig. 3A–C. Histamine-immunoreactive endocrine cells in the gastric epithelium of the daddy sculpin (**A** and **B**). The cells, which are elongated, are characteristically dispersed in the surface epithelium and sometimes occur at the exits of the fundic glands (see also Fig. 2B). Paraffin sections, DEPC fixation, antiserum HIST II, PAP staining. The cells are invariably of the open type and their varying shape is illustrated in **C. A, B** ×250

exocrine cell type that is thought to produce both pepsin and hydrochloric acid (Andrew 1959; Ito 1981).

Frog (Rana temporaria) and toad (Bufo bufo). Fairly numerous, intensely immunoreactive endocrine cells were located in a narrow zone at or close to the mucosal surface, preferentially at the base of the gastric pits (Fig. 4A and B). At least a proportion of the histamine-storing endocrine cells seemed to be of the open type. They were rounded, elongated or irregular in shape, sometimes with a fairly long process descending along the basal membrane to the more basal parts of the gland. As a rule, the immunoreactive material in the cells was accumulated basally. The pyloric glands and the mucosa of the upper small intestine were devoid of histamine-immunoreactive endocrine cells. Mast cells were moderate in number and scattered with a preferential localization at or close to the mucosal surface.

Reptiles

The stomach of reptiles is an elongated sac-like dilatation of the alimentary tract. The surface epithelium resembles that described for fish and amphibia. The gastric pits of the body of the stomach open into glands which harbour mucous neck cells similar to those seen in amphibia. The rest of the gland is composed of but one type of exocrine cell that produces both pepsin and hydrochloric acid (cf. Ito 1981).

Lizard (Lacerta vivipara). Large histamine-immunoreactive endocrine cells with an oval shape were seen in the epithelium at or immediately below the surface of the proximal part of the stomach; they often had an ascending or descending process and many seemed to be of open type. Such cells were lacking in the pyloric gland area. Mast cells were few and scattered.

Birds

In birds, the crop precedes the stomach, which is characteristically divided into two portions a thin-walled "glandular" stomach (proventriculus) and a thick-walled "muscular" stomach (gizzard). The mucosa of the proventriculus is made up of a type of gland not seen in any of the animals previously discussed (Fig. 1). These tubular, compound glands are spherical in shape with a central cavity into which a large number of individual tubules open. The central cavity opens onto the surface of the proventriculus through a duct-like exit. The surface epithelium is villous and consists of mucus-secreting columnar cells. The cells that line the central cavity and the duct-like exit are reminiscent of mucous neck cells in other phyla. The exocrine cells of the composite glands are oxyntico-peptic cells (cf. Ito 1981).

Chicken (Gallus gallus). Numerous histamine-immunoreactive endocrine cells, usually extremely elongated and "paracrine-like", were found along and in close contact with the basal lamina of the tubular glands (Fig. 4C and D). They were all of the closed type (without luminal contact) and gave off long, slender, basal processes which often ended in club-like swellings. The immunoreactive material was often accumulated in the periphery of the cells, which were characteristically much more numerous in the peripheral parts of the compound glands than in the more central parts. No immunoreactive endocrine cells were found in the surface epithelium of the proventriculus. There was no evidence of histamine-storing endocrine cells in the crop, gizzard, gizzard-duodenal junction (antrum) and duodenum. Mast cells were rather many beneath the surface epithelium of the proventriculus (few in the compound glands), somewhat less numerous in the mucosa of the gizzard and rather few in the gizzard-duodenal junction.

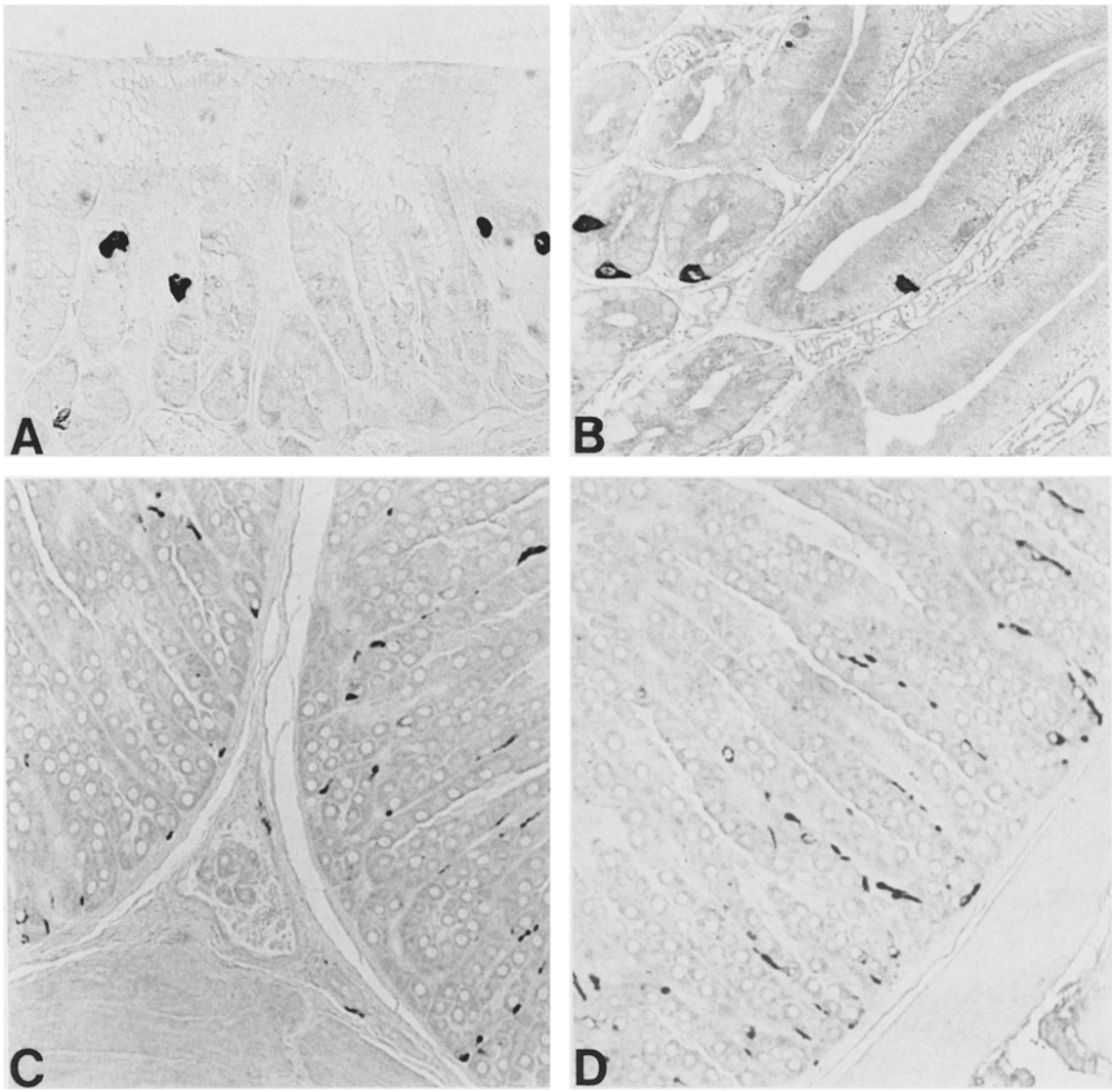


Fig. 4 A–D. Histamine-immunoreactive endocrine cells in the stomach of the toad (**A**) and frog (**B**), and in the deep compound glands of the chicken proventriculus (**C**) where they are found in large number at the periphery. **A** paraffin section, **B, C** plastic sections, DEPC fixation, antiserum HIST II, PAP staining. For comparison a plastic section of chicken proventriculus was stained for chromogranin A (**D**). The chromogranin-immunoreactive cells were similar in number and distribution to those demonstrated by histamine antibodies. **A–D** × 200

Mammals

The stomach shows a great variety in shape among mammals, with many rodents (mouse, rat, hamster) having a rumen immediately distal to the oesophagus. The gastric glands in the body of the stomach differ from those of lower vertebrates in containing two exocrine cell types, chief cells and parietal cells, respectively (Fig. 1). The surface epithelium in mammals consists of columnar mucus-producing cells. Mucous neck cells, finally, are found at the junction of the glands and the pits and sometimes also high up in the gland proper.

Mouse. A moderate number of intensely histamine-immunoreactive endocrine cells of the closed type were found in the basal third of the fundic (oxyntic) glands (Fig. 5A). They were oval or oblong, often with a thin ascending or descending process, running along the basal membrane. Histamine-immunoreactive endocrine cells could not be detected in the pyloric glands or in the mucosa of the duodenum. Mast cells were quite numerous in the submucosa but few in the mucosa.

Hamster. Histamine-immunoreactive endocrine cells of the closed type were found in moderate number in the basal half of the oxyntic mucosa (Fig. 5B); they were absent from the pyloric and duodenal glands. Mast cells were moderate in number in the submucosa and few in the mucosa.

Rat. Numerous histamine-immunoreactive endocrine cells were seen in the basal half of the oxyntic mucosa (Fig. 5C, D, E). They were of the closed type and often displayed long thin, basal processes. Very rarely, a few immunoreactive endocrine cells were seen also in the pyloric gland area; none were seen in the duodenum. Mast cells were numerous in the submucosa but few in the mucosa where they were restricted to the superficial layer.

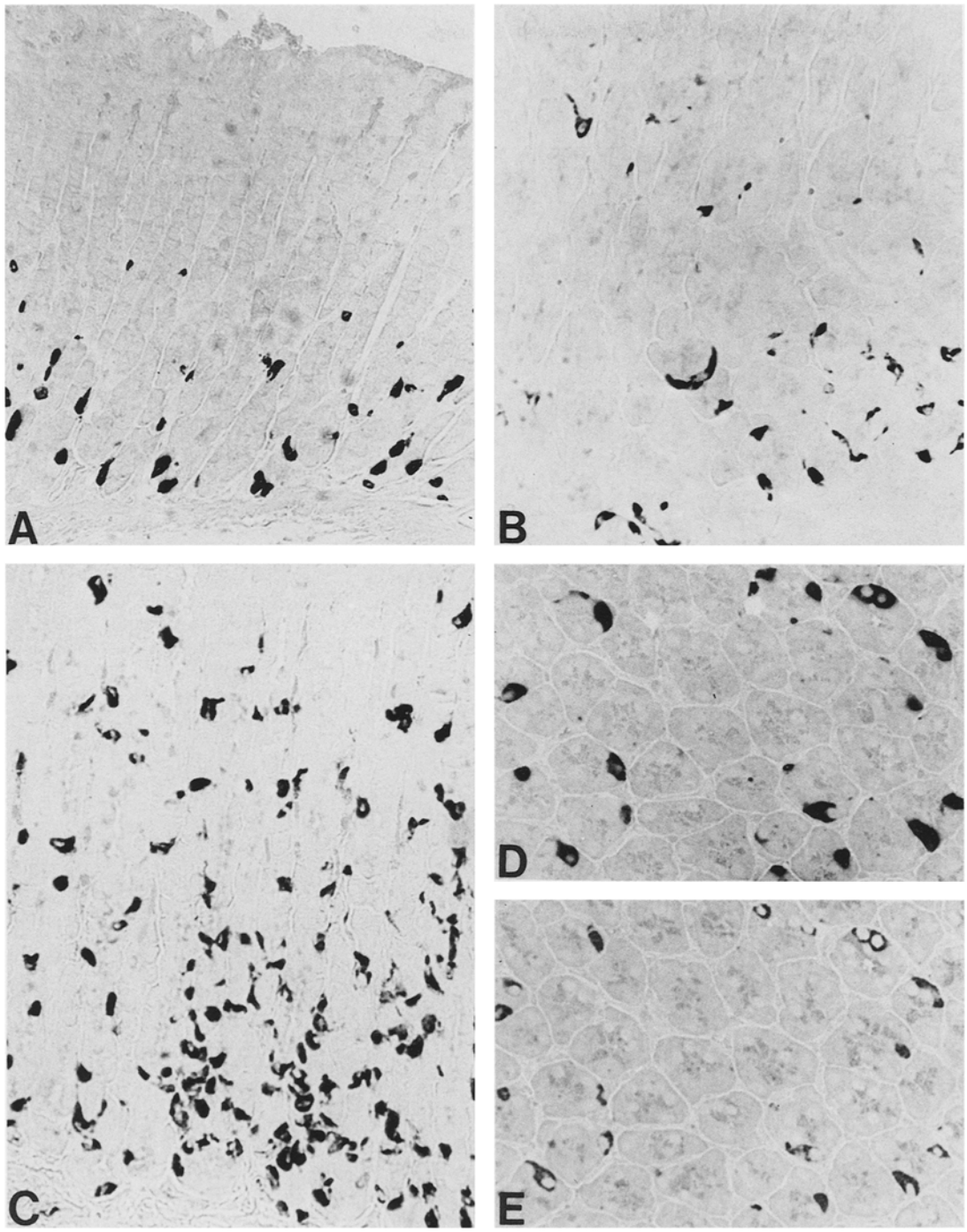


Fig. 5. Histamine-immunoreactive endocrine cells in the gastric mucosa of mouse (**A**), hamster (**B**) and rat (**C**). Transverse paraffin sections, DEPC fixation, antiserum 8431, PAP staining. In all three species the cells are located preferentially at the bottom of the glands. **D** and **E** shows two consecutive plastic sections of rat stomach (through the base of the glands), immunostained for histamine (**D**) and chromogranin A (**E**) respectively. It is evident that most of the chromogranin-immunoreactive cells stain with the histamine antibodies. **A–C** × 175, **D, E** × 250

Guinea-pig. A moderate number of rounded or polygonal, strongly histamine-immunoreactive endocrine cells of the closed type, some of which having a paracrine appearance (descending processes) (Fig. 6), were observed at the very base of the oxyntic glands (Fig. 7A). Occasionally immunoreactive endocrine cells were seen also in the pyloric gland area; none were seen in the duodenum. A moderate number of mast cells occurred in the submucosa and scattered in the upper third of the glands.

Hedge-hog. Histamine-immunoreactive, oval endocrine cells of the closed type occurred in moderate number in the oxyntic mucosa where they were scattered throughout the glands. Mast cells were equally numerous. The pyloric gland area and the duodenum harboured fairly many mast cells but no histamine-immunoreactive endocrine cells.

Rabbit. A moderate number of histamine-immunoreactive endocrine cells (closed type) were found scattered basally

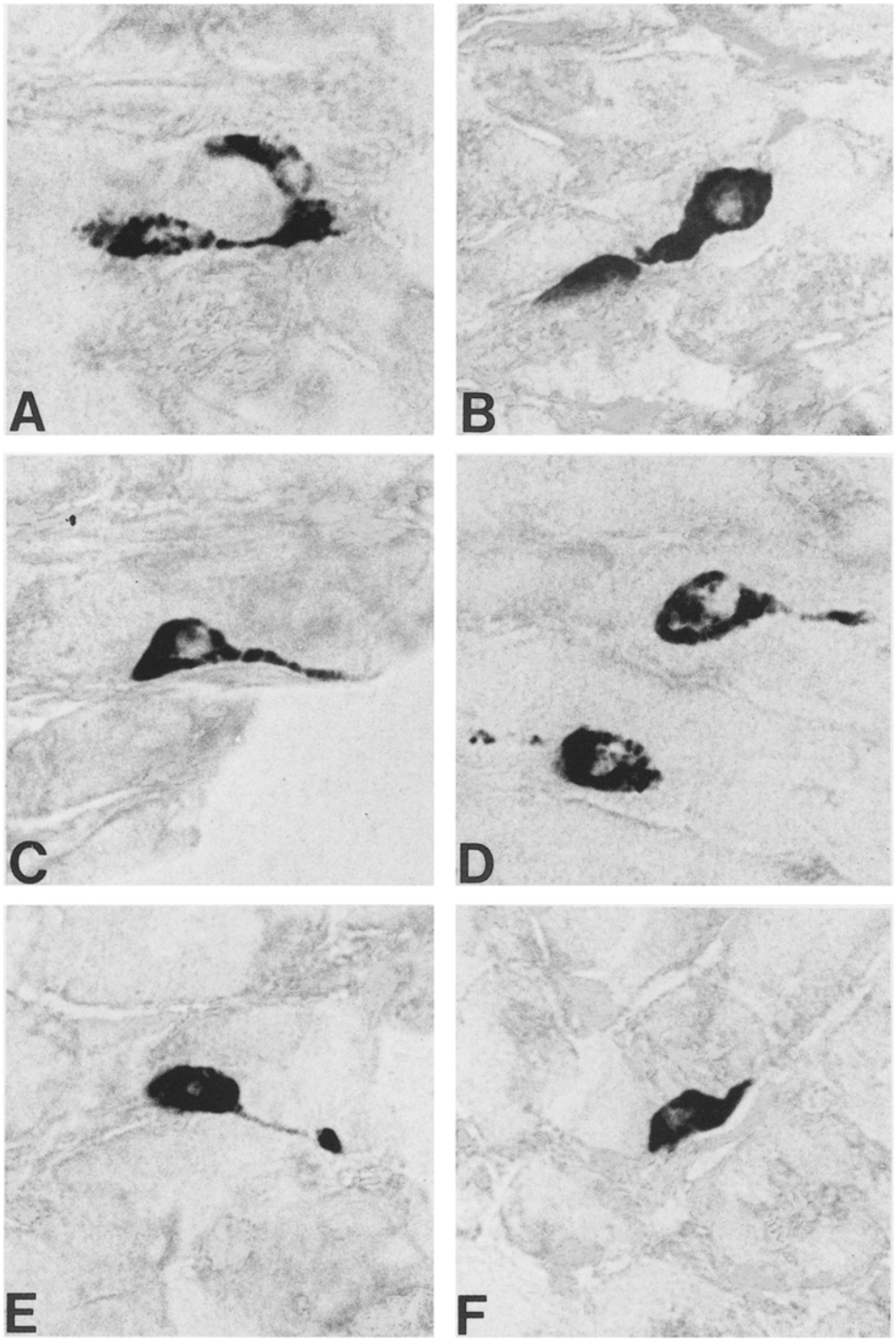


Fig. 6. Examples of histamine-immunoreactive endocrine cells of "paracrine" type in the guinea-pig stomach. The cells are selected to illustrate the varying shapes of such cells and the long thin processes issued basally, often ending in a club-like swelling. Paraffin sections, DEPC fixation, antiserum 8431, PAP staining. × 600

in the oxyntic glands (Fig. 7B). None were observed in the antropyloric or duodenal mucosa. Mast cells were moderate in number.

Dog. The oxyntic glands in the dog gave the appearance of being invaded by mast cells, many of which seemed to have an intra-epithelial localization (Fig. 8A). In addition, it was possible to discern a low number of weakly or moderately histamine-immunoreactive endocrine cells basally in the oxyntic glands (Fig. 8B). They were fewer in number than the endocrine cells that could be demonstrated by chromogranin A immunostaining (Fig. 8C). The shape of the endocrine cells was difficult to ascertain because the immunoreactive material was usually confined to the base of the cell. Histamine-immunoreactive endocrine cells were not found in the pyloric gland area or in the duodenum.

Cat. The oxyntic mucosa in this species harboured very many mast cells displaying a variety of shapes, usually elongated and with one or two rather long processes running along and close to the glands (Fig. 8D and E). Most of the mast cells were located in the connective tissue surrounding the glands. However, some gave the appearance of having an intra-epithelial localization. Comparatively

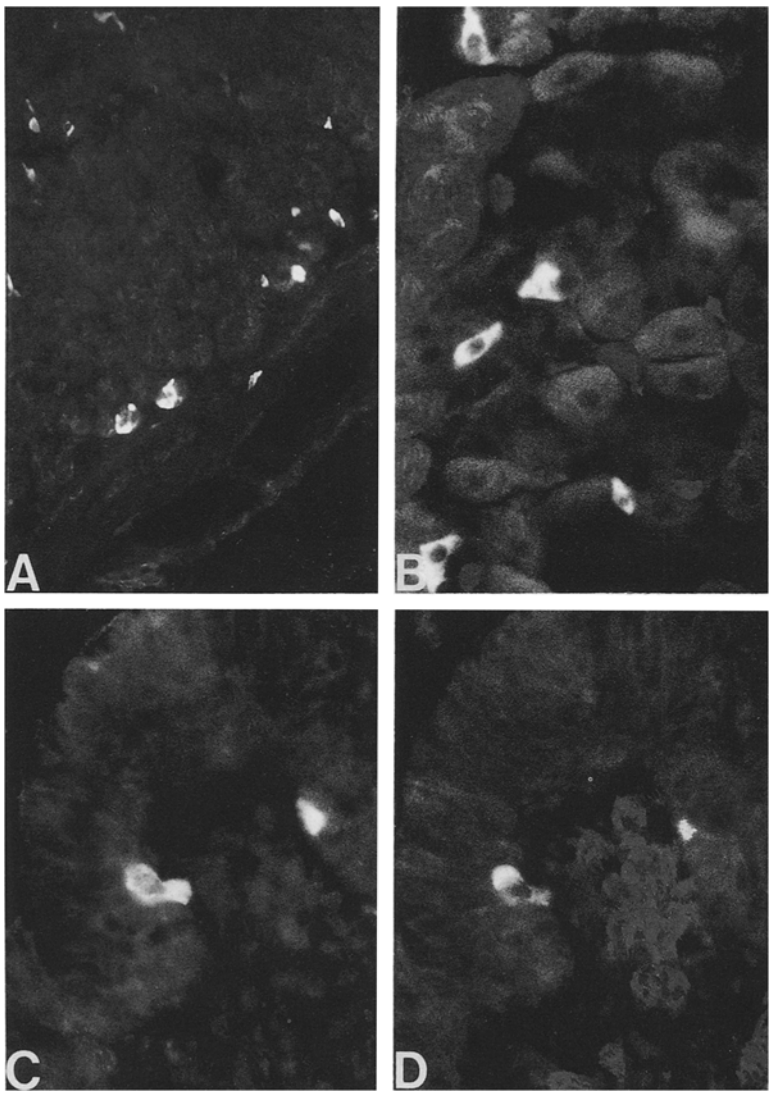


Fig. 7. Histamine-immunoreactive endocrine cells at the very base of the fundic (oxyntic) glands of a guinea-pig (**A**) and in the basal portion of such glands in a rabbit (**B**). Two histamine-immunoreactive endocrine cells were detected in the gastric mucosa of a human foetus, 24 weeks gestational age (**C**). The two cells stained also with antiserum to chromogranin A (**D**). Paraffin sections, DEPC fixation, antiserum HIST II, immunofluorescence. **A** × 125, **B–D** × 300

few, weakly to moderately histamine-immunoreactive endocrine cells of the closed type were seen scattered basally in the oxyntic glands. They sometimes had a paracrine appearance in that they issued long basal processes. New-born cats (one and three days old, respectively) had rather few mast cells and about the same density of histamine-immunoreactive endocrine cells as adult cats (not shown). As a consequence histamine-containing endocrine cells were easy to demonstrate. No histamine-immunoreactive endocrine cells were detected in the antropyloric or duodenal mucosa.

Pig. Histamine-immunoreactive rounded or triangular endocrine cells could be detected in a fair number in the oxyntic glands (Fig. 9A and B); none were found in the pyloric glands or in the duodenal mucosa. Rather many mast cells were scattered throughout the oxyntic glands. They were large and had an irregular shape and were located in the lamina propria. The picture was similar in the pyloric gland area.

Squirrel monkey. Immunoreactive endocrine cells were observed in the basal two thirds of the oxyntic mucosa; they were few in number and fairly uniformly distributed. They were absent from the pyloric gland area. Mast cells were numerous in the submucosa and moderate in number in the mucosa; they were quite numerous in the antrum.

Man. The oxyntic mucosa in man is rich in mast cells having an extra-epithelial localization (Fig. 10A). In addition a low number of weakly to moderately histamine-immunoreactive endocrine cells could be seen scattered in the oxyntic mucosa (Fig. 10A and B). These cells often issued thin ascending or descending processes. they were seen also in the gastric mucosa of foetuses, gestational age 17–24 weeks (Fig. 7C and D). In fact, they were easier to detect in the foetal mucosa because the immunostaining intensity of the endocrine cells was stronger and the mast cells much fewer than in adult gastric mucosa. Immunoreactive endocrine cells were exceedingly rare at the 17 week stage; they were regularly seen at the 18 week stage, and at the 23–24 week stage their number (per visual field) was similar to that in adults. Immunoreactive endocrine cells were notably numerous in the stomach of a patient with gastrinoma (Fig. 10C) and of two patients with atrophic gastritis. None were found in the pyloric glands.

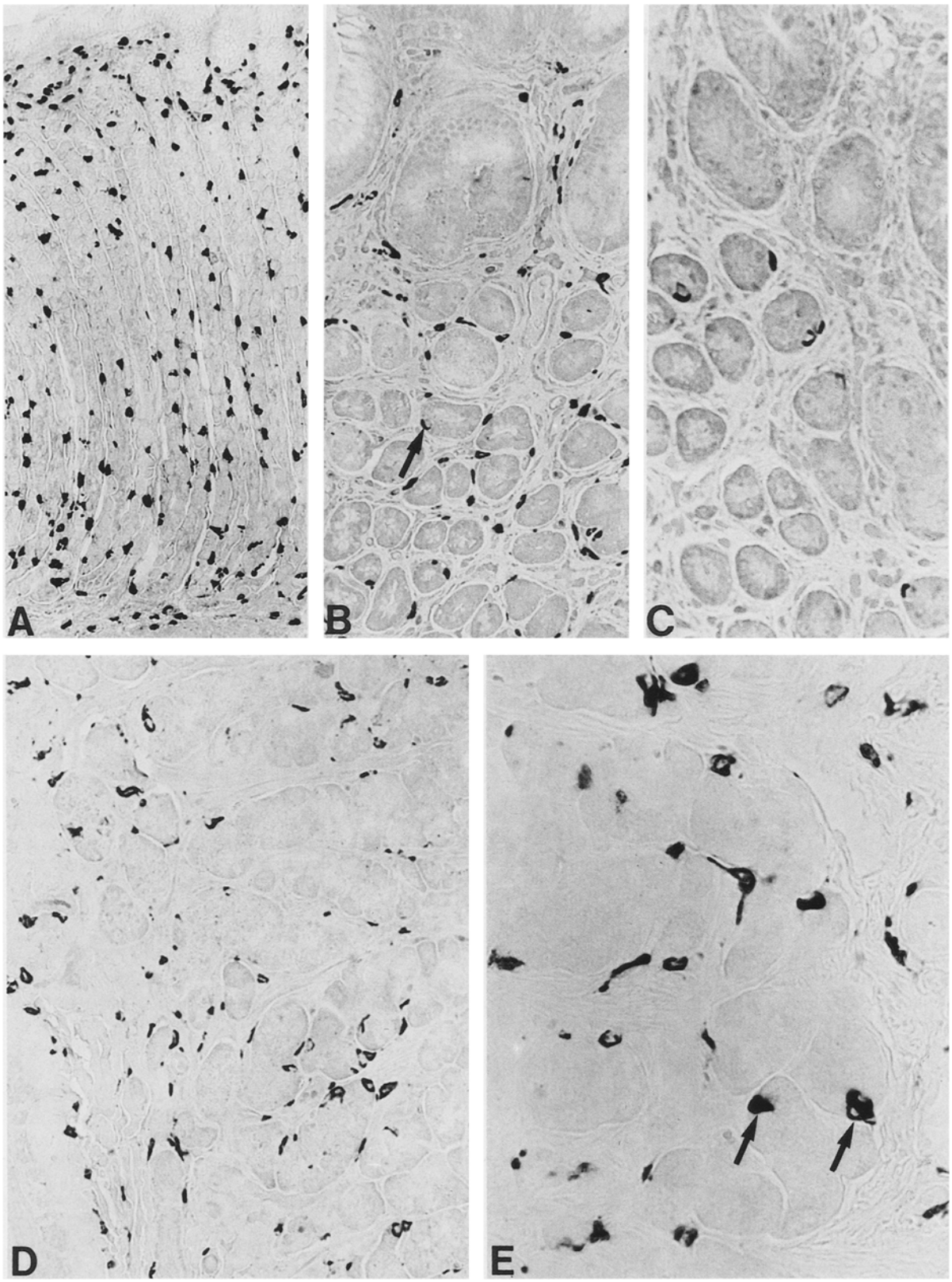


Fig. 8. Histamine-immunoreactive cells in the gastric mucosa of dog (**A**, **B**) and cat (**D**, **E**). In both species the great majority of the immunoreactive cells are mast cells, endocrine cells are comparatively few (**B, E**) (*arrow*). The relative scarcity of endocrine cells in general is illustrated by staining for chromogranin A in the stomach of the dog (**C**). In the cat histamine-immunoreactive endocrine cells are somewhat more numerous (**D**) than in the dog (**B**). This is also illustrated at high magnification in E where endocrine cells are indicated by arrows and where mast cells can be seen to issue long thin processes. (**A**) Paraffin section, (**B–E**) plastic sections, DEPC fixation, antiserum 8431, PAP staining. **A** ×100, **B–D** ×175, **E** ×250

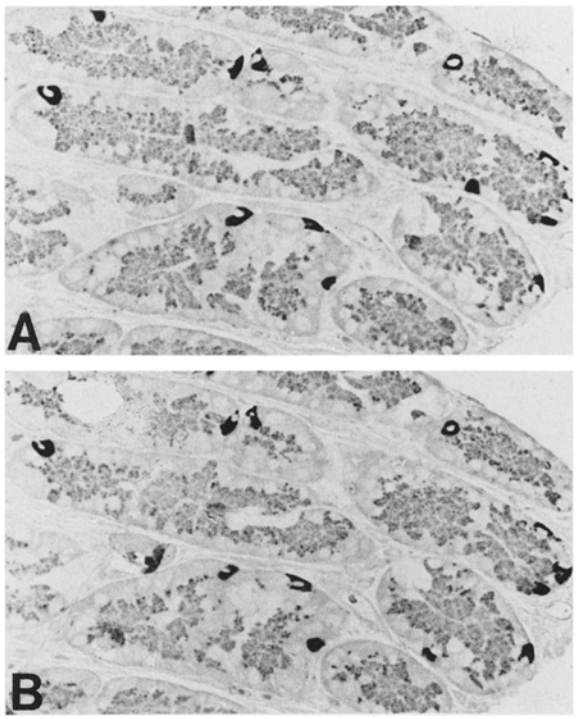


Fig. 9. Histamine-immunoreactive cells in plastic section from pig stomach (**A**) identified as endocrine by staining the consecutive section for chromogranin A (**B**). Most of the chromogranin-immunoreactive cells stained with the histamine antibodies. DEPC fixation, antiserum 8431, PAP staining. × 225

Discussion

The gastric mucosa represents a prominent endocrine organ (Håkanson 1970), in as much as it is rich in endocrine/paracrine cells of various types (Solcia et al. 1975), only few of which have been "identified" in terms of their secretory products. These cells can be more or less collectively demonstrated by certain silver staining procedures, notably the Grimelius (1969) technique for staining argyrophil cells, or by exposure to L-dopa or L-5-hydroxytryptophan (cf. Håkanson 1970; Håkanson et al. 1970), a treatment which makes it possible to visualize such cells by the Falck-Hillarp monoamine histofluorescence method (cf. Björklund et al. 1972). Alternatively, immunocytochemical methods using antibodies to chromogranin A and chromogranin-related polypeptides (cf. Nolan et al. 1985) or to the enzyme neuron-specific enolase (Marangos et al. 1982) seem to demonstrate the great majority of endocrine cells in the gastric mucosa. Finally, they are readily recognized by electron microscopy on the basis of their characteristic ultrastructure (Solcia et al. 1975).

The gastric endocrine cells fall in one of two major subgroups based on their histochemical features: one is the group of argentaffin (enterochromaffin) cells which stain also with argyrophil stains. These cells contain 5-hydroxytryptamine (more rarely dopamine) which explains the argentaffin (and chromaffin) properties of these cells (Håkanson 1970). The other group comprises the argyrophil, non-argentaffin cells which do not contain 5-hydroxytryptamine (or dopamine) but which can be made to accumulate dopamine (or 5-hydroxytryptamine) after administration of L-dopa (or L-5-hydroxytryptophan) (Håkanson 1970). The enterochromaffin cells in the stomach prevail in the pyloric gland area of many mammalian species (mouse, rat, hamster, dog and pig), but they are not always confined to that region. In the rabbit, enterochromaffin cells are abundant in the oxyntic mucosa, and in the guinea-pig, cat, monkey and man they are uniformly distributed in all regions of the gastric mucosa. The argyrophil, non-argentaffin cells occur in all regions of the stomach in all species studied (Håkanson et al. 1970).

The two sub-types of endocrine cells can be subdivided further. 5-Hydroxytryptamine-containing enterochromaffin cells in the stomach of the rabbit differ greatly in their response to the amine-depleting drug reserpine (Zbinden et al. 1957). Moreover, it is possible to differentiate between at least two types of enterochromaffin cells at the ultrastructural level. Also the argyrophil, non-argentaffin cells seem to be a heterogenous group of cells. Electron microscopic examination has revealed a variety of such cells in all species studied. Two cell types that seem to occur in the oxyntic mucosa of all species studied so far are the so-called ECL cells and the A-like cells (or X cells) (Forssmann et al. 1969; Capella et al. 1971; Håkanson et al. 1971; Solcia et al. 1975).

Immunocytochemistry has demonstrated gastrin cells in the pyloric glands of all mammals and somatostatin cells throughout the gastric mucosa but the great majority of the endocrine cells in the stomach have not yet had a peptide hormone ascribed to them. This is the case with most of the argentaffin (enterochromaffin) cell types and this is the case also with the majority populations of the argyrophil, non-argentaffin cells.

Since almost two decades histamine is known to be a constituent of an endocrine cell population in the stomach of the rat and mouse (Håkanson and Owman 1967; Håkanson et al. 1969). This was shown by the OPT method which subsequently was found to have a fairly low sensitivity, so that for instance hypothalamic histamine could not be detected. In no other species but the rat, mouse and Mastomys was it possible to detect histamine by the OPT method in gastric endocrine cells (Håkanson 1970) although claims to the contrary have been made (Hui et al. 1985). In the rat it could be shown that the histamine-storing endocrine cells were identical with a population of argyrophil, non-argentaffin cells (Håkanson and Owman 1967, 1969).

The histamine-storing endocrine cells were first thought to incorporate both the ECL cells and the A-like cells (Håkanson et al. 1971). Subsequently, Kubota et al. (1984) demonstrated histidine decarboxylase by immunocytochemistry in the ECL cells but not in the A-like cells. Recently Panula et al. (1985) demonstrated histamine-storing endocrine cells in the rat, thus confirming earlier histochemical findings. In addition, however, histamine-storing endocrine cells were observed also in the stomach of the guinea-pig, and histamine-storing neurones were demonstrated in the hypothalamus, suggesting that the immunocytochemical method for demonstrating histamine is greatly superior to the OPT method in sensitivity and probably in specificity as well (cf. Brody et al. 1972). This encouraged us to re-examine the question of where gastric histamine is to be found in various species (see also Panula et al. 1985). The results of the present study suggest that mast cells – identified by metachromasia upon toluidine staining – represent an important pool of gastric histamine in most if not all animal species. There is considerable species variation in

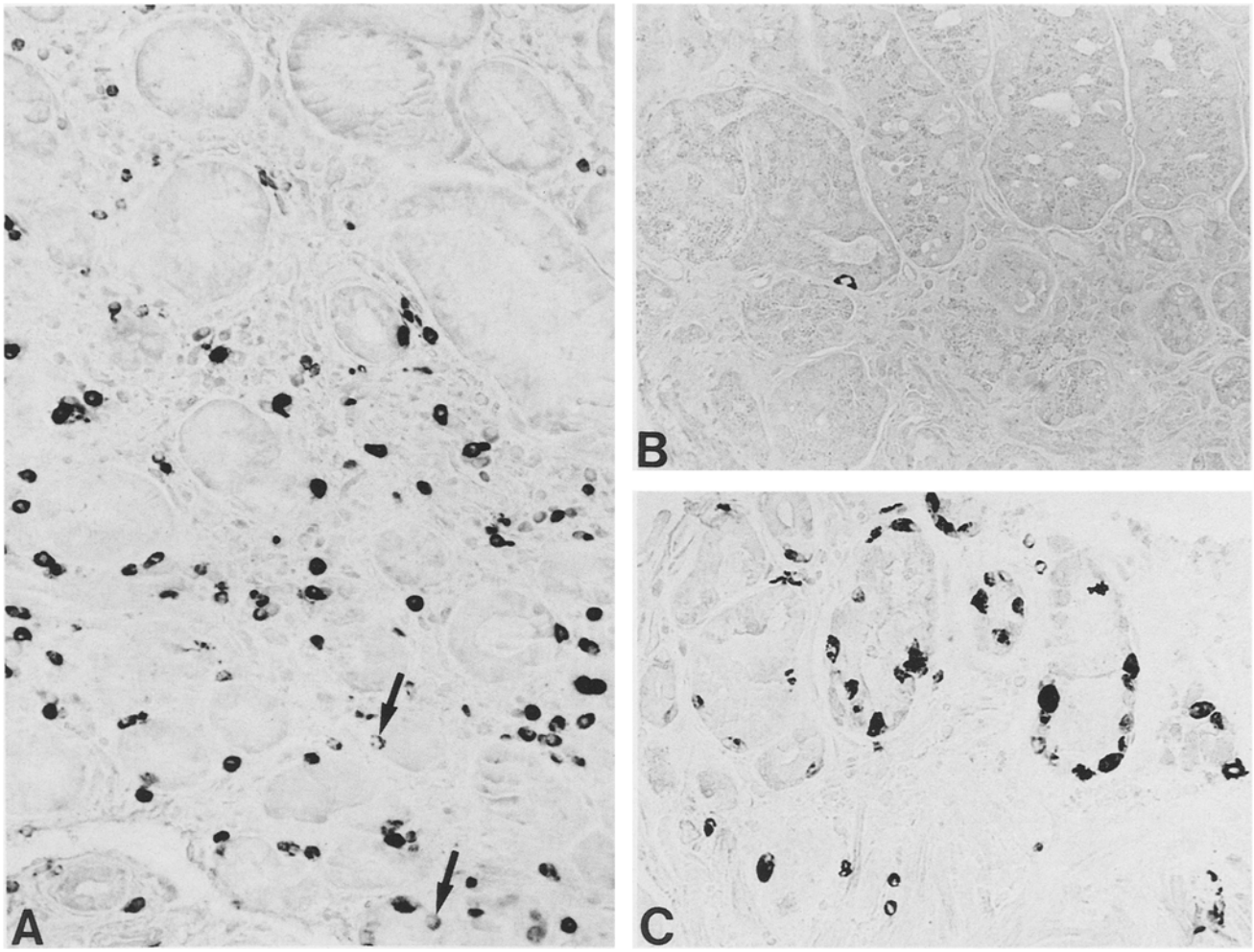


Fig. 10. Histamine-immunoreactive cells in a paraffin section from oxyntic mucosa of man (**A**). The majority of them are mast cells, those believed to be endocrine are indicated by arrows. For comparison, a plastic section was stained for chromogranin A, illustrating the relative scarcity of endocrine cells in the oxyntic glands (**B**). The number of histamine-immunoreactive endocrine cells is increased in conditions of hypergastrinemia (**C**), here examplified by a paraffin section from a patient with a gastrin-producing tumour, showing endocrine cells outlining cross sectioned oxyntic glands. DEPC fixation, (**A**, **C**) antiserum HIST II, PAP staining. **A–C** × 200

the density of mast cells in the gastric mucosa, however. They are few in fish, amphibia and reptiles, in mammals they are numeorus in the cat, dog, and man (see also Håkanson et al. 1970). Sometimes their shape and general appearance makes it very difficult to distinguish them from endocrine cells. The distinction becomes even more difficult with mast cells that have an intra-epithelial localization as seems to be the case with a proportion of the gastric mast cells in dog, cat and man. Also gastric endocrine/paracrine cells seem to store histamine in surprisingly many species. This is so in lower species, such as cartilaginous and bony fish, amphibia, reptiles and birds but it is so also in many mammals. In man such cells are few and difficult to find under normal conditions, but they are easy to demonstrate in patients with pernicious anaemia or gastrinoma, possibly because histamine-storing endocrine cells in such patients are more numerous and contain more histamine than they do in normal individuals. It was fairly easy to detect histamine-storing endocrine cells in the gastric mucosa of human foetuses at 18–24 weeks of gestation, because here mast cells were few. Although histamine can be detected in endocrine cells in many mammals, it is known from previous studies that the activity of the histamine-forming enzyme, histidine decarboxylase, is low in mammals except in the rat, mouse, Mastomys and hamster (Aures et al. 1969). This suggests a low rate of histamine formation in the histamine-storing endocrine cells in species other than those referred to above. Interestingly, the histamine-storing cells in the lower species had a localization high up in the mucosa, usually at the junction of the pits and the gastric glands, where mucous neck cells are numerous. Further, the endocrine cells in these species appeared to be of the open type, i.e. they had a direct luminal contact. This was not so in the mammals. In many species the histamine-containing endocrine cells had a characteristic "paracrine" appearance in that they possessed long thin basal extensions often ending in club-like swellings. This was particularly noticeable in chicken, mouse, rat, hamster and guinea-pig. Here the histamine-containing endocrine cells were of the closed type with a basal localization in the oxyntic glands, where chief cells are numerous. Unlike the mast cells, the histamine-immunoreactive endocrine cells were invariably stained with the antiserum to chromogranin A, a feature that was of great help in identifying these cells in those species where mast cells were numerous.

From the results of the present survey it is perhaps to

be expected that histamine will turn up in a population of gastric endocrine/paracrine cells in all amimal species. The functional significance of these cells and of the histamine they contain remains to be elucidated. So far, the histamine-storing endocrine cells have been identified as ECL cells in the rat only. From the preliminary results of electron microscopic observations it is not unlikely that the histamine-storing endocrine cells can be classified as ECL cells also in other mammals, birds and lower vertebrates. Previous reports indicate the existence of endocrine cells in the stomach of fish (Giraud and Yeomans 1982), amphibia (Giraud and Yeomans 1981), reptiles (Giraud et al. 1979) and birds (Usellini et al. 1983) that are strikingly similar to the ECL cell in mammals (Capella et al. 1971; Håkanson et al. 1971).

In addition, we have immunocytochemical evidence for the presence of histamine in intramural nerve fibers in the stomach wall. This information will be presented in a separate publication.

Acknowledgement. The study was supported by grants from the Swedish Medical Research Council (1007, 4499).

References

Andrew W (1959) Textbook of comparative histology. Oxford University Press, New York

Aures D, Håkanson R, Owman C, Sporrong B (1968) Cellular stores of histamine and monoamines in the dog stomach. Life Sci 7:1147–1153

Aures D, Davidson W, Håkanson R (1969) Histidine decarboxylase in gastric mucosa of various mammals. Eur J Pharmacol 8:100–107

Björklund A, Falck B, Owman C (1972) Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic monoamines. In: Berson SA (ed) Method of investigative and diagnostic endocrinology. Vol 1: Rall JE, Kopin IJ (eds) The thyroid and biogenic amines. North-Holland, Amsterdam, pp 318–368

Brody MJ, Håkanson R, Owman C, Sundler F (1972) An improved method for the histochemical demonstration of histamine and other compounds producing fluorophores with o-phthalaldehyde. J Histochem Cytochem 20:945–948

Capella C, Vassallo G, Solcia E (1971) Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in murine stomach and of its equivalent in other mammals. Z Zellforsch 118:68–84

Cohn DV, Elting JJ, Frick M, Elde R (1984) Selective localization of the parathyroid secretory protein-I/adrenal medulla chromagranin. A protein family in a wide variety of endocrine cells of the rat. Endocrinology 114:1963–1974

Coons AH, Leduc EH, Conolly JM (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J Exp Med 102:49–60

Forssmann WG, Orci L, Pictet R, Renold AE, Rouiller C (1969) The endocrine cells in the epithelium of the gastrointestinal mucosa of the rat. J Cell Biol 40:692–715

Giraud AS, Yeomans ND (1981) Fine structure of the gastric mucosa and endocrine cells of the toad, *Bufo marinus*. Cell Tissue Res 218:663–668

Giraud AS, Yeomans ND (1982) Cell types of the gastric mucosa of a teleost, the river blackfish, *Gasopsis marmoratus*. Aust J Mar Freshwater Res 33:1103–1108

Giraud AS, Yeomans ND, St John DJB (1979) Ultrastructure and cytochemistry of the gastric mucosa of a reptile. *Tiliqua scincoides*. Cell Tissue Res 197:281–294

Grimelius L (1969) A silver nitrate stain for α_2 cells in human pancreatic islets. Acta Soc Med Upsal 73:243–270

Håkanson R (1970) New aspects of the formation and function of histamine, 5-hydroxytryptamine and dopamine in gastric mucosa. Acta Physiol Scand (Suppl) 340:1–134

Håkanson R, Owman C (1967) Concomitant histochemical demonstration of histamine and catecholamines in enterochromaffin-like cells of gastric mucosa. Life Sci 6:759–766

Håkanson R, Owman C (1969) Argyrophilic reaction of histamine-containing epithelial cells of murine gastric mucosa. Experientia 25:625–626

Håkanson R, Lilja B, Owman C (1969) Cellular localization of histamine and monoamines in the gastric mucosa of man. Histochemie 18:74–86

Håkanson R, Owman C, Sjöberg NO, Sporrong B (1970) Amine mechanisms in enterochromaffin and enterochomaffin-like cells of gastric mucosa in various mammals. Histochemie 21:189–220

Håkanson R, Owman C, Sporrong B, Sundler F (1971) Electron microscopic identification of the histamine-storing argyrophil, enterochromaffin-like cells in the rat stomach. Z Zellforsch 122:460–466

Håkanson R, Larsson L-I, Owman C, Snell KC, Sundler F (1973) Fluorescence and electron microscopic histochemistry of endocrine-like cells in gastric mucosa and argyrophil tumor of *Praomys (Mastomys) natalensis*. Histochemie 37:23–38

Hui WM, Liu HC, Lam SK (1985) Human gastric enterochromaffin-like (ECL) cells – demonstration of histamine-content and its cholinergic nerve supply. Gut 26:A1145

Ito S (1981) Functional gastric morphology. In: Johnson LR (ed) Physiology of the gastrointestinal tract. Raven Press, New York, pp 517–550

Kapoor BG, Smith H, Verighina IA (1975) The alimentary canal and digestion in teleosts. Adv Mar Biol 13:109–239

Kubota H, Taguchi Y, Tohyama M, Matusuura N, Shiosaka S, Ishihara T, Watanabe T, Shiotani Y, Wada H (1984) Electron microscopic identification of histidine decarboxylase-containing endocrine cells of the rat gastric mucosa: an immunohistochemical analysis. Gastroenterology 87:496–502

Marangos PJ, Polak JM, Pearse AGE (1982) Neuron-specific enolase: a probe for neurons and neuroendocrine cells. Trends Neurosci 5:193–196

Nolan JA, Trojanowsky JQ, Hogue-Angeletti R (1985) Neurons and neuroendocrine cells contain chromagranin. J Histochem Cytochem 33:791–798

O'Connor DT, Burton D, Deftos LJ (1983) Chromogranin A: Immunohistology reveals its universal occurrence in normal polypeptide hormone producing endocrine glands. Life Sci 33:1657–1663

Panula P, Yang H-YT, Costa E (1984) Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci USA 81:2572–2576

Panula P, Kaartinen M, Mäcklin M, Costa E (1985) Histamine-containing neuronal and endocrine systems. J Histochem Cytochem 33:933–941

Pearse AGE, Polak JM (1975) Bifunctional reagents as vapour- and liquid-phase fixatives for immunohistochemistry. Histochem J 7:179–186

Romeis B (1948) Mikroskopische Technik. R Oldenbourg, München

Solcia E, Capella C, Vassallo F, Buffa R (1975) Endocrine cells of the gastric mucosa. Int Rev Cytol 42:223–286

Sternberger LA (1979) Immunocytochemistry, 2nd edn. Wiley, New York

Usellini L, Tenti P, Fiocca R, Capella C, Buffa R, Terenghi C, Polak JM, Solcia E (1983) The endocrine cells of the chicken proventriculus. Bas Appl Histochem 27:87–102

Wilson BS, Lloyd RV (1984) Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. Am J Pathol 115:458–468

Zbinder G, Pletscher A, Studer A (1957) Regionäre Unterschiede der Reserpinwirkung auf enterochromaffine Zellen und 5-Hydroxytryptamin-Gehalt im Magendarmtrakt. Schweiz Med Wochenschr 87:629–631