CHAPTER 5

ULTRASTRUCTURAL MORPHOLOGY OF GLYCOGEN BODY OF CHICK OF DOMESTIC FOWL AND ADULT PIGEON.

The fine structure of glycogen body cells were first described by Revel *et al.* (1960) and Revel (1964). However, their studies were concerned mainly with the preservation and appearance of glycogen in this tissue. They reported the glycogen body cells to be densely packed with beta-granules of glycogen, with majority of cell organelles occurring in the vicinity of the peripherally placed nucleus. Matulionis (1972) later worked out the ultrastructural details of the developing glycogen body cell in the chick embryo. He observed the prevalence of golgi complex to which were associated multivesicular bodies and rough endoplasmic reticulum (RER). Occurrence of ribosomes in close proximity of glycogen particles in the electron micrographs was implicated to the cells being involved in glycogen synthesis. Lyser (1973) studying the ultrastructural morphology of glycogen body of the *ex-ovo* chicks found the cells of this tissue to abut directly on the adjacent neural tissue and ependyma. She observed the perinuclear cytoplasm as containing extensive, RER and numerous free polyribosomes, mitochondria as well as golgi complex. Both Welsch and Wachtler (1969) and Lyser (1973) consider

these cells to be a mass of specialized astroglia. Electron microscopic studies of Paul (1973) showed the presence of nerve fibres in the glycogen body tissue. The nerve fibres seemed to be following the course of blood vessels and were possibly adrenergic and innervating them.

More recent studies of Sansone (1980) on glycogen rich cells surrounding the ependymal lining of central canal, extending both caudally and rostrally through all the spinal segments upto the floor of the 4th ventricle of brain revealed the cells to be similar to glycogen body cells except that the former cells had fewer glycogen particles and more gliofilaments whereas the latter had densely packed glycogen particles with fewer gliofilaments. In spite of such information on glycogen body, the significance of this tissue in the C.N.S. of bird remains unclear. Astrocytes are known to play an important role in neuronal maintainence by providing materials for their metabolism as well as dealing with unwanted products like ammonium ions etc. However, one of the possible function of glycogen body could be secretion of certain products for extracellular transport. Astrocytes else where in the nervous system has been known to have secretory function. The aim of the present

work is to study the morphological aspect of glycogen body of the two week post-hatched chick of domestic fowl and adult feral pigeon ultrastructurally and, also, to discuss the possible function of these glial cells in the central nervous system. Histochemical localization of RNA was carried out in the glycogen body of chick and pigeon with a view to understand the nature of perinuclear cytoplasm.

MATERIAL AND METHOD

Electron Microscopy :

Day old chicks of domestic fowl (RIR variety), purchased from Government poultry farm were placed in well aerated cages. The chicks had an access to water and chick mash till the day of experimentation. Two week post-hatched chicks were killed by decapitation, glycogen body tissue was quickly dissected out along with lumbosacral spinal cord and placed in Karnovsky fixative buffered with 0.2M sodium phosphate solution. While the tissues were immersed in aldehyde fixative, they were cut into thin transverse slices with a razor blade. Following 2 to 3 changes in Karnovsky fixative, the tissues were rinsed in 0.1M phosphate buffer twice to remove excess of glutaraldehyde from the tissues. Post fixation was done in 1% osmium tetroxide in 0.1M phosphate buffer for 1 hour, dehydrated

in a graded series of acetone to be followed by embedding in Araldite 502 for transmission electron microscopy. All the steps were carried out at room temperature.

Semithin sections (1.0 µm) were cut, stained with 1% Toluidine blue to delimit glycogen body and for region selection. Tissues were sectioned with glass knives on LKB Bromma 2128 Ultramicrotome. Ultrathin sections were collected on copper grids (200 mesh size), stained with uranyl acetate and lead citrate and viewed with Phillips EM 400 electron microscope, photographed at different magnifications and enlarged as desired.

Adult feral pigeon, *Columba livia* was also used as an experimental model for studying the glycogen body at electron microscope level. However, the pigeons had to be deeply anesthetised with ether prior to decapitation to avoid stress involved in handling the bird. Other procedure used is same as for the developing chicks.

Histochemical staining for RNA :

RNA was studied histochemically in the glycogen body of the chick according to methyl green-pyronin-Y method of Brachet (1940a & b). Controls were pretreated with RNase.

Birds were decapitated, tissue removed rapidly and 15-20μ sections taken at -20°C in a cryostat microtome. Photographs were taken immediately.

RESULTS

The present ultrastructural study on glycogen body of the two week post-natal chicks revealed their cells to closely abut with each other with very little intercellular space (Fig. 1). Tight junctions were observed between two adjoining endothelial cells of the blood vessels (Fig. 1). Some of glycogen body cells were placed in such a manner that their perinuclear regions apposed each other and lay side by side, facing each other (Fig. 2). Whereas few of the cells had the nucleus with their perinuclear region on opposite sides. The perinuclear area was electron dense and therefore very dark in appearance (Fig. 2, 3). Staining glycogen body tissue of the chick for RNA indicated the material to be RNA. Treatment of the tissues sections with RNase led to juxtanuclear region losing the RNA and therefore gave an empty appearance. Glycogen occupied major portion of the cell's cytoplasm and had a dense profile (Fig. 4). Ribosomes were seen both in free as well as polysomal cluster in the vicinity of the small eccentric nucleus i.e the surrounding region of the nucleus and the ectoplasmic area (Fig. 5, 6).

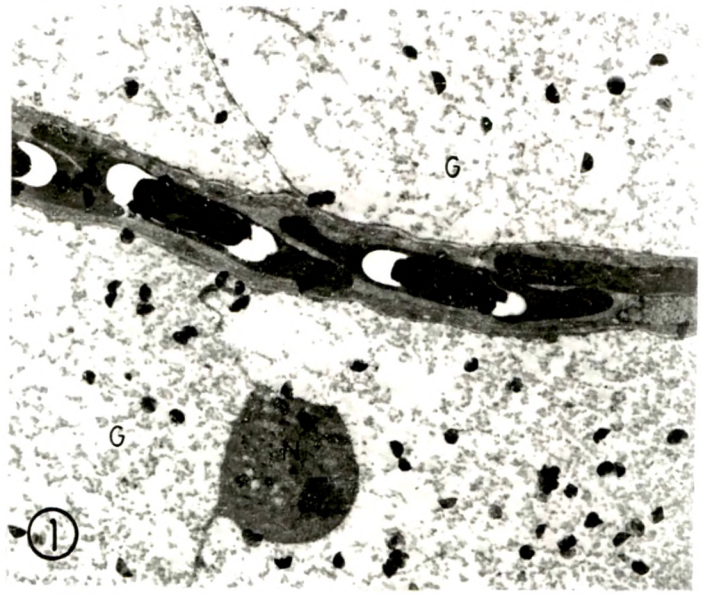
EXPLANATION OF FIGURES

Fig. 1-3 Electron micrographs of glycogen body cells from two week post-hatched chick of domestic fowl.

Fig. 1 Low magnification micrograph of glycogen body cells showing a peripherally placed nucleus (N), with rest of the cytoplasm filled with glycogen granules (G). Note the presence of blood vessel.X 3350.

Fig. 2 Two glycogen body cells with perinuclear area facing each other. N- nucleus, M-mitochondria, G-glycogen particles.X 17750

Fig. 3 Part of three glycogen body cells of the chick. Note the dense perinuclear area of the right cell and dark cytoplasmic glycogen granules of the left cell. I-intercellular space.X 24300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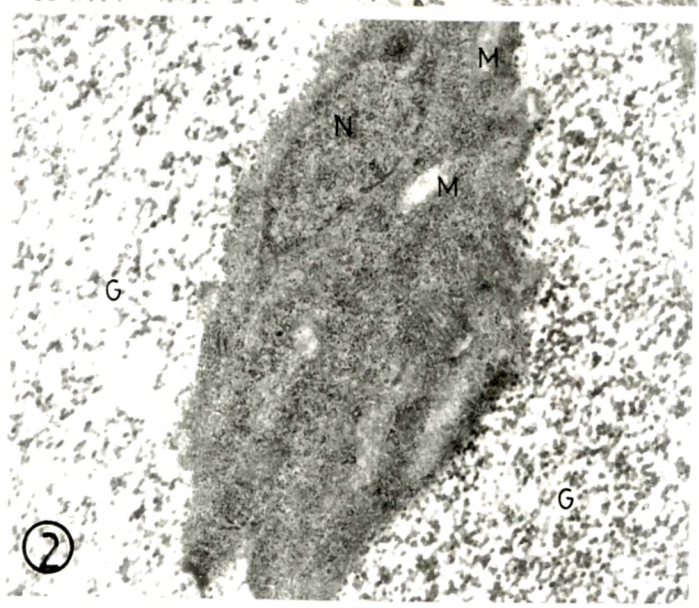
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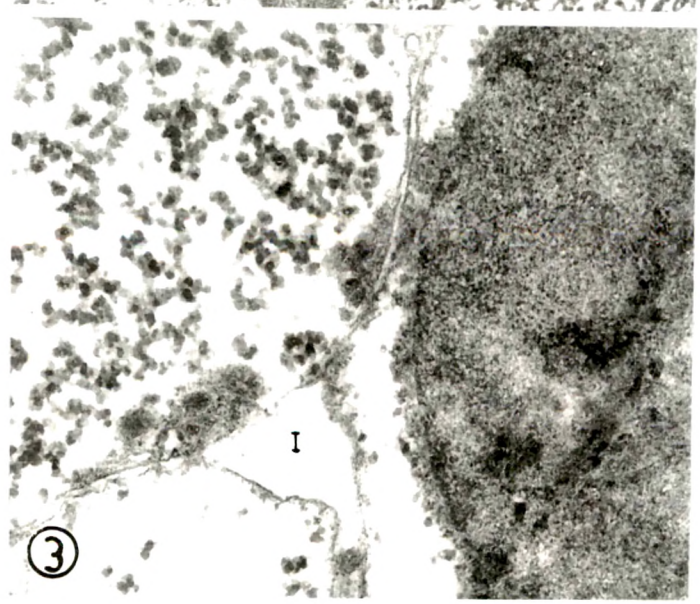
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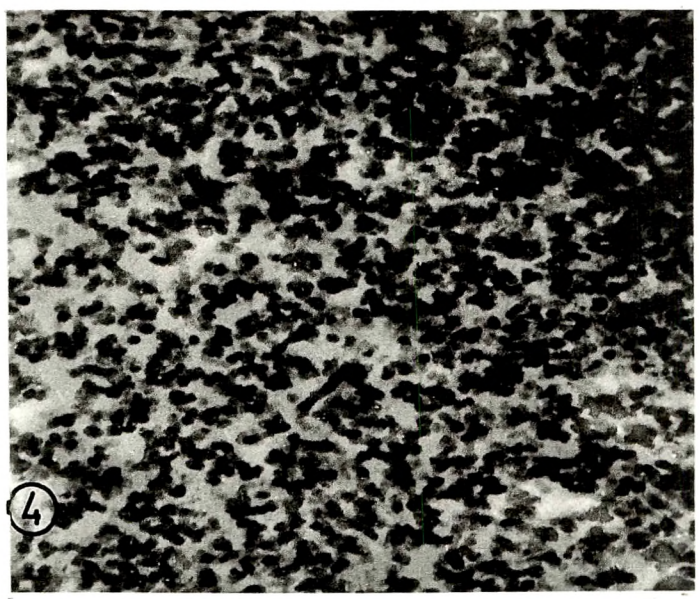
EXPLANATION OF FIGURE

Fig. 4 Electron micrograph of part of glycogen body cell from a 15 day old post-natal chick showing accumulation of dense granular glycogen particles.X 32400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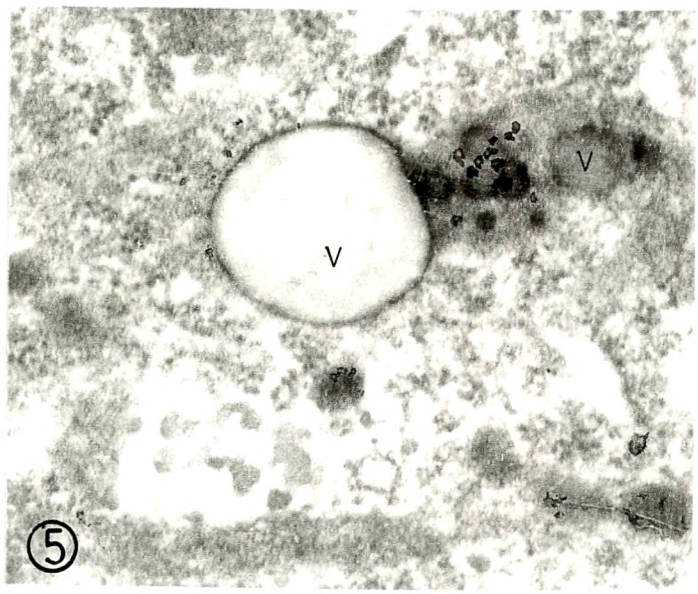
EXPLANATION OF FIGURES

Fig. 5 & 6 High magnification micrograph of glycogen body cell of the chick.

Fig. 5 Portion of the perinuclear cytoplasm containing numerous vesicles (V). X 59800

Fig. 6 Electron micrograph of the glycogen cell showing the dense ectoplasmic area containing ribosomes (R). Note the proximity of ribosomes to the glycogen granules (arrows). X 82900



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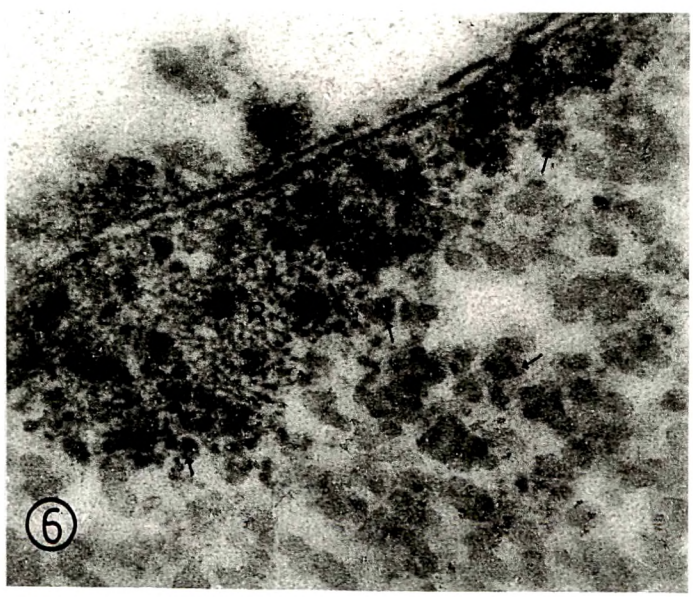

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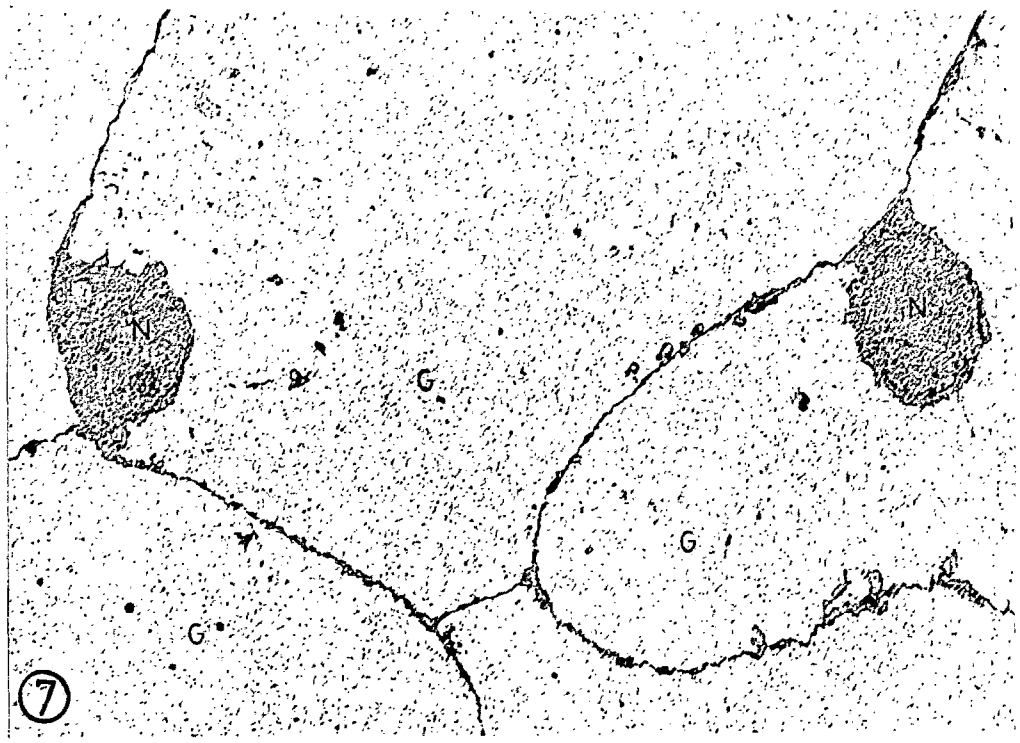
EXPLANATION OF FIGURES

Fig. 7 & 8 Electron micrographs of glycogen body cells from adult pigeon.

Fig. 7 Low magnification micrograph of glycogen body cells. Note the presence of a small nucleus (N) with narrow perinuclear area towards the border of the cell. Glycogen granules (G) fills the rest of the cytoplasmic area.X 4750

Fig. 8 Higher magnification of the glycogen body cell placed to the left in fig. 7. Note the presence of various cell organelles like mitochondria(M) and golgi complex (GC).X 16950



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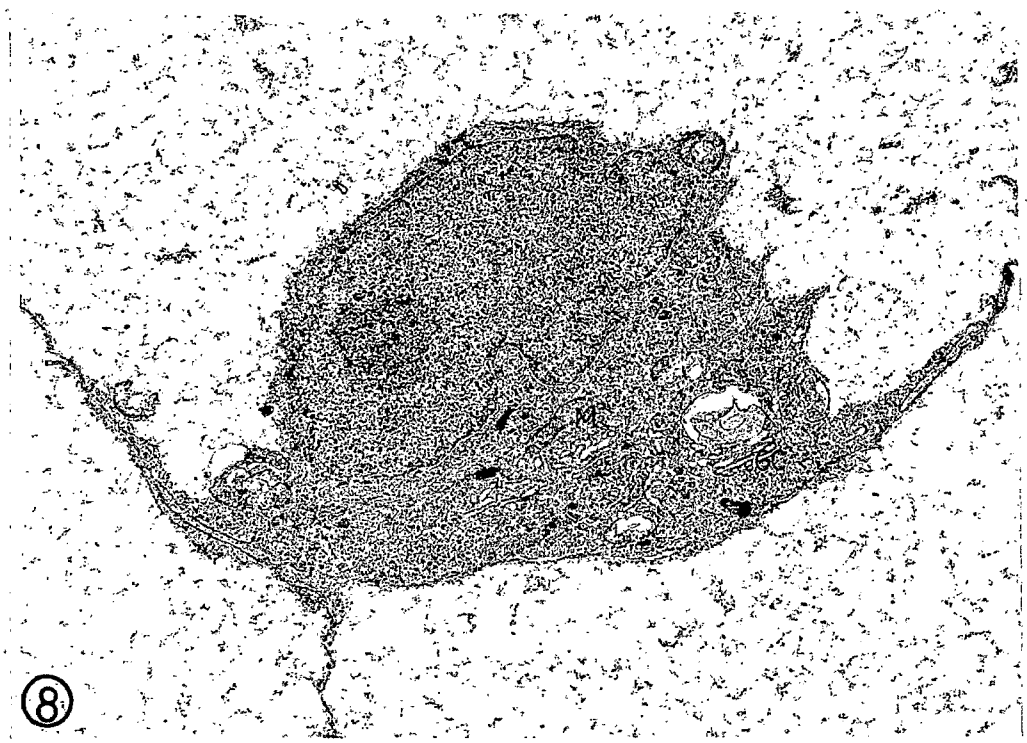

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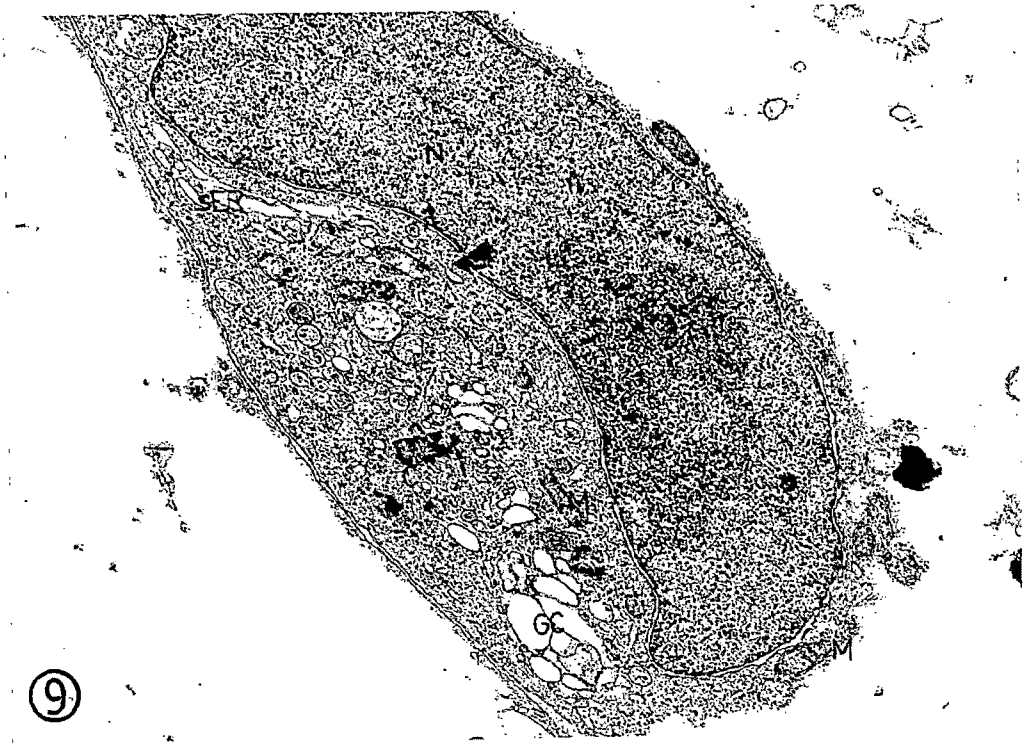
EXPLANATION OF FIGURES

Fig. 9 & 10 Electron micrograph of glycogen body cell from adult pigeon.

Fig. 9 The glycogen body cell's perinuclear region shows presence of cell organelles like centriole (C), mitochondria (M), golgi complex (GC), smooth endoplasmic reticulum (sER) and various golgi vesicles in different formative stages. Note that this cell has a long and elongated nucleus (N). X 18200

Fig. 10 Higher magnification of the same as in fig. 9. X 45200



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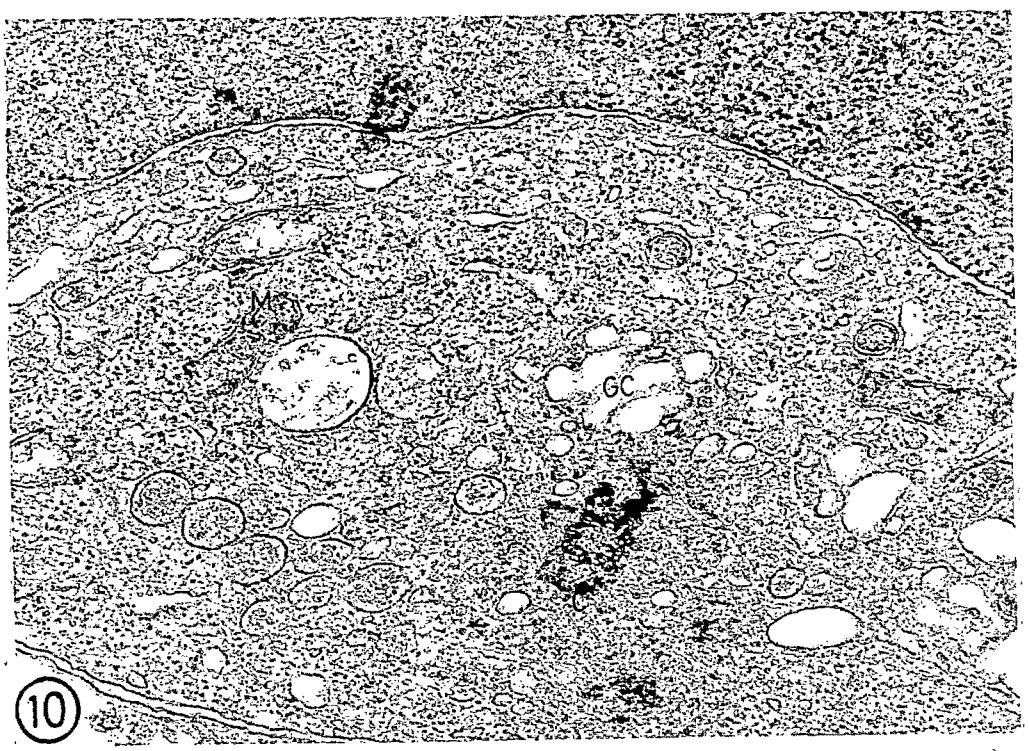

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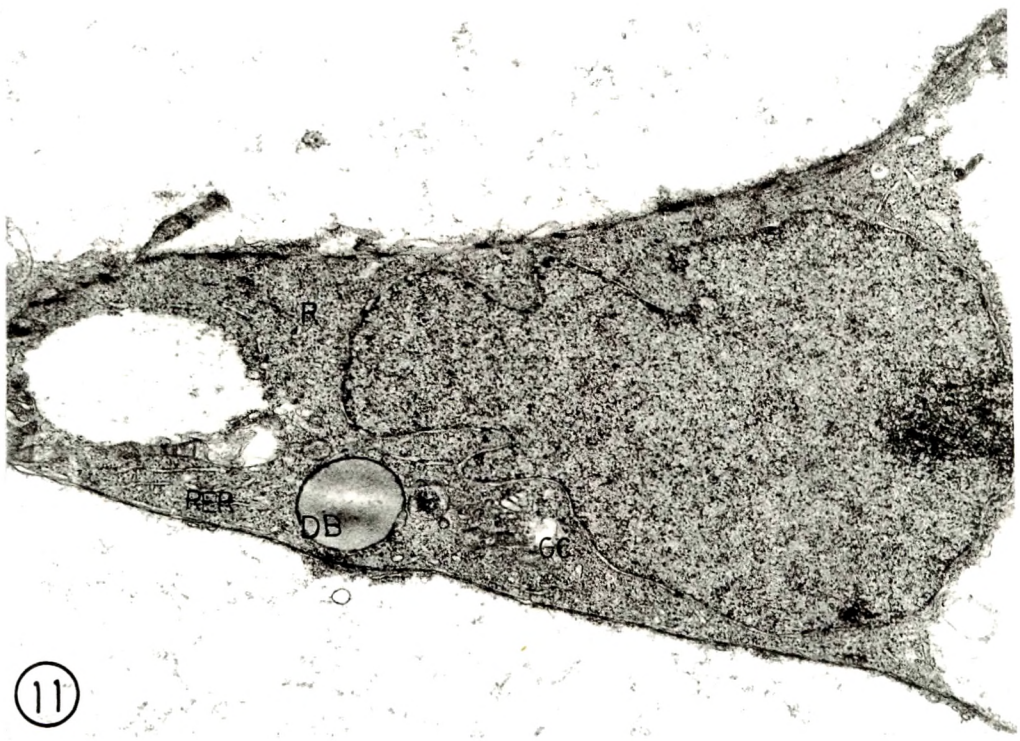
EXPLANATION OF FIGURES

Fig. 11 & 12 Electron micrograph of glycogen body cell of adult pigeon.

Fig. 11 The electron dense perinuclear cytoplasm contains various organelles like ribosomes (R), rough endoplasmic reticulum (RER), golgi (GC) and a double membraned electron dense body (DB). Note that glycogen like formation seen in the large electron lucent area within the perinuclear area. X 16950

Fig. 12 Higher magnification of the same as in fig. 11. X 33000



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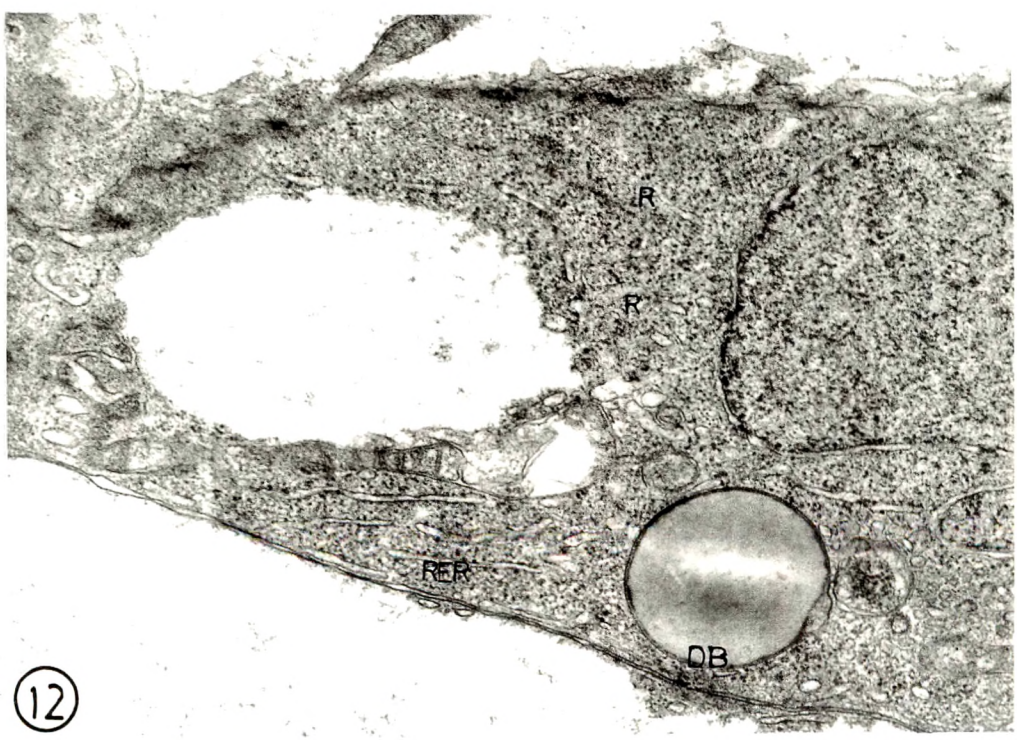

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Electron microscopic examination of adult pigeon glycogen body revealed their cells to tightly abut with each other. Fig. 7 represents a low power view of the same showing a very small peripherally placed nucleus with majority of cytoplasm filled with glycogen. Cell organelles like centriole, mitochondria, rough endoplasmic reticulum, ribosomes and golgi complex were visible very clearly in the juxtanuclear cytoplasm (Fig. 8, 9). The cells also had a well developed stack of golgi complex with numerous vesicles of various sizes, that is, they were present in different formative stages (Fig. 10). The large empty electron lucent area (Fig.11, 12) containing material similar to that of the cytoplasmic glycogen was observed in some of the electron micrographs. An electron dense double membraned structure was also observed in the pigeon glycogen body (Fig. 11, 12).

DISCUSSION

The fine structure of chicken glycogen body has been well documented (Matulonis, 1972; Lyser, 1973; Paul, 1973; DeGennaro and Benzo, 1978; Sansone, 1980). The electron microscopic observations made on the glycogen body of chicks and adult pigeon revealed the occurrence of cytoplasmic organelles like ribosomes, rough endoplasmic reticulum, golgi, centrioles, mitochondria etc. The

presence of dense cored vesicles in different formative stages amidst the golgi complex was a prominent feature of glycogen body of both the chick and the pigeon. The vesicles contained material that had appearance more or less similar to glycogen granules. Some of the cell's perinuclear area contained large empty electron lucent region also containing glycogen like material. The presence of such structures and dense cored double membraned bodies suggests that the glycogen-like material and other secretory substances may be getting secreted in to this area to gradually enlarge and fuse with the plasma membrane of the cell. The nature of component of the vesicles could not be determined. Azcoitia *et al.*(1987) have observed glycogen granules in the arachnoid space above the glycogen body tissue and have contended the cells to secrete these granules directly into the space above as pia mater was missing in this region. They were of the view that granules, due to their high osmotic pressure would draw the liquids from glycogen body towards them and help maintain a constant flow of CSF from neural canal to arachnoid space as was done in the cranial region where Foramina of Magendie and Luschka enable the CSF from the 4th ventricle to enter the outer subarachnoid space. Lyser (1973) proposed the involvement of golgi in the production of certain packaged enzymes related to glycogen metabolism. The *in vivo* studies of

Snedecor *et al.* (1963) that labelled glycosyl units are being added to as well as removed from the glycogen of glycogen body suggests that synthesis and breakdown of glycogen does occur in this tissue. Not much of glucose can be released freely due to lack of enzyme glucose-6-phosphatase, it seems likely that G-1-P released by phosphorylase action may be getting converted into any of the uronic acids (glucuronic acid or galacturonic acid) and into amino sugars (glucosamine or galactosmine). These sugar derivatives are the components of glycosaminoglycan, a polysaccharide built up from such repeating disaccharide units. Most glycosaminoglycans are found attached to protein by covalent bonds so as to form protein-carbohydrate complexes known as proteoglycans. Other sugar derivative that can be formed are N-acetylglucosamine or N-acetylgalactosamine which are constituents of various glycoproteins. Steindler *et al.* (1989) are of the view that the sugars of these glycoconjugates themselves may function as signals for different states of morphogenesis. It is known that immature astrocytes express chondroitin sulfate proteoglycans that have neurite growth inducing properties (Faissner *et al.*, 1994). Over and above, astroglial cells are known to secrete large number of neurotrophic factors including nerve growth factor (NGF), basic fibroblast

growth factor (b-FGF), platelet derived growth factor (PDGF), ciliary neurotrophic factor (CNTF) etc. [Norenberg, 1994]. Survival of motor neurons is largely dependent on bFGF and CNTF (Arakawa *et al.*, 1990: Sendtner *et al.*, 1990). CNTF is also needed for the survival of both neurons and oligodendroglia. In chicken spinal cord, the neurons and glia arise from single precursors over relatively long periods of development (Leber *et al.*, 1989). CNTF and PDGF are thought to stimulate the production of specific glial phenotypes in the rat optic nerve during development (Raff, 1989). Also differentiation of O2A progenitor cells into astrocytes also require an extracellular matrix constituent in addition to CNTF (Lillien *et al.*, 1990). In the lumbar spinal cord of the chick embryo, between E6-E12, death of motorneurons occurs. It is probable that glycogen body develops at about this time in this part of the cord, as a compensatory structure against loss of huge mass of ventral motor neurons (chapter 4). The specialized feature of these astrocytes is that they have the capacity to store enormous quantities of glycogen in its cytoplasm. This feature is not commonly found in astrocytes elsewhere in the nervous system. Astrocytes are known to proliferate and hypertrophy following damage to the C. N. S., and fill up small spaces left by the degenerated tissue (Reier and Houle, 1988). It is known that survival of developing

neurons depends to some extent on the glial cell-derived factors. Extra cellular matrix (ECM) also seems to be the source of trophic support for the survival of neurons. The glycogen body at least during early embryonic development, may be providing CNTF needed for the protection of both neurons and oligodendroglia. Oligodendroglia that survive, differentiate fully and begin to myelinate fibre tracts. It is known that myelination begins from E13 onwards (Hasan *et al.*, 1993) and this is the time when death of motoneurons decline. It can be said that glycogen body may be indirectly assisting in the myelinization process. by way of supply of various proteoglycans and neurotrophic factors.

Two decades earlier ECM was an unknown element in the central nervous system. Extensive work on ECM from nervous system has been carried out in recent years. This has shown that the ECM associated molecules are derived predominantly from glia. Generally, extracellular matrix from commonly studied tissues is a complex set of specific collagens and noncollagenous glycoproteins and proteoglycans with ECM of each organ of the body displaying a unique composition (Venstrom and Reichardt, 1993). Within C. N. S., ECM plays an important role in the regulation of cell migration, survival, differentiation

and axonal pathfinding (Reichardt and Tomaselli, 1991). ECM also has influence on synaptogenesis and glial differentiation (Sanes, 1983). Proteoglycans are component of ECM that function as cell surface receptors (Ruoslahti, 1989) and modulate growth factor action, most notably b-FGF and TGF-β (Rifkin and Moscatelli, 1989). Gould *et al.*(1995) demonstrated immuno-histochemically the presence of Syndecan-3 in the brain and neural tube of embryonic chicks. Immunoreactivity was abundant in the ventral half of neural tube with striking expression in the floor plate at stage 25 (E5) in the embryonic chick. Synedcans are cell surface transmembrane proteoglycans with heparan sulfate and chondroitin sulfate glycosaminoglycan chain. Syndecan has the capacity to bind various growth factors especially FGF which is known to stimulate proliferation of many types of cells *viz.* mesoderm, neuroectodermal cells, endothelial cells etc (Choy *et al.*, 1996). Binding of FGF to syndecan brings about a conformational change that enable FGF to bind to its receptors. This binding also prevents the degradation of FGF by proteases (Ruoslahti and Yamaguchi, 1991).

During early development glycogen body tissue is richly supplied with capillaries. If substances are to diffuse in and out, presence of ECM with its body of water will

have to fill the gaps between the cells. This material seems to be secreted by glycogen body cells. In absence of substantiating evidence regarding chemical composition of the material within ER or golgi, all one can do at the present moment is to hypothesize regarding the nature of secretory product. This may be in the form of export material such as ECM proteoglycan and glycoprotein, which means the protein core must be getting formed by RER located in perinuclear space and glycosylated further either by ER or golgi. The golgi vesicles so formed then move towards plasma membrane and discharge the contents into the intermembrane space. As against this, the polyribosomes perhaps help in the formation of glycogenin needed to initiate formation of glycogen and the enzymes needed by the cell to carry out various metabolic activities. In addition to this, several growth factors will also have to be supplied by RER and golgi during early developmental stages. In addition to this it will also be obliged to form cell-surface proteoglycan. The glycogen body tissue therefore appears to be a reservoir and supplier of sugar groups. As and when necessary the sugar groups are modified into specific sugars, to be used for glycosylation of the protein components of either proteoglycan and glycoprotein as the case may be. Once these ECM components fill up the intercellular spaces.

growth factors formed later could be acquired or supplied through mediation of these ECM compoments. On the basis of results obtained in adult pigeon, it seems that the same trend (namely of supporting proteoglycans and glycoproteins) continues even in the adult.