Plasticity of myenteric neurons

N Saini and M Gupta

Department of Anatomy, Postgraduate Institute of Medical Education and Research, Chandigarh, India- 160012

Corresponding author: Madhur Gupta, Professor in anatomy, Gian sagar Medical college and hospital, Ramnagar, Banur 140401, Patiala, India, e-mail: madhurg1@rediffmail.com

ABSTRACT

The nervous system exerts a profound influence on all digestive processes. The wall of digestive system is endowed with its own, local nervous system referred to as the enteric or intrinsic nervous system which is responsible for the modulation of the rhythmic gastrointestinal peristaltic activities along with other functions. The principal components of the enteric nervous system are two neuronal networks: myenteric and submucosal, embedded in the wall of the digestive tract and extend from esophagus to anus. The musculature of different parts of gastrointestinal tract is differently disposed to perform different functions. Thus the aim of present study was to demonstrate the presence of neurons and to quantify the number of collections of neurons, number of neurons in each collection and area of the neurons of the plexus. One cm piece from all the parts of GIT containing entire wall was taken from the same region of 5 adult cadavers of postmortem cases which were embalmed in the Department of Anatomy, Postgraduate Institute of Medical Sciences and Research, Chandigarh and were processed for paraffin sectioning. 5 and 10µ thick serial sections were obtained and 6th and 7th slides were stained with: Hematoxylin and Eosin and Marsland, Glees and Erikson's silver stain. The slides were photomicrographed using digital camera. The morphometrical analysis was done using Image Pro Express software. Aggregations of 1-31 neurons present in myenteric network located between longitudinal and circular muscle layers of the GIT whose size varied from 10.263-259.660µ m². They were oval or round; multipolar, arranged in two rows and dispersed in groups in connective tissue of muscularis propria. The collections of neurons were appeared to be more numerous in appendix and ileoceacal valve.

Keywords: autonomic ganglia, smooth muscle tunica, collection of neurons, size and number of neurons.

INTRODUCTION

The enteric nervous system is a large and complex neuronal network embedded in the wall of the gut.[1] One of the most developed ganglionated plexuses of the gastrointestinal tract is the myenteric plexus (or Auerbach's plexus) located between the inner circular layer and outer longitudinal layer of smooth muscle tunica.[2] The musculature of different regions of the gastrointestinal tract is differently disposed and they perform different functions.[3] Hence morphometric and quantitative assessment of neurons was carried out in all the different parts of GIT by counting the number of neurons, presence of neurons in each collection, area of neuronal cell body to find out if the neuronal population of these parts differ according to their functions.

MATERIAL AND METHODS

One cm piece from all the parts of GIT containing entire wall was taken from the same region of 5 adult cadavers of postmortem cases which were embalmed in the Department of Anatomy, Postgraduate Institute of Medical Sciences and Research, Chandigarh and were processed for paraffin sectioning.[4] Ten micrometer thick serial sections were obtained and serial sections were arranged in slides having three sections on each slide. 6th and 7th slides having three consecutive sections each of 10µ thick were stained with: Hematoxylin and Eosin and Marsland, Glees and Erikson's silver stain. The 60 slides of each structure of GIT were photomicrographed using digital camera. The morphometrical analysis was done using Image Pro Express software. The number of collections of neurons in entire circumference of each section, presence of neurons in each collection and area of neuronal cell bodies were tabulated and statistically analyzed. The circumference of neuronal bodies was marked with the help of curser and readings were automatically stored in excel file by pro-image software. The neurons were classified on the basis of their area into small, medium and large. The neurons with areas smaller than the mean minus its standard deviation were considered small, those above the mean plus its standard deviation were considered large, and those intermediate to these values were considered medium neurons.[2]

RESULTS

Myenteric plexus of gastrointestinal tract present between inner circular and outer longitudinal layers of muscular tunica contained 4.64±1.41 number of ganglia ranging from 1-15 in entire circumference of 10 µ thick section of GIT (Table-1). The neurons in ganglia were

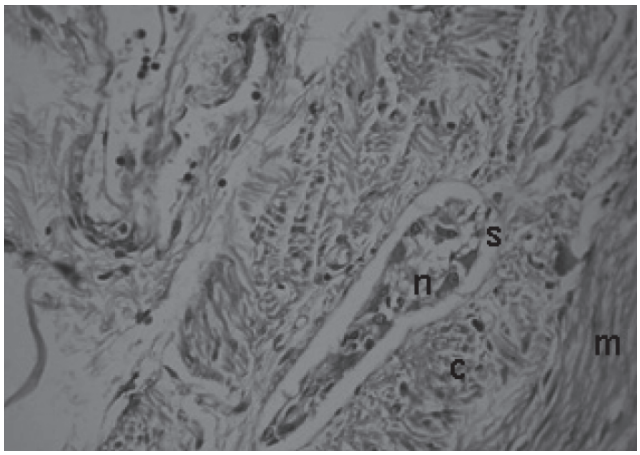

Fig. 1. Photomicrograph of oesophagus showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

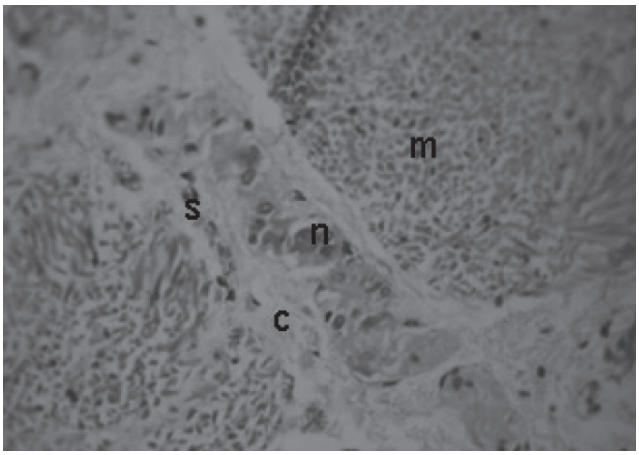

Fig. 2. Photomicrograph of fundus of stomach showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

multipolar and of various sizes and shapes with eccentric nucleus scattered through out the organ.

In esophagus (Fig. 1), myenteric plexus contained small ganglia with elongated or pear like neurons with an eccentric nucleus and scattered throughout the organ. 2.72±1.75 collections were present in entire circumference (Table-1) and each collection contained 1-15 numbers of neurons with an average of 7.71±3.41 (Table-2). Mean area was found to be 118.44±43.61 with a range of 51.75-178.32 (Table-3).

In fundus of stomach (Fig. 2), large elongated ganglia having neurons packed in a characteristically tight fashion in the connective tissue surrounded by satellite cells were observed. 4.52±2.55 collections were present in entire circumference (Table-1) and each collection contained 1-31 numbers of neurons with an average of 5.270±5.684 (Table-2). Mean area was found to be 99.472±35.739 with a range of 10.263-254.994 (Table-3).

In body of stomach (Fig. 3), the ganglia were elongated and triangular present in the connective tissue surrounded by satellite cells. 3.29±1.26 collections were present in entire circumference (Table-1) and each collection contained 1-14 with an average of 3.198±2.588 (Table-2). Mean area of neurons was 127.547±43.929 with a range of 14.184-317.527 (Table-3).

In pylorus of stomach (Fig. 4), the ganglia appeared compressed in most of the sections, the long axis of which lay perpendicular to the circular muscle layer. The mean number of collections in entire circumference of pylorus was found to be 3.88±1.72 (Table-1). The mean value of neurons was found to be 4.111±4.287 in these collections (Table-2). Mean area of neurons was

Table-1: Number of collections of neurons in myenteric plexus of GIT

Part of GIT	Mean	Range collections (1-3)	% age of collections (4-7)	% age of collections (8-12)	% age of collections (13-15)	% age of
Oesophagus	2.72±1.75	1-7	75	25	-	-
Fundus	4.52±2.55	1-9	39.13	43.47	17.39	-
Body	3.29±1.26	1-8	63.41	34.14	2.43	-
Pylorus	3.88±1.72	1-9	50	45.23	4.76	-
Duodenum	6.7±4.08	1-15	25.64	33.33	28.20	12.82
Jejunum	6.22±3.49	1-12	25	36.11	38.88	-
Ileum	5.41±3.22	1-10	25.80	41.93	32.25	-
Colon	3.10±1.86	1-6	57.14	42.85	-	-
ICV	5.14±2.92	1-10	32.14	46.42	21.42	-
Appendix	5.47±2.60	1-9	20.58	50	29.41	-

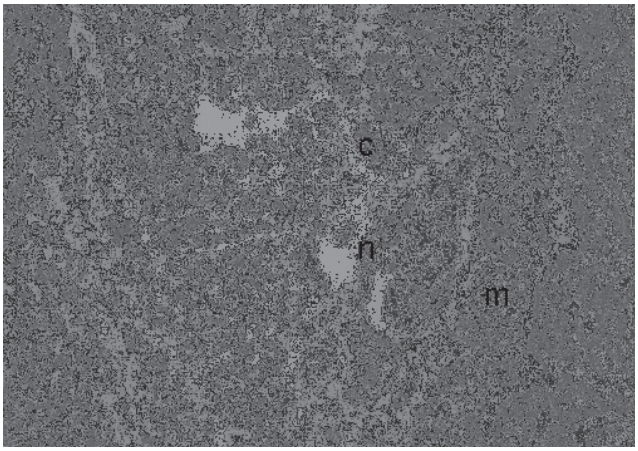

Fig. 3. Photomicrograph of body of stomach showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

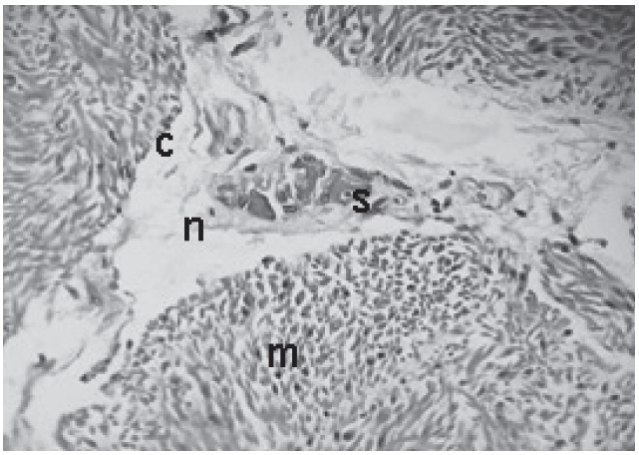

Fig. 4. Photomicrograph of pylorus of stomach showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

131.431±35.962 ranging from 65.111-259.660 (Table-3).

In duodenum (Fig. 5), 6.7±4.08 collections were present (Table 1); each collection contained 1-13 numbers of neurons in entire circumference with an average of 6.73±4.06 (Table 2). Mean area of neurons was found to be 178.54±36.18 with a range of 88.559-287.491 (Table-3).

In jejunum (Fig. 6), neurons are gathered in long chords in the connective tissue surrounded by satellite cells. 6.22±3.49 collections were present (Table-1); each collection contained 4-14 numbers of neurons with an average of 9.64±3.24 (Table-2). Mean area of neurons was found to be 178.36±46.25 with a range of 48.456-275.747 (Table-3).

In ileum (Fig. 7), a meshwork of neuronal cell bodies was present containing 5.41±3.22 number of collections (Table-1) and 2-9 numbers of neurons in each collection with an average of 5.36±2.33 (Table-2). Mean area of neurons was found to be 212.10±69.02 with a range of 83.315-381.276 (Table-3).

In colon (Fig. 8), the patterns of plexus were irregular with thin ganglia present between inner circular and outer longitudinal layers of smooth muscle tunica and were surrounded by satellite cells. 3.10±1.86 collections were observed in entire circumference (Table-1), each collection containing 3-5 neurons with an average of 3.89±0.73 (Table-2). The mean area of neurons was found to be 143.20±31.76 with a range of 87.109-246.554 (Table-3).

In appendix (Fig. 9), the collections of neurons were appeared to be more numerous (5.47±2.92) (Table-1) containing an average of 5.47 having neurons with a range of 1-12 with an average of 5.25±2.60 (Table-2). The mean area of neurons was found to be 75.77±42.61 with a range of 16.25-203.95 (Table-3).

Table-2: Number of neurons present in each collection of myenteric plexus of GIT

Part of GIT	Mean	Range
Oesophagus	7.71±3.41	1-15
Fundus	5.270±5.684	1-31
Body	3.198±2.588	1-14
Pylorus	4.111±4.287	1-16
Duodenum	6.73±4.06	1-13
Jejunum	9.64±3.24	4-14
Ileum	5.36±2.33	2-9
Colon	3.89±0.737	3-5
ICV	3.44±1.78	1-6
Appendix	5.25±2.60	1-12

Table-3: Area of neuron (μm²) in each part of the stomach

Part of GIT	Mean area	Range
Oesophagus	118.44±43.61	51.75-178.32
Fundus	99.472±35.739	10.263-254.994
Body	127.547±43.929	14.184-317.527
Pylorus	131.431±35.962	65.111-259.660
Duodenum	178.54±36.18	88.55-287.49
Jejunum	178.36±46.25	48.45-275.74
Ileum	212.10±69.02	83.31-381.27
Colon	143.20±31.76	87.10-246.55
ICV	146.45±37.00	92.90-241.34
Appendix	75.77±42.61	16.25-203.95

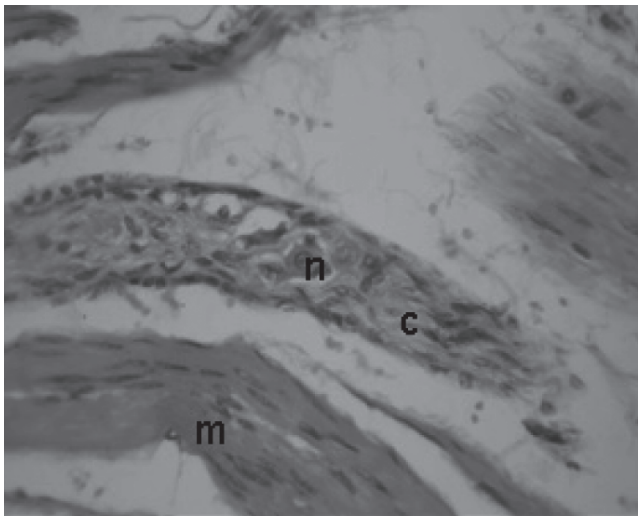

Fig. 5. Photomicrograph of duodenum showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

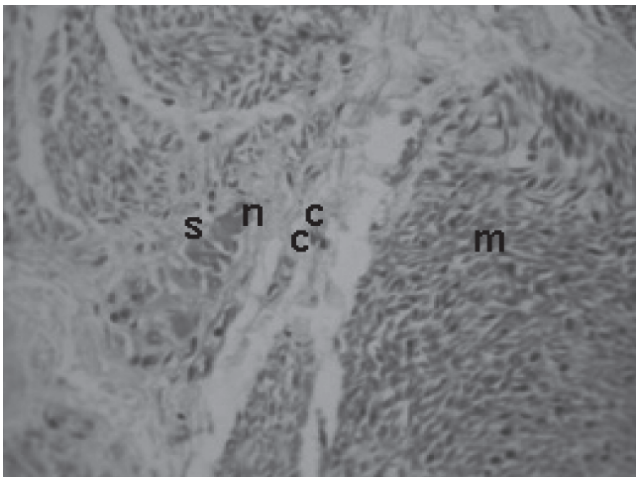

Fig. 6. Photomicrograph of jejunum showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

In ileo-caecal (Fig. 10), large numbers of evenly distributed neurons lying singly and in clusters were found having mean area 146.45±37.00 with a range of 92.90-241.34 (Table-3). The numbers of collections of neuron was found to be 5.14±2.60 (Table-1) and each collection contained an average of 3.44±1.78 neurons with a range of 1-6 (Table-2).

DISCUSSION

The neuronal population was observed in the connective tissue between inner circular and outer longitudinal layers of smooth muscle layers in all the parts of the GIT and was described as ganglia. Bowen[5] described these complex amalgamations of neurons as ganglia. While[6] stated that these neurons in ganglia are gathered into long cords which fuse into one another lacking a clear demarcation into ganglia. The present study showed that the neuronal density was found maximum in the jejunum (9.64±3.24) as compare to other parts of GIT including the small intestine. The neuronal density in each part of the stomach was found to be different. It was observed 5.270±5.684 in fundus, 3.198±2.588 in body and 4.11±4.28 in pylorus which is reported earlier[7]. The neuronal density is a subject to variation according to the species and age of the animal. Gabella[8] and Santer[9] employed NADPH-Diphorase and cuprolinic blue stains and a decrease were observed in the neuronal density in the older animals. They also verified greater neuronal density in undernourished animals and related this finding to the smaller body growth of these animals and to the reduced intestinal wall and smooth muscle tunica which lead to a smaller spread of the nerve cells and greater concentration per area. Neto[10] verified that maternal proteic malnutrition does not cause decrease on the number of myenteric neurons per unit area of

Table-4: Incidence of small, medium and large neurons in each part of GIT

Part of GIT	Small (%)	Medium (%)	Large (%)
Oesophagus	18.75	50	31.25
Fundus	11.34	69.50	19.14
Body	8.75	80.62	11.25
Pylorus	11.11	74.35	14.52
Duodenum	12.19	75.60	12.19
Jejunum	11..76	68.62	19.60
Ileum	11.76	76.47	11.76
Colon	9.02	77.27	13.63
ICV	8	80	12
Appendix	21.21	65.15	13.36

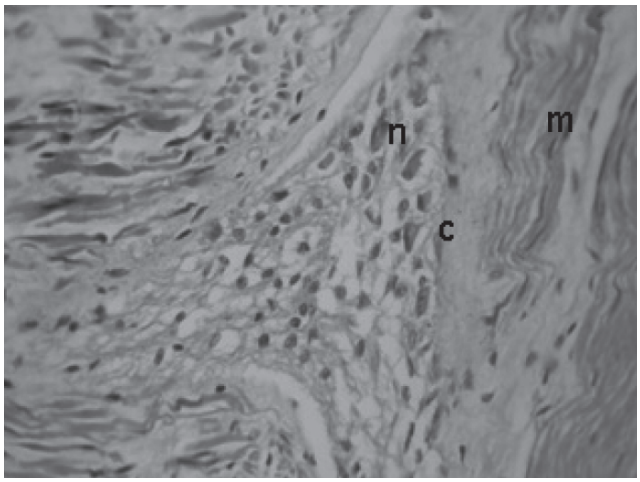

Fig. 7. Photomicrograph of ileum showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

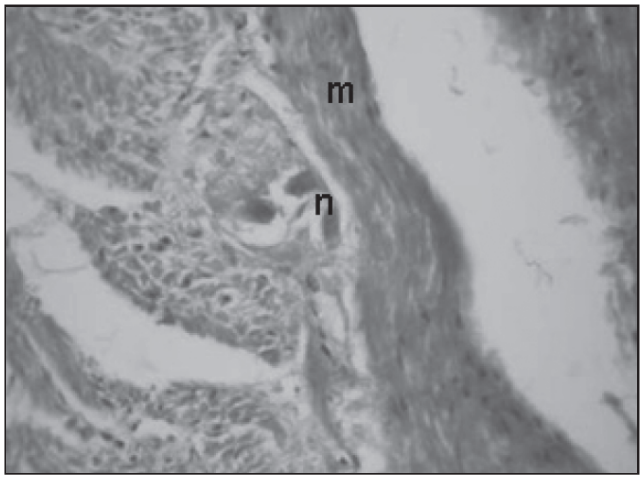

Fig. 8. Photomicrograph of colon showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

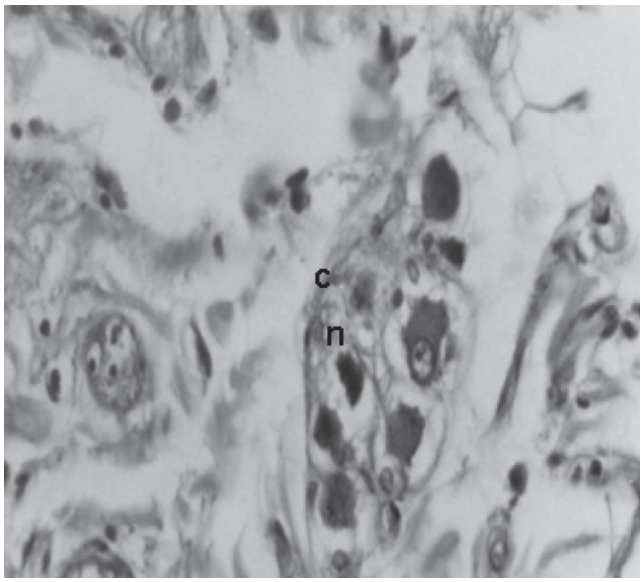

Fig. 10. Photomicrograph of appendix showing collections of large, medium and small neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

jejunum in rats and concluded that when the animal gain receives normal proteic level diet, there occurs proteic material on the cytoplasm of the neurons, rendering them larger and strongly basophilic. In the present study, the size of the neurons varied from 10.263-317.527 μm² and neurons were surrounded by satellite cells. Schofield[11] and Cook[12] studied these satellite cells ultrastructurally and stated that they resemble those of Schwann cells and are derived from neural crest. Natali[2] divided the neurons into small, medium and large because of the presence of large range of neuronal size and also stated that the large neurons make up the major neuronal population. Gabella[13] observed large neurons make up larger neuronal population in duodenum of rat whereas in present study, medium sized neurons make up the larger portion of neuronal population. Though in the present study, the neurons were similarly described in man as large, medium and small using the same methodology but the medium size neurons were more in number than large. Large and medium size neurons of the present study were multipolar neurons which are described to control gastrointestinal motility; secretion and possibly absorption and considered to be a part of sympathetic and parasympathetic nervous system[5]. There are two secretomotor neurons in myenteric plexus, one are cholinergic and the other non-cholinergic neurons.[14]

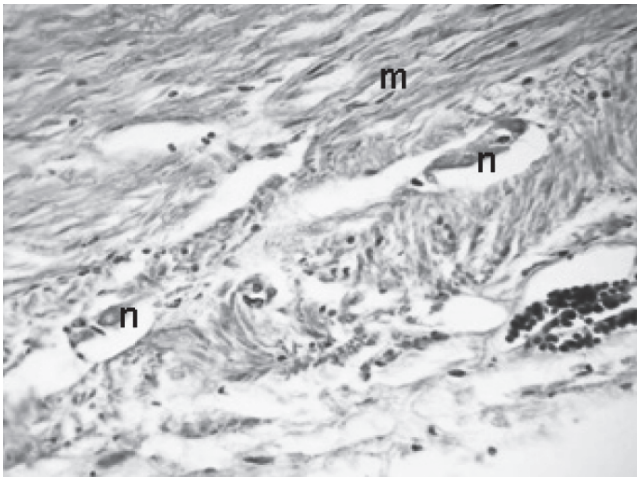

Fig. 9. Photomicrograph of ileo-ceacal valve showing collections of neurons in muscularis propria (m); neurons (n), connective tissue ©, satellite cells (s). (H&E X650).

The small neurons having area 10.263 μm² of the present study are probably sensory neurons as described earlier which respond to mechanical, thermal, osmotic and chemical changes. Though some of these small neurons are described to be interneurons and are largely responsible for integrating information from sensory neurons and providing it to enteric neurons.[5] 7% of the total neurons are interneurons.[15,16] Reflex function of bowel independent of the central nervous system arises because of the coordinated activity in their sensory neurons, interneurons and excitatory and inhibitory motor neurons. The neuronal mechanisms that coordinate gut function rely on a complex interplay between many neuroactive mediators and their receptors.[5,2] Natali[2] also stated that probably number of smaller neurons was more but they remained unstained. Heinicke[17] observed that number of myenteric neurons stained with NADPH-diaphorase is smaller than that found with cuprolinic blue stain. This difference is due to the fact that cuprolinic blue and similar stains reveal the affinity of these stains for acidic cellular structures which are abundant in the neuronal cell body. The size of neuronal population may depend upon the eating habbits of an individual as Natali[2] described the reduction of the cell body size and an increase in the population of small neurons on the rat

subjected to hypoproteic chow. Cellular hypertrophy, including in neurons, can be considered as a basic response mechanism in instances where the cells are under aggression, such as decrease of nutrient supply or of necessary stimuli for their functioning. As a result the cells get adapted, with decreased metabolism, leading to a decrease in the turnover of their own structures and then to a reduction in their volume.[18] Kaur[19] stated that the lip of the ileocaecal valve has a well developed muscularis propria and a proportionate complement of nerve elements which are in accord with a predominantly afferent function in the lip of the valve.

The variation in the neuronal population between different parts of GIT may be probably due to different functions of its each part. The data of the present study elicited us to carry out an experimental study to contribute to the knowledge concerning the effect caused by any adverse condition on the myenteric plexus such as malnutrition and poisonous conditions etc.

REFERENCES

1. Karaosmanoglu T, Aygun B, Wade PR, Gershon MD. Regional difference in the number of neurons in the myenteric plexus of the Guinea Pig small intestine and colon: An evaluation of markers used to count neurons. *Anat Record* 1996; 244: 470-80.
2. Natali MMR, Neto M, Orsi M. Morphometry and quantification of the myenteric neurons of the duodenum of adult rat fed with hypoproteic chow. *Int'l J Morphol* 2003; 21: 273-7.
3. Bannister LH, Berry MM, Collins P, Dyson M, Dussek JE, Ferguson MWJ. Alimentary System. In: Gray's Anatomy. 38th Ed. Edinburg: Churchill Livingston 1995: 1775.
4. Machado EMM, Junior DJC, Pinheiro SW *et al*. Morphometry of submucous and myenteric esophagic plexus of dogs experimentally reinfected with Trypanosoma Cruzi. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 2001; 96: 545-8.
5. Bowen R. The Enteric Nervous System. In: Oesophagus Anatomy and Development: GI Motility Online 2006.
6. Maifrino LBM, Prates JC, De-Souza RR, Liberti EA. Morphometry and acetylcholinesterase activity of the myenteric plexus of the wild mouse Calomys Callosus. *Brazilian J Med Biol Res* 1997; 30: 627-32.
7. Saini N, Gupta M. Morphometry of myenteric neurons in stomach. *Nepal Med Coll* J 2007; 9: 96-9.
8. Gabella G. Fall in the number of myenteric neurons in aging guine a pig. *Gastroenterol* 1989; 96: 1487-93.
9. Santer, RM. Survival of the population of NADPH-Diphorase stained myenteric neurons in the small intestine of aged rats. *J Auton Nerv Syst* 1994; 49: 115-21.
10. Neto MHDM, Molinari SL, Stabille SR, Sant'ana DDMG, Natali MRM. Morphologic and quantitative study of the myenteric neurons of the jejunum of malnourished rats (Rattus Norvegicus). *Arq Neuropsiquiatr* 1999; 57B: 387-91.
11. Schofield GC. Handbook of Physiology. 4th ed. (Code, GF); Williams and wilkins Baltimore 1968: 1579-627.
12. Cook RD, Burnstock G. J. Neurocytol 1976; 5: 195-206. Pubmed; ISI: ChemPort.
13. Gabella G. Neuron size and number in the myenteric plexus of the newborn and adult rat. *J Anat* 1971; 109: 81-94.
14. Costa M, Brookes SJH, Henning GW. Anatomy and physiology of the enteric nervous system. *Gut* 2000; (Suppl 4th) 47: IV 15-9.
15. Costa M, Brookes SJH, Steele PA *et al*. Neurochemical classification of myenteric neurons in the Guinea-Pig ileum. *Neuroscience* 1996; 75: 949-67.
16. Song ZM, Brookes SJH, Costa M. Projection of specific morphological types of neurons within the myenteric plexus of the small intestine in the guinea-pig. *Cell Tissue Res* 1996; 285: 149-56.
17. Heinicke EA, Kiernan JA, Wijsman J. Specific, selective and complete staining of the neurons of the myenteric plexus using cuprolinic blue. *J Neurosci Methods* 1987; 21: 45-54.
18. Bogliolo, Patalogia L. 3rd ed. Rio de janeiro, Guanabara Koogan 1981: 1236.
19. Kaur U, Goyal N, Gupta M. Disposition of muscularis propria and nerve elements in the lip of the ileocaecal valve. *Nepal Med Coll J* 2005; 7: 125-8.