Clusters of endocrine-like cells in the Proventriculus of three small passerines

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Abstract— *The proventriculi of three small passerines have been studied by electron microscopy. Based on morphology, location of cells and size of granules, ten different types of gastric endocrine-like cells GELCs in the proventriculus were identified. GELCs were observed singly and in clusters in the glandular tubules of the proventriculi. Clustered GELCs in connective tissue, with a blood and nerve supply, is reported for the first time in the avian proventriculus. All GELCs were closely associated with capillaries and many had unmyelinated axons in close proximity. There was a considerable range in the size, electron density and distribution of the granular vesicles within the GELCs suggestive of colocalization and storage of differennt hormones in the same cell. Although most types of GELCs were round to oval, some had elongated cytoplasmic processes that contained numerous vesicles. The GELCs also varied in that some had specific membrane associations with adjacent cells while others did not. The identification of several types of cell suggests that there is considerable local hormonal control of activity within these regions of the avian digestive system. The study attempts to provide a baseline for future ultrastructural, immunocytochemical studies on the content of the granules in these cells.*

Keywords: Clusters, Endocrine-Like Cells, Gastric Proventriculus.

I. INTRODUCTION

Morphological and histochemical studies have described gastric endocrine-like cells GELCs, including argentaffin and argyrophilic cells, in the proventricular epithelium.[1,2,3] Immunocytochemical studies have identified several types of GELCs in the gastrointestinal tract of the domestic chicken including the proventriculus.[1,3] Other immunocytochemical studies have identified GELCs in the common finch, pigeon, quail, duck, gull, kite[4], New Holland honeyeater[5]; ostrich[6] and three other honeyeaters: brown honeyeater, singing honeyeater and spiny-cheeked honeyeater.[7]

GELCs tend to be oval with long or short cytoplasmic process, adjacent to the basement membrane and usually covered by epithelial cells. Some, the open type of GELC, make contact with the lumen via long cytoplasmic process or with other GELCs while the closed type of GELC make contact with parietal or chief cells.[2,4,5,7]

GELCs in the gastrointestinal tracts of birds and mammals have been reported as individual cells[8], located between glandular or duct cells and the basement membrane.[9]

Andrew[1] identified two types of enterochromaffin cells based on whether the secretory vesicles had a maximum diameter of 290 nm or 510 nm but there is relatively little information on the ultrastructure of GELCs in birds.

This study describes the ultrastructure and location of these cells in the proventriculi of three species of small passerines *Lichmera indistincta*, the Brown Honeyeater; *Poephila guttata*, the Zebra Finch and

Zosterops lateralis, the Silvereye that have different dietary regimens. The results show that several morphological types of GELC are present in this region of the digestive system of these birds. The different types of GELCs are described to provide an ultrastructural basis for future immunocytochemical studies.

II. METHODOLOGY

Tissue from the proventricular apparatus of granivorous, nectarivorous and omnivorous passerine birds were fixed in half strength Karnovsky fixative and post osmicated for electron microscopy. The specimens were processed for routine electron microscopy.[10]

III. RESULTS

Several types of GELCs, based on morphology and location, were identified in the proventriculi of the three species examined; some types were observed in more than one region. Individual or clusters of GELCs were common within the glandular epithelium and connective tissue surrounding the proventricular glands. Generally, the GELCs had pale to moderately electron-dense cytoplasm with few organelles and numerous membrane-bound, cytoplasmic granules of varying morphology and electron density.

3.1 Cells within the secretory epithelium:

Type 1. Large, rounded or slightly elongated cells that sometimes extended towards the luminal surface of the secretory epithelium within the tubule and occurred individually or in small clusters. The homogenous, membrane-bound, granules of medium to dark electron-density measured 230-490 nm in diameter (Fig. 1.) There were no obvious interdigitations with adjacent chief cells. Axons, with large, dense-cored, granular vesicles, were sometimes associated with these cells.

Figure 1

Electron micrograph of a cluster of type 1 endocrine-like cells adjacent to blood vessels and surrounded by chief, parietal-like cells of the glandular epithelium in the proventriculus of *Lichmera indistincta*. Bar, 1μm

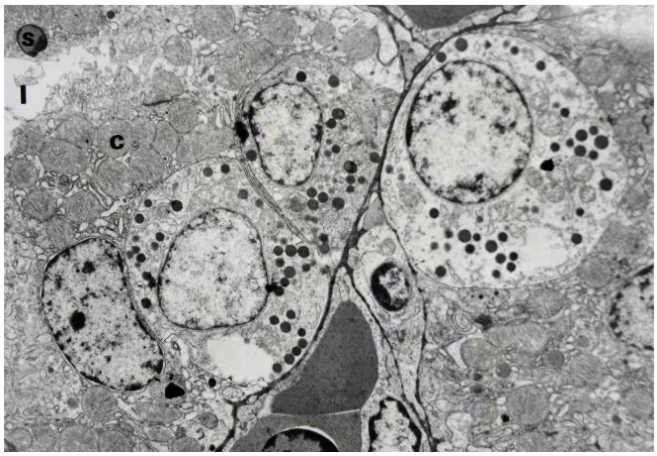


Figure 2

Type 2 GELC in the proventriculus of *Lichmera indistincta* having an attenuated process, with an atypical cytoplasmic swelling, extends from the nuclear region of the cell and passes adjacent to the upper blood vessel. The arrowhead indicates an axonal profile. Bar, 1μm

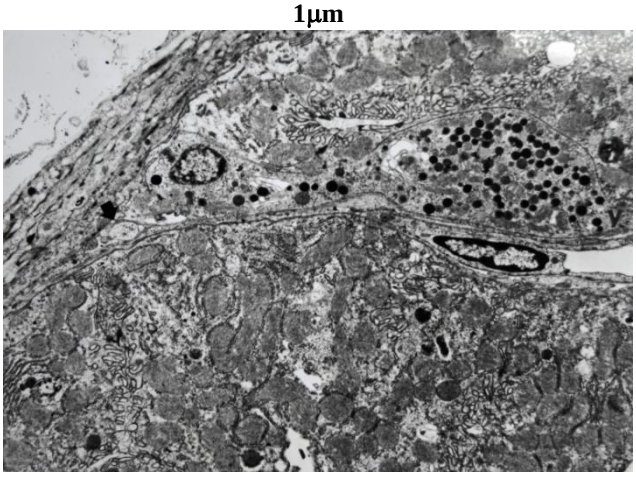


Figure 3

Type 3 GELC has an attenuated cytoplasmic process in contact with a chief cell of the proventriculus of *Lichmera indistincta*. The cytoplasmic granules are considerably smaller than those of the type 2 GELC. Bar, 1μm

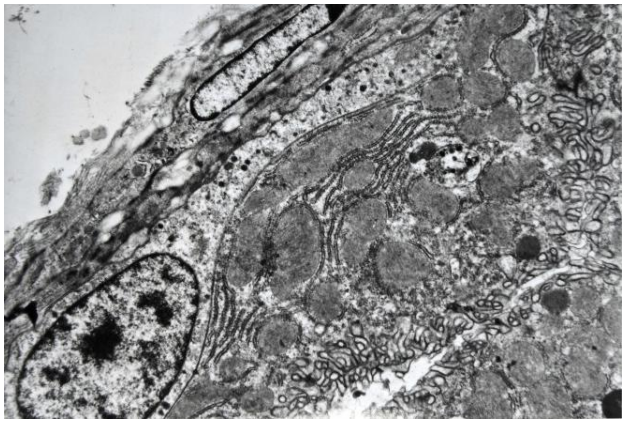
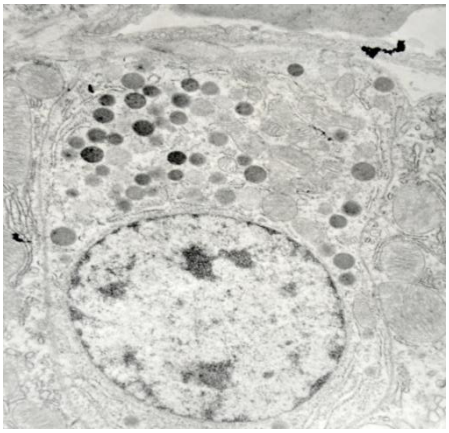


Figure 4

The micrograph shows a type 4 GELC between the chief, parietal-like cells and adjacent to the basal lamina of the proventriculus of *Zosterops lateralis*. Bar, 1μm



Type 2. These flattened cells, which had cellular projections that contained the majority of the granular vesicles (Fig. 2), were basal in the secretory epithelium, had no interdigitations with adjacent cells but did show occasional membrane junctions with adjacent chief cells. The 280-490 nm granules varied considerably in appearance from almost electron-lucent to very electron-dense vesicles Fig. 2. These GELCs were often adjacent to continuous capillaries.

Type 3. These contained small, dense-cored, granular vesicles 70-120 nm in diameter (Fig. 3). The cells were basal to the secretory cells, had attenuated cytoplasmic processes and showed specialised membrane contacts with chief cells or other GELCs. Small bundles of axons were often present in the adjacent connective tissue.

Type 4. These, round to elongate cells contained mostly basal, pale to electron-dense granules, about 370-420 nm in diameter (Fig. 4). They were located between chief cells, adjacent to capillaries and occasionally extended from the basal lamina towards the lumen of the epithelium. No interdigitations or membrane specialisations with adjacent cells were observed.

Figure 5

Type 5 GELC from the proventriculus of *Poephila guttata* is located between the chief, parietal-like cells and the basal lamina. Many of the dense-cored secretory vesicles have distinct halos

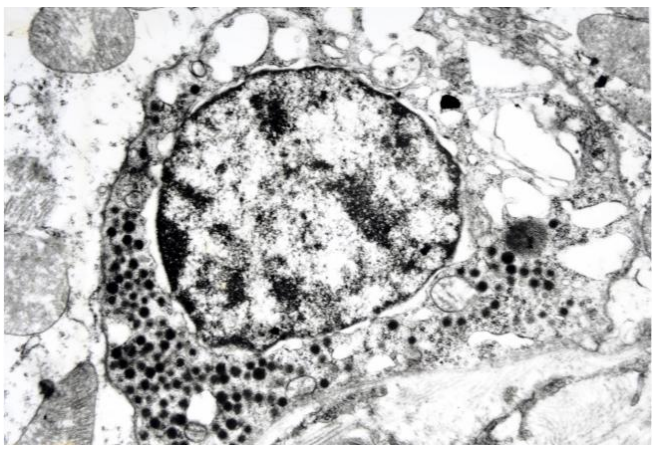


Figure 6

Type 6 GELC is also located at the base of the secretory epithelium of the proventriculus of *Zosterops lateralis.* An unmyelinated axon arrowhead is close to the basal lamina associated with this cell. Bar, 1μm

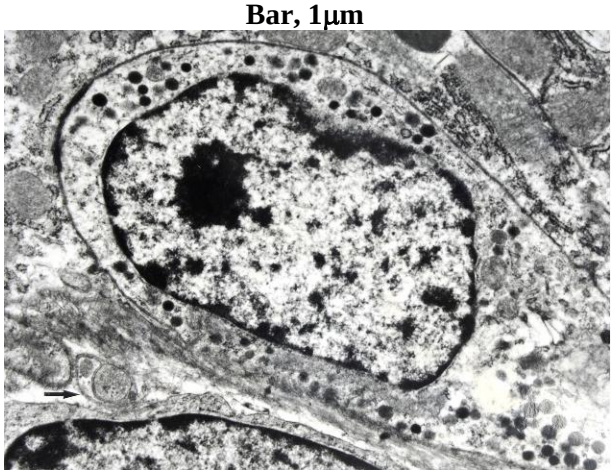


Figure 7
This electron micrograph shows the type 7 7 & 8 8 GELC in the alveolar epithelium between the glandular neck cells G and the surrounding connective tissue in the proventriculus of *Poephila guttata*. Unmyelinated axons a and a capillary C are close to the GELCs. Bar, 1μm

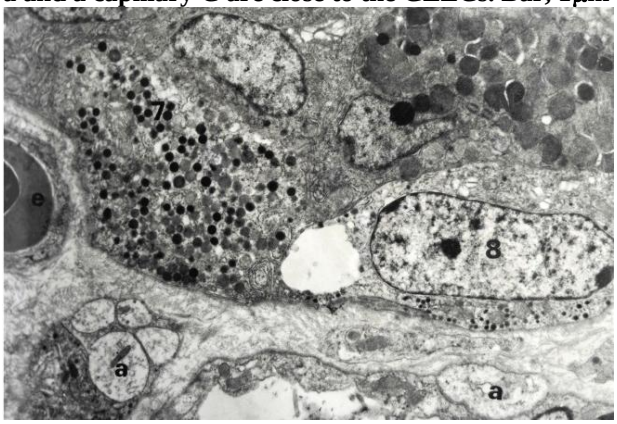
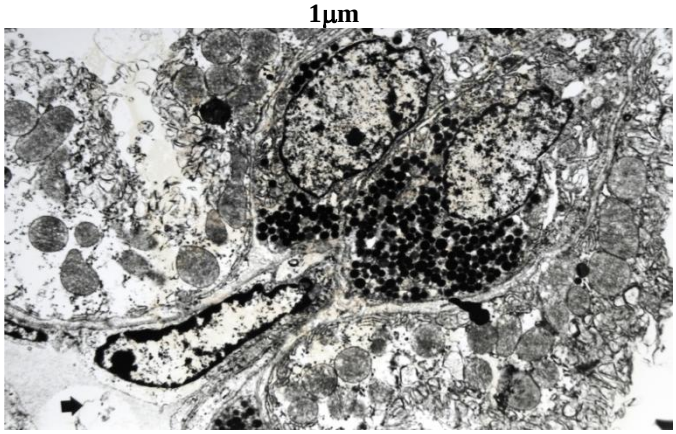


Figure 8
This micrograph shows a cluster of type 9 GELCs in the periglandular connective tissue of the proventriculus of *Poephila guttata*. Note that the electron-dense granules are aggregated in the cytoplasm closest to the capillary C. Bar 1μm



Type 5. Located basal to the chief cells these round to elongate cells had relatively little cytoplasm and contained160-230 nm dense-cored granular vesicles with distinct halos (Fig. 5). The RER in these cells was usually swollen and they showed desmosome-like contacts, but no cytoplasmic interdigitations, with chief cells.

Type 6. These elongate to flattened cells fig. 6 had relatively little cytoplasm that contained membrane-bound, homogeneously filled vesicles about 125-185 nm. The cells were basal to chief cells with which they had desmosome-like contacts. Small bundles of unmyelinated axons were often seen in the connective tissue adjacent to these cells.

Type 7. This type contained granular vesicles of varying electron-density, about 280-380 nm in diameter (Fig. 7). These round to oval cells with dense cytoplasm were basal to the glandular neck cells and close to capillaries and axon bundles but did not show membrane specialisations with adjacent cells.

Type 8. These were elongate, pale-staining with small, dense-cored granules, about 65-100 nm (Fig. 7). The granules, which lacked obvious halos, were usually aggregated in the basal cytoplasm. The cells were basal to glandular neck cells and did not show membrane specialisations with adjacent cells.

3.2 Cells exterior to the secretory epithelium:

Some of these cells were morphologically similar to those described above but the location of the cells within the lamina propria was considered sufficient to classify them separately from the previous types.

Type 9. Flattened, elongate to oval shaped cells that usually occurred in clusters or individually in the connective tissue external to the basal lamina of chief cells and adjacent to capillaries. The numerous 260-300 nm granules showed varying electron density (Figs. 8 and 9). Occasionally, apparent exocytosis of granules into the extracellular space between these GELC and chief cells was seen. No membrane specialisations were observed.

Type 10. This type includes two possible variants: one with a paler cytoplasm than the other. These, round to elongate cells occurred in clusters in the connective tissue external to the glandular neck cells. The average diameter of the dense-cored, granular vesicles was 220 nm in the paler cells and 240 nm in

the darker cells (Figs. 9 and 10). Axons that contain either dense-cored granular vesicles, 50-60 nm in diameter, or small agranular vesicles, 45-60 nm in diameter were associated with these cells.

Figure 9

These type 9 9 and 10 10 GELCs occurred in clusters in the periglandular connective tissue of the proventriculus of *Poephila guttata*. The two GELCs shown are adjacent to a capillary. Bar 1μm

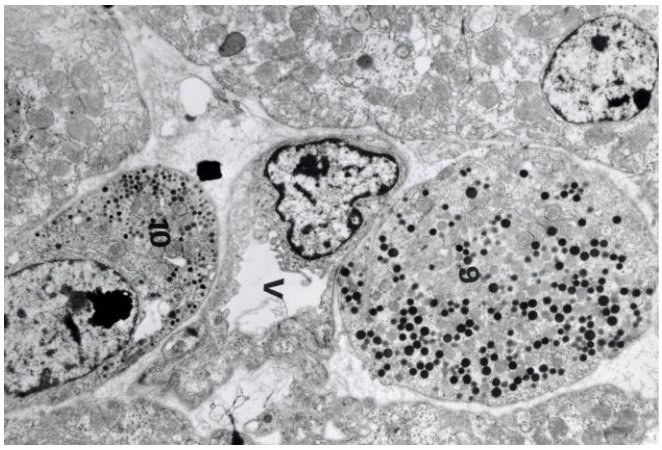
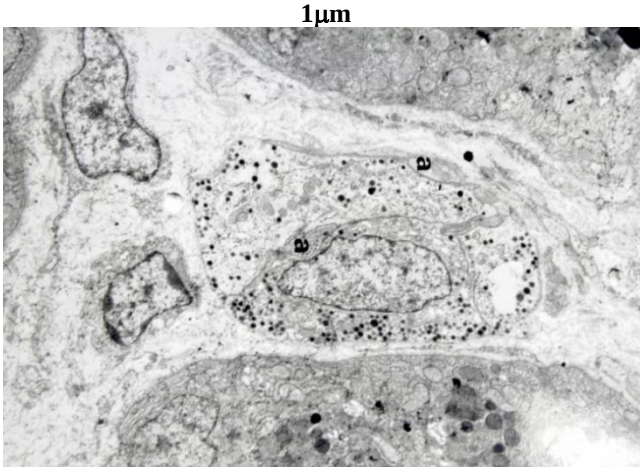


Figure 10

Electron micrograph showing a cluster of type 10 GELCs in the interglandular connective tissue of the proventriculus of *Poephila guttata.* Unmyelinated axons arrowheads are closely associated with these cells. Bar, 1μm



IV. DISCUSSION

This ultrastructural study of the proventiculus of three species of small passerines has identified 10 morphologically different types of GELCs based on the appearance and arrangement of secretory vesicles and associations with adjacent cells. The general appearance of these cells is comparable to that of both argentaffin cells (enterochromaffin or Amine Precursor Uptake and Decarboxylation, APUD, cells) that have pleomorphic granules and argyrophil cells that have regular, round granules as described in mammals.[11,12]

In ultrathin sections most GELCs in the proventriculus appeared round, elongate and flattened in contrast to the oval cells previously described in thicker sections.[4,5] Endocrine-like cells in the avian proventriculus are located between the oxynticopeptic cells and the basal lamina.[5,13] In mammals, gastric GELCs have been described either between parietal cells and/or chief cells and the basal lamina.[9] The present study confirms these locations. However, in contrast to previous reports, which have described GELCs as occurring singly, this study has shown that GELCs occur either singly or in clusters.

The clusters of GELCs were present in the glandular epithelium of the proventriculus, adjacent to chief cells, and the intertubular connective tissue. Those in the connective tissue were separated from the glandular epithelium and were closely associated with blood vessels. Their presence in connective tissue is comparable with reports on endocrine cells in mammals[14-17] and birds.[3]

This study has utilized the fact that secretory granules are the main intracellular storage sites for secretory products[18] and they show morphological variations that allow classification. The identification of several types of GELC based on the granule and cellular features provides a morphological basis for future ultrastructural immunocytochemical studies aimed at establishing the function of these cells.

Despite the present lack of immunological results, the following attempts to correlate previous descriptions with some of the present classification.

Three types of immunoreactive cells somatostatin-, gastrin releasing polypeptide [GRP] and serotonin-immunoreactive cells have been described in the basal epithelium of gland lobules of the avian proventriculus. These closed GELCs[17] are characterized by long cytoplasmic process that terminate on other GELCs or chief or parietal cells. The ends of these processes are enlarged into synaptic bouton-like swellings prior to terminating on the effecter cells.[5,7,19] The morphology of these closed GELCs is comparable with the current type 2, 3, and 4 cells.

The granules 150-490 nm in the current type 2 cells are similar to those in both mammalian and avian G cells which produce gastrin.[18,20] The diameter of the secretory granules of G cells in chick embryos ranges from 190-250 nm[3]and 150-400 nm in mammals.[18] The localization of GRP-immunoreactive cells[24] in the proventriculus suggests that avian G cells may produce and secrete gastrin similar to their mammalian counterparts.

D cells produce somatostatin in both mammals and birds.[3,7,21] The diameter of the secretory granules of D cells varies from 250-500 nm in mammals, 320 nm in chick embryos to 700 nm in humans. [1,18] The present type 4 cells have ultrastructural similarities to D cells.

Alumets et al.[2] identified APP cells in the proventricular, duodenal and rectal epithelia of newly hatched chicks. According to Dayal [18], granules in APP cells are 150-170 nm in diameter while those in P cells are 90-150 nm in diameter. The granules of type 6 cells have diameter of 125-180 nm and are similar to those of both APP and P cells.

"A" cells of both the stomach and pancreas in mammals secrete glucagon.[22] The present type 9 cells have ultrastructural features similar to "A" cells of both the mammalian stomach and the avian proventriculus[1] which resemble the pancreatic and gastric A cells.

The current study observed the presence of polymorphic secretory granules in the cytoplasm of some individual endocrine cells. Earlier workers[17,23] observed and reported that an individual endocrine cell is capable of synthesizing and storing more than one hormone in its cytoplasm. Modern researchers[24,25] working on normal and pathologic endocrine tissues have reported the presence of polyhormonal secretory granules in a single endocrine cell. The above reports coincide with the observations of the current study.

The current study has shown that within one type of GELC the granules varied mophologically from moderate to electron-dense or homogenous, membrane-bound, dense-cored granular vesicles with or without halos. It is logical to assume that one type of endocrine cell is polygenic rather than monogenic and it is highly probable that some GELCs observed secrete more than one type of hormone.

V. CONCLUSION

This study has identified 10 possible types of endocrine-like cells based on their location, and their ultrastructural appearance. The possible endocrine function of these cells is supported by the presence of dense–cored vesicles of varying size and appearance in the cytoplasm. The presence of polymorphic secretory granules within the cytoplasm of some GELCs portrays their multihormonal function. The study has revealed the presence of clusters of endocrine-like cells in the glandular epithelium, interglandular and periglandular connective tissue.

CONFLICT OF INTEREST

None declared till now.

REFERENCES

[1] Andrew, J. 1976. Endocrine cells of the stomach of chicks around the time of hatching. *Journal of Cell and Tissue Research***172,**557-561.

[2] Alumets,J. Hakanson, R. Sundler, F. Chang, K. J. 1978. Leukoenkephalin-like material in the nerves and enterochromaffin cells in the gut. *Histochemistry* **56,** 187-196.

[3] Hodges, R. D. 1981. Endocrine glands. In *Form and Function in Birds.* eds. A. S. King, J. McLelland, vol. 2. pp. 186-191 London Academic Press.

[4] Yamada, J.Kitamura, N. Yamashita, T. Yanaihara, N. 1986. Immunohistochemical studies on the endocrine cells in avian gizzard. Biomedical Research **7,** 39-45.

[5] Richardson, K. C. Yamada, J. Wooller, R. D.1988. An immunocytochemical study of the gastrointestinal endocrine cells of the new Holland honeyeater, Phylidonyris novaehollandiae. Australian Journal of Zoology **36,** 483-496.

[6] Bezuidenhout, A.J., van Aswergen, G. A. 1990. A light microscopic and immunocytochemical study of the gastro-intestinal tract of the ostrich Struthio camelus L. Onderstepoort Journal of Veterinary Research **57,** 37-48.

[7] Hashimoto, N. Yamada, J., Richardson, K. C., Kitamura, N., Yamashita,T. 1993. An immunohistochemical study on the gastrointestinal endocrine cells of three honeyeaters singing honeyeater Meliphagavirescens, spiny-cheeked honeyeater Acanthogenys rufogularis, and brown honeyeater Lichmera indistincta. European Journal of Histochemistry **37,** 233-240.

[8] Schonhoff, S. E., Giel-Moloney, M., Leiter, A. B. 2004. Minireview Development and differentiation of gut endocrine cells. Endocrinology **456,** 2639-2644.

[9] Stinson, A. W., Calhoun,M. L. 1981. Digestive system. In Textbook of Veterinary Histology eds. H. Dellman, E. M. Brown, pp. 232-233. Philadelphia Lea & Febiger.

[10] Ogunkoya, Y. O., Cook, R.D., Fallon, P. O. 2009.Histomorphology of the proventriculus of three species of Australian PasserinesL*ichmera indistincta*, *Zosterops lateralis* and *Poephilaguttata*. Anat. Histol .Embryol **38,**246-253.

[11] Rubin, W., Schwartz,B., 1981. An electron microscopic radioautographic identification of the APUD endocrine cells in the rat gastric pyloric glands. *Gastroenterology***81,**311-320.

[12] Pearson, G. R., Logan,E. F. 1983. Observations on the ultrastructural appearance of endocrine cells of the small intestine of the calf. *Zentralblatt fuer Veterinaermedizin Reihe C. Anatomia Histologia Embryologia* **12,**1-6

[13] Saito, T., Yamada, J. 1989..An immunohistochemical study of the distribution of endocrine cells in the gastrointestinal tract of domestic pigeon, *Columbia livia var domestica. Zeitschrift fur mikroskopisch-anatomische Forschung* **103,** 237-246.

[14] Rubin, W. 1972. Endocrine cells in the normal human stomach. A fine structural study. *Gastroenterology***63,** 784-800.

[15] Bock, P., Gorgas, K. 1976. Enterochromaffin cells and enterochromaffin-like cells in the cat pancreas. In *Endocrine Gut and Pancreas.* ed. T. Fujita, pp. 13-24. Amsterdam Elsevier Scientific Publishing Co.

[16] Matsuo, Y., Seki, A., Fukuda, S. 1976. Neuro-endocrine cells in the lamina propria of rat stomach. In *Endocrine Gut and Pancreas* ed. T. Fujita, pp 159-165. Amsterdam Elsevier Scientific Publishing Co.

[17] Dellman, H. 1981. Endocrine system. In *Textbook of Veterinary Histology* eds. H. Dellman, E. M. Brown, pp. 388-399. Philadelphia Lea & Febiger.

[18] Dayal, Y. 1991. Neuroendocrine cells of the gastrointestinal tract introduction and historical perspective. In *Endocrine Pathology of the Gut and Pancreas*. ed. Y. Dayal, pp. 2-31. Boca Raton, Florida CRC Press.

[19] Yamanaka, Y., Yamada, J., Kitamura, N., Yamashita, T 1989. An immunohistochemical study on the observations on each intestinal segment in chickens and water fowls. Zeitschrift fuer Mikroskopisch-Anatomische Forschung. **103,** 437-446.

[20] Fujita, T., Kobayashi,S. 1974. The cells and hormones of the GEP system. The current of studies. In *Gastro-Entero-Pancreatic system. A Cell Biological Approach* ed. T. Fujita, pp 1-16. Stuttgart, Georg Thieme.

[21] Dockray, G. J. 1994. The gut endocrine system and its control. *Experimental Physiology***79**, 607-634.

[22] Forssmann, W. G., Ito, S. 1976. Ultrastructural analysis of the GEP endocrine system in primates. In *Endocrine Gut and Pancreas.* ed. T. Fujita, pp. 25-41. Amsterdam Elsevier Scientific Publishing Co.

[23] Lukinius, A., Ericsson, J. L., Grimelius, L., Korsgren, O. 1992.Ultrastructural studies of the ontogeny of fetal human and porcine endocrine pancreas, with special reference to colocalization of the fourmajor islet hormones. Dev Biol **153,2** 376-385

[24] Minuto, M., Varaldo, E., Marcocci, G., de Santanna, A., Ciccone, E., Cortese, K. 2018.ERBB1- and ERBB2- Positive medullary thyroid carcinoma A case report. Acta Histochemica et Cytochemica **511,** 9-20.

[25] Robert, T., de Mesmaeker, I., Stange, G. M., Suenens, K. G., Ling, Z., Kroon, E. J. and Pipeleers, D. J. 2018. Functional beta cell mass from device-encapsulated hESC-derived pancreatic endoderm achieving metabolic control, Stem Cell Reports **103,** 739-750.