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Development of the glycogen body in turkey (*Meleagris gallopavo*) embryo

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ABSTRACT

Development of the glycogen body was investigated in turkey (*Meleagris gallopavo*) embryo. The glycogen body is formed by cells rich in glycogen. To date, neither the origin of these cells nor the function of the glycogen body have been entirely clarified. The glycogen body in turkey embryo develops from the paired primordia which appear in the dorsal part of the spinal cord on both sides of the ependymal septum on the 11th day of embryonic development. On the 12th day of development the number of cells increases and fusion of the paired primordia begin dorsally and is completed on the 14th day. On the 13th day of development the paired primordia of the ventral part of the glycogen body appear ventrolaterally from the central canal. In the further course of development they gradually enclose the central canal. The total development of the glycogen body in turkey is completed on the 25th day of development. The dorsal and ventral parts are joined and the central canal of the spinal cord lies within the glycogen body.

Key words: glycogen body, spinal cord, development, turkey embryo

Introduction

The glycogen body is present only in birds and lies within the spinal cord, in the area of its lumbosacral sinus. Situated deeply between the dorsal horns it divides the spinal cord into left and right halves, mutually interconnected by the ventral commissura (WATTERSON, 1949). The dorsal surface of the glycogen body is covered by the spinal pia mater from which the pial septum enters the interior of the organ, separating its larger, dorsal part from the smaller, ventral part. The central canal passes through the ventral part of the glycogen body. The glycogen body position is partly intrapial and partly subpial

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(DICKSON and MILLEN, 1957), and is formed by a mass of polygonal cells with narrow cytoplasmatic rim and the nuclei moved towards the cellular periphery (HODGES, 1974). The central part of these cells is filled with glycogen (WATTERSON, 1949; DeGENNARO, 1961, 1974), which is presumed to be functionally active in the processes of lipid synthesis and in myelin formation in the avian nervous system (BENZO and DeGENNARO, 1974, 1981; BENZO et al., 1975).

Glycogen body cells are of glial origin (DeGENNARO, 1993), possibly astrocytes, having undergone extreme differentiation (LYSER, 1973; SANSONE, 1980; LEE et al., 2001).

The glycogen body develops from the paired primordia, which in the chicken embryo develop after 7-8 days of incubation (WATTERSON, 1952; MATULIONIS, 1972; UEHARA and UESHIMA, 1982). These primordia appear on the roof plate of the spinal cord on both sides of the ependymal septum. Because of cellular proliferation in the further course of development, the primordia approach each other and gradually fuse into a unique organ, which, in the final stage of development, encloses the central canal. A similar development of the glycogen body has been described in the Japanese quail (DeGENNARO and BENZO, 1987).

All that has been learned so far about the avian glycogen body, its position, structure, origin and development, is largely based on research performed in the domestic chicken. To obtain a full insight into this strange structure, investigations should extend to other avian species. Therefore, the present article describes the structure and development of the glycogen body in turkey (*Meleagris gallopavo*), the bird in which this organ has not been studied so far and which is rarely the subject of embryonic investigations.

Materials and methods

Fertilised eggs of the "Nicholas" turkey hybrid were obtained from the "Puris" hatchery, Pazin, Croatia. They were incubated in the laboratory incubator at 37.5 °C, at a relative humidity of 60%. The eggs were daily rotated and from the 7th day of incubation onwards embryos were killed at precisely 24-hour intervals. In the investigation of the glycogen body, three normally developed turkey embryos per day of embryonic development were used. The eggs were removed from the incubator and, according to recommendation for euthanasia of experimental animals (CLOSE et al., 1996), cooled and decapitated. The lumbosacral part of the vertebral column and spinal cord were isolated and fixed in buffered 10% formaldehyde, dehydrated and embedded in formalin. From the 8th incubation day, the vertebral column was carefully dissected from the spinal cord. The isolated spinal cord with the glycogen body was also fixed in buffered 10% formalin, dehydrated and embedded in paraffin. The tissue was serially cut into 10 μm sections. The sections were stained with hematoxylin-eosin (HE) stain for routine histological examination, treated

using the periodic acid-Schiff reagent (the PAS method) for polysaccharide detection (PEARSE, 1968) and incubated in diastase prior to treatment, with PAS as a control. Serial sections were stained in a precise order: firstly, using the PAS method, then PAS-diastase control, and finally, HE staining. A Nikon Microphot-FXA light microscope was used for microscopic analysis.

Results

First indications of the glycogen body in turkey appeared in the sections of the lumbosacral part of the spinal cord on the 10th day of incubation. Using the PAS method for polysaccharide detection, only a small number of glycogen body cells could be identified on the very edge of the dorsal spinal cord, on both sides of the ependymal septum. Within these cells, individual PAS-positive granules were found, whereas PAS-diastase control was negative. In HE-stained sections, glycogen body cells could not be distinguished from the surrounding cells that formed the spinal cord tissue in that developmental stage.

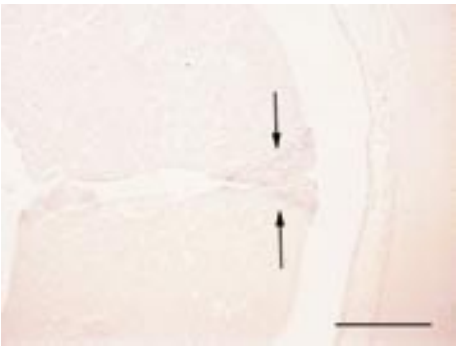

Fig. 1. Turkey embryo, 11th day of incubation. Paired primordia of the glycogen body are seen bilateral to each side of ependimal septum (arrows). PAS, 10 × 2.5, scale bar = 200 μm.

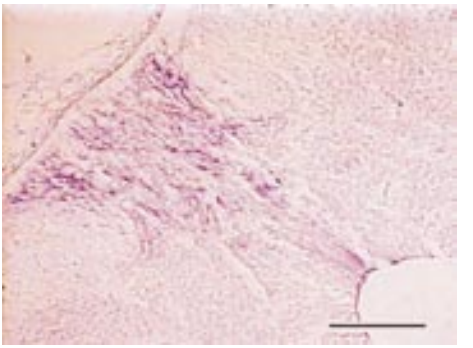

Fig. 2. Turkey embryo, 12th day of incubation. Increase in the number of the PAS-positive cells in the glycogen body. Fusion of the paired primordia spread in a dorsoventral direction. PAS, 20 × 2.5, scale bar = 100 μm.

Glycogen body cell count increased after 11 days of incubation and the paired primordia were clearly formed (Fig. 1). In sections stained using the PAS-method, the primordia were seen to be conical in shape, with the base at the dorsal edge of the spinal cord and the narrowed spike directed towards the central canal. PAS-diastase control was negative, which means that these cells are capable of accumulating glycogen. In HE-stained sections of the lumbosacral spinal cord, glycogen body cells still did not differ from the surrounding cells, although elongated ventricular cells of the spinal cord's roof plate, forming the ependymal septum, were clearly seen.

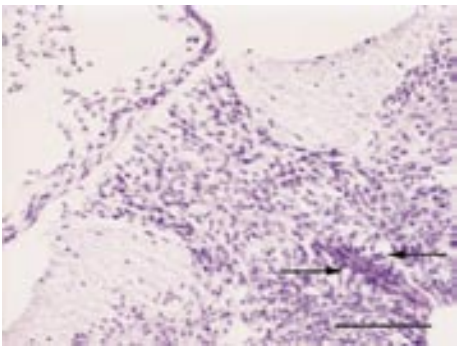

Fig. 3. Turkey embryo, 12th day of incubation. Some glycogen body cells are polygonal with nuclei pushed towards the cellular edge. Ependimal septum is reduced (arrows). H&E, 20 × 2.5, scale bar = 100 μm.

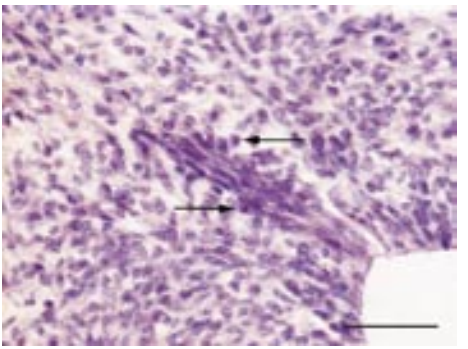

Fig. 4. Turkey embryo, 12th day of incubation. Elongated ventricular cells which form the ependimal septum. H&E, 4 × 2.5, scale bar = 50 μm.

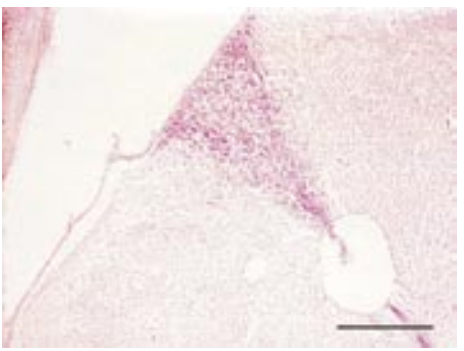

Fig. 5. Turkey embryo, 13th day of incubation. The cuneiform-shaped glycogen body is present. Intensive PAS-positive reaction demonstrates the high level of glycogen accumulation in the cells. PAS, 10 × 2.5, scale bar = 200 μm.

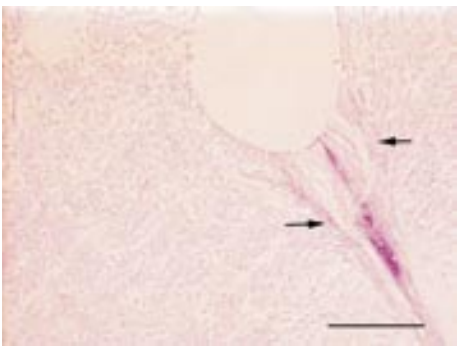

Fig. 6. Turkey embryo, 13th day of incubation. Paired primordia of ventral part of glycogen body extended ventrolaterally from the central canal of the spinal cord (arrows). PAS, 20 × 2.5, scale bar = 100 μm.

On the 12th day of incubation the number of PAS-positive cells in the glycogen body increased (Fig. 2). Diastase control was negative. On that day, dorsal fusion of the paired primordia commenced (Fig. 3) and the number of cells forming the ependymal septum decreased. On the same day the ependymal septum was reduced to a short beam of elongated cells located dorsally from the central canal (Fig. 4). These cells were clearly visible in

HE-stained sections. Some glycogen body cells became polygonal, with their round nuclei pushed towards the cellular perimeter. Mitoses could be observed in the nuclei.

On the 13th day of incubation, as visible in tissue sections stained using the PAS method, the glycogen body assumed cuneiform shape (Fig. 5). The intensity of PAS reaction was higher, which indicates that the quantity of accumulated glycogen had increased. The process of the dorsoventral fusion of the paired primordia was not yet completed: several elongated cells forming the ependymal septum could still be seen close to the central canal. After the completion of 13 days of incubation two lines of PAS-positive cells aligned ventrolaterally from the central canal were observed (Fig. 6). These were the paired primordia of the ventral part of the glycogen body. In the HE-stained sections increasing numbers of polygonal cells were observed, with round or elongated nuclei moved toward the cellular edge. The ingrowth of blood vessels into the spinal cord, and their ramification within the glycogen body, was apparent (Fig. 7).

On the 14th day of incubation, in histological PAS-stained sections for glycogen detection, the glycogen body was visible to the naked eye as a small triangular structure embedded in the dorsal part of the lumbosacral spinal cord. It was markedly larger, with cells having accumulated more PAS-positive substances. Complete fusion of the glycogen body paired primordia occurred at the same developmental stage. Elongated cells of the ependymal septum were no longer observable in HE-stained sections.

On the 15th day of incubation the glycogen body was markedly larger and protruding somewhat above the dorsal surface of the spinal cord (Fig. 8). PAS reaction was severe and diastase control negative. In HE-stained sections polygonal cells with narrow cytoplasmatic rims and the nuclei pushed towards the cellular edge were seen. The glycogen body tissue was pervaded with numerous capillaries filled with blood components.

Over the following days of development the glycogen body grew and further divided the spinal cord into left and right halves. From the 18th day of embryonic development onwards, the cells appeared ever larger and were rich in PAS-positive granules. In HE-stained sections the glycogen body tissue assumed a reticular appearance. The only essential developmental characteristic of the glycogen body in the final days of development was completion of development of its ventral part, which completely enclosed the central canal and fused with the dorsal part. On the 25th day of incubation the central canal of the spinal cord extended through the substance of the glycogen body (Fig. 9).

On the final (28th) day of development the glycogen body lay within the spinal cord in the area of its lumbosacral sinus. It was oval and slightly protruded above the dorsal surface of the spinal cord, completely transparent and of gelatinous consistency. Even to the naked eye the transverse section yielded a presentation of a large wedge inserted in the widened dorsal fissure, surrounding the central canal.

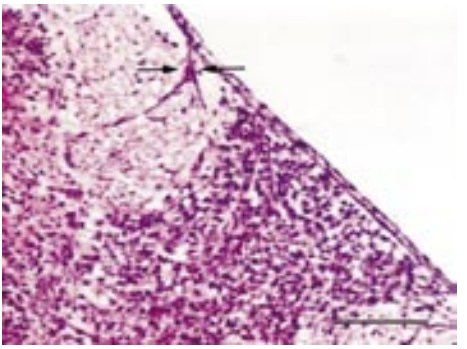

Fig. 7. Turkey embryo, 13th day of incubation. Penetration of blood vessels (arrows) in the spinal cord and rich vascularisation of the glycogen body. H&E, 20 × 2.5, scale bar = 100 μm.

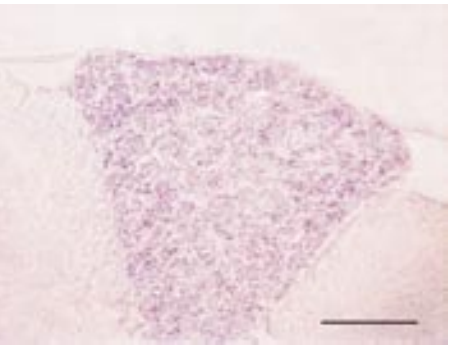

Fig. 8. Turkey embryo, 15th day of incubation. The glycogen body protrudes from the dorsal surface of the spinal cord. PAS, 10 × 2.5, scale bar = 200 μm.

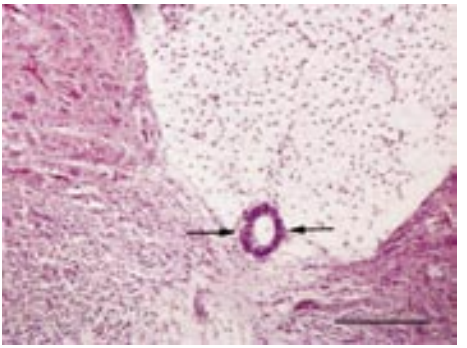

Fig. 9. Turkey embryo, 25th day of incubation. Development of the glycogen body is complete and the central canal lies inside the glycogen body (arrows). H&E, 10 × 2.5, scale bar = 200 μm.

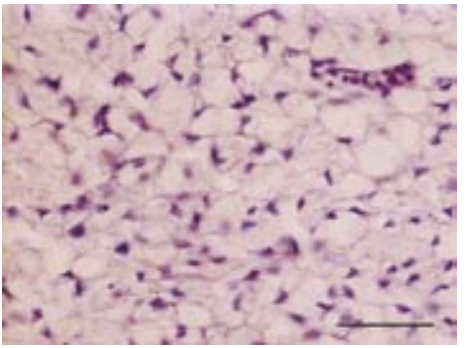

Fig. 10. Turkey embryo, 28th day of incubation. HE, Large polygonal cells of the glycogen body have a thin layer of the cytoplasm and nuclei which are located at the edge of the cell. Nuclei are round or oval in shape with irregular borders. H&E, 40 × 2.5, scale bar = 50 μm.

Again on the final day of development the tissue of the glycogen body contained a mass of large, mainly polygonal cells, densely pressed together. The cells had narrow cytoplasmatic rims and their nuclei were pushed towards the cellular perimeter (Fig. 10). The nuclei were round or oval and with irregular edges. Glycogen particles accumulated in the cell centre. The connective tissue was not found, except in very small quantities close to the blood vessels. The glycogen body was well vascularised, which was substantiated by numerous longish diagonal and transverse capillary sections.

Discussion

In comparison to other Vertebrata the avian spinal cord is atypical because it contains an unusual cellular mass known as the glycogen body.

The microscopic structure of the glycogen body has been described in chicken (WATTERSON, 1949; DeGENNARO, 1959; HODGES, 1974; SANSONE, 1980), Japanese quail (DeGENNARO and BENZO, 1987), pigeon (SCHROEDER, 1987) turkey (VUKOVIĆ, 1993) and different species of wild birds (PETERNEL, 1994). In all previously described birds the glycogen body is formed by large, round-to-oval cells which seem polygonal because of their proximity. Each cell contains a peripheral cytoplasmatic frame encircling a centrally positioned glycogen mass and peripherally placed nuclei. The nuclei are mainly elongated, with irregular edges and, in the pigeon, lobular (SCHROEDER, 1987). The connective tissue is very sparse. On the final (28^{th}) day of development the structure of the turkey glycogen body resembles that of the chicken, consisting of large polygonal cells with narrow cytoplasmatic edges and, generally, oval nuclei pushed towards the cellular perimeter. The cells are filled with glycogen granules. The small quantity of connective tissue is mainly associated with ramified blood vessels.

Using light microscopy WATTERSON (1952) investigated development of the glycogen body in chicken up to the 10^{th} day of incubation. Development of this structure along the whole length of the spinal cord, with special mention of a typical glycogen body, was analysed by UEHARA and UESHIMA (1982). MATULIONIS (1972) investigated the morphology of the glycogen body ultrastructure in chicken, with the aim of identifying subcellular structures involved in glycogen synthesis. Development of the glycogen body was also described in Japanese quail (*Coturnix japonica*) (DeGENNARO and BENZO, 1987).

In the present paper, development of the glycogen body was first published in turkey (*Meleagris gallopavo*).

The glycogen body in chicken develops from the paired primordia, which appear on the roof plate of the lumbosacral spinal cord on both sides of the ependymal septum. There is slight disagreement among the cited authors as to the reported times of appearance of the paired primordia. Thus, the shortest duration of incubation, 7-7.5 days, was reported by UEHARA and UESHIMA (1982) which would correspond to HAMBURGER and HAMILTON (1951) stage 31 of chicken embryo development. MATULIONIS (1972) described a small number of PAS-positive cells in the bilateral primordia of the glycogen body, observed in a 7.5-7.75-day incubation period. The earliest time when the glycogen body primordia can be discerned is, according to WATTERSON (1952), 7.5-8 days of incubation. The number of glycogen body cells grows and gradually, after 8-9 days of incubation, the paired primordia fuse (WATTERSON, 1952; MATULIONIS, 1972). This corresponds to stage 35 of chicken embryo development (UEHARA and UESHIMA, 1982). After ten days of incubation, or at

stage 37 of chicken development, the paired primordia close completely. At stage 42 or later (16 days or more), as described by UEHARA and UESHIMA (1982), fusion of the dorsal and ventral parts of the glycogen body occurs.

Although Japanese quail embryo development lasts 16 days, and chicken incubation lasts 21 days, the paired primordia of the glycogen body appear in both kinds of birds after 7-8 days of incubation. On the 9th day the paired primordia of the glycogen body of the quail begin to fuse and, up to the 16th day, the glycogen body assumes its final form and size (DeGENNARO and BENZO, 1987).

Results of the present investigation show that development of the glycogen body in turkey is very similar to that in chicken and Japanese quail. The paired primordia of the glycogen body in turkey are visible on the 11th day of incubation. In this investigation, the cells of the glycogen body primordia could not be differentiated from adjacent cells in HE-stained sections. In contrast, UEHARA and UESHIMA (1982) described spindly sharp and radially arranged cells of the paired primordia of the glycogen body. Elongated cells forming the ependymal septum were clearly identified in the present investigation. On the 12th day of embryonic development the number of cells increases and the fusion of the paired primordia begins dorsally. Some of the glycogen body cells can be identified in HE-stained sections due to their polygonal shape and the nuclei positioned towards the cellular border. On the 13th day the paired primordia of the ventral part of the glycogen body appear ventrolaterally from the central canal. On the same day blood vessels grow into the spinal cord and ramify in the glycogen body into a rich net. DeGENNARO and BENZO (1987) explained the appearance of the glycogen body paired primordia in chicken and Japanese quail between the 7th and the 8th day of incubation by the simultaneous penetration of blood vessels into the roof plate of the spinal cord in both kinds of bird. The blood vessels ingrowth into the turkey spinal cord on the 13th day of incubation, when the process of glycogen body development has advanced, cannot be associated with the above statement. On the 14th day of development the paired primordia are completely fused. From the 18th day onwards glycogen body cells in turkey are larger. According to MATULIONIS (1972), up to the 15th day of chicken embryo development the glycogen body grows by hyperplasia. After the 16th day, cellular volume increases due to the accumulation of large quantities of glycogen. Development of the glycogen body in turkey is completed on the 25th day, when its ventral part entirely encircles the central canal and fuses with the dorsal part into a single organ.

The course of development of the glycogen body in turkey corresponds to the described development of this organ in chicken and Japanese quail. In all the described avian species, paired primordia appear on the dorsal side of the spinal cord. They grow and fuse. When they fuse with the ventral part the glycogen body encircles the central canal. This completes the development process. However, as could be expected, the time periods necessary for

individual developmental stages to take place differ, depending on the duration of the whole embryonic development of the investigated avian species

References

BENZO, C. A., L. D. DEGENNARO (1974): Glycogen syntethase and phosphorilase in developing chick glycogen body. J. Exp. Zool. 88, 375-380.

BENZO, C. A., L. D. DeGENNARO (1981): Glycogen metabolism in the accesory lobes of Lachi in the nerve cord of the chick. Metabolic correlations with the avian glycogen body. J. Exp. Zool. 215, 47-52.

BENZO, C. A., L. D. DeGENNARO, S. B. STEARNS (1975): Glycogen metabolism in the developing chick glycogen body. Functional significance of the direct oxidative pathway. J. Exp. Zool. 193, 161-166.

CLOSE, B., K. BANISTER, V. BAUMANS, E.-M. BERNOTH, N. BROMAGE, J. BUNYAN, W. ERHARDT, P. FLECKNELL, N. GREGORY, H. HACKBARTH, D. MORTON, C. WARWICK (1996). Recommendations for euthanasia of experimental animals: Part 1. Laboratory Animals 30, 293-316.

DeGENNARO, L. D. (1959): Differentiation of glycogen body of the chick embryo under normal and experimental conditions. Growth 23, 235-249.

DeGENNARO, L. D. (1961): The carbohydrate composition of the glycogen body of the chick embryo as revealed by paper chromatography. Biol. Bull. 20, 348-352.

DeGENNARO, L. D. (1974): Differentiation of the glycogen body of the chick embryo. Studies on glucose ^{14}C incorporation, chorioallantoic grafting, histochemistry and electrophoresis. Growth 38, 1-15.

DeGENNARO, L. D. (1993): Origin of the avian glycogen body. 2. Observations in support of a glial nature in the chick embryo. Growth, Dev. & Aging 57, 275-281.

DeGENNARO, L. D., C. A. BENZO (1987): Development of the glycogen body of the Japanese Quail, Coturnix japonica. I. Light microscopy of early development. J. Morph. 194, 209-217.

DICKSON, A. D., J. W. MILLEN (1957): The meningeal relationships of the glycogen body in the chick. J. Anat. 91, 47-51.

HAMBURGER, V., H. L. HAMILTON (1951): A series of normal stage in the development of the chick embryo. J. Morphol., 88, 49-92.

HODGES, R. D. (1974): The Histology of the Fowl. Academic Press, London, New York, San Francisco. 609-611.

LEE, K., S. MAKINO, T. IMAGAWA, M. KIM, M. UEHARA (2001): Effects of adrenergic antagonists on glycogenolysis in primary cultures of glycogen body cells and telencephalon astrocytes of the chick. Poult. Sci. 80, 1736-1742.

LYSER, K. M. (1973): The fine structure of the glycogen body of the chicken. Acta Anat. 85, 533-549.

MATULIONIS, D. H. (1972): Analysis of the developing avian glycogen body. I. Ultrastructural morphology. J. Morph. 137, 463-482.

PEARSE, A. G. E. (1968): Histochemistry, theoretical and applied. J & A Churchill, London.

PETERNEL, R. (1994): Poredbena istraživanja želatinoznog (glikogenskog) tjelešca u različitih vrsta ptica. Disertacija. Veterinarski fakultet. Zagreb.

SANSONE, F. M. (1980): Ultrastructural study of cranio-caudal continuation of the glycogen body. J. Morph. 163, 45-58.

SCHROEDER, D. M. (1987): The glycogen body in the spinal cord of the pigeon. Anat. Rec. 218, 121 A. American Association of Anatomists, 100th Meeting, George Washington University, School of Medicine, Washington D. C. May, pp. 10-14, 1987.

UEHARA, M., T. UESHIMA (1982): Development of the glycogen body through the whole length of the spinal cord. Anat. Rec. 202, 511-519.

VUKOVIĆ, S. (1993): Razvoj glikogenskog tijela purana. Disertacija. Veterinarski fakultet. Zagreb.

WATTERSON, R. L. (1949): Development of the glycogen body of the chick spinal cord. I. Normal morphogenesis, vasculogenesis and anatomical relationships. J. Morphol. 85, 337-389.

WATTERSON, R. L. (1952): Development of the glycogen body of the chick spinal cord. III. The paired primordia as revealed by glycogen specific stains. Anat. Rec. 113, 29-52.

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SAŽETAK

Istražen je razvoj glikogenskog tijela u zametcima purana (*Meleagris gallopavo*). Glikogensko tijelo građeno je od stanica ispunjenih glikogenom, a podrijetlo stanica i njegova uloga nije do danas potpuno jasna. U purana se glikogensko tijelo razvija iz parnih primordija koji se pojavljuju na dorzalnom dijelu kralježnične moždine sa svake strane ependimalnog septuma jedanaestog dana razvoja. Dvanaestog dana razvoja broj stanica raste i dorzalno započinje spajanje parnih primordija koje završava četrnaestog dana. U trinaestom danu razvoja ventrolateralno od centralnog kanala javljaju se parni primordiji ventralnog dijela glikogenskog tijela koje, postupno, u daljnjem tijeku razvoja, okružuje centralni kanal. Cjelokupni razvoj glikogenskog tijela u zametku purana završava dvadeset i petog dana razvoja kada su dorzalni i ventralni dio spojeni, a centralni kanal kralježnične moždine leži unutar glikogenskog tijela.

Ključne riječi: glikogensko tijelo, kralježnična moždina, razvoj, zametak purana