Scientific Papers. Series D. Animal Science. Vol. LXV, No. 1, 2022
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

PROTEIN METABOLISM IN EPITHELIOCYTES OF THE LARGE INTESTINE IN FETUSES OF BLACK-SPOTTED CALVES

**Vladimir ZDOROVININ, Natalia PUGACHEVA,
Julia KULIKOVA, Tatiana DOROFEEVA**

Penza State Agriculture University, 30 Botanicheskaya, Penza, Russia

Corresponding author e-mail: kulikova.y.n@pgau.ru

Abstract

The article shows the dynamics of total proteins and the features of protein metabolism at the fetal stage of calf development. This is important for diagnosing intestinal diseases in newborn calves due to their rather high mortality from such diseases. It was revealed that at the early fetal stage of development, a villous-cryptal gradient of the distribution of total proteins in the cytoplasm of epithelial cells is formed. With the growth of the fetus, at the midfetal stage of development, an increase in the content of total proteins is observed in the epithelial cells of the villi. In a differentiated study, it was found that the decrease in the color of total proteins in 4-5-month-old fetuses is due to the action of acidic proteins. The intensity of the reaction to the main proteins does not decrease, but even increases. In fetuses of 6 months, acidic proteins are again accumulated in epithelial cells, by birth, the ratio of acidic and basic proteins levels off. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring.

Key words: *epitheliocytes, fetal stage of development, large intestine, protein metabolism, total proteins.*

INTRODUCTION

The study of the patterns of development of the digestive system, including the large intestine, is an important prerequisite for the development of a nutrition system, prevention and diagnosis of various diseases (Reid, 1993).

A significant number of studies on the digestive system of animals touch upon general issues of the structure and patterns of growth of the gastrointestinal tract in ontogeny. The morphology of the small and large intestines of cattle has been studied quite well. At the same time, the histochemistry of the large intestine has not been studied deeply enough, in particular, we are talking about protein metabolism in enterocytes (Furness, 2000; Teltsov, 2002).

An analysis of the dynamics of total proteins and the characteristics of protein metabolism at the fetal stage of calf development is important for diagnosing intestinal diseases in newborn calves due to their rather high mortality from such diseases (Zdorovinin et al., 2021).

MATERIALS AND METHODS

The material of the study was the fruits of the black-motley breed of cattle, their large intestine (its components: blind, colon, rectum). Pieces of tissue were taken from different sections of the colon: cranial, middle, and caudal. The material was fixed by a standard method for histological and histochemical studies. For the statistical analysis of all results, such indicators as the arithmetic mean, the error of the arithmetic mean, the correlation coefficient, and the probability of error were used.

RESULTS AND DISCUSSIONS

At the early fetal stage of fetal development (2-5 months), simultaneously with the formation of true intestinal villi, a villous-cryptal gradient in the distribution of total proteins in the cytoplasm of epitheliocytes is formed. The nuclei of epithelial cells at the top of the villi contain granules of total proteins, which are distributed throughout the karyoplasm. In the center of the karyoplasm, small granules are visible, and larger, intensely colored ones are located near the nuclear envelope. The cytoplasm is filled with small granules, which are located closer to the periphery of the cell and therefore the perinuclear region remains free. A dark band is visible in the area of the brush border. In the epithelial cells of the lateral surface of the villus,

the color intensity in the area of the brush border is preserved. In the karyoplasm, an increase in the number of large, intensely stained granules of total proteins is observed. In the cytoplasm of epitheliocytes, the granules are distributed evenly throughout the cell, in contrast to the epitheliocytes of the villus apex. Goblet cells have large granules of total proteins in their contents, which are distributed throughout the cell and form a light blue staining in the form of a mesh. With the growth of the fetus, at the midfetal stage of development (5-7 months), an increase in the content of total proteins is observed in the epithelial cells of the villi. This is evidenced by an increase in the number of large intensely colored granules. The granules are distributed throughout the cell. Closer to the cell periphery, an increase in the intensity of the reaction to proteins is noted. In the region of the brush border, a dark-colored band is visible, which loses its intensity of reaction in epitheliocytes lying in the region of the base of the villus. In a 7-month-old fetus, epithelial cells are characterized by an increased content of total proteins. In the nucleus, large granules are visible, distributed throughout the karyoplasm. A dark band with thickenings is visible along the nuclear membrane.

Goblet cells also contain large amounts of total protein granules. Some of the cells have a light content, which, apparently, is due to the fact that the cells have had time to secrete the contents of their glands into the intestinal cavity and have not yet had time to accumulate a sufficient amount of protein to give an intense staining. In the epithelial cells of the villi, there is a decrease in the color intensity from the base of the villus to its apex (Figure 1). In the area of the brush border, there is also an increase in the intensity of color in the cryptal-villous direction.

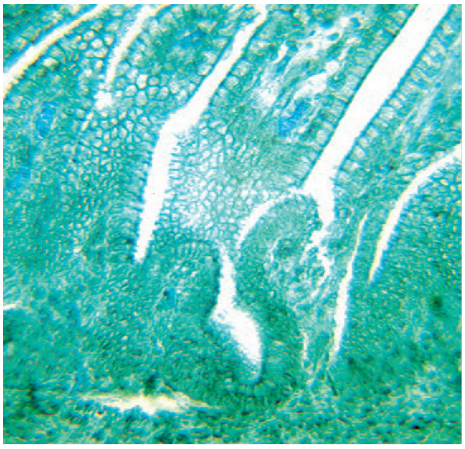

Figure. 1. Section of the wall of the caecum of a 5-month-old fetus.
Positive reaction for proteins according to Bonheg. About 20 × Ok.10

In the colon at the early fetal stage of development (2-5 months), the nuclei of villi enterocytes contain intensely stained clumps of total proteins. In the cytoplasm of epithelial cells, total proteins are presented in the form of granularity. The grains are distributed throughout the cytoplasm. The highest intensity of the content of total proteins is noted in the area of the brush border. A similar picture is observed in the epithelial cells of the lateral surfaces of the villi. In the region of the base of the villi, the nucleus and cytoplasm of epitheliocytes contain a large amount of total proteins. They are presented in the form of large grains, colored in dark blue, in the region of the brush border, the color intensity disappears.

By the age of 5 months, there is an increase in the content of total proteins in the epithelial cells of the villus apex. The most intense staining is noted in the nucleus and cytoplasm of epithelial cells, where total proteins are presented in the form of large granules. A large number of total proteins are observed in the region of the nuclear envelope and brush border. The nuclei of the epitheliocytes of the lateral surface of the villus also contain a large amount of total proteins, but

the intensity of their coloration is weaker than in the nuclei of the epithelial cells of the apex of the villi. The cytoplasm of the epithelial cells of the lateral surface of the villus, as well as the cytoplasm of the epithelial cells of the apex of the villus, contain a large amount of total proteins. In the area of the brush border, the color intensity is preserved.

In the epithelial cells of the base of the villi, the intensity of the reaction to proteins is preserved. In the nucleus and cytoplasm, total proteins are isolated in the form of large colored granules. Crypt epithelial cells are characterized by a high content of total proteins. In the nucleus, there is intense staining of granules of total proteins distributed throughout the karyoplasm.

A large amount of total proteins is observed in the region of the nuclear membrane, where they are presented in the form of a uniform band. In the cytoplasm of epithelial and goblet cells, total proteins in the form of large granules are distributed throughout the cell. In the area of the basement membrane, there is an intense reaction to proteins. At the midfetal stage of development (5-7 months), in the nuclei of the epitheliocytes of the top of the villi of the colon, an intense reaction to granule proteins is noted. This indicates an increased content of total proteins. In the cytoplasm of epitheliocytes, total proteins are presented in the form of small grains distributed over the entire area. A particularly intense reaction to total proteins is observed in the area of the brush border. A similar picture of the content of total proteins is observed in the epithelial cells of the lateral surface and the base of the villus. The nuclei of crypt epithelial cells have granules of total proteins located in the region of the nuclear envelope. In the karyoplasm, granules occur in separate groups. In the cytoplasm, total proteins are detected in the form of fine grains, which occupy the area of the entire cell.

Goblet cells are also characterized by an increased content of total proteins distributed throughout the cell. In the area of the basement membrane, an intense reaction is noted in the form of a dark blue stripe.

At the late fetal stage of development (7-9 months) and before birth, an increased content of total proteins persists. They are detected in the nucleus and cytoplasm of epithelial and goblet cells in the form of large dark blue granules. The granules fill the area of the entire cell, which gives the cells a dark shade. In the region of the nuclear envelope, total proteins are presented as an intensely colored band. In the region of the brush border, a reaction is observed, the intensity of which is maintained from the mouth to the bottom of the crypts.

In the epithelial cells of the crypts, with the growth of the fetus, an increase in the content of total proteins in the nucleus and cytoplasm is noted. A similar picture is observed in the intestinal endocrinocytes of the crypts. The persistence of the intensity of the reaction of the granules is also noted in the neonatal period in the goblet cells of the crypts, which, apparently, is associated with the specific functions of these cells.

In the rectum of 3-month-old fetuses, in the nuclei of epithelial cells of the tops of the villi, total proteins are detected in the form of small grains distributed throughout the karyoplasm. The most intense reaction of total proteins is noted in the region of the nuclear envelope. Throughout the cytoplasm of epitheliocytes, total proteins appear as a delicate light blue mesh. A narrow dark stripe is noted in the region of the brush border.

In the epithelial nuclei of the lateral surfaces of the villi, an intense reaction of protein granules is visible. An increase in the intensity of the reaction of protein granules is also noted in the cytoplasm. To the base of the villi in the karyoplasm and cytoplasm of epithelial cells, an increase in the intensity of color is noted. Dark blue granules are visible.

Fruits 4-5 months. in epithelial cells, in contrast to the epitheliocytes of a 3-month-old fetus, there is a slight decrease in the intensity of the reaction to proteins. Obviously, this is due to a decrease in the content of total proteins in the nucleus and cytoplasm of epitheliocytes. Goblet cells, like epithelial cells, contain granules of total proteins, some goblet cells have a light content. Apparently, by the time the material was taken, the cells had managed to release the secret of their glands into the intestinal cavity. A decrease in the intensity of the reaction is also noted in the area of the brush border, which indicates a decrease in the amount of total proteins.

At the midfetal stage of development (5-7 months), the nuclei of the epithelial cells of the apex of the rectal villi show an increase in the intensity of the reaction to proteins. This is

confirmed by the appearance of intensely stained granules of total proteins in the karyoplasm. They are distributed throughout the karyoplasm, and near the nuclear membrane, the granules seem to merge and form a darkly colored strip. In the cytoplasm of epitheliocytes, granules of total proteins are distributed throughout the cell. A dark colored strip is visible in the area of the brush border.

Epithelial cells of the lateral surfaces of the villi are characterized by an increased content of total proteins. This can be judged by the intense staining of epithelial cells. Large, intensely stained granules are visible in the nucleus and cytoplasm. An increase in color intensity is also observed in the area of the brush border. Goblet cells have a similar intensity of reaction. An increased content of total proteins is also observed in the epithelial cells of the base of the villi.

The epithelial cells of the crypts are dark blue in color. In the nuclei of epitheliocytes and goblet cells, a high protein content is noted, which gives the cells a dark color. A dark stripe is visible near the nuclear membrane, but it is not the same along the entire length, some thickenings are noted in places. In the cytoplasm of epitheliocytes, total proteins are presented in the form of medium-sized granules distributed throughout the cell. The highest staining intensity is observed at the apical pole of the cells. In some goblet cells, the cytoplasm is lighter and granules of total proteins are visible in it, located in separate granules, forming a mesh. Consequently, at the midfetal stage of fetal development, a cryptal-villous distribution gradient of total proteins is formed in the epithelial cells of the colon mucosa.

At the late fetal stage of development (7-9 months) and before birth, destruction of epithelial cells is noted (Figure 2). Large clumps of total proteins of dark blue color are visible in the nuclei. A dark band with separate thickenings is visible near the nuclear envelope. A large number of granules is also observed in the cytoplasm of epitheliocytes. A similar picture is observed in goblet cells. On the lateral surface of the crypts, goblet cells have a darker staining. At the apical pole, a dark, almost black coloration is seen. Obviously, the cells are preparing to release a secret with a high protein content into the intestinal cavity. The epithelial cells of the base of the crypts are characterized by a slight decrease in the color intensity of the granules of total proteins distributed throughout the cell. The crypts contain a large number of dark-colored granules of total proteins. In the nuclei of epitheliocytes and goblet cells, dark blue granules of total proteins are observed, distributed throughout the karyoplasm. In the cytoplasm, the total protein is secreted in the form of small granules distributed throughout the cell and forming a dense network. At the apical pole of the cells, more intense staining is observed.

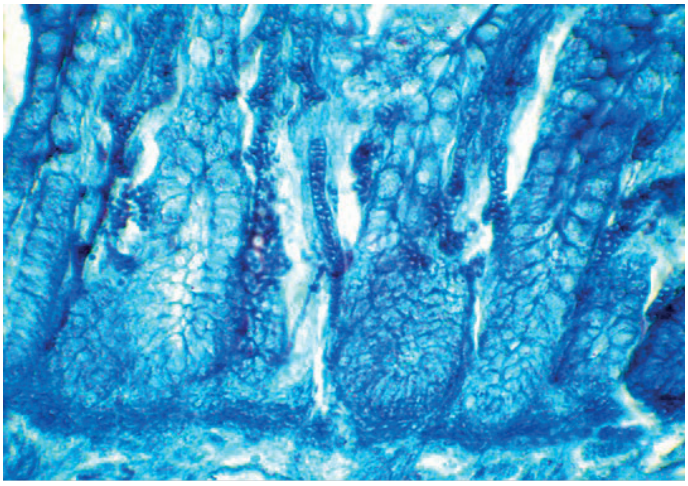

Figure. 2. A section of the wall of the rectum of a 9-month-old fetus. Positive reaction for proteins according to Miquel-Calvo. About. 40 × Approx. 15

In epithelial cells, the crypts of the nucleus contain separate large dark blue granules. The entire karyoplasm is filled with small granules. Closer to the nuclear envelope, they merge and form a dark band. This indicates an increased content of total proteins in this region of the nucleus. Small light-colored granules of total proteins are observed in the cytoplasm. Their number is greater at the periphery of the cell, as evidenced by intense staining in this area. The perinuclear region remains free of granules of total proteins. Goblet cells have dark-colored granules of total proteins that are evenly distributed throughout the cell. A dark band is observed in the region of the basement membrane.

At the late fetal stage of development (7-9 months) and before birth, the epithelial cells of the caecum are characterized by an increased content of total proteins. The nuclei of epitheliocytes contain large granules of dark blue color, they are especially numerous in the region of the nuclear membrane. Darkly stained granules of total proteins are also found in the cytoplasm of epitheliocytes. A dark band is visible in the area of the brush border. A similar picture of the content of total proteins is observed in goblet cells. In the cell secretion, intensely stained granules are visible, distributed throughout the cell. In the epithelial cells of the bottom of the crypts, a slight increase in the intensity of staining is observed. In the karyoplasm, individual granules of total proteins are visible, the color intensity of which is somewhat higher than in the nucleus of the epithelial cells of the mouth of the crypts. In the cytoplasm, small intensely stained granules of total proteins are visible, distributed throughout the cell. In the region of the brush border, their greatest accumulation is observed.

In the nuclei of the epithelial cells of the crypts, separate large granules of a dark blue color are visible, which form an intense staining in the form of a strip near the nuclear membrane. The karyoplasm is occupied, small dark blue granules. In the cytoplasm, granules of total proteins form a dark blue mesh. The most intense staining of the granules is noted at the apical pole of the cell.

In goblet cells, the color intensity of the granules is similar to the color intensity of the granules of epithelial cells. Only along the cell periphery is a darker band visible.

In epithelial cells, basic proteins create a blue, soft background. Acidic proteins are intensely stained in the karyoplasm, the nucleolus of the nucleus, the cytoplasm of crypt enterocytes, and in the brush border of epitheliocytes. The main proteins are presented in the form of a homogeneous network in the nucleus and cytoplasm. As the epitheliocytes move from the bottom of the crypts to its mouth, they accumulate.

In a differentiated study, it was found that the decrease in the color of total proteins in 4-5-month-old fetuses is due to the action of acidic proteins. The intensity of the reaction to the main proteins does not decrease, but even increases. In fetuses of 6 months, the accumulation of acidic proteins in epithelial cells is again taking place, by birth the ratio of acidic and basic proteins is leveling off. However, the villous-cryptal gradient in the distribution of total proteins in epitheliocytes in fetuses disappears after 4.5-5 months. Intestinal endocrinocytes have the same reaction to total proteins as epithelial cells. The cytoplasm of goblet cells contains much less total proteins, while the nuclei of goblet cells practically do not differ from the nuclei of epitheliocytes.

Thus, in the dynamics of the content of total proteins in enterocytes, three successive stages are revealed: up to 3-4 months - accumulation of total proteins; from 3-4 months up to 6 months – decrease in acidic proteins and gradual accumulation of basic proteins; from 6 months before birth - accumulation of total proteins in epitheliocytes.

According to the measurement of the average histochemical coefficient, the intensity of the reaction to SH-groups of proteins in fetuses before birth is 4.1 points, while in newborn calves it is 3.7. Goblet cells of the rectum contain -SH-groups, while in the colon and cecum they are not detected in goblet cells. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring. It is characterized by the restructuring of the nucleoprotein, carbohydrate and protein metabolism of epitheliocytes.

CONCLUSIONS

In the dynamics of protein substances of cellular different of enterocytes of the large intestine at the fetal stage of development, successive stages can be traced. The first - in fetuses up to 3-4 months. there is an increase in the concentration of acidic and total (basic) proteins.

The second - in fetuses from 3-4 to 6 months. the concentration of acidic acids decreases and the content of total proteins increases.

The third - in fetuses from 6 months. before birth, accumulation of total proteins in enterocytes is observed.

The formation of a cryptal-villous gradient in the distribution of total proteins occurs at 2-5 months. development of the fetus, and the caudal-cranial gradient - in fetuses for 5-7 months. The epithelium of the large intestine at the neonatal stage undergoes adaptive histochemical restructuring. It is characterized by the restructuring of the nucleoprotein, carbohydrate and protein metabolism of epitheliocytes.

REFERENCES

Furness, J.B. (2000). Types of neurons in the enteric nervous system. *Journal of the Autonomic Nervous System*, 81(1-3), 87-96. .

Reid, P.E., Owen D.A., et al. (1993) Histochemical studies of intestinal epithelial goblet in glycoproteins during the development of the human foetus. *Histochem. J.,* 2, 81-86.

Teltsov, L.P., Kizim, E.V., & Rodin, V.N. (2002). The development of the nervous tissue of the large intestine wall in cattle in early ontogenesis. *Morphological statements*, 1-2, 47-50.

Teltsov, L.P., Romanova, T.A., & Zdorovinin, V.A. (2004). Histochemistry of the epithelium of the small and large intestine in embryogenesis. *Morphological journals*, 1-2, S. 88.

Zdorovinin, V., Kulikova, J., Mavlydov, I., & Dorofeeva, T. (2021). Development and differentiation of the intermuscular and submucosal nerve plexus of cow embryos large intestine. *Scientific papers Series D. Animal science,* LXIV(1), 100-108.

TECHNOLOGIES OF THE AGRO FOOD PRODUCTS PROCESSING