

Structural Insights of the Glycogen Body in Domestic Chicken

Ebraheim L*

Department of Histology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt

*Corresponding author: Ebraheim L, Department of Histology, Faculty of Veterinary Medicine, Zagazig University, zagazig, Egypt, Tel: +201061896031; Fax: +20552283683; E-mail: lamiaavet@yahoo.com

Received date: Nov 22, 2015; Accepted date: Jan 20, 2016; Published date: Jan 22, 2016

Copyright: © 2016 Ebraheim L. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

There is a special unusual cellular mass in birds' spinal cords known as glycogen body (*corpus gelatinosum*). Because of the specific topographical situation of this circumventricular organ, the structure of this organ on the cellular and subcellular level is of special interest with respect to the still unsolved functional problems aiming to find a relation between the structure and the function of this organ. Twenty domestic chickens were used in this study to demonstrate the structural peculiarities of this body from the histological point of view. Our results revealed that the glycogen body is a very delicate, small, ovoid circumventricular, transparent and gelatinous consistency structure embedded in the dorsal part of the lumbosacral region of spinal cord. The body has two cell zones; Peripheral one had regularly arranged large cells with metachromatic faint red cytoplasm and peripheral basophilic nuclei and central one with irregularly arranged cells that had different basophilic degrees, variable sizes, and peripheral nuclei. By transmission electron microscope, the cells of both zones showed deeply infolded electron dense peripherally located nuclei with prominent nucleoli that were rounded in the outer zone and ovoid in the central one. The cytoplasm was almost occupied with large dense masses of glycogen. No connective tissue was observed in the body except in the vicinity of the blood vessels that were more distributed in the center of the body than in its periphery.

Conclusion: It could be concluded that the histophysiological relationship of the glycogen body still a mystery hoping that the incoming research can solve it.

Abbreviations:

TEM: Transmission Electron Microscope

L: Lumber Region of Spinal Cord

CNS: Central Nervous System

H&E: Haematoxylin and Eosin Stain

Keywords: Glycogen body; Domestic chicken; Fine structure

Introduction

The domestic chicken vertebral column has a unique structure known as glycogen body occupying the dorsal rhomboid sinus and extends from the level of 26 to 29 spinal nerves [1,2]. This body is partly intrapial and partly subpial and its dorsal surface is covered by the spinal pia mater, from which a pial septum enters the interior of the organ, separating it into a larger dorsal part and a small ventral part. The central canal of the spinal cord passes through the ventral part of the glycogen body [3]. Histologically, the body classified into central and peripheral parts. The cells in the peripheral portion showing a more regular arrangement than the cells in the central portion [2]. The nature of these cells is distinctive as it composed from highly specialized and morphologically modified astroglial cells [3-5]. In general, these cells are polygonal with narrow cytoplasmic rim and peripheral nuclei [6]. The cytoplasm is fully laden with glycogen which has strong positive reaction to both Best's carmine stain and PAS reaction [3,4]. At the subcellular level, these cells have few short processes, dense irregular-shaped nuclei and fully loaded cytoplasm with glycogen. The cytoplasmic rim is rich in ribosomes, rough

endoplasmic reticulum, and free heterogeneous lysosomes [7,8]. The blood supplies of the glycogen body ramify from a sinusoidal network which is better developed in the central portion than in the peripheral one [6]. The physiology of glycogen body is a controversial and very complicated issue and there is no clear-cut function till now, but many literature had suggested that the function of the glycogen body is mainly related to transmission of hydrostatic pressure changes during movements of the bird on the ground [9,10], distributing substances originating in the glycogen body to the CNS [11], playing a role in the metabolism of the neurons of the spinal cord [2,11-13], and sharing in the processes of myelin formation in the central nervous system [14].

Material and Methods

Twenty white Leghorn chicken of 8-month-old of 2000-2200 gm were used for glycogen body in vitro assay. The birds were obtained from the faculty of agriculture, Zagazig University, Zagazig, Egypt. They were housed separately, one in each cage and fed synthetic ration and water ad libitum. They were kept in the laboratory for 2 days before euthanization. They were euthanized by injection of sodium Phenobarbital (100 mg/kg body weight) in the wing vein [15]. Spinal cord lumbosacral region segments were collected, some of them were fixed in Bouin's solution and others were fixed in 10% buffered neutral formalin for 48 hours. All Tissue samples were dehydrated in ascending grades of alcohols, cleared in xylene and embedded in paraffin then some segment sectioned in 5 microns and stained with H&E and PAS. Others samples were sectioned at 10 microns serial sections and stained with H&E to demonstrate the extension of the glycogen body and the cross-sectional area of it using a calibrating lens [16]. For E/M study, the samples were, fixed in 3% glutaraldehyde and

processed for resin preparation; Glycogen body tissues washed in phosphate buffer, post-fixation in 2% osmium tetroxide, Then washed again in the same buffer, dehydrated in ascending grades of alcohol followed by propylene oxide. Finally, infiltrated in a mixture of 50% propylene oxide and 50% resin then embedded in 100% resin. Semithin sections were obtained using Leica ultracut ultratome and stained with toluidine blue. Ultrathin sections were obtained from different areas after using semithin sections as a guide, then mounted and stained with uranyl acetate and lead citrate and studied in a Jeol 1010 TEM [17,18].

Results

Gross appearance of the glycogen body

Dissection of the vertebral column revealed; delicate, ovoid circumventricular, transparent and gelatinous glycogen body which embedded in the dorsal part of the lumbosacral region of spinal cord at the level of L2, L3, L4, S1 and S2 segments (Figure A1). It is separate structure and upon removal it leave widened dorsal fissure without any injury to the spinal cord tissue. Its cross-sectional area was maximal at L4. Rostral to L2 and caudal to S2 the body is much smaller, leaving a large space dorsally.

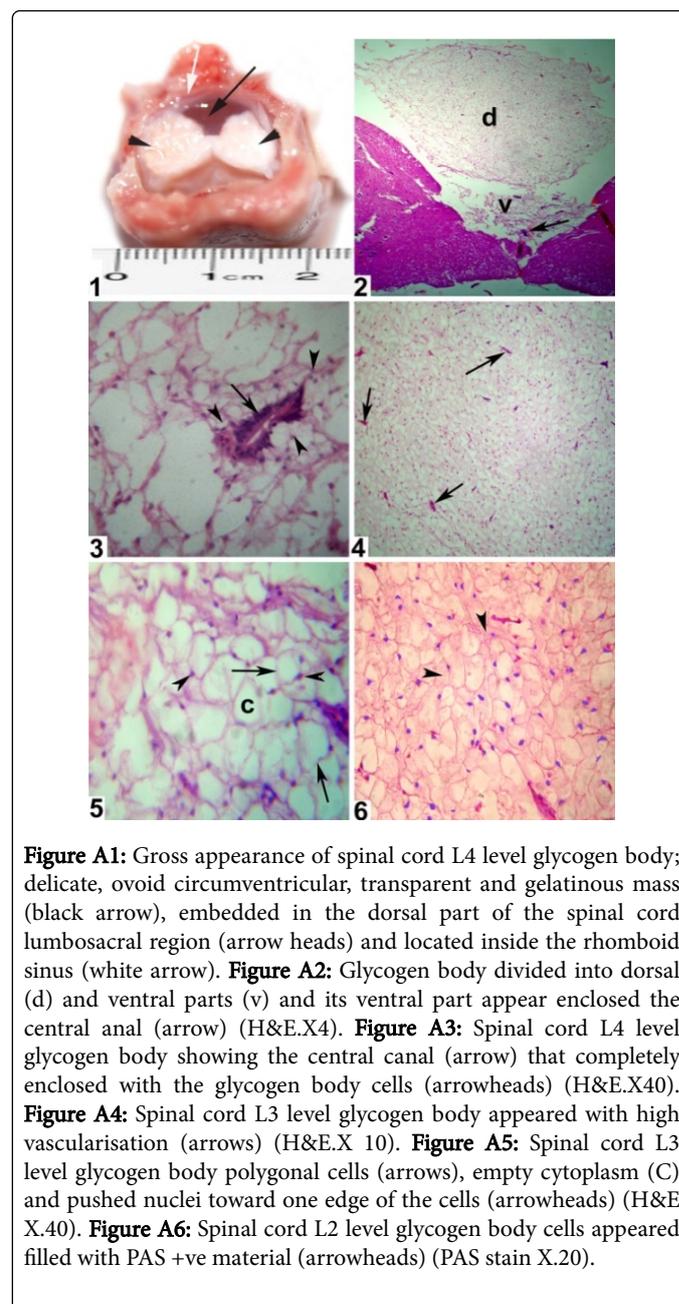
Light microscopic picture of the glycogen body

Histological examination of serial paraffin sections stained with H&E declared that glycogen body located dorsal to the spinal cord and divided into dorsal and ventral parts (Figure A2). The ventral part appears enclosed the central canal (Figure A3). It appeared highly vascularised particularly, in the central portion and no connective tissue were detected except in the vicinity of the blood vessels (Figure A4). Its cells are closely pressed polygonal cells with narrow cytoplasmic rim and pushed nuclei toward one edge of the cells (Figure A5). The cells appeared filled with PAS +ve material and there was zonal variation in the positive PAS reaction intensity (Figure A6). Plastic section stained with toluidine blue revealed two cell zones; peripheral cells zone and central one. The peripheral cells were large and regularly arranged with metachromatic faint red color with peripherally located basophilic nuclei. Most cells in this zone appeared occupied with granular eosinophilic material, but few cell clusters filled with homogenous translucent eosinophilic content. These cells were found most closely to the blood vessels (Figure B1). The cells in the central zone were irregularly arranged, had different basophilic degrees, variable in sizes, and peripherally located nuclei. The cells basophilia was greater around the blood vessels. The cytoplasm was occupied with a basophilic granular material in most cells but, few cells contained dark homogenous basophilic circular bodies (Figure B2). Most cells in the central part of the central zone had very light basophilic cytoplasm and others appeared evacuated with an intact cell membrane and peripheral nuclei (Figure B3).

Transmission electron micrograph of the glycogen body

Transmission electron micrograph showed that the peripheral zone cells had a small amount of juxtannuclear cytoplasm and very thin cytoplasmic rim around the cell perimeter (Figure B4). The central zone cells had more voluminous juxtannuclear cytoplasm and broader cytoplasmic rim around the cell perimeter (Figure B5). Both zones cells showed electron dense nuclei that were deeply infolded and peripherally located toward one side of the cell with prominent

nucleoli. The cytoplasm was almost occupied with large dense masses of glycogen which were free of the usual cell organelles with the exception of few; small clear membrane-limited vacuoles and membrane-bounded masses of glycogen. Most cellular organelles were present but almost located at the juxtannuclear regions between the glycogen masses and the nucleus; numerous mitochondria, well developed Golgi apparatus, round lysosomes, numerous ribosomes mainly polysomes and rough endoplasmic reticulum was widely distributed. Clear cell junctions appeared between adjacent cells at the cells perimeters that facing the voluminous cytoplasmic parts (Figure B6).



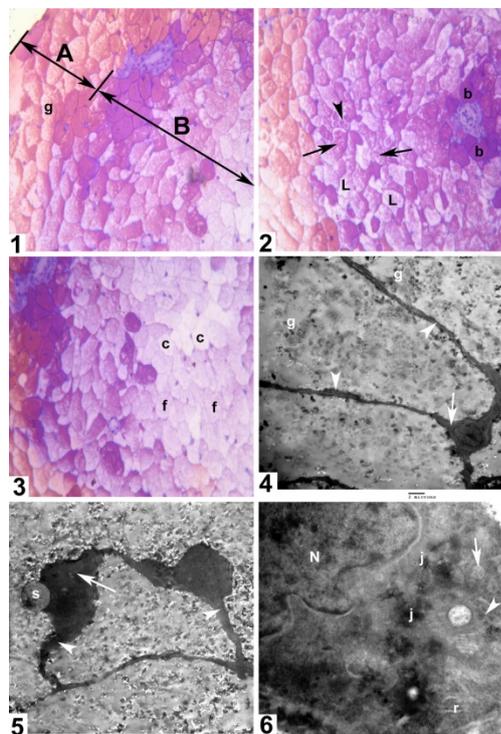


Figure B1: Spinal cord L4 level glycogen body plastic section revealed peripheral faint red metachromatic cell zone (A) filled with granular eosinophilic material (g), and central basophilic cell zone (B) (Toluidine blue X40). **Figure B2:** Spinal cord L3 level glycogen body plastic section showing the outer part of the central zone with deep basophilic perivascular cells (b) and less basophilic outer ones (L). The cells are variable in size, most of them contain granular material (arrows) and few contain circular basophilic bodies (arrowhead) (Toluidine blue X40). **Figure B3:** Spinal cord L4 level glycogen body plastic section central zone most inner cells that had very light basophilic cytoplasm (f) and some appeared evacuated with intact cell membrane and peripheral nuclei (c) (Toluidine blue X40). **Figure B4:** Transmission electron micrograph showing the peripheral zone cells with small amount of juxtannuclear cytoplasm (arrow) and very thin cytoplasmic rim around the cell perimeter (arrowheads) and most of the cell filled with glycogen particles (g) (E/M X5000). **Figure B5:** Transmission electron micrograph showing the central zone cells with voluminous juxtannuclear cytoplasm (arrow) and broader cytoplasmic rim around the cell perimeter (arrowheads) and perinuclear circular membranous structure (s) (E/M X4000). **Figure B6:** Transmission electron micrograph showing the peripheral zone cells with electron dense nucleus (N), numerous polysomes clusters (arrowhead), mitochondria (arrow) rough endoplasmic reticulum (r) and cell junctions (j) (E/M X3000).

Discussion

Although some litterateurs reported that the glycogen body extended along the whole length of the spinal cord [2], this study was designed to investigate the glycogen body in the lumbosacral region only because the typical circumscribed gelatinous mass present only in

this region [13,19]. Upon dissection, the vertebral column glycogen body tissue was completely separate and easily removed from the nervous tissue of the spinal cord. This indicates that there is no interdigitation between the cord and the body [3]. Based up on the present findings; the area of the vertebral canal increases from L2 to reach its maximum at L4 with a subsequent decrease toward more caudal segments. This enlargement in the vertebral canal is not due to an increase in the size of the spinal nervous tissue but to the large glycogen body embedded in the dorsal rhomboid sinus [20]. The best method for soft tissue description and proper locations determination of the glycogen body was done by using razor blade sections because fixation in formalin cause soft tissue (spinal cord and glycogen body) shrinkage [21]. This is confirmed by the presence of a free space between the spinal cord and the glycogen body in paraffin sections and between dorsal and ventral part of the body. Although the glycogen body cells are of glial origin properly astrocytes [5] yet its morphology is completely different as they undergo extreme differentiation [22]. They are compressed polygonal cells of variable sizes, with narrow cytoplasmic rim and the nuclei pushed toward one edge of the cells. These cells are filled with PAS +ve material. The nature of this material has been approved histochemically, and by electrophoreses that it is glycogen [3,4,23,24]. According to previous reports and the present results the glycogen body cells could be classified morphologically into peripheral cell zone and central one [6,21]. The cells in peripheral zone are regularly arranged, large sized with peripheral basophilic nuclei and metachromatic faint red color with toluidine blue. This metachromasia might be due to interaction between carbohydrate macromolecules and toluidine blue [16,24]. The cells in the central zone are irregularly arranged, have variable sizes with peripheral nuclei. Its cytoplasm has different basophilic degrees with toulidine blue [2]. The glycogen accumulation in glycogen body cells may act as a possible source of energy for the avian central nervous system [25,26]. Many reports hypothesized that the transition in glycogen contents indicate that this body might be metabolically active and play a role in the metabolism of the neurons in the chicken spinal cord [2,12,13]. Other reports mentioned that these cells may be metabolically inert and their function related to the processes of myelin formation in the avian central nervous system in which such glycogen may serve as a source of organic acids which might provide alternate substrates to the CNS under conditions of metabolic stress [14]. In contrary few reports hypothesized that this organ involved in the equilibrium of locomotion and transmission of hydrostatic pressure changes during movement on the ground [8,19].

References

1. Sansone FM (1977) The craniocaudal extent of the glycogen body in the domestic chicken. *J.Morphol* 153: 87-105.
2. Uehara M, Ueshima T (1982) Extent of the glycogen body and the glycogen content of the chicken spinal cord. *Jpn J Vet Sci* 44: 31-38.
3. Vukovic S, Lucic H (2005) Development of the glycogen body in turkey (*Meleagris gallopavo*) embryo. *Veterinarski Arhiv* 75: 101-110.
4. Lyser KM (1973) The fine structure of the glycogen body of chicken. *Acta Anat (Basel)* 85: 533-549.
5. De Gennaro LD (1993) Origin of the avian glycogen body. II. Observations in support of a glial nature in the chick embryo. *Growth Dev Aging* 57: 275-281.
6. Hodges RD (1974) *Histology of the Fowl* (1st Edition) Academic Press Inc.
7. Uehara M, Ueshima T, Kudo N (1982) The fine structure of the glycogen containing cells in the chicken spinal cord. *Jpn J Vet Res* 30: 1-10.

8. Rosenberg J, Necker R (2002) Ultrastructural Characterization of the Accessory Lobes of Lachi in the Lumbosacral Spinal cord of the Pigeon with Special Reference to Intrinsic mechanoreceptors. *The Journal of Comparative Neurology* 447: 274-285.
9. Necker R (1999) Specializations in the lumbosacral spinal cord of birds: morphological and behavioural evidence for a sense of equilibrium. *Eur J Morphol* 37: 211-214.
10. Necker R, Janssen A, Beissenhirtz T (2000) Behavioral evidence of the role of lumbosacral anatomical specializations in pigeons in maintaining balance during terrestrial locomotion. *J Comp Physiol A* 186: 409-412.
11. Wilhelm M, Wolfgang K (2003) The blood-brain barrier of the chicken glycogen body (corpus gelatinosum) and its functional implications. *Cell Tissue Res* 313: 71-80.
12. Benzo CA, De Gennaro LD, Stearns SB (1975) Glycogen metabolism in the developing chick glycogen body: Functional significance of the direct oxidative pathway. *J Exp Zool* 193: 161-166.
13. Azcoitia I, Fernandez-Soriano J, Fernandez-Ruiz B (1985) Is the avian glycogen body a secretory organ? *Journal fur Hirnforschung* 26: 651-657.
14. Benzo CA, De Gennaro LD (1983) An hypothesis of function for the avian glycogen body: A novel role for glycogen in the central nervous system. *Med Hypotheses* 10: 69-76.
15. American Veterinary Medical Association (2013) AVMA Guidelines for the Euthanasia of Animals: 2013 Edition.
16. Suvarna SK, Layton C, Bancroft JD (2013) Bancroft's Theory and Practice of Histological Techniques, 7th edition. Churchill Livingstone, London.
17. Hayat MA (1990) Principles and Techniques of Electron Microscopy, 3rd (edn). CRC Press Inc., Baco Raton, Florida, USA 19-137.
18. Bozzola JJ, Russell LD (1998) Electron Microscopy. Principles and Techniques for Biologists. 2nd (edn). John and Bartlett Publisher, Boston, Toronto, London, and Singapore 148-200.
19. Necker R (2006) Specializations in the lumbosacral vertebral canal and spinal cord of birds: evidence of a function as a sense organ which is involved in the control of walking. *J Comp Physiol* 192: 439-448.
20. Baumel JJ, Witmer LM (1993) Osteology. In: Baumel JJ (edn) Handbook of avian anatomy: nomina anatomica avium. Mass 2nd (edn). Nuttal Ornithology Club, Cambridge, 45-132.
21. Necker R (2005) The structure and development of avian lumbosacral specializations of the vertebral canal and the spinal cord with special reference to a possible function as a sense organ of equilibrium. *Anat. Embryol* 210: 59-74.
22. Lee K, Makino S, Imagawa T, Kim M, Uehara M (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.
23. De Gennaro LD (1974) Differentiation of the glycogen body of the chick embryo. Studies on glucose-14C incorporation, chorioallantoic grafting, histochemistry and electrophoresis. *Growth* 38: 1-15.
24. Gokul S, Akhil AS (2012) Toluidine blue: A review of its chemistry and clinical utility. *J Oral Maxillofac Pathol* 16: 251-255.
25. Benzo CA, De Gennaro LD (1981) Glycogen metabolism in the developing accessory lobes of Lachi in the nerve cord of the chick: metabolic correlations with the avian glycogen body. *J Exp Zool* 215: 47-52.
26. De Gennaro LD (1982) The glycogen body. In: Farner DS, King JR, Parkers KC (eds) Avian Biology 6. Academic, New York, 341-371.