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Original Research

Identification of Gut Endocrine Cells in Intestine of Post Hatch Broiler Chicken: A Histological and Histochemical Study

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Abstract

A comparative histological and histochemical study was undertaken to evaluate different staining techniques for identification of gut endocrine cells in post hatch broiler chickens. Fifty (50) day old broiler chicks were reared in experimental pens up to day 43. Histological and histochemical studies were carried out on different segments of gastro-intestinal tract of the birds at different ages (from day 1 to day 43 at weekly intervals). Different light microscopic staining methods were employed to elucidate nine morphological types of gut endocrine cells. Out of the staining techniques followed, ferric ferricyanide reduction test and argentaffin reaction elucidated the histomorphology of the endocrine cells better. The former one was the best among these methods, followed by the argentaffin reaction and lead haematoxylin technique. The detailed and systematic study in this regard authenticates the very importance and usefulness of special staining techniques for localization and identification of endocrine cells along the length of the gut in broiler chickens.

Keywords: Broiler Chicken, Gut Endocrine Cells, Histological and Histochemical Study, Identification

Introduction

The gastrointestinal tract is considered as the largest endocrine organ of the body. Enteroendocrine cells are diffusely distributed in the gastric, intestinal and pancreatic tissue. These cells synthesize peptide hormones and amines that are released in response to appropriate stimulation (Argenzio, 2004). At least 12 different endocrine cell types were described in mammalian gastrointestinal tract (Frappier, 1998). In poultry, gut endocrine cells were categorized into 6 types depending on their histomorphology (Mishra and Das, 2006). Despite much information in the field of histochemistry, immunohistochemistry and ultrastructural study on the diffuse endocrine system, light microscopy is still an important tool in general histological study as well as diagnostic histopathology (Grimelius, 2008).Paler cytoplasm of the gut endocrine cells as compared to that of surrounding enterocytes (Fawcett, 1994), isolated and diffuse distribution of these endocrine cells render them more difficult to be visualized in routine histological staining. Poor visibility of enteroendocrine cells in traditional haematoxylin and eosin staining was also suggested by Dellmann and Eurell(1998) and Samuelson (2007). Most of the earlier studies were carried out either at or before hatching of the chicks, or in adult birds. The same at different age groups during post- hatch period of broiler chicken up to marketable age is scant. However, few reported for ferric ferricyanide reaction (Lillie and Donaldson, 1974, Dey *et al*.,1983), argentaffin and argyrophil techniques (Polak *et al*.,1974, Okamoto, 1981, Polak and Bloom,1982, Dellmann and Eurell,1998, Samuelson, 2007 and Mandal and Das, 2015), in different species. In view of the aforesaid facts, the present investigation was undertaken.

Materials and Methods

Fifty day old broiler chicks were reared in experimental pens up to day 43. Standard management practice was followed uniformly for all the birds. A total of thirty-five birds were sacrificed, five birds each at weekly intervals, i.e. on days 1, 8, 15, 22, 29, 36 and 43 for collection of specimens (Intact gastro-intestinal tract). For histological and histochemical study, three small tubular pieces (initial, mid and last portion) of each gut segment starting from esophagus up to colo-rectum were collected, fixed in 10% buffered neutral formalin (BNF) for 2 – 3 days and processed for paraffin block making (Bancroft and Stevens, 1996). Sections at 5 –7 µ were obtained and subjected to the following staining techniques.

a. Lead haematoxylin method for endocrine cells (Bancroft and Stevens, 1996),
b. Singh's modification of the Masson-Hamperl argentaffin technique (Bancroft and Stevens, 1996) for argentaffin cells,
c. Grimelius' silver method for argyrophil cells (Bancroft and Stevens, 1996),
d. Chromaffin reaction for endocrine cells (Barka and Anderson, 1963),
e. Ferric ferricyanide reduction test (Bancroft and Stevens, 1996),
f. Vulpian's reaction for endocrine cells (Pearse, 1985),
g. Ninhydrin-Schiff method for peptide secretory cells (Humason, 1962),

Results and Discussion

Tinctorial Methods for Demonstration of Gut Endocrine Cells

In H& E staining, the endocrine cells were not distinctly visible. They appeared as "clear" cells mostly at the crypt epithelium (Crypt of Lieberkuhn) of the intestine close to the basement membrane. Some endocrine cells were located in the lining epithelium of the intestinal villi. But morphological features of these cells were not discernible. Endocrine cells of the gastrointestinal tract of post – hatch Vencob broiler chicken reacted selectively to several staining methods employed. Different types of endocrine cells were found to occupy each segment of the gut. Based on histomorphological features of the endocrine cells, reducing ability of ferric ferricyanide solution, degree of argentaffin affinity, and intracytoplasmic distribution of secretory granules, different types (categories) of gut endocrine cells were localized and identified. In the present investigation, both argentaffin and argyrophil reactions were adopted. The secretory granules of the gut endocrine cells were stained black in argentaffin method or brownish black by argyrophilia reaction (Fig.1,2,3). In lead haematoxylin technique, the endocrine cell granules appeared dark blue to bluish black in colour (Fig. 4,5). Different morphological and granulation pattern of these cells were better elucidated after ferric – ferricyanide reduction test. In the present investigation, ferric ferricyanide reduction test demonstrated the maximum number of endocrine cells.

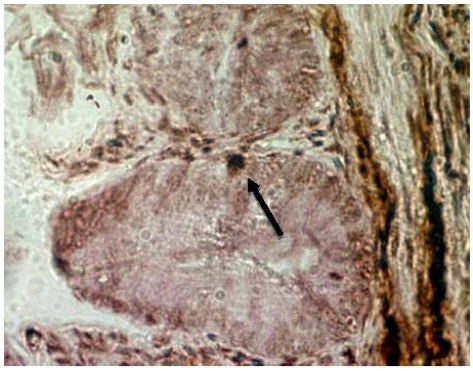

Figure 1: Micrograph of caecum in 8days old broiler chicken, showing an argentaffin cell (arrow)at crypt. Singh's method x 400

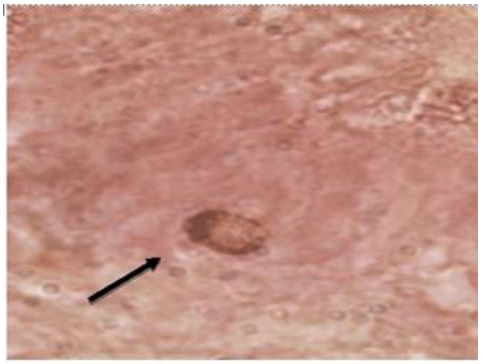

Figure 2: Micrograph of duodenum in 16 days old broiler chicken, showing an argentaffin cell (arrow). Singh's method x 1000

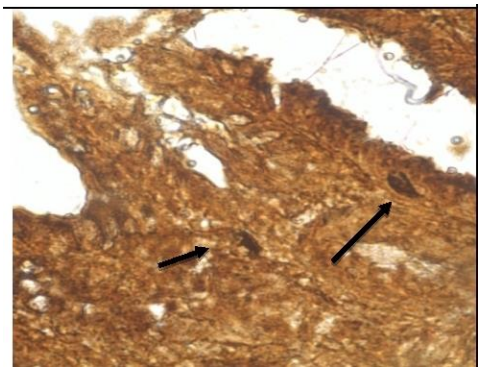

Figure 3: Micrograph of jejunum in day old broiler chicken, showing argyrophil cells (arrow) at crypt & villus. Grimelius method x 400

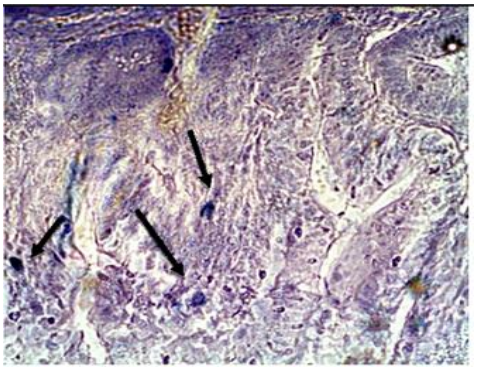

Figure 4: Micrograph of jejunum in 16 days old broiler chicken, showing different endocrine cells (arrow). Lead haematoxylin stain x 400

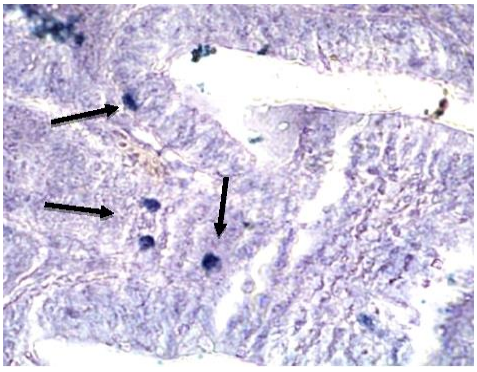

Figure 5: Micrograph of duodenum in day old broiler chicken, showing different endocrine cells (arrow). Lead haematoxylin stain x 400

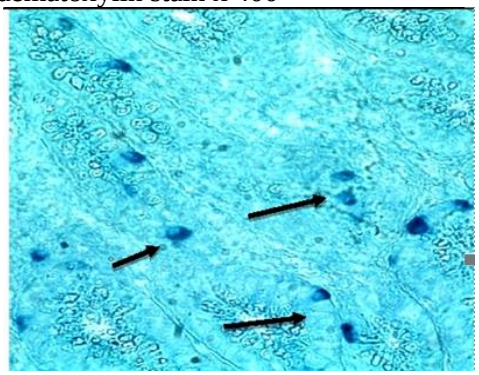

Figure 6: Micrograph of jejunum in 24 days old broiler chicken, showing different endocrine cells (arrow) at crypts. Ferric ferricyanide test x 400

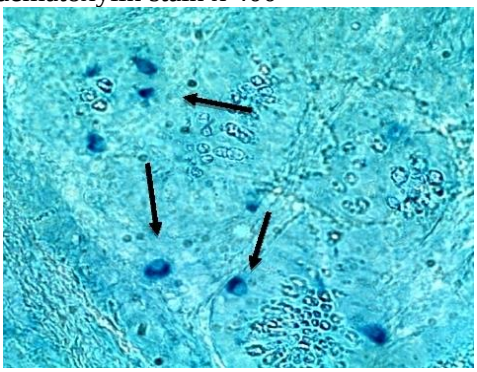

Figure 7: Micrograph of ileum in 24 days old broiler chicken, showing different endocrine cells (arrow) at crypts. Ferric ferricyanide test x 400

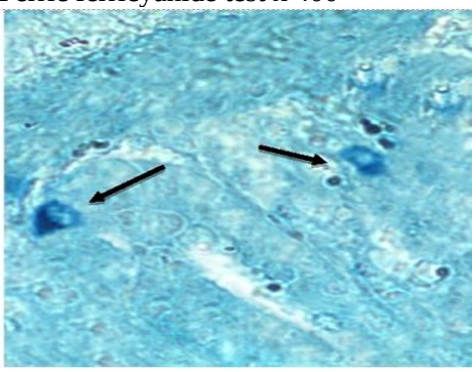

Figure 8: Micrograph of jejunum in 24 days old broiler chicken, showing different endocrine cells (arrow) at crypts. Ferric ferricyanide test x 400

After ferric ferricyanide treatment of the tissue sections (with and without counter stain) the endocrine cells appeared dark blue in colour (Fig. 6,7,8). The nuclei and the secretory granules were also clearly evident. This method even revealed the cytoplasmic processes of the enteroendocrine cells clearly. It was the technique of choice after due standardization of the reaction time. The following morphological types of endocrine cells were identified. Type I (Pyramidal cells), Type II (Piriform cells), Type III (Spindle shaped cells), Type IV (Oval cells), Type V (Spherical or Rounded cells), Type VI (Lance or Arrow head shaped cells), Type VII (Elongated cells), Type VIII (Flask shaped cells) and Type IX (Comma or Sickle shaped cells). The details of these cell types are not mentioned in this text.

Differential Staining Characteristics of Endocrine Cell Types

The endocrine cell types reacted to different staining techniques to a varying degree are detailed in Table 1. The ferric ferricyanide test elucidated the highest number of endocrine cells, followed by argentaffin reaction and lead haematoxylin method. It is noteworthy that Vulpian reaction showed nonspecific result. Ninhydrin Schiff method stained few endocrine cells. But due to the diffusing nature of the stain and poor differentiation from the surrounding enterocytes, no cell typing could be done on the basis of this reaction.

Table 1: Differential staining characters of different gut endocrine cell types

Cell Types	Ferric ferricyanide reduction test	Argentaffin reaction	Argyrophil reaction	Lead haematoxylin stain	Chromaffin reaction	Ninhydrin Schiff method	Vulpian reaction
I	+++	++	_	++	++	+	_
II	+++	++	_	++	_	_	_
III	+++	++	_	++	_	_	_
IV	+++	++	++	++	++	+	_
V	+++	++	++	++	_	+	_
VI	+++	++	_	++	_	_	_
VII	++	++	++	_	_	_	_
VIII	++	_	_	_	_	_	_
IX	++	_	_	_	_	_	_

+++ = strong, ++= moderate, += very low, - = non-reactive

Appearance of the gut endocrine cells in routine haematoxylin and eosin (H & E) staining as "clear cells" without any distinct morphology either in the epithelium of crypts or villi in the present study was based on the previous observations (Polak and Bloom, 1982; Fawcett, 1994;Bancroft and Stevens, 1996; Dellmann and Eurell, 1998; Samuelson, 2007). In the present investigation, ferric ferricyanide reduction test demonstrated the maximum number of endocrine cells. After ferric ferricyanide treatment of the tissue sections (with and without counter stain) the endocrine cells appeared dark blue in colour. The nuclei and the secretion granules were also clearly evident. This histochemical method even revealed the cytoplasmic processes of the enteroendocrine cells efficiently. Lillie (1965) gave a detailed view on the use of ferric ferricyanide reduction test in histochemistry. According to him, this reaction is much convenient as it can be easily performed. Moreover, sharp morphologic localization of different substances that reduce ferricyanide can be achieved by this test. The secretory substances of the enteroendocrine cells promptly reduce ferricyanide to ferrocyanide and subsequent production of Prussian blue. He also reported that Schmorl's reaction (a modification of original ferric ferricyanide reduction test) can be used for demonstration of melanin, lipofuscins, bile pigments as well as neuroendocrine granules. Melanin and lipofuscin reduce ferricyanide mixture very quickly (within 5 minutes or so), whereas reaction time for enterochromaffin cells is well beyond 10 minutes. In the present work, reaction time was standardized to 12 – 13 minutes. This showed the best possible result. Pearse (1985) also suggested formation of Prussian blue by the enterochromaffin cell granules. Later on, Lillie and Donaldson (1974) opined that ferric ferricyanide reaction depends on the binding of ferric ions and their subsequent reduction to ferrous ions. They ascribed the resultant blue colouration to Turnbull's blue precipitate. Further, the present finding on staining character of enteroendocrine cells with this method agrees well with those of Humason (1962), Dey *et al*. (1983) and Pearse (1985).

One of the very useful and authentic staining techniques for demonstration of neuroendocrine cells is the silver

impregnation. In the current investigation, both argentaffin and argyrophil reactions were followed to selectively stain argento / silver – reducing granules of the gut endocrine cells. The argentaffin technique was found to be better than the latter one. This method effectively and distinctly identified and localized the secretion granules of these cells. Though the morphology of the argentaffin cells were well visualized, the cell processes did not appear clearly like those seen after ferric ferricyanide reduction test. A number of argentaffin methods are available (Humason, 1962; Barka and Anderson, 1963; Singh, 1966; Luna, 1968). According to their views, this reaction was based on the intrinsic capacity of different tissue components including poly phenols, amino phenols and aldehydes to reduce silver salt solution to black metallic silver.

On the other hand, the argyrophilia needs addition of an external reducing agent for formation of metallic silver. Lundqvist *et al*. (1990) reported that the argentaffin reaction was the result from the reaction products of aldehydes and biogenic amines. Tryptophan and tyrosine metabolites, dopamine, norepinephrine, 5–HT etc. caused strong argentaffin reaction. Previously, many workers (Toner, 1964; Okamoto, 1981; Inokuchi *et al*., 1983; Martinez *et al*., 1993; Mishra and Das, 2006 and Vaccaro *et al*., 2009) advocated the usefulness of argentaffin and argyrophil reaction to demonstrate argento – reducing gut endocrine cells in different avian species including chicken. Similarly, Carvalheira *et al*. (1968) attributed the cytochemical features of argyrophil cells to polypeptide hormone producing APUD cells and that the argentaffin cells differed from the argyrophil cells in four species of mammals. These were two distinct cell types rather than being functional or developmental stages of a single variety of cell. Similarly, a clear distinction between these cell types was established by Singh (1966) in guinea pig, rabbit and human. Again, some other researchers (Capella *et al*., 1971 and Grimelius, 2008) also emphasized the selectivity and specificity of this silver reaction to elucidate a number of enteroendocrine cells. Few Ninhydrin – Schiff positive endocrine cells were also noticed in the intestinal crypts to reveal the presence of amino group containing proteins (peptides). Same technique was advocated by Barka and Anderson (1963), Pearse (1985), and Bancroft and Cook (1996). Mishra and Das (2006) also reported few Ninhydrin sensitive cells in gastrointestinal tract of broiler chicken. However, the reaction did not show any specificity like other important staining methods used. Moreover, the cells were too weakly stained. The Vulpian reaction also could not demonstrate any specific endocrine cell type in the present study.

Conclusion

Out of different light microscopic staining techniques followed in the present study, ferric ferricyanide reduction test and argentaffin reaction revealed the histomorphology of the gut endocrine cells better. The former one was the best among these methods, followed by the argentaffin reaction and lead haematoxylin technique. Considering the availability of the modern techniques, a conventional and logical light microscopic identification of gut endocrine cells in broiler chicken is still of much relevance, that could be an interesting prospect.

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Conflict of Interests

There is no conflict of interest.

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References

1. Argenzio, R.A. (2004). General functions of the gastrointestinal tract and their control. In: Dukes Physiology of Domestic Animals, (Ed.) Reece, W.O., 12th Edn, Panima Publishing Corporation, New Delhi, Bangalore, pp 381-390.

2. Bancroft, J.D. and Cook, H.C. (1996). Proteins and nucleic acids. In: Theory and Practice of Histological Techniques, (Eds.) Bancroft, J.D. and Stevens, A., 4th Edn, Churchill Livingstone, New York, London and Tokyo, pp139- 150.
3. Barka, T. and Anderson, P.J. (1963). Histochemistry: Theory Practice and Bibliography. Harper and Row Publishers Inc., London, pp182-202.
4. Capella, C., Vassallo, G. and Solcia, E. (1971). Light and electron microscopic identification of the histamine-storing argyrophil (ECL) cell in murine stomach and its equivalent in other mammals. *Z. Zellforsch.*, 118, 68 – 84.
5. Carvalheira, A. F., Welsch, U. and Pearse, A. G. E. (1968). Cytochemical and ultrastructural observations on the argentaffin and argyrophil cells of the gastro-intestinal tract in mammals and their place in the APUD series of polypeptide-secreting cells. *Histochemie*, 14, 33 – 46.
6. Dellmann, H.D. and Eurell, J.A. (1998). Digestive system. In: Textbook of Veterinary Histology,12thEdn, Williams & Wilkins, Philadelphia, London, Paris, Bangkok, Hong Kong, Tokyo, pp164- 202.
7. Dey, R., Shannon, W.A. Jr., Hagler, H.K. and Said, S.I. (1983). Histochemical and ultrastructural characterization of serotonin- containing cells in rabbit tracheal epithelium. *J.ournal of Histochemistry and Cytochem*istry, 31(4), 501 – 508.
8. Fawcett, D.W. (1994). A Text Book of Histology. 12th Edn., Chapman and Hall, pp 617-651.
9. Frappier, B.L. (1998). Digestive system. In: Textbook of Veterinary Histology, (Eds.) Dellmann, H.D. and Eurell, J.A., 5th Edn., Williams and Wilkins, Philadelphia, London, Paris, Tokyo, pp.164-202.
10. Grimelius, L. (2008). Methods in neuroendocrine histopathology: A methodological overview. *Upsala Journal of Medical Sciences*, 113 (3), 243–260.
11. Humason, G.L. (1962). Animal Tissue Techniques. W.H. Freeman and Company, San Francisco and London.
12. Inokuchi, H., Kawai, K., Takeuchi, Y. and Sano, Y. (1983). Identification of EC cells in the human intestine: A comparative study between immunohistochemical and silver impregnation techniques. *Histochemistry*, 79, 9 – 16.
13. Lillie, R.D. (1965). Histopathologic Technic and Practical Histochemistry. 3rd Edn. Blackiston Div., McGraw – Hill, New York.
14. Lillie, R. D. and Donaldson, P. T. (1974). The mechanism of the ferric ferricyanide reduction reaction. *The Histochemical Journal*, 6, 679 – 684.
15. Luna, L.G. (1968). Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd Edn, McGraw Hill Book Company, New York.
16. Lundqvist, M., Arnsberg, H., Candell, J., Malmgren M., Wilander, E., Grimelius, L. and Oberg, K. (1990). Silver stains for identification of neuroendocrine cells. A study of the chemical background. *Histochemical J*., 22(11), 615 – 623.
17. Mandal, A.K. and Das, R.K. (2015). Innervations pattern of the intestinal wall with reference to gut endocrine cells in post hatch broiler chickens. *Indian Journal of Animal Sciences*,85(4), 379-381.
18. Martinez, A., Lopez, J., Sesma, P. (1993). Development of the diffuse endocrine system in the chicken proventriculus. *Cell Tissue Research*, 271(1), 107-13.
19. Mishra, U.K. and Das, R.K. (2006). Cytochemical characterization of the endocrine cells of gastrointestinal muscosa of broiler chicken. *Orissa Veterinary Journal*, 21(4), 25-30.
20. Okamoto, T. (1981). Light and electron microscopic studies on the endocrine cells in the duck proventriculus. *Japanese Journal of Veterinary Sci*ence, 43, 863 – 870.
21. Pearse, A.G.E. (1985). Histochemistry Theoretical and Applied. Vol.2., Churchill Livingstone, Edinburg.
22. Polak, J.M. and Bloom S.R. (1982). Localization of regulatory peptides in the gut. *British Medical Bulletin*, 38 (3), 303 - 307.
23. Polak, J.M., Pearse, A.G.E., Adams, C. and Garaud, J.C. (1974). Immunohistochemical and ultrastructural studies on the endocrine poly peptide (APUD) cells of the avian gastrointestinal tract. *Experientia*, 30 (5), 564 – 567.
24. Samuelson, D.A. (2007). Textbook of Veterinary Histology.Saunders Elsvier, pp329.
25. Singh, I. (1966). Argyrophile and argentaffin reactions in individual granules of enterochromaffin cells of the guinea pig. *Zeitschrift Zellforschung.*, 73 (4), 549 – 552.
26. Toner, P. G. (1964). Fine structure of argyrophil and argentaffin cells in the gastrointestinal tract of the fowl. *Zeitschrift Zellforschung.*, 63, 830 – 839.
27. Vaccaro, R., Parisi Salvi, E., Nofroni, I., D'Este, l., Baglaj, S.M. and Renda, T. (2009). Endocrine cells in atresic chick embryo intestine: histochemical and immunohistochemical study. *European Journal of Histochemistry*, 53 (3),143-150.