THE FINE STRUCTURE OF THE GLYCOGEN CONTAINING CELLS IN THE CHICKEN SPINAL CORD

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Transmission electron microscope has been used to observe the glycogen body, the major marginal nuclei, the ventral margins of the lumbosacral cord and the lower coccygeal cord of chickens in which large amounts of glycogen accumulate.

The typical glycogen body cells are located in the classically described glycogen body. They have a dense, irregular-shaped nucleus and also a dense juxtanuclear cytoplasm containing numerous free ribosomes, and a rather long cisternae of the rough endoplasmic reticulum, as well as Golgi apparatus and mitochondria. Their cytoplasm is almost completely occupied by large masses of sharply delimited glycogen.

The glycogen body cells in the cervical and thoracic cords have a round, light nucleus and a light cytoplasm containing the usual component of organelles. The interface between the glycogen areas and the juxtanuclear regions in more obscure than in the typical glycogen body cells.

The glycogen containing cells located in the ventral margins and in the major marginal nuclei of the lumbosacral cord belong to the same structures as the typical or the other glycogen body cells. The cell structures of the above-mentioned glycogen containing cells seem to show a gradual transition according to glycogen content per cell.

The glycogen containing cells located in the lower coccygeal cord are found throughout the entire areas of the cord. They contain a round nucleus and have a low electron density. The prominent cytoplasmic component of this cell is the numerous gliofilaments; these may be considered as fibrous astrocytes.

INTRODUCTION

The existence of large amounts of glycogen in the glycogen body and in the major marginal nuclei of the chicken spinal cord is a well known fact. The chicken glycogen

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body has been regarded as a prominent egg-shaped mass restricted to the level of spinal nerves 26 to 29,\textsuperscript{14,15} although it extends rostrally and caudally into all levels of the spinal cord beyond the boundaries indicated above. Its rostral and caudal continuations mainly occupy the dorsal median region to the central canal.\textsuperscript{16,17} Electron microscopic studies of the classically described glycogen body in the lumbosacral cord have been made by several workers,\textsuperscript{4,15,16,26} among whom Sansone (1980) reported the fine structure of the glycogen body cell at all cord levels.

The major marginal nuclei lie on each side of the spinal cord with a definitive segmental arrangement. They bulge out from the lateral border of the cord and the inner border merging gradually into the white substance of the cord. These nuclei amount to ten or eleven pairs which are located from the 23rd to 32nd or 33rd segments. Glycogen is seen not only in the major marginal nuclei but also in the caudal two or three pairs of the minor marginal nuclei to the major nuclei.\textsuperscript{23} Up to now, electron microscopic studies of the major marginal nuclei in the chick have been reported only by De Gennaro (1976), and De Gennaro & Benzo (1978).

Glycogen is also observed in the ventral margins of the lumbosacral cord,\textsuperscript{1,4,15,16,23,26} and in the lower coccygeal cord.\textsuperscript{25}

These findings suggest that there are various glycogen containing cells in the chicken spinal cord. The present study was undertaken to elucidate and compare the structure of each glycogen containing cell in detail.

**Materials and Methods**

Eight chickens, aged 3 days to 12 months, were deeply anesthetized with ethyl ether and perfused through the left ventricle with aldehyde fixative buffered to pH 7.4 with 0.1 M phosphate. The fixative used was either 3% glutaraldehyde or 2% glutaraldehyde-2% paraformaldehyde. Following the perfusion, the spinal cord was removed from the vertebral canal, and freehand transverse slices were made and placed in cold fixative for at least 2 to 3 hours. The samples were rinsed briefly in phosphate buffer and post-fixed for two hours in a 1% osmium tetroxide solution, buffered to pH 7.4 with 0.1 M phosphate. The samples were next dehydrated in a graded series of ethyl alcohol solution and embedded in Epok 812. Semithin sections stained with toluidine blue were cut for orientation of these samples. Thin sections were stained with uranyl acetate, lead citrate, lead acetate and lead nitrate, and then examined by an Hitachi H-500 electron microscope.

**Results**

Glycogen body cells

The typical, classically described glycogen body cells in the lumbosacral cord showed a rather dense nuclei and cytoplasm in the electron micrographs (figs. 1 & 2). The
glycogen body cells had only a few short processes. The nucleus was deeply infolded and highly irregular in outline, and it occupied a peripheral position (figs. 1 & 2). The cytoplasm was almost completely occupied with large dense masses of glycogen which were free of the usual cell organelles, with the possible exceptions of a few small clear membrane-limited vacuoles and membrane-bounded masses of glycogen. The interface between the glycogen masses and the juxtanuclear regions containing the usual organelles was clear (figs. 1 & 2). Usually occurring organelles were observed in the juxtanuclear regions and in the narrow, peripheral rim of the cytoplasm (figs. 1 & 2). In the narrow cytoplasmic rim, the organelles were composed of numerous free ribosomes, a few mitochondria and a few heterogenous lysosomes. In the juxtanuclear regions, numerous ribosomes, many in polysomal clusters, were distributed, and mitochondria were numerous or moderate in number and sometimes large in size. The rough endoplasmic reticulum consisted of rather long, flattened cisternae and occasionally formed the lamellar stacks of the cisternae (fig. 2). The Golgi apparatus was well developed. Lysosomes were usually round or oval and homogenous, but were often heterogenous as well. Gliofilaments could not be recognized in most of the glycogen body cells, but rarely were observed as small bundles.

The glycogen body cells in the cervical and thoracic cords were mainly distributed just dorsal to the central canal in the dorsal gray commissure and partly extended into the dorsal median septum (figs. 3 & 4). The cell organelles, in general, resembled their counterparts described for similar cells in mammals and birds, with the exception of the densely packed glycogen granules. The nucleus and cytoplasm possessed a low electron density. The nucleus was round or oval with a prominent nucleolus (fig. 5). The glycogen content per cell was less than that of the typical glycogen body cell, and the interface between the glycogen areas and the juxtanuclear regions was also more obscure than typical glycogen body cells. Gliofilaments characteristically occurred in bundles but were not abundant.

Major marginal nuclei

The glycogen containing cells in the major marginal nuclei were distributed both in the protrusive parts and in the white substance parts (figs. 6 & 8). There was a greater variation in the glycogen content per each glycogen containing cell of the major marginal nuclei. The cells had many processes filled with glycogen granules. Located mainly in the superficial layer of the protrusive regions, the cells contained about the same amounts of glycogen as typical glycogen body cells. The nucleus, which was round to highly irregular in outline, displayed a dense matrix with one or two prominent nucleoli, and it was located in one pole of the cell. The cytoplasm was occupied by large masses of glycogen, and the usual organelles were restricted within the narrow juxtanuclear regions. A distinct contrast was observed between the glycogen masses and the glycogen-free juxtanuclear regions (figs. 8 & 9). The gliofilaments were
as few as the typical glycogen body cells (fig. 7). The clear membrane-limited vacuoles and membrane-bounded masses of glycogen were occasionally encountered in the midst of the densely packed glycogen granules (fig. 8). The cells located in the deeper layers of the protrusive portions and in the white substance portions were low in density with a round or oval nuclei. The gliofilaments were usually observed of the bundles (fig. 10). Golgi apparatus was fairly well developed, and the cisternae of rough endoplasmic reticulum were rather long in the glycogen containing cells located throughout the major marginal nuclei (fig. 10). Some glycogen containing processes in the protrusive portions appeared to be enveloped by eccentric layers of membrane forming a whorl (fig. 11).

Another type of astrocytes was observed in the major marginal nuclei. These were distinguished by their low electron density of cytoplasm containing no glycogen (fig. 12). The cytoplasm was relatively wide and generally was scant of organelles. Cisternae of the rough endoplasmic reticulum were extensively dilated and contained amorphous materials with moderate electron density. Clusters of free ribosomes were sparcely distributed throughout the cytoplasm. Gliofilaments were diffusely distributed, but these filaments did not tend to occur in bundles. The nucleus was round or oval and clearer than that of the glycogen containing cell. This type of astrocytes was also observed in the minor marginal nuclei of the cervical and thoracic cords.

Ventral margins of the lumbosacral cord

In the ventral margins of the lumbosacral cord, large amounts of glycogen were seen at the level of the spinal cord including the classically described glycogen body. The highest concentration of glycogen in the ventral margins was found in the middle of the classical glycogen body level, and the glycogen concentration decreased gradually rostral and caudal from the above-mentioned level. In the middle of the classical glycogen body level, the ventral margins consisted of several layers of the cell bodies, and the processes contained large amounts of glycogen (fig. 13). Glycogen containing cells were also located among the myelinated fibers in the superficial layer of the ventral funiculi. The glycogen containing cells resembled the glycogen body cells in the cervical and thoracic cords. The nuclei and cytoplasm had generally a low electron density, but a few cells were high in electron density (fig. 14). At the rostral and caudal levels of the glycogen-rich ventral margins, the glia limitans was about similar to the structures of the other spinal cord level. Moreover, numerous glycogen granules were observed in the cell bodies and their processes including the glia limitans processes (fig. 15).

Small amounts of glycogen occasionally appeared in the glia limitans processes through the other cord level (fig. 16).

Lower coccygeal cord

There were large accumulations of glycogen in whole areas of the lower coccygeal cord (fig. 17). The glycogen containing cells of the lower coccygeal cord showed rather
low electron density with a round or oval nucleus. The prominent cytoplasmic components of these cells were the numerous glifilaments and the densely packed glycogen granules (fig. 19). The cells had many long processes whose cytoplasm contained numerous glifilaments, glycogen granules and mitochondria (figs. 18 & 19). There were occasionally clear membrane-bounded vacuoles and membrane enveloped glycogen masses in the midst of the glycogen masses (fig. 18). With the exception of the mitochondria, the usual organelles were mainly confined to the juxtanuclear region.

Small to moderate amounts of glycogen were occasionally seen in the synaptic terminals of the cervical and thoracic cords in which the α-particles of glycogen occupied relatively large areas (fig. 20).

**DISCUSSION**

In the present study, it was noted that the ultrastructure of the classically described glycogen body was essentially similar to that of other descriptions. The glycogen containing cells, including the glycogen body cells in the cervical to lumbosacral cords and the cells located in the ventral margins and the major marginal nuclei of the lumbosacral cord, were considered to be a special type of astrocytes. The cell structures seemed to show a successive transition according to the glycogen content per cell. The structures of the cells containing small amounts of glycogen were similar to the usual astrocytes found in birds or mammals. As the cells contain more glycogen, the nucleus is located peripherally, most of the organelles are concentrated in the juxtanuclear regions and the cytoplasm is largely occupied by the densely packed glycogen. The demarcation between the juxtanuclear cytoplasmic regions and the glycogen areas is obvious. Furthermore, the cells with large amounts of glycogen had high electron density with a nucleus of highly irregular outline. The nucleus was peripherally located with the cytoplasmic organelles located in juxtanuclear positions. The rough endoplasmic reticulum was well developed, and its rather long cisternae or the stack of cisternae tended to be arranged in a circumferential manner around the nucleus. The free ribosomes were distributed densely through the juxtanuclear regions. These successive transitions of the cell structures are in agreement with the changes which occur in the developing glycogen body cells.

According to Matsulionis (1972), avian glycogen body ribosomes may contain cellular entities which are most closely related to glycogen synthesis. On the basis of morphological findings, some authors have suggested that the specified organelles may play some intimate role in glycogen metabolism, although other authors have not observed these close topographic relationships. In the present study, the topographic relations of glycogen accumulations to specified organelles were not observed in the cells heavily laden with glycogen.

The glycogen body cells and the cells located in the major marginal nuclei of the
lumbosacral cord had highly irregular-shaped nuclei. Studies have shown that the epithelium lining a distal segment of the tubuli recti in the guinea-pig stores large amounts of glycogen in which a comparable amount of glycogen with the chicken glycogen body cell is contained. The nucleus of those epithelial cells is also deeply infolded and irregular in outline. Generally, nuclei of elaborate shape occur in long and fully differentiated cells that are metabolically very active, but nuclear lobulation may occur in end-stage cells in which protein synthesis is minimal. The typical glycogen body cells are metabolically inactive. Therefore, the significance of the highly irregular shape of the nuclei in abundant glycogen containing cells remains unexplained. One possibility, however, is that the cells laden heavily with glycogen, such as the typical glycogen body cells and the glycogen containing cells in the major marginal nuclei, possess a remarkably different nature or role from the usual astrocytes.

The functional role of abundant glycogen located in the cervical to lumbosacral cords of chickens has been unknown up to now. The presence of abundant glycogen in the central nervous system is a characteristic feature of the lower vertebrates including birds. In the synaptic terminals, numerous glycogen granules were occasionally found in the present materials. This might also be a characteristic feature of the central nervous system in the lower vertebrates. Although in the adult mammalian central nervous system glycogen occurs in small amounts, some regions of the brain are known to contain considerable amounts of glycogen, and particularly in rat brain glycogen, the highest concentration occurs in the medulla and the lowest in the hypothalamus. The functional role of glycogen is only insufficiently explained as it relates to the central nervous system of all vertebrates. Thus comparative anatomical and physiological researches of glycogen located in the central nervous system of different vertebral classes are important in order to resolve the functional relationships between the glycogen accumulation and the metabolism of the neurons in the chicken spinal cord.

The large accumulation of glycogen in the chicken lower coccygeal cord has been studied histochemically in our previous experiments. In comparison with other glycogen containing cells, those of the lower coccygeal cord are abundant in the gliofilaments and in the long processes, and they have fewer glycogen granules. They are thus considered as fibrous astrocytes. In the chicken lower coccygeal cord the gray matter is obscure or disappears. The nerve cell bodies are few, and in the last coccygeal segment it is difficult to see any nerve cell bodies. The myelinated and unmyelinated fibers also decrease continuously in number throughout the cord. In contrast with the decrease of the neuronal elements, the regions which are occupied by the glycogen containing cells are relatively increased throughout the cord. The significance of the glycogen accumulation in the lower coccygeal cord remains obscure, but some evidence suggests that the glycogen containing cells in the lower coccygeal cord may play a
different function from that of the glycogen body cells.

In the major marginal nuclei many glycogen containing cells and a few glycogen-free cells were observed. The prominent cytoplasmic components of the glycogen-free cells were the gliofilaments and the remarkably dilated rough endoplasmic reticulum. The gliofilaments varied in quantity. Although some of the cells possessed a large number of filaments, the latter did not tend to occur in bundles as they do in astrocytes in other regions. We therefore postulated that the glycogen-free glial cell may be an immature or regressive astrocyte.

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EXPLANATION OF PLATE

PLATE I

Fig. 1 Electron micrograph of the typical and classically described glycogen body cells in the lumbosacral cord. Notice the high electron density of the nucleus and juxtanuclear cytoplasm. Glycogen granules are almost missed. 3-day-old chicken ×9,300

Fig. 2 Electron micrograph of the classically described glycogen body cells. Notice the long cisternae of rough endoplasmic reticulum. Glycogen granules are almost missed. 3-day-old chicken ×12,000

Fig. 3 Light micrograph of semithin transversal section of the glycogen body in the thoracic cord. 160-day-old chicken Toluidine blue ×300

Fig. 4 Low magnification electron micrograph of the glycogen body in the thoracic cord. 160-day-old chicken ×5,200
**PLATE II**

Fig. 5  Electron micrograph of the glycogen body cell in the thoracic cord. 75-day-old chicken  ×8,800

Fig. 6  Light micrograph of semi thin section of the protrusive portion of the major marginal nucleus. The protrusive portion is essentially constituted of large nerve cell bodies and glycogen containing cells. Toluidine blue 300-day-old chicken  ×520

Fig. 7  Electron micrograph of a glycogen containing cell in the protrusive portion of the major marginal nucleus showing the glial filaments. 103-day-old chicken  ×29,400

Fig. 8  Low magnification electron micrograph of the major marginal nucleus. Upper right side is the protrusive portion and lower left side is the white substance portion. 6-day-old chicken  ×4,400

Fig. 9  Electron micrograph of the glycogen containing cells in the protrusive portion of the major marginal nucleus. Notice the irregular shaped nucleus. 103-day-old chicken  ×12,000
PLATE III

Fig. 10 Electron micrograph of the glycogen containing cell in the white substance portion of the major marginal nucleus. Notice the long cisternae of rough endoplasmic reticulum and the oval nucleus. 103-day-old chicken ×9,600

Fig. 11 Electron micrograph of the protrusive portion of the major marginal nucleus showing a glycogen containing cell process enveloped by concentric layers of membrane forming a whorl. 6-day-old chicken ×22,000

Fig. 12 Electron micrograph of the glycogen-free glial cell in the major marginal nucleus. 6-day-old chicken ×6,600

Fig. 13 Light micrograph of semithin transversal section through the middle of the classically described glycogen body level showing the ventral margin of the spinal cord. 103-day-old chicken Toluidine blue ×400

Fig. 14 Electron micrograph of the glycogen containing cell in the ventral margin of the lumbosacral cord through the middle of the classically described glycogen body level. 75-day-old chicken ×12,000
PLATE IV

Fig. 15  Electron micrograph of the ventral margin of the spinal cord through the rostral part of the classically described glycogen body. 160-day-old chicken ×8,400

Fig. 16  Electron micrograph of the glia limitans processes located in the thoracic cord. 160-day-old chicken ×16,400

Fig. 17  Light micrograph of semithin transversal section through segment 40. 50-day-old chicken Toluidine blue ×160

Fig. 18  Electron micrograph of the superficial layer of the lower coccygeal cord. 160-day-old chicken ×12,000

Fig. 19  Electron micrograph of the glycogen containing cell in the lower coccygeal cord. 160-day-old chicken ×9,600

Fig. 20  Electron micrograph showing an accumulation of glycogen particles in the synaptic terminal. 300-day-old chicken ×32,000