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Age-related changes in antral endocrine cells in mice

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Summary. Antral endocrine cells in four age groups of mice, namely prepubertal (1 month old), young (3 months old), ageing (12 months old) and senescent (24 months old), were detected by immunocytochemistry and quantified by computerized image analysis. A statistical difference was detected between the different age groups regarding the numbers of gastrin-, somatostatin-, and serotonin-immunoreactive cells. The number of gastrin-immunoreactive cells significantly increased between 1 and 12 months, whereas they became significantly fewer between 12 and 24 months. Somatostatin-immunoreactive cell number increased significantly in 1-, 12- and 24-month-old mice, compared with young mice (3 months old). The number of serotonin-immunoreactive cells also increased significantly in 1- and 12-month-old mice as compared with young mice. There was a statistical difference between different age-groups regarding the cell secretory index (CSI) of somatostatin- and gastrin-immunoreactive cells, the CSI of both somatostatin- and serotonin-immunoreactive cells increased significantly in 1-, 12-, and 24-month-old mice, compared with young mice. There was no statistical difference between the different age-groups regarding the CSI of gastrin-immunoreactive cells, nor between males and females regarding the number and CSI of all the endocrine cell types investigated. It is suggested that the large number of somatostatin-immunoreactive cells in ageing and senescent mice might have an impact on the gastric delay seen in the elderly. It was concluded also that the changes in the antral endocrine cells could be involved in the development of dysfunction of the gastrointestinal tract inherent in ageing, or could be secondary to structural and functional changes in the alimentary tract caused by ageing.

Key words: Computerized image analysis, Immunocytochemistry, Gastrin, Serotonin, Somatostatin

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Introduction

The incidence of gastric and duodenal ulcer increases with advancing age (Bonnevie, 1975; Ostensen et al., 1985). It has been speculated as to whether this increased incidence is due to a reduction in the turnover rate of gastric mucosa, to reduction of the lymphatic reticulum and consequently of the immunological defence reaction of the stomach, or to atrophic mucosa and intestinal metaplasia (Wanke and Schwan, 1982). Moreover, slow gastric emptying of liquid has been reported in the elderly (Moore et al., 1983). Since gastric endocrine cells play an important role in regulating gastric acid secretion, stomach motility and local immune defence (O'Dorisio, 1987; Allescher, 1991; Ekblad et al., 1991; Rangachari, 1991), it is conceivable that they are involved in the development of gastric ulcer and slow gastric emptying.

The purpose of the present study was therefore to ascertain what the possible age-related changes could be in the antral endocrine cells in a random-bred mouse line.

Materials and methods

Animals

NMRI/Bom mice (Bomholtgård Bredding and research Centre, Denmark) were used The mice were kept in our vivarium conditions, 60 mice (30 males and 30 females) for 24 months. The males and females were housed separately in cages, 5 to a cage, in a room with a 12/12 h light-dark cycle, and fed on a standard pellet diet (Astra-Ewos AB, Södertälje, Sweden) with free access to water. Under these conditions, the mean lifetime was 16.7±1.2 months for female, 20.1±1.2 months for male mice and 17.8±0.9 for all together. The 50% survival point was 19 months. The downswing of the survival curve occurred at 8 months. All the female mice died before the age of 24 months.

Three groups of mice, 10 in each (5 males and 5 females) aged 1, 3 and 12 months as well as a group of the 5 surviving males, aged 24 months were sacrificed.

The animals were fasted overnight, and then killed by cervical dislocation and the antrum was excised.

Immunocytochemistry

Tissue specimens were fixed overnight in 4% buffered formaldehyde, embedded in paraffin wax and were sectioned at 5 μm. The sections ere immunostained by the avidin-biotin complex (ABC) method (DAKO A/S, Glostrup, Denmark) as described previously in detail by El-Salhy et al. (1993). Briefly, to block the endogenous peroxidase, the sections were immersed in 0.5% H_2O_2 in Triss-buffer, pH 7.6 for 30 min. They were then incubated with 1% bovine serum albumin for 10 min to occupy the non-specific binding sites. The sections were incubated with primary antisera for 20 h at room temperature. The primary antisera had been raised in rabbit against synthetic human gastrin-17 (diluted 1:10000, code no. R-783511, specific for gastrin/CCK C-terminus, Eurodiagnostica, Malmö, Sweden), against synthetic human somatostatin (diluted 1:1600, code no. A566, Dakopatts, Glostrup, Denmark), and against serotonin (diluted 1:600, code no. B45-1, Eurodiagnostica). Incubation with the secondary antibody, biotinylated swine anti-rabbit IgG, diluted 1:200 was carried out at room temperature for 30 min. The sections were then incubated with avidin-biotin-peroxidase complex and diluted to 1:200 at room temperature for 30 min. The immune reaction was detected by immersing the sections in 50 ml Tris-buffer containing 25 mg diaminobenzidine tetrahydrochloride (DAB) and 10 μl of 30% H_2O_2, followed by light counterstaining in Mayer's haematoxylin.

Specificity controls included replacing the primary antibodies with non-immune rabbit serum and preincubating the antisera for 24 h at 4 °C with the corresponding or structurally related antigens (75 μg/ml diluted antibody). Positive controls were obtained by immunostaining sections of human antrum.

Computerized image analysis

Image analysis was performed with the Quantimet 500 MC Image Processing and Analysis System (Leica, Cambridge, England) linked to an Olympus microscope, type BX50. The software used in this system was the Leica Windows-based image analysis program «QWIN» (version 1.02) and the interactive programming system QUIPS (version 1.02). When x4 and x20 objectives were used, each pixel corresponded to 2.12 or 0.414 μm, respectively, and each field viewed in the monitor represented 1.3 or 0.047 mm^2 of tissue area, respectively. The immunostained sections of the antrum from different ages of the mice were coded and mixed and measurements were made without knowledge of animal's age. For endocrine cell quantification a x20 objective was used, in 20 randomly chosen fields from 2-5 sections, which were at least 50 μm apart.

The numbers of various endocrine cell types and their cell secretory index (CSI) were measured as described previously (El-Salhy et al., 1997) using an automated standard sequence analysis operation. Briefly, the cells were counted using field measurements. The areas of gastric gland and the immunoreactively stained areas were measured using a threshold setting. The data from each field were tabulated, computed and statistically analysed automatically. The CSI was calculated (El-Salhy et al., 1997) as follows: CSI= VS/CN, where VS= the immunoreactively-stained volume/mm^3 of the epithelial cells, and the CN= the number of cells/mm^3 measured in the same fields.

The area of epithelium corresponding to 1 mm baseline was measured for each mouse at four different sites in 2-4 perpendicularly cut sections, 50 μm apart. This was done by using the interactive measurements in the manual menu and a x4 objective.

Statistical analysis

The four age-groups were compared with the Kruskal-Wallis non-parametric ANOVA test and post-tested with the Mann-Whitney non-parametric U-test. Comparisons between male and female mice were also performed with the Mann-Whitney non-parametric U-test. P-values less than 0.5 were considered significant.

Results

Immunocytochemistry

Gastrin- (Fig. 1), somatostatin- and serotonin-immunoreactive cells were found in gastric gland of the antrum in all age groups. The endocrine cells were flask-shaped or basked-shaped. There was no immunostaining when the primary antisera were replaced by non-immune rabbit serum or when it was pre-incubated with the corresponding antigen. Pre-incubation of the primary antisera with structurally-related antigen had no effect on the immunostaining. The antisera stained endocrine cells in sections from human antrum.

Computerized image analysis

The numbers of various endocrine cell types and their CSI in the antrum of different age groups are given in Fig. 2. The proportion of each endocrine cell type of the total number of endocrine cells is reported in Fig. 3. There was a statistical difference between the different age groups regarding the number of gastrin-, somatostatin-, and serotonin-immunoreactive cells (P= 0.04, <0.0001 and <0.0001, respectively). The number of gastrin-immunoreactive cells significantly increased between 1- and 12-month-old mice but decreased significantly between 12- and 24-month-old mice. The number of somatostatin-immunoreactive cells was significantly increased in 1-, 12- and 24-month-old mice, compared with mature (3-months-old) mice. The number of serotonin-immunoreactive cells increased significant-

ly in 1- and 12- month-old mice as compared with young mice. Different age-groups differed statistically regarding the CSI of somatostatin- and gastrin-immunoreactive cells ($P<0.0001$ and 0.002, respectively). The CSI of both somatostatin- and serotonin-immunoreactive cells, was significantly increased in 1-, 12-, and 24-month-old mice, but there was no statistical difference between different age groups regarding the CSI of gastrin immunoreactive cells ($P=0.13$), nor was there any statistical difference between males and females regarding the number and CSI of all the endocrine cell types investigated (Table 1). The mucosal area/mm baseline (mean±SEM) of 1-, 3-, 12-, and 24-month-old mice were 0.28±0.02, 0.28±0.02, 0.31±0.04 and 0.37±0.02 mm^2/mm, respectively. There was no statistical difference between the different age groups regarding the mucosal area/1 mm baseline ($P=0.07$).

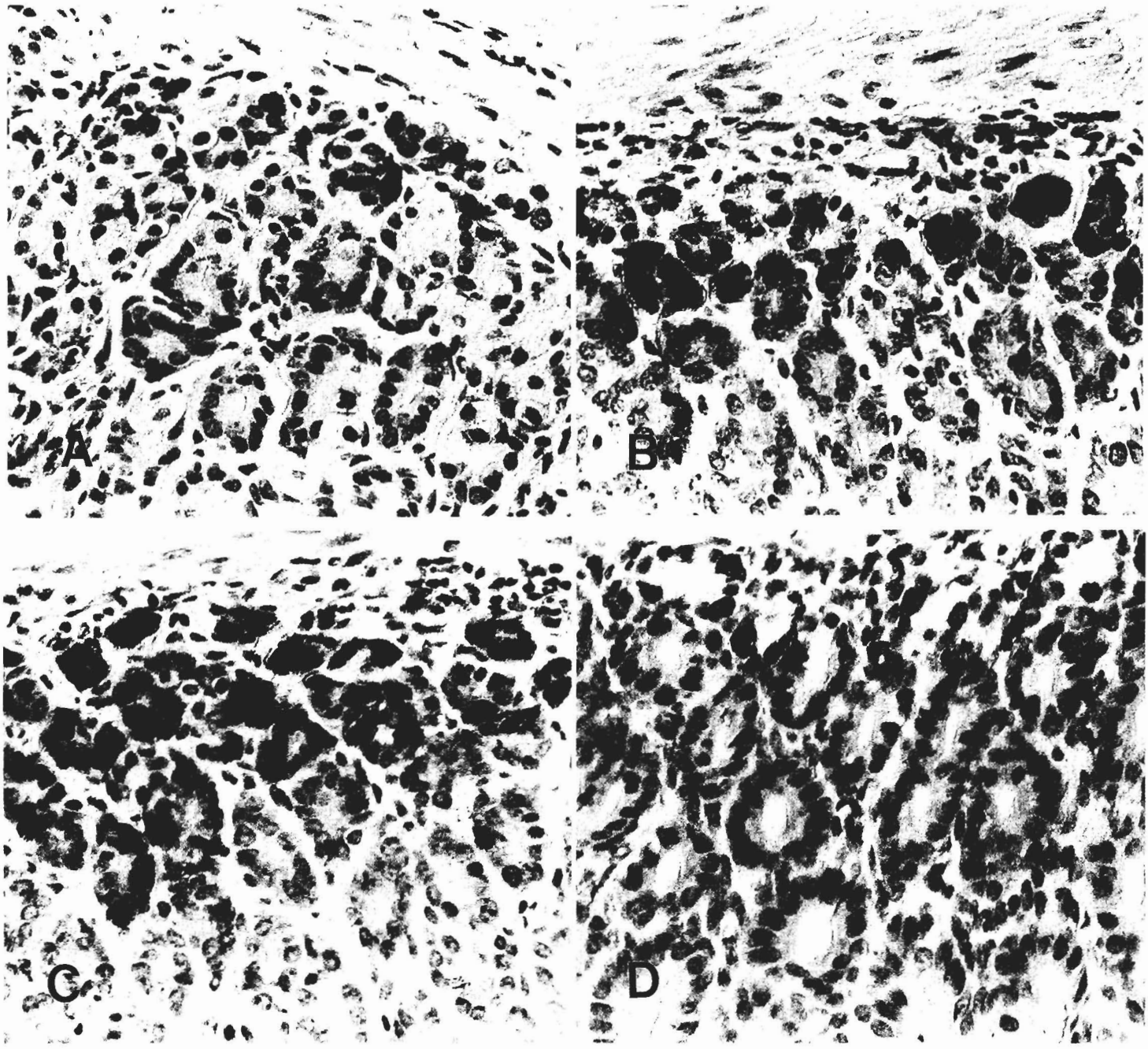

Fig. 1. Gastrin-immunoreactive cells in the antrum of 1-month **(A)**, 3-month **(B)**, 12-month **(C)** and 24-month-old mice. x 250

Discussion

In the present study, antral endocrine cells were investigated in a rodent animal model, namely the MNRI mouse. These mice are a random-bred line having a degree of genetic variance similar to that which would be expected in the human population. The endocrine cells were studied in four age groups, namely pre-pubertal (1 month old), young (3 months old), ageing (12 months old) and senescent (24 months old).

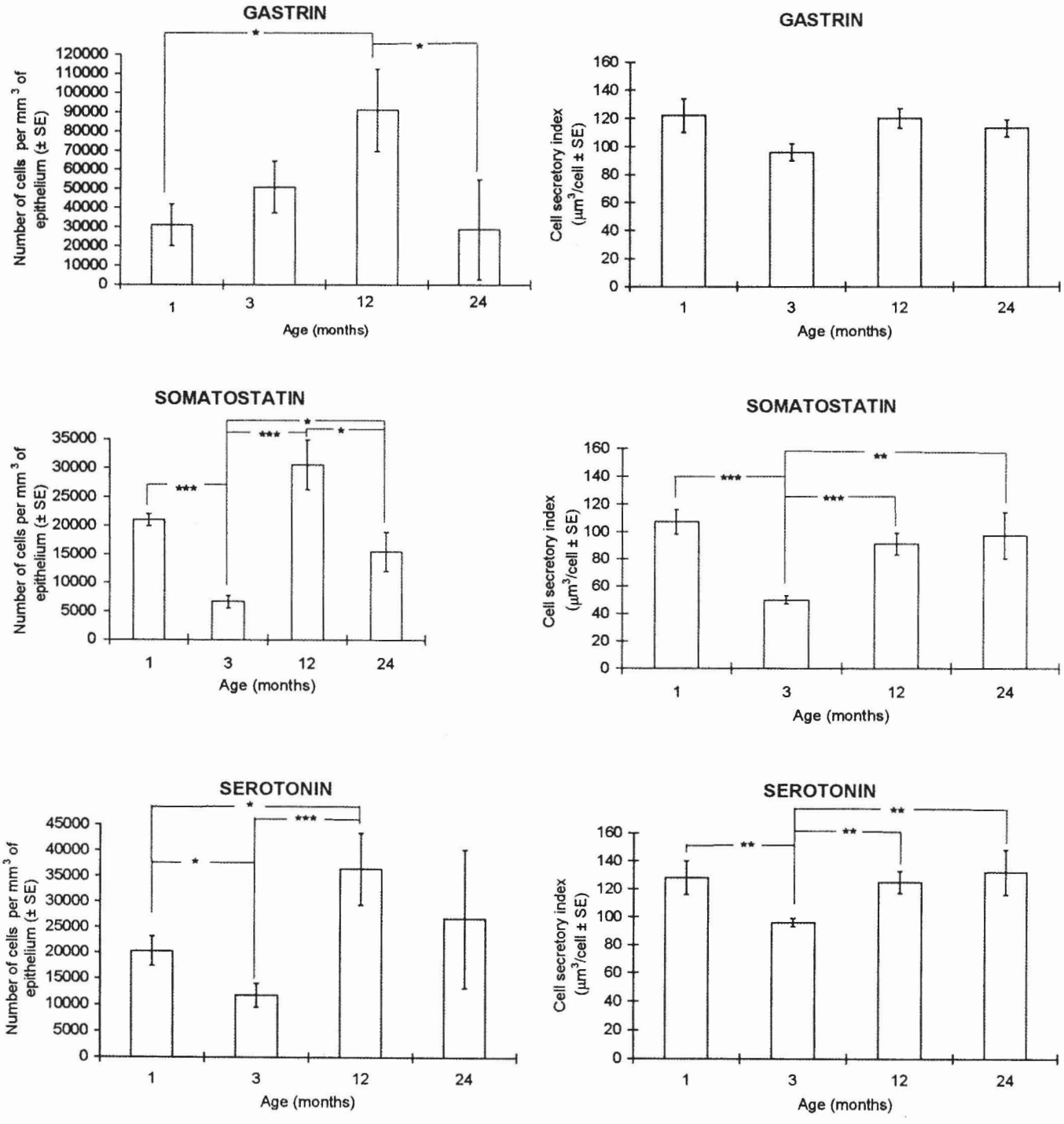

Fig. 2. The number of various endocrine cell types and their cell secretory index in different age groups.

Table 1. The number and cell secretory index of various antral endocrine cells (mean±SEM) of males and females from different age groups.

	ENDOCRINE CELL NUMBER			CELL SECRETORY INDEX		
	Male	Female	p-value	Male	Female	p-value
Gastrin	44265±14391	71548±13354	n.s.	112±8	119±8	n.s.
Somatostatin	17316±2572	19230±3363	n.s.	86±9	79±8	n.s.
Serotonin	19346±3151	28726±5554	n.s.	116±12	178±23	n.s.

n.s.: not significant

The density of all the antral endocrine cell types investigated changed with age. Thus, the number of gastrin-immunoreactive cells decreased significantly in senescent mice. This observation is in line with earlier findings in rat antrum (Lehy et al., 1979). It also agrees with the previously reported decreased serum and antral gastrin content in old rats and humans, as detected by radioimmunoassay (Borgström et al., 1973; Khalil et al., 1988; Kogire et al., 1993). The number of somatostatin-immunoreactive cells was significantly higher in prepubertal mice, and in the ageing and senescent stages than in young mice. This finding agrees with that reported for the antrum of opossum (Krause et al., 1985), where a greater number of somatostatin-cells was found in the antrum of young animals. It disagrees, however, with earlier reports that there are fewer antral somatostatin cells in calves (Kitamura et al., 1985) and that the number of these cells is the same in old as in mature rats (Lehy et al., 1979). Serotonin-immunoreactive cells were more numerous in number prior to puberty, and in ageing mice, than young animals. This observation is consistent with a previous finding that serotonin cells are more numerous in young opossum and calf than in mature animals (Kitamura et al., 1985; Krause et al., 1985).

The CSI is an index used to indicate the immunoreactive secretory content of the endocrine cells (El-Salhy et al., 1997). It roughly indicates the secretory activity of the cell, namely the summation of peptide synthesis and secretion. In order to determine the peptide/amine synthesis and secretion more precisely, *in situ hybridization* and determination of the content of the complementary mRNA (Mulder et al., 1995), and measurement the circulating peptides/amines should be performed. It is well known, however, that endocrine cells with a high physiological activity (high rates of peptide synthesis and release) show a small amount of cytoplasmic secretory content (Pinto et al., 1995; El-Salhy et al., 1997). It was assumed in the present investigation, therefore, than an increase in the CSI represented a low physiological activity in the endocrine cells and viceversa. Whereas the CSI of gastrin did not change in the different age groups, that of somatostatin- and serotonin-immunoreactive cells increased significantly in mice prior to puberty, and in the ageing and senescent stages. This increase may represent a decline in the secretory activity of these cells.

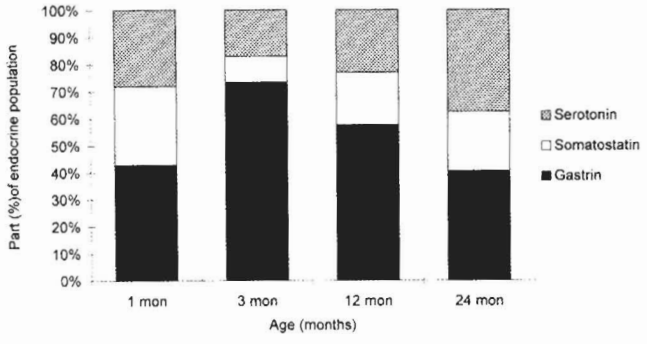

Fig. 3. The percentage of each antral endocrine cell type in different age groups.

Gastrin stimulates gastric acid secretion and is considered to be the major hormone regulating oxyntic mucosal growth (Walsh, 1994). Somatostatin inhibits the secretion of acid, pepsinogen and gastrin (Chiba and Yamada, 1994). In patients with gastric ulcus, gastric acid secretion is normal or low (Seensalu, 1994). The present findings of a decreased number of gastrin-immunoreactive cells and an increased number of somatostatin immunoreactive cells in senescent vis-à-vis mature mice, together with the reported observation of decreasing basal and gastrin-stimulated gastric acid secretion with age both in rat and man (Borgström et al., 1973; Khalil et al., 1988) make it likely that an endocrine factor might be one of the factors responsible for the increased incidence of gastric and duodenal ulcer in the elderly. Gastrin has an excitatory effect on the corpus and antrum by exerting a direct effect on the smooth muscles, and by an indirect effect mediated by the release of acetylcholine (Allescher, 1991). Despite this stimulatory motor activity of the antrum, gastrin delayed gastric emptying of liquid and solids (Allescher, 1991). Somatostatin inhibits the late phase of gastric emptying (Chiba and Yamada, 1994). The high somatostatin content in ageing and senescent mice might have an impact on the gastric delay seen in the elderly.

The present study of antral endocrine cells of mice establishes that these cells change with age. Such a change may be involved in the development of dysfunction of the gastrointestinal tract observed in the elderly. It is also possible that these changes are secondary to structural and functional changes in the alimentary tract, caused by ageing.

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