

**Immunolocalisation of the serotonin in the fundus ventriculi and duodenum of the Asia minor ground squirrel (*Spermophilus xanthoprymnus*)**

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**TIMURKAAN, S., E. ÖZKAN, R. İLGÜN, F. M. GÜR: Immunolocalisation of the serotonin in the fundus ventriculi and duodenum of the Asia minor ground squirrel (*Spermophilus xanthoprymnus*). Vet. arhiv 79, 69-76, 2009.**

**ABSTRACT**

**ABSTRACT:** The endocrine cells of the proximal duodenum and fundus ventriculi of the citellus (*Spermophilus xanthoprymnus*) were investigated using the immunohistochemical method. Serotonin immunoreactive cells were located and distributed in the fundus and duodenum with variable frequencies. They were spherical or spindle-shaped and the highest frequency serotonin immunoreactive cells were detected in the whole fundic region. The regional distribution of the endocrine cells in the fundus and duodenum of the citellus resembled other mammalian species.

**Key words:** immunohistochemistry, serotonin, endocrine cells, gastrointestinal tract, Asia minor ground squirrel

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**Introduction**

Anatolian ground squirrels (*Spermophilus xanthoprymnus*), which are Asia Minor ground squirrels, are from the Sciuridae family and which was described by Aksaray, range from Anatolia up to the Caucasus (WILSON and REEDER, 1993).

It is well known that regulation of the motility, secretion and absorption of the fundus and duodenum is coordinated by neural and hormonal controls (STEVENS and HUME, 1995). Many studies have described the types and distribution of hormone-producing cells in the gastrointestinal tract of mammals at light and electron microscopic levels (SUNDLER and HAKANSON, 1988; KAWANO et al., 1983).

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Endocrine cells have an important role in the gastrointestinal tract. Many different cell types have been identified, which produce a variety of biologically active peptides (SJÖLUND et al. 1983). The gastrointestinal endocrine system has been studied with immunocytochemistry (CASTALDO and LUCINI, 1991; KITAMURA et al., 1985). Different gut hormones have a significant role in the digestive functions of the stomach and intestine (FUJITA and KOBAYASHI, 1977). The distribution of endocrine cells in the gastrointestinal tract has been widely investigated in the gastrointestinal tract of many domestic (CAPELLA and SOLCIA, 1972; CASTALDO and LUCINI, 1991; CECCARELLI et al., 1987 and 1990; MIMODA et al., 1998) and wild animals (KRAUSE et al., 1985). The distribution of serotonin immunoreactive cells has previously been studied in the proventriculus and intestinal tract of many avian species (ADAMSON and CAMPBELL, 1988; RAWDON and ANDREW, 1981 and 1994; USELLINI et al., 1983; WATANABE et al., 1987), reptiles (PEREZ-TOMAS et al., 1989) and rodents (TIMURKAAN et al., 2005).

However, there are no data indicating their presence related to the immunohistochemical distribution of serotonin in the fundus and duodenum of the Asia Minor ground squirrel (*Spermophilus xanthoprimum*). Therefore, the present study was to improve the understanding of the functional role and localization of serotonin in the fundus and duodenum of the Asia Minor ground squirrel.

#### Materials and methods

Four adult Asia Minor ground squirrels (*Spermophilus xanthoprimum*) were anesthetized and killed using ether. Small pieces of tissues were dissected from the duodenum and the fundic gland region was removed immediately and placed in 10% formalin in phosphate-buffered saline (PBS), pH 7.4, for 18 hours before paraffin embedding. Tissue samples were routinely processed through a graded series of alcohols, cleared in xylol and embedded in paraffin. 5 µm thick sections were obtained and processed for immunohistochemical staining.

Immunohistochemistry. Immunohistochemical staining was carried out using the peroxidase-antiperoxidase (PAP) method (STERNBERGER, 1979). Blocking of endogenous peroxidase activity was carried out with % 0.08 hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in methanol for 5 minutes. In order to block unspecific binding, incubation with (1:10) normal goat serum in 0.1 M, PBS, pH 7.2, was performed.

*PAP technique.* Sections were incubated for 16-20 hours at 4 °C in rabbit anti-serotonin (Sigma). The antibody was diluted to 1:500 with PBS containing % 0.25 sodium azide and % 2.5 bovine serum albumin respectively. Sections were then incubated in goat anti-rabbit Ig G (Dako), followed by rabbit peroxidase anti-peroxidase complex (Sigma), both at a dilution of 1:50 in PBS for 1 hour at room temperature. Sections were washed in PBS for 30 minutes after each incubation and finally immersed in nickel ammonium

sulphate (GDN) substrate (SHU et al., 1988) for 10 minutes. After washing in distilled water, sections were counterstained with eosin. Sections were dehydrated and coverslips mounted with mounting medium. Sections were examined with a light microscope and photographs were taken.

### Results

In the present study, immunoreactive endocrine cells to the antisera against serotonin were detected in the fundus ventriculi, duodenum, jejunum of the Asia Minor ground squirrels (*Spermophilus xanthoprimum*). The endocrine cells were distributed throughout the gastric glands and intestine. No positive reactions were detected in the negative control sections.

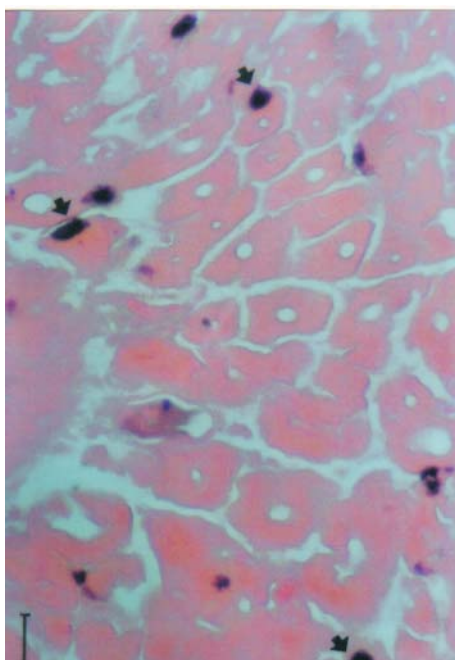


Fig. 1. Microphotograph of the immunohistochemical localization of serotonin in the fundic gland region of the *Spermophilus xanthoprimum*. Serotonin-containing cells in the fundus epithelium (arrows).  
20 × 5; scale bar = 40 µm.

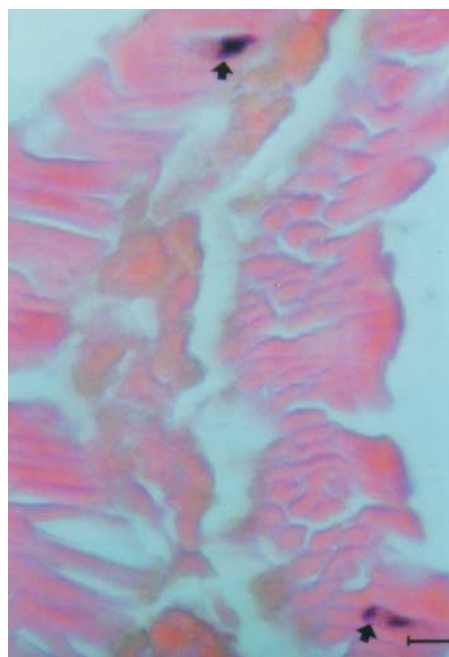


Fig. 2. Microphotograph of the immunohistochemical localization of serotonin in the villus intestinalis of the duodenum of the *Spermophilus xanthoprimum* (arrows).  
40 × 5; scale bar = 20 µm.

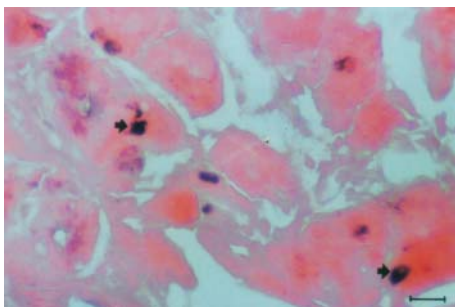


Fig. 3. Microphotograph immunohistochemical localization of serotonin in the intestinal glands of the duodenum of the *Spermophilus xanthoprimum*. Serotonin-containing cells in the duodenum (arrows).  
20 × 5; scale bar = 40 μm.

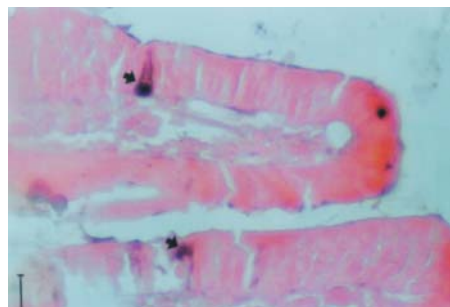


Fig. 4. Microphotograph illustrating the localization of serotonin in the jejunum of the *Spermophilus xanthoprimum*. Serotonin-containing cells in the jejunum (arrows).  
20 × 5; scale bar = 40 μm.

Serotonin immunoreactive cells were identified in the stomach and intestine. Most of the gastrointestinal endocrine cells were located in the basal portions of the fundus. The immunoreactive cells were round or oval in shape or triangular, spindly, long or slender. The immunoreactive cells were either of 'open' or 'closed' type. They appeared as close-type cells as they did not possess lamina contact with their apical cytoplasmic processes and distributed at the bases of the crypts and some open-type cells with apical cytoplasmic processes that reached the intestinal lumen (Figs 2, 4).

In the fundus, the endocrine cells were distributed widely from the base to the apical portion of the glands. Serotonin immunoreactive cells were located between the gland cells and the basement membrane at the basal portion of the glands and they were close-type cells (Fig. 1). They then decreased gradually from moderate numbers in the villus intestinalis of the duodenum (Fig. 2) and a moderate number of serotonin immunoreactive cells was detected in the base of the intestinal glands of the duodenum (Fig. 3). In the jejunum, a few serotonin immunoreactive cells were found in the base of the intestinal glands (Fig. 4).

### Discussion

There have been many studies on the distribution and frequency of endocrine cells in the gastrointestinal tract of mammalian species (CAPELLA and SOLCIA, 1972; CALINGASAN et al., 1984; KAWANO et al., 1983; KITAMURA et al., 1984 and 1985). Recent advances in immunohistochemistry have made it possible to demonstrate the presence of a number of endocrine cells in the gut of mammals. Also our similar study revealed

serotonin immunoreactive cells in the gastrointestinal tract of porcupines (TIMURKAAN et al., 2005).

In the present study, the distribution was revealed of serotonin immunoreactive cells of the fundus and intestine of Asia Minor ground squirrels (*Spermophilus xanthoprimum*). This study is the first to clarify immunohistochemically, the distribution of serotonin in the fundus and intestinal tract in the *Spermophilus xanthoprimum*. Immunohistochemical studies on the fundus and intestinal tract of the citellus have revealed a range of endocrine cell types similar to that of mammals and avian species.

The immunolocalization of serotonin has been reported throughout the gastrointestinal tract. The general distribution of serotonin immunoreactive cells in Asia Minor ground squirrels is similar to that described in other mammals (FACER et al., 1979; NEMOTO et al., 1983) and other vertebrates (EL-SALHY et al., 1985). These data are substantially in agreement with those observed in the gastrointestinal apparatus of chickens (RAWDON and ANDREW, 1981 and 1994; USELLINI et al., 1983). Serotonin is known to stimulate strongly the smooth musculature of the gut and secretion of the exocrine gland (FUJITA et al., 1988; FURNESS and COSTA, 1982). This report suggested that serotonin immunoreactive cells distributed in the gastrointestinal tract contract the smooth muscle in the mucosa of the glandular epithelium. These cells might also control the mucous secretion of the glandular epithelium. The duodenum contains the greatest variety of endocrine cell types in the digestive tract (SOLCIA et al., 1987).

In conclusion the regional distribution of serotonin immunoreactive cells of the fundus and intestine of the citellus were clarified in this study. The distribution pattern of endocrine cells in the fundus and duodenum of the citellus was fundamentally similar to those of mammals rather than those of avian species.

In summary, in order to obtain a better understanding of the functional role of serotonin, we then investigated their immunocytochemical localization in the souslik gastrointestinal tract. The immunocytochemical localization of the serotonin in the souslik gastrointestinal tract was described for the first time in the present study. This study showed similarity to that observed in the rat, and other mammalian and avian species. The results of the present study may contribute to the extension of data in this field of science.

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**TIMURKAAN, S., E. ÖZKAN, R. İLGÜN, F. M. GÜR: Imunolokalizacija serotonina u želučanom dnu i dvanaesniku male azijske vjeverice (*Spermophilus xanthoprymnus*). Vet. arhiv 79, 69-76, 2009.**

**SAŽETAK**

Endokrine stanice proksimalnoga dvanaesnika i želučanoga dna vjeverice *Spermophilus xanthoprymnus* istražene su imunohistokemijskom metodom. Ustanovljeno je da su stanice koje reagiraju na protutijela za serotonin rasprostranjene s različitom učestalošću na dnu želuca i u dvanaesniku. Kuglasta su ili vretenasta oblika s najvećom učestalošću u fundusnom području. Takva rasprostanjenost endokrinih stanica na dnu želuca i u dvanaesniku vjeverice slična je raspodjeli u drugih sisavaca.

**Ključne riječi:** imunohistokemija, serotonin, endokrine stanice, probavni sustav, azijska vjeverica

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