Autofluorescent vesicular structures in hematoxylin and eosin stained duodenal mucosa of the domestic cat

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Abstract
Sections of formaldehyde-fixed, paraffin-embedded and routinely haematoxylin and eosin stained duodenal tissue of the domestic cat were inadvertently observed under blue excitation visible light of a fluorescence microscope, while undertaking histological studies on normal mammalian gastrointestinal tract. The sections revealed yellow fluorescing vesicular droplets in basally located cells within mucosal epithelium of the villi and the intestinal glands. The droplets had pink coloration under light microscopy, while with non-routine staining methods of Periodic acid – Schiff reagent and silver (Fontana method), they were negatively stained. An evaluation for similar structures in duodenal tissue of dogs, cattle and rats proved negative. A review of the literature indicates that these structures may be unique to the cat’s duodenum, and that they may represent serotonin secretory product of cells that can be classified as a member of the diffuse neuroendocrine system.

Key words: Diffuse neuroendocrine cells; cat duodenum; serotonin secretory cells; autofluorescence.

Introduction
The duodenum is the initial segment of the small intestine, and in mammalian species, it is grossly characterized by a typical u-shaped morphology with two arms generally designated as descending and ascending loops. The pancreas is normally positioned between the two duodenal loops. Microscopically, the duodenal wall, apart from conforming to the typical four-layered arrangement of its tunics, namely: tunica mucosa, tela submucosa, tunica muscularis, and tunica serosa, also presents classical features of the small intestine. These features range from the presence of villi, permanent or non-permanent circular folds termed plicae circulares, to the presence of intestinal glands within its mucosa. In the normal duodenum, the intestinal glands, rich in goblet cells, and the submucosal glands termed Brunner’s glands have been described as aiding the digestive process by elaborating mucus-rich product which neutralizes the acidic chyme, as well as form slippery visco-elastic gel that protects the mucosal lining.1, 2. There are also a few reports of pathologies associated with the duodenal mucosa in domesticated animals and man. In dogs and cats, such conditions include: lymphocytic-plasmacytic enteritis, viral and non-viral associated intestinal lymphomas, and immunodeficiency-associated syndromes3, 4.

Our present focus on the duodenum of the domestic cat (Felis cattus) stem from an unexpected observation of autofluorescing vesicular droplets associated with basal cells of the duodenal mucosal lining, in hematoxylin and eosin-stained sections. The fluorescing structures were so unique in morphology that we were prompted to expand our investigative procedure by staining with other non-routine histological methods. We were also compelled to examine duodenal tissue of other mammalian species, such as man, cattle, rat and dog. Our observation, so far, indicates the absence of similar structure in the duodenal mucosa of these mammalian species we have investigated. An investigation into the possible existence of the structure within the mucosa of the remainder of the small intestine of the domestic cat also proved negative.

The present report is, therefore, aimed at bringing this observation to the awareness of the scientific community. In our opinion, the existence of the glandular structure is quite unique to the domestic cat, and this observation may not have been documented in the past.
Materials and Methods

Duodenal tissue specimens were collected from a total of 4 domestic cats (2 young and 2 adults), 2 dogs, 2 cows, and 2 rats. The specimens were mostly collected at post mortem, either following euthanasia of the normal animals (cat and rat), or during routine post mortem on the animals (dog and cattle) for conditions unrelated to intestinal problems. The human specimen was in the form of already prepared sections from post mortem subjects unrelated to intestinal conditions.

Routine specimen preparative procedure involved fixing in 10% buffered-neutral formalin, dehydration in graded ethanol, infiltration and embedment in paraplast, sectioning, mounting and staining by standard haemotoxylin and eosin (H&E) procedure; all according to standard methods.

Non-routine specimen preparative procedure involved tissue processing and staining by periodic acid-Schiff (PAS) reagent, staining with silver stains according to Fontana methodology, and staining separately with hematoxylin, methylene blue, and eosin, according to standard methods.

Fluorescence microscopy viewing procedure involved the use of a Riechert epifluorescence microscope interfaced with an Olympus digital image acquisition camera (Olympus DP12 system). Fluorescent absorption was at blue spectrum while emission was at green spectrum of the visible light.

Results

The autofluorescing structures appeared as typical spherical clusters of condensed droplets with vesiculated morphology (Figs. 1, 2). The vesicular droplets were of variable sizes, ranging from 0.3 to 1.2 μm. They were intracytoplasmic in location; each typical cluster being reminiscent of a grape bunch, and occurring within the basal cytoplasmic region of the lining enterocytes. The distribution of the structures was that of isolated patches within the mucosal epithelial lining, as well as the lining of the intestinal glands. Under H&E stained sections, the structures were pink and could easily be confused for blood capillaries. However, blood vessels were distinguishable by virtue of their contents of brick red erythrocytes which were non-fluorescent under the fluorescent microscope (Fig. 2).

The fluorescing images of the droplets were characterized by colour hues that ranged from dull green to bright yellow; the spread of the hue tending to vary with the size of the droplets. Thus, the smaller droplets were more of a dull green hue while the larger droplets were a brighter yellow (Figs. 1C, 2B). Within the cell containing the droplets, the single nucleus was eccentric in position and non-fluorescent (Fig. 2B), however, the nucleus displayed attributes of functionality such as reasonable euchromasia. Unlike the more basally-located nucleus within the cell, the droplets occupied mostly the apical cytoplasm.

The droplets in sections prepared by non-routine staining methods of PAS and Fontana were observed to be negative for the respective stains (Figs. 3, 4, 5). Within the sections prepared by the PAS staining method, goblet cells were clearly observed to be PAS positive by their typical dark red colouration, compared with the cells containing the droplets that revealed the droplets as pink coloured structures (Fig 3). Similarly, with the Fontana staining method, the droplets did not exhibit signs of argyrophilia, unlike the dark reticular meshwork within the stroma of the section (Fig. 5).

Table 1 summarizes the observations made for the staining attributes of the droplets with the different stains.
**Figure 1.** H&E-stained images (Panels A and B) and fluorescent image (Panel C) of a section of cat duodenal villi (Cap_19-8-05). Multiple foci of orange-coloured vesicular clusters are apparent within deeper regions of the lining epithelium in H&E-stained section (panel A). These vesicular clusters autofluoresce as shown in panel C. Panels B and C are equivalent to magnified rectangular area shown in panel A. The smaller cluster of fluorescing vesicles at the upper left of Panel C represents the cytoplasmic region of the cell with formative stage of the vesicular clusters (circle in panel B). This smaller cluster appears to be initial stages of condensation of the vesicles. Fluorescent absorption and emission was respectively at blue and green wave lengths. Bars =6 μm.
Figure 2. H&E-stained image (Panel A) and autofluorescent image (Panel B) of another section of cat duodenal mucosa (Cap_19-8-05-36y; Cap_19-8-05-37y). Panel B is equivalent to magnified rectangular area shown in panel A. The fluorescing vesicles are located in cells within the basal epithelial lining of the villi. Note that the superimposed oval nuclei within the cells do not fluoresce. Also note that red blood cells which appear brick red coloured within the core of the villus in Panel A (green arrows) are non-fluorescent. Bars =6 µm.
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Figure 3. PAS-stained image of a section of the cat duodenal villi (Cap_6-9-05). The fluorescing vesicles, unlike contents of the goblet cells, are PAS negative as shown (Arrows). The vesicles do not fluoresce with PAS stain. Note that the PAS-positive inclusions of the goblet cells are located towards the apical portion of the epithelial lining, while the fluorescing structures are located at the basal regions. Bar =15 mm

Figure 4. PAS-stained image of another section of the cat duodenum showing PAS positive contents in goblet cells within the lining of the intestinal glands (Cap_6-9-05). The enterocytes are PAS negative. Bar =15 μm.
Figure 5. Silver-stained (Fontana method) image of a section of the cat duodenal mucosa (Cap_24-2-06-003x). The fluorescing vesicles, pointed to by the arrows, are negative for argentaffin reaction. Note the fine reticular meshwork of the surrounding tissue which is argyrophilic. Bar =15 μm.

Table 1. Stain types and observation of fluorescence by the vesicular structures in the cat duodenal mucosa.

<table>
<thead>
<tr>
<th>Stain Type</th>
<th>Reaction to stain</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>H &amp; E</td>
<td>Pink vesicles</td>
<td>+++</td>
</tr>
<tr>
<td>PAS</td>
<td>Pink vesicles</td>
<td>nil</td>
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<tr>
<td>Methylene Blue</td>
<td>Light blue vesicles</td>
<td>nil</td>
</tr>
<tr>
<td>Hematoxylin</td>
<td>Light blue vesicles</td>
<td>nil</td>
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<tr>
<td>Eosin</td>
<td>Light blue vesicles</td>
<td>++</td>
</tr>
<tr>
<td>Silver Stain</td>
<td>Red vesicles</td>
<td>nil</td>
</tr>
</tbody>
</table>
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Discussion

The microscopic features of the duodenum in cats as revealed in the present investigation are similar to those of the classical duodenum of most mammals described in the literature. The observation of the fluorescing vesicular structures mostly within cells of the basal epithelial lining of the villi and the intestinal crypts, however, suggests extended functionality of the duodenal mucosa in the cat. The profile of the normal cell population of the typical mammalian duodenal mucosa includes: enterocytes, goblet cells, Paneth cells and neuroendocrine cells belonging to the diffuse neuroendocrine system (DNES). Within the profile, the cells with the autofluorescing droplets appear to have features that could enable their classification as members of the DNES family. Notable among the features is the deeper location of the cells within the mucosa, which would be necessary if they were associated with the direct release of the secretory products into extracellular spaces of the well vascularized core of the villi and the underlying subepithelial tissue. DNES cells were originally classified as amine precursor uptake and decarboxylation (APUD) cells owing to their affinity for amine uptake as well as being argyrophilic5. Forty different types of DNES cells dispersed all over the different organs of the mammalian body were described by Pearse6. Our observation under Fontana staining method did not reveal any argyrophilic property for the droplet-containing cells.

Duodenal microanatomy of six domestic cats studied by Henry and Al-Bagdad7 showed the presence of different epithelial cells but did not include the droplet-containing cells observed in this study. A report by Krause1 on duodenal glands in 14 primates indicated that the glands which are mucous in nature may elaborate mucopolysaccharides. The droplet-containing cells of our observation are non-mucous as they are negative with the PAS staining method. A more recent classification of the DNES cells specifies two types: open and closed8,9. The open type includes DNES cells that have apical surfaces exposed to the lumen of the gastrointestinal tract whereby enabling reception to the gastrointestinal tract content materials. The closed type, on the other hand, are more deeply located and do not have surfaces that are exposed to the GIT luminal contents. This latter category of DNES cells are ascribed the function of reception for changes within the local tissue environment. Under fluorescence microscopy, formaldehyde-fixed DNES cells have been reported to have a characteristic yellow fluorescence if they are serotonin-secreting cells, while green fluorescence has been associated with formaldehyde-fixed DNES cells secreting cathecolamines8. Our observed cells with the autofluorescing droplets appear to belong to the closed type of DNES cells. Given the magnitude of the yellow fluorescence of the autofluorescing droplets, the cells with the droplets may be serotonin-secreting DNES type. Further studies, such as staining for chromaffin attributes through the use of chromium stains, are recommended to fully clarify the nature of these cells.

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References