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INTRANUCLEAR BODIES AND OTHER CYTOMORPHOLOGIC FEATURES OF THE HORSE JEJUNUM MUCOSA

H. A. EL-HABBACK

Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

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SUMMARY

Investigation of the jejunum of horse at the ultrastructure level has clarified some of the unique features of the equine jejunum.

It has a long blunt villi. The intestinal crypts open at the base of the villi. Three types of cells were demonstrated at these crypts, granular cells, mucous cells and paneth cells; in addition to the columnar and goblet cells. The granular cells form the majority of cells at the initial parts of the crypts and decreased basely. The cell membrane of these cells showed desmosomal junctions. The nuclei of these cells were irregular and some of them showed mitotic figures. The karyoplasm of some nuclei revealed a membrane bound intranuclear dense bodies (average diameter 0.2 µm). Some of these bodies had the same electron density as the secretory granules found in the apical cytoplasm and others were electron -lucent. The possible origin of these bodies was discussed. They could be originated as cytoplasmic granules that may be trapped in the nucleus during mitosis. The mucous cells form most of the cell population at the base of the crypt. Their nuclei were devoid of intranuclear bodies. A condensation of intermediate filaments was noticed in the mucous cells. They appeared associated with the mucous globules, some of them form a demilune at the edge, some form a dot like at the pole and others form a diameter across the globule. These filaments could play a role in transportation of these globules and or help in evacuation of the cell contents.

INTRODUCTION

The gastrointestinal tract of the horse has not been subjected to extensive ultrastructure investigation.

Only few reports deal with the normal morphology of the equine small intestine (Roberts and Hill, 1974). Furthermore, the intestinal mucosa presents some characteristics, which are uncommon in other species. The most obvious were the presence of the granular cells that contain apical granules and intranuclear bodies (Doyle, 1980; Pfeiffer, Murray and Fainter, 1987; Kaup and Deege, 1994). Nuclear bodies were first reported by De-The, Riviere and Bernhard (1960) and HinglaisñGuilland, Moricord and Bernhard (1961).

Since this time, intranuclear bodies have been reported in a variety of tissues in different animal species, under normal and diseased conditions (Foster, Young, Allanson and Cameron, 1965; Bouteille, Kalifat and Delarue, 1967; Buttner and Horstmann, 1967; Buttner, 1968a; Simar, 1969; Dupuy-Coin, Kalifat and Bouteille, 1972; Doyle, 1980; Fournier, Privat and Bouteille, 1981; De-Oliviera, Rosenbruch and Schulz, 1985; Pfeiffer et al., 1987 and Kaup and Deege, 1994). The present communication is focused on several morphological features of the jujenal granular epithelial cells. In particular, we wish to standardize the intranuclear bodies that occur at high frequency within the nuclei of these cells.

MATERIALS AND METHODS

Tissue samples were taken from the mucosa of the jejunum of normal adult horses under thiamylal sodium anesthesia. Specimens were immediately placed in cold, buffered (pH 7.4) glutaraldehyde for rapid fixation, which was subsequently followed by osmium postfixation, ethanolic dehydration, and embedding in Epon (Hayat, 1981). Thick sections were studied after staining with methylene blue-azure basic fuchsin (Humphrey and Pittman, 1974) for orientation, and thin sections were doubly stained with lead citrate and uranyl acetate. Transmission electron microscopy (TEM) was undertaken with a JEOL 100-CX-II electron microscope operating 80 kV.

RESULTS

The jejunum had the same histological architecture as seen in the other mammalian species. It consisted of mucosa, submucosa, muscularis and covered by the mesentery serosa.

The jejunum had long, blunt wide villi lined by columnar cells and dispersed goblet cells inbetween (Fig. 1). The columnar cells had basely situated nuclei with prominent nucleolus, and peripheral heterochromatin. The cytoplasm showed free ribosomes, few individual cisternae of rough endoplasmic reticulum (rER), dispersed tonofilaments and numerous electron dense mitochondria. The mitochondria showed more electron dense granules in their matrix and increased in number toward the apical part of the cytoplasm. The columnar cell plasmalemma showed apical and lateral modifications (Fig. 1). The apical one formed a long, blunt cylindrical microvilli. The lateral

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modifications included tight junctions and desmosomes with the adjacent columnar cells, and or goblet cells (Fig. 1).

The intestinal crypts opened at the base of the villi. They were simple, branched tubular invaginations. Three types of cells: granular cells, mucous cells and paneth cells (Fig. 2) were demonstrated in these crypts in addition to the columnar and goblet cells. The granular cells were numerous at the initial region of the crypt and decreased toward the base. The cells were truncated in shape with basal nuclei. The nuclei were irregular in shape, some of them showed indentations. They had a peripheral heterochromatin, which sometimes extended to the nuclear centers (Fig. 3). The karyoplasm of these nuclei contained consipicuous nuclear bodies (Figs 3 & 4). The Intranuclear bodies were spherical or ovoid shaped homogenous structure. Within the nucleus, the nuclear bodies were often found grouped together in clusters of average 5-25 individual bodies (Figs. 5 & 6). They were varied between electron dense and electron lucent (Figs. 5 & 6) with fine granular contents. Some of these bodies had the same electron density as the secretory granules in the apical cytoplasm (Fig. 7) and approximately the same diameter 0.2 µm. Although most of the nucleoplasm was occupied by dense bodies, all the transitional stages could be observed. Most of the nuclear bodies were surrounded by a double membrane delimiting them from the rest of the nucleoplasm (Figs. 5 & 6). Some of these bodies showed a remnant of membrane, while no limit-

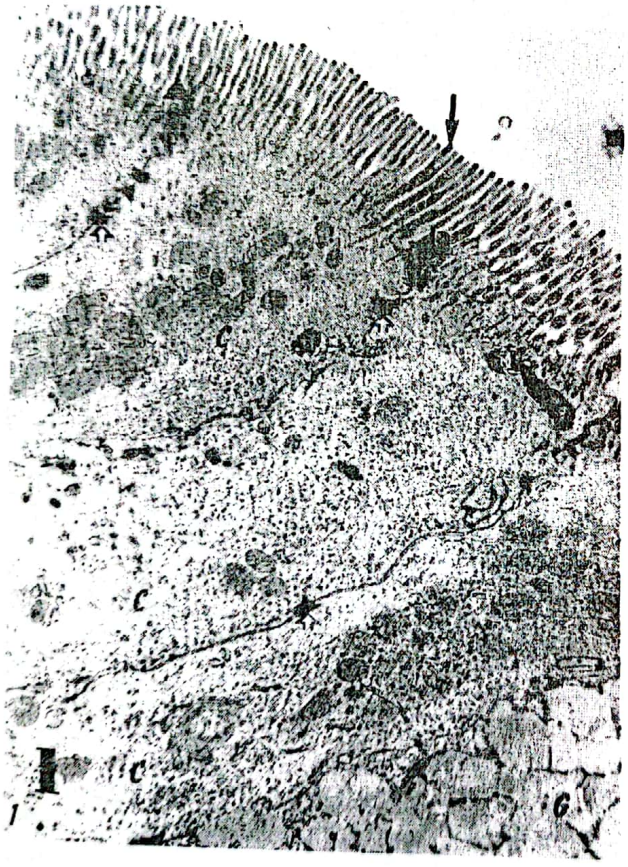

Fig. (1): Electron micrograph of horse Jejunum showed the columnar cells (C) and Goblet cell (G) lined the Jejunal villi. Note the microvilli (arrow), tight Junction (arrowheads) and desmosomes (open arrows) between the adjacent columnar cells and or goblet cells
Uranyle acetate & lead citrate. 19,440.

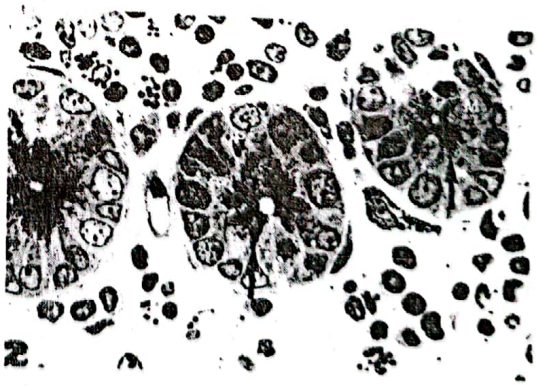

Fig. (2): Cross section of the apical part of the crypt of horse Jejunum showed granular cells (arrows), mucous cell (M) and paneth cell. (p).
Toluidin blue, X 100.

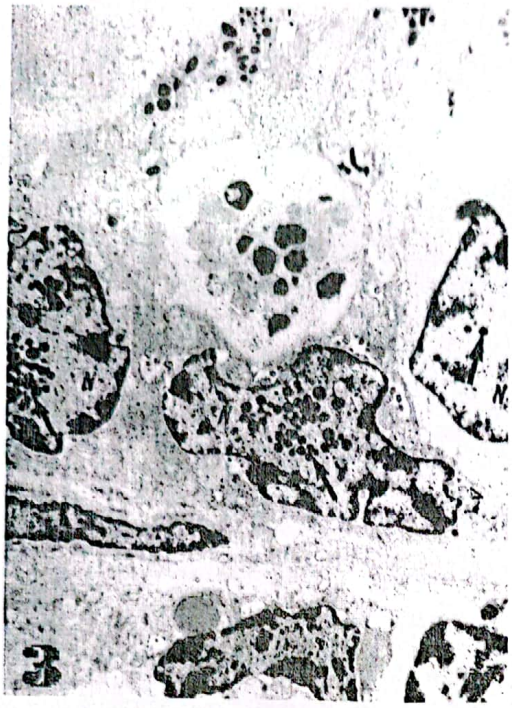

Fig.(3): Electron micrograph of Jejunal glands of horse showed the nucleus of the granular cells (N). Note the intranuclear bodies (arrows).
Uranyle acetate & Lead citrate 12,960

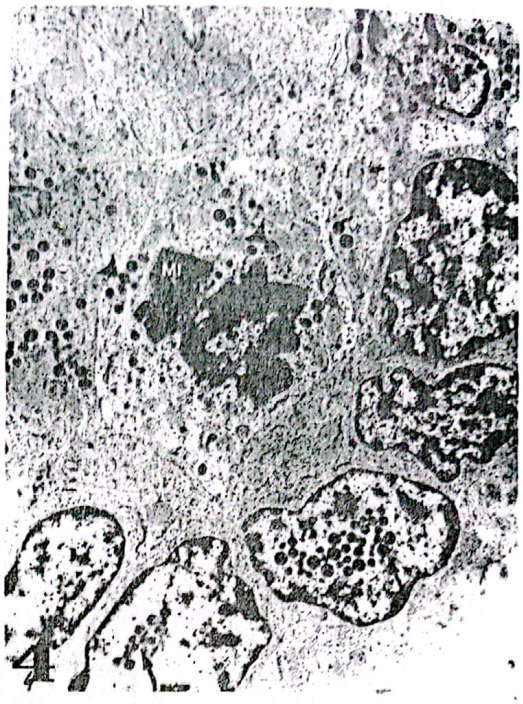

Fig.(4): Electron micrograph of Jejunal glands of horse. Note intranuclear bodies (arrows). Some nuclei showed mitotic figures (MI) and the electron dense granules (arrowheads) distributed all over the cytoplasm and near to the nucleus.
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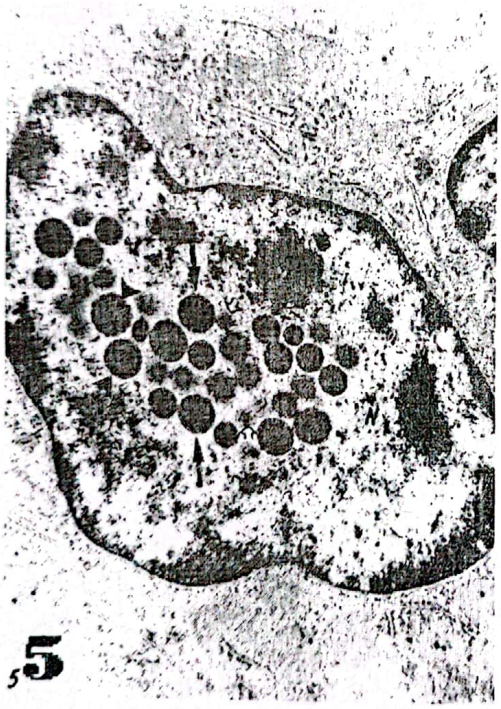

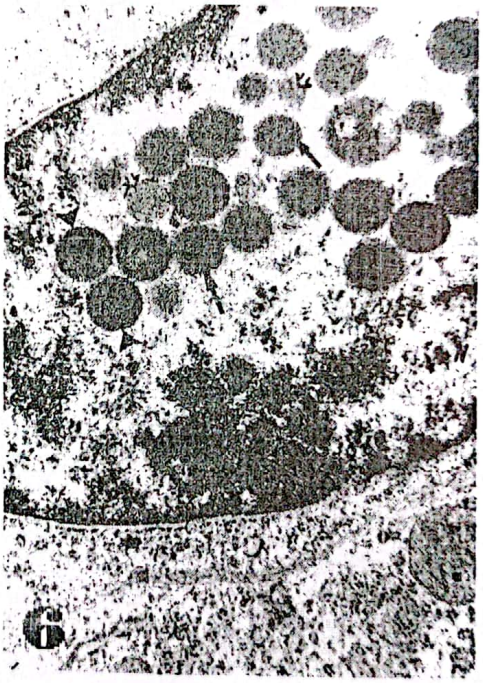

Fig.(5&6): Electron micrograph of the Jejunal glands of horse showed high magnification of a nuclei (N) of granular cells. Most of the intranuclear bodies are surrounded by membrane (arrowheads). Some of these bodies are electron dense (arrows) and others are electron - lucent (arrowheads).
Uranyle acetate & Lead citrate 37,800 & 51,300.

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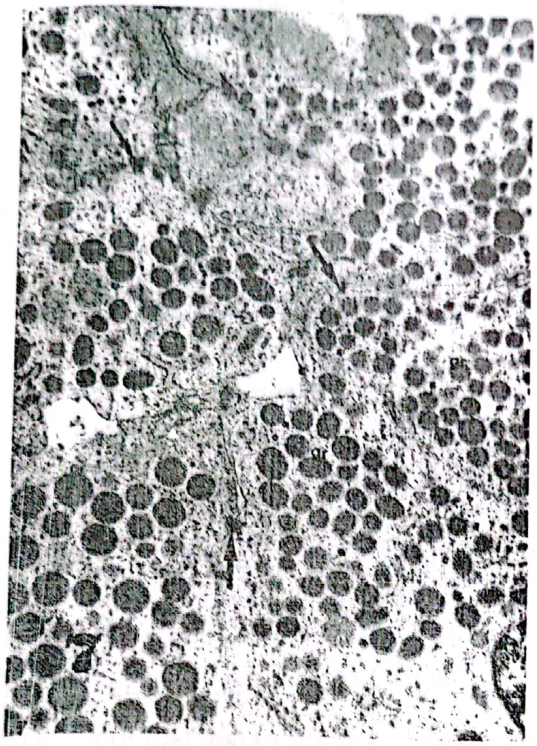

Fig.(7): Electron micrograph of horse Jejunum showed the cytoplasmic granules (gr) that have the same electron density as the intranuclear bodies noticed in Figs. (5&6). Note the desmosomal Junctions between the granular cells (arrows)
Uranyle acetate & Lead citrate. 27,000.

Fig.(9): Electron micrograph of the horse Jejunum showed high magnification of mucous cells (mu). Note that the euchromatic nucleus (N) are Free of intranuclear bodies. Moderate Number of rER (arrowhead) cistern are found.
Uranyle acetate & Lead citrate. 7,830.

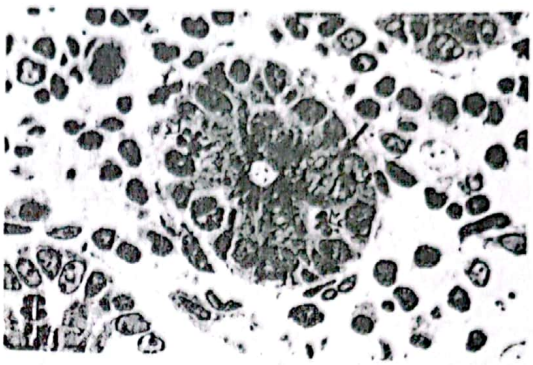

Fig. (8): Cross section at the base of the crypt of the horse Jejunum showed marked increase in the mucous cell (arrows). Note the granular cells (arrowheads) and paneth cells (open arrows)
Toluidine blue, X100.

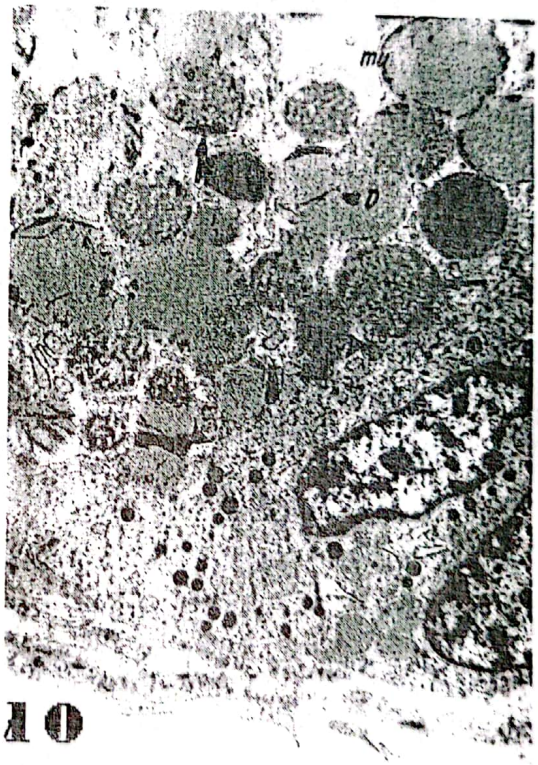

Fig.(10): Electron micrograph of horse Jejunum showed the mucous cell (mu). Note the condensation of intermediate filaments at the periphery of the mucous globule (arrows) or form dot like (D) or cross the diameter of the globule (arrowheads).
Uranyle acetate & Lead citrate. 19,440.

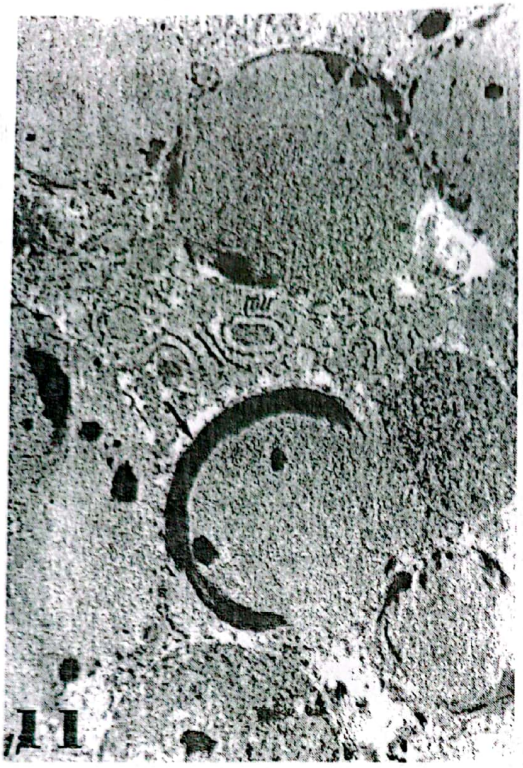

Fig. (11): Electron micrograph of horse Jejunum showed a high magnification of mucous cells (mu). Note the intermediate Filaments form a demilune (arrow) around the mucous globule.
Uranyle acetate & Lead citrate. 37,800.

ing membrane was noticed in the others. The latter was electron lucent (Fig. 6) and had single shadow granules. Some of these nuclei showed mitotic figures and the cytoplasm of these cells appeared electron-lucid and the electron dense granules distributed all over the cytoplasm near to the nucleus (Fig. 4). The cytoplasm of the granular cell showed a low electron dense mitochondria, a well-developed Golgi apparatus, dispersed cisternae of rER, and electron dense granules at the apical region. The cell membrane of these cells showed desmosomal junctions (Fig. 7). Wandering white blood cells were detected inbetween these cells.

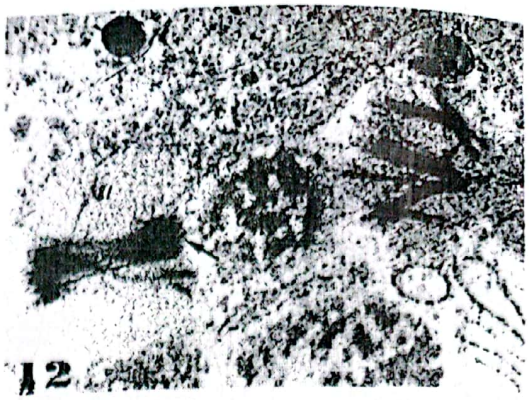

Fig. (12): Electron micrograph of horse Jejunum showed the intermediate filament (arrow) cross the diameter of mucous globule (gl).
Uranyle acetate & Lead citrate. 78,300.

The mucous cells were few at the initial part of the crypt but increased distally forming the entire base of the crypt (Fig. 8). The mucous cells were spherical to oval in shape. They had light euchromatic nuclei devoid of intranuclear bodies. The nuclei of these cells were always pushed to a side in the cell by the mucous granules. The cytoplasm had moderate number of rER cisternae in the form of vesicles (Fig. 9), well-developed Golgi apparatus and mucous globules. The latter showed a peripheral condensation of intermediate filaments of 10-20 µm (Fig. 10). Some of them formed a demilune at the edge of the globule (Figs. 10 & 11), some formed a dot-like at the pole (Fig. 10) and others formed the diameter across the globule (Figs. 10 & 12). The mucous cell also showed desmosomal junctions with the adjacent cells.

The paneth cells were observed in the distal half of the crypt. They had small, spherical to oval membrane bound granules. The granules were variable in density and distributed all over the cytoplasm (Fig. 13). The nuclei were irregular in

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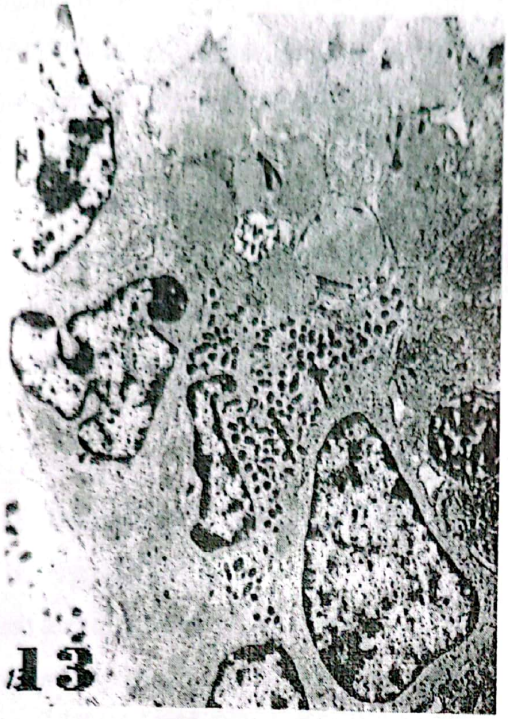

Fig. (13): Electron micrograph of horse Jejunum showed the paneth cell (p) at the distal half of the crypt. Note the cytoplasmic granules (arrows) are varied in density and distributed all over the cytoplasm. Uranyle acetate & Lead citrate. 15,660.

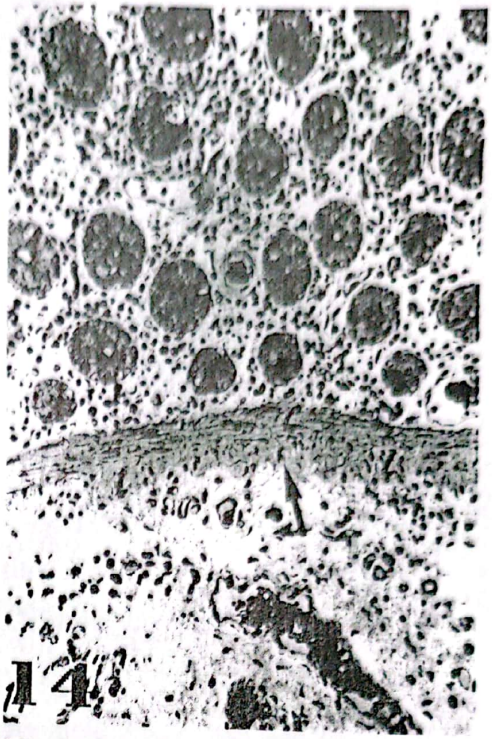

Fig.(14): A photomicrograph of the Jejunum of horse showed that the muscularis mucosa (arrow) formed of 2 layers of smooth muscles, outer longitudinal (L) and inner circular (C). Toluidine blue, X40.

shape with peripheral heterochromatin. The cytoplasm showed mitochondria, rER at the marginal area of the cytoplasm (Fig. 13) and well developed Golgi complex. Desmosomal junctions with adjacent cells were also detected.

The muscularis mucosa of the jejunum was thick, formed mainly of two layers. The outer one was longitudinal and ran perpendicular to the inner circular one (Fig. 14).

DISCUSSION

Previous light microscope studies of the columnar cells of equine small intestine were insufficient to reveal clearly the morphology of the granular cells. In this study, these cells appeared very similar at the ultrastructural level to the columnar epithelial cells lining the equine small intestine described by Doyle (1980) and also to equine colon mentioned by Kanakaudis (1973) and Pfeiffer et al., (1987).

The role of this cell type remains unclear, although the ultrastructure of the granules suggested some secretory function. Using histochemistry, it was possible to demonstrate that the granules contain neutral protein - polysaccharide complexes

with terminal amino groups (Doyle, 1980). As in that of Doyle (1980); mitotic activity was demonstrated in the granular cell at the present study, therefore, we suggested that this cell might act as a stem cell for the other cell types.

The present investigation has focused on the nuclear bodies, which were frequently observed in columnar granular cells of the equine jejunum. Nuclear bodies were firstly demonstrated in tumors by De-The et al., (1960) and Hinglais - Guillaud et al., (1961). Many reports dealing with nuclear bodies have been done in normal and diseased tissues (Bouteille et al., 1967; Buttner and Horstmann, 1967; Simar, 1969; Dupuy-Coin et al., 1972; Doyle, 1980; Pfeiffer et al., 1987 and Kaup and Deege, 1994). Intranuclear bodies were also seen in the granular epithelial cells of equine small intestine by Doyle (1980) and Kaup and Deege (1994). Moreover, the former and Pfeiffer et al., (1987) noticed these bodies in equine intestinal goblet cells.

A system of classification of nuclear bodies has been proposed by Bouteille et al., (1967) covering five types of nuclear bodies in degrees of increasing complexity. Buttner and Horstmann (1967) have presented similar classification, based on four morphologically distinct types. They added that the evolution of these bodies was being from the simple fibrillar to the more complex and membrane bound.

Very little informations have been reported regarding the content of nuclear bodies. RNA was probably a frequent component of the nuclear body core (Han, 1967, Dupuy-Coin et al., 1972), though glycogen has been mentioned (Caramia, Ghergo and Menghini, 1967) and also virus like particles (Zelickson and Lynch, 1961; Granboulan, Tournier, Wicker and Bernhard, 1963 and Sonoda and Marshak, 1970).

Weber and Frommes (1963); Popoff and Stewart (1968) and Kierszenbaum (1969) suggested that degenerated RNA in the nucleolus forms the nuclear bodies and the latter act as some kind of organizer for the functioning of the nucleolus. Regarding the possible origin of these bodies from the nucleolus may be not accepted in our observation; as a non- membranous organelles could not be a forerunner for a membranous one.

The structural similarity of the granular cell nuclear bodies with the apical secretory granules in the same cell type in this study raises the question whether they are identical or they undergo transport into or out of the nucleus. Pfeiffer et al., (1987) showed by x-ray microanalysis of colonic mucosal granular cells that there was a great similarity between the nuclear bodies and the small cytoplasmic granules. Furthermore, Doyle (1980) proposed that the nuclear bodies originated as cytoplasmic granules, which have been trapped in the nucleus during mitosis.

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On the other hand, Ross, Scott and Gardner (1985) suggested that these bodies may be produced in the cytoplasm of the cell and pass into the nucleus where they were encapsulated by the nuclear membrane and accumulated as large clusters. They added that the nuclear bodies might enter a developmental pathway proceeding from this stage.

In the present work, the density similarity between the nuclear bodies and the cytoplasmic granules and the presence of some cytoplasmic granules near to the nuclear envelope during mitosis in the equine jejunum may support Doyle's (1980) idea. Moreover, the central position of the nuclear bodies in this study seemed to be a result of nuclear remodeling after mitosis. In such a way, the delayed degeneration after mitosis and maturation of undifferentiated granular cells to goblet cells might explain the occasional observation of intranuclear bodies in the nuclei of goblet cells (Doyle, 1980 and Pfeiffer et al., 1987).

The fate of these nuclear bodies in horse is difficult to ascertain because of the rapid loss of intestinal epithelial cells and the high rate of proliferation. Furlan and Jericijo (1967) reported the presence of hydrolytic enzymes within the nucleoplasm. They added that the nucleus possesses a hydrolytic capability to degrade the unwanted foreign materials within its boundaries. If such was the case in the present work, the nucleus may respond to the presence of foreign bodies (intranuclear bodies) by producing these enzymes when needed. This could explain the appearance of a membrane around some bodies and others not, as well as, the variety of density of these granules that may indicate a stage of evolution.

No satisfactory explanation for the function of intranuclear bodies has been made, but several attempts have been done based on morphological observations. Sugimura, Ohataishi, Kudo and Mifune (1969) and Kierszenbaum (1969) suggested that they may segregate material foreign to the nucleus from the rest of the karyoplasm. Simar (1969), Dupuy-Coin et al., (1972) and Ross et al., (1985) emphasized that the nuclear bodies are protein in nature and increased during the course of antibody synthesis. As the immunological function of the intestine is well known, there may be an important relationship between the presence of nuclear bodies and protein synthesis in the granular cells of the horse jejunum where antibody synthesis and secretion are occurring (Ross, 1982).

Intermediate cytofilaments in the goblet cells at this study formed a demilune at the edge of the mucous globule, dot-like at the pole and others formed the diameter across the globules. This distribution of the cytofilaments might indicate the role of this cytoskeleton in transportation of these globules to the apical part of the cell and or help in the evacuation of the cell contents.

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