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A LIGHT MICROSCOPICAL STUDY OF THE INTESTINAL TRACT OF THE NILE CROCODILE (*CROCODYLUS NILOTICUS*, LAURENTI 1768)

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ABSTRACT

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Although the histology of the intestinal tract of *Crocodylus niloticus* is touched on in overall studies on reptilian intestinal tract, a more comprehensive light microscopical study on this area is lacking. Specimens for histological examination were taken from the duodenum, the jejunum, the ileum and the rectum. The data obtained revealed that the mucosa is thrown into folds and simple, slightly branched tubular intestinal glands. The mucosal folds diminished in height and eventually disappeared upon reaching the ileorectal junction. The epithelium covering the folds and crypts was of the simple columnar type. Clear marginal cells, goblet cells and argentaffin cells were observed throughout the intestinal tract. No Paneth cells were seen in this study. The lamina propria was rich in lymphocytic infiltrations while the muscularis mucosa consisted mainly of an outer longitudinal layer, the inner circular layer being rudimentary or absent. The submucosa was extremely narrow, and the circular and longitudinal layers of the tunica muscularis contained distinct layers of dense fibrous connective tissue. The histology of the intestinal tract of *C. niloticus* is shown to be in line with the situation in crocodilians and also exhibits a resemblance to that of carnivorous mammals.

INTRODUCTION

Studies on the histology (Luppa, 1977; Beguin, 1902; Jacobsagen, 1937), the intestinal relief (Jacobshagen, 1920; Parsons & Cameron, 1977) and the physiology (Skockzylas, 1978) of the intestinal tract of *C. niloticus* form part of general overview studies of the reptilian intestinal tract. Specific studies on *C. niloticus* include studies on the histoenzymology of the digestive tract (Avry & Bonichon, 1958a,b).

Törö (1930) addressed some aspects of the histology of the crocodilian intestinal tract but failed to specify the species under investigation. A histological study on the intestinal tract of the Florida alligator was carried out by Reese (1913).

The growth in intensive crocodile farming in South Africa in recent years created the need for the formulation of cost efficient rations for optimal growth of the Nile crocodile (Smith & Marais, 1990). The latter reverted the attention to the histology of the digestive tract of this species. The aim of this study is to record the histology of the intestinal tract of the Nile crocodile on the light microscopic level.

MATERIALS AND METHODS

The intestinal tracts of 6 two-year-old Nile crocodiles of both sexes were used in this study. Four of the crocodiles were anaesthetised and exsanguinated while two were shot. Samples for histological examination were taken from the duodenum, jejunum, ileum and rectum. These specimens were fixed in Bouin's fluid for 8 hours at room temperature, dehydrated and routinely imbedded in paraffin wax. Sections were then cut at a thickness of 6 µm, stained with haematoxylin and eosin, examined and photographed using a light microscope. In addition, similarly cut sections were stained with Phloxine, tartrazine to detect Paneth cells, Verhoeff's iron-haematoxylin stain for elastic fibers, periodic acid Schiff reaction (PAS) for demonstrating mucus in goblet cells and the Masson-Fontana method for the detection of argentaffin cells.

RESULTS

In the duodenum the mucosa consisted of short mucosal folds and simple and slightly branched tubular glands which opened in crypts between the bases of the folds (Fig. 1a). In the proximal jejunum a gradual increase in height of the folds was observed and continued towards the distal jejunum. Branching and anastomosing of folds were observed along the small intestine starting from the distal duodenum and continued into the proximal part of the ileum (Fig. 1b). Both the height and the branching and anastomosing of the folds decreased in the distal ileum towards the ileorectal junction. A gradual disappearance of mucosal folds finally occurred in the rectum where only crypts were observed (Fig. 1c).

The mucosal folds and glands were lined by a simple columnar epithelium which consisted of clear absorptive cells with well developed striated borders (Fig. 1d). These cells exhibited a clear pale-staining cytoplasm and basally situated oval nuclei (Fig. 1d). Typical flask-shaped goblet cells stained PAS positive and were found to be scattered abundantly throughout the entire intestinal tract (Fig. 1e). Using Masson-Fontana stain, scattered agentaffin cells could be demonstrated throughout the intestinal tract, but were found to be most abundant in the crypts of the rectum (Fig. 1f). In this study no Paneth cells were observed in any region of the intestine. In some areas the mucosal surface was partly covered by a layer of mucus, cell debris, desquamated epithelial cells and lymphocytes.

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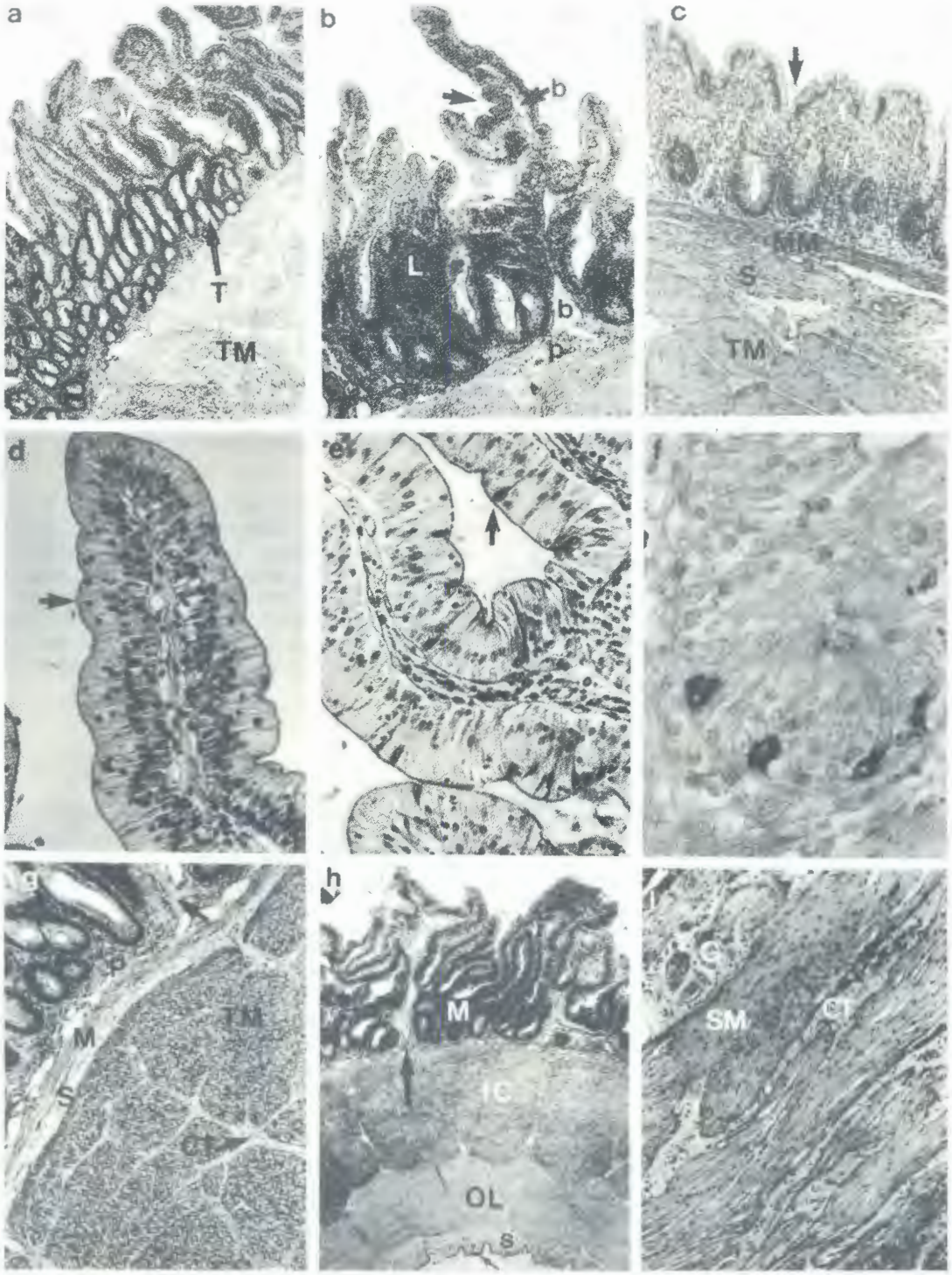

FIG. 1 (a) Jejunum. The mucosa consisted of mucosal folds (V) and simple tubular glands (T). The inner circular layer of the tunica muscularis (TM) is visible. H&E 8 ×

(b) Jejunum. Diffuse lymphocytic infiltration into the mucosa is marked (L). Branched mucosal folds (arrow) and blood vessels (b) extending into the mucosal folds can be seen. H&E 20 ×

(c) Rectum. Absence of mucosal folds can be observed with crypt openings (arrow). The muscularis mucosa is marked (MM). The submucosa (S) containing blood vessels is slightly wider in the rectum. The circular layer of the tunica muscularis is marked (TM). H&E 20 ×

(d) Duodenum. The tip of a mucosal fold showing the brush border (arrow) of the enterocytes cells with basally situated nuclei. H&E 80 ×

(e) Jejunum. Goblet cells, one of which is marked with an arrow occurred throughout the intestinal tract. PAS 80 ×

(f) Rectum. Argentaffin cells were observed in the bases of the crypts. Masson Fontana 200 ×

(g) Jejunum. The lamina propria (P) containing a blood plexus separated from the submucosa (S) by the muscularis mucosa (M), the inner layer from which muscle strands run into the cores of the mucosal folds (arrow). The circular layer of the tunica muscularis (TM) showing septa of dense fibrous connective tissue (CT). H&E 20 ×

(h) Duodenum. The mucosa is marked (M) while the arrow points to the blood plexus in the lamina propria. The inner circular (IC) and outer longitudinal (OL) layers of the tunica muscularis are visible. The submucosa is marked (S) while the arrow points to the mesothelial serosal layer. H&E 8 ×

(i) Jejunum. The circular layer of the tunica muscularis showing the bundles of smooth muscle (SM) with layers of dense fibrous connective tissue (CT) in between. Ganglionic nerve cells are marked (G). H&E 20 ×

The lamina propria consisted of typical reticular connective tissue, and revealed fine reticular and elastic fibers, fibroblasts and plasma cells. Areas of diffuse and prominent localized lymphocytic infiltrations were frequently observed in the lamina propria (Fig. 1b). Occasionally the localized lymphocytic infiltrations displaced and in some cases obliterated the underlying muscularis mucosa thus making it unclear whether it occurred in the lamina propria or the submucosa. A well develop periglandular capillary network was present which extended into the mucosal folds (Fig. 1b).

The muscularis mucosa was present and consisted mainly of longitudinal fibres (Fig. 1g). An inner circular layer of the muscularis mucosa was poorly developed and in most cases absent. Strands of smooth muscle extended from the muscularis mucosa into the lamina propria of the mucosal folds reaching their tips (Fig. 1g).

The submucosa was thin and revealed collagen and reticular fibres. A capillary network was also present in the submucosa (Fig. 1g). In the rectum the submucosal layer appeared slightly thicker and contained larger blood vessels than observed in the duodenum, jejunum and ileum (Fig. 1c).

The tunica muscularis consisted of a well developed inner circular layer and a narrower outer longitudinal layer. (Fig. 1h). Well developed sheets of dense fibrous connective tissue separated the muscle layers into distinct bundles (Fig. 1g & i). This phenomenon was more obvious in the circular layer but could also be observed in the longitudinal layer to a lesser degree.The connective tissue in these strands was continuous with connective tissue in the submucosa. Capillaries and ganglionic nerve cells were present in the connective tissue between the circular and longitudinal layers as well as in the connective tissue sheets within the muscle layers.

The subserosa was well developed and consisted of a loose connective tissue containing collagen and reticular fibres. Blood vessels, adipose tissue and nerve bundles were abundant in this layer. The serosa consisted of a layer of typical mesothelial cells resting on the subserosal loose connective tissue (Fig. 1h).

Discussion

The mucosa of reptiles in general is said to consist of intestinal folds and to be devoid of villi and tubular intestinal glands (Patt & Patt, 1969; Andrew, 1959). In the present study, however, tubular intestinal glands, which resembled the typical crypts of Lieberkühn of mammals, were observed. In a study on an unknown species of crocodile, Törö (1930) did not mention the occurrence of villi but made reference to longitudinal mucosal folds which became higher distally in the small intestine. In the same study he reported anastomosing of these folds. In the present study, distinct mucosal folds became higher towards the middle of the small intestine. They branched and anastomosed. Törö (1930) reported the presence of crypts of Lieberkühn in the crocodile similar to the crypts seen in the present study. The present study is the first report on the diminishing in height of the mucosal folds towards the ileum and ileorectal junction and their final disappearance in the rectum.

In the present study the intestinal mucosa was lined by simple columnar epithelium and not the stratified columnar type Reese (1913) reported in the Florida alligator. Structurally the enterocytes seen in the present study are in agreement with those described by Luppa (1977) and with those observed in the mammalian intestine (Bloom & Fawcett, 1975). Well developed microvilli observed in the present study are in line with the findings on enterocytes of reptiles (Andrew, 1959). The topographic distribution and structure of goblet cells observed in the present study were similar to those described by Luppa (1977) but in contrast to the study by Reese (1913), who did not observe goblet cells at all. The failure to demonstrate Paneth cells in the present study confirms the findings of Törö (1930) but conflicts with the report cited by Luppa (1977), who demonstrated Paneth cells in the crypts of all regions of the intestine of *C. niloticus*. According to Luppa (1977) the granules of the Paneth cells may be dissolved by acid in the fixative and this may account for their absence in the present study. The presence of argentaffin or enterochromaffin cells in this study is confirmed by the findings of Gabe (1973) who demonstrated these cells in the duodenum of *C. niloticus*. Further investigation is needed to determine whether or not the cells seen in the present study correspond to the first enterochromaffin type described by Gabe (1973) or the second, non-indolic type. In the same paper he warned that negative results for argentaffin reactions may be obtained in tissues fixed in Bouin's fluid which may be the reason for the relative scarcity of these cells in the present study. This may be why the distribution of argentaffin cells in the present study is in agreement with the distribution in mammals (Bloom & Fawcett, 1975).

Observations on the lamina propria in the present study are in line with findings by Törö (1930) who reported both lymph follicles and blood plexuses in

the lamina propria of a crocodile. Evenly distributed lymphocytic infiltrations were observed in the lamina propria of the entire intestinal tract of the crocodile. This is in contrast to the findings of Avry & Bonichon, (1958a) who observed an increase of lymphocytic infiltrations toward the rectum of the same species. The lamina propria seen in the present study also resembled the mammalian type (Bloom & Fawcett, 1975). However, lymphocytic infiltrations seemed to occur mostly in the lamina propria in the present study, in contrast to the situation observed in the small intestine of mammalian carnivores where lymphocytic infiltrations occur in the submucosa (Bloom & Fawcett, 1975).

The structure of the muscularis mucosa in this study is in agreement with the findings of Törö (1930) who also found it not to be a clear double layer. The smooth muscle bundles extending from the rudimentary inner circular layer of the muscularis mucosa into the cores of the mucosal folds seen in the present study were confirmed by the findings of Törö (1930).

The findings of the present study, as well as Reese's (1913) on the Florida alligator, are in agreement that the submucosa is poorly developed, and thus seems to be typical for crocodilians.

The structure of the tunica muscularis seen in the present study is in line with reports on other crocodilians (Törö, 1930; Reese, 1913). However, the present study is the first to report distinct sheets of connective tissue between, and in, both layers of the tunica muscularis. Although the arrangement of these sheets is more haphazard than the laminar distribution of dense fibrous connective tissue in the stratum compacta of the carnivore stomach (Banks, 1986), it may be possible that these sheets have a similar function in preventing perforation of the gut by sharp objects. The occurrence of ganglionic nerve cells between the muscle layers is consistent with Luppa's (1977) findings.

From the results of the present study it is clear that the histology of the intestinal tract of the *C. niloticus* does not differ dramatically from that seen in carnivorous mammals. This study serves as a basis for histopathological investigations which are neccessary from time to time at crocodile farms.

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