GENERAL CONSIDERATIONS

Although presence of glycogen loaded cells especially in the region immediately around the central canal, fourth ventricle, Iter and third ventricle is known, formation of a compact mass of glycogen loaded cells has not been reported in any class of vertebrates other than Aves. Within the C. N. S. of birds is located the glycogen body (GB), a specialized mass of astroglial cells (Welsch and Wachtler, 1969; Lyser, 1973) whose cytoplasm is heavily filled with glycogen particles. Accessory lobes (DeGennaro and Benzo, 1976; 1978) are similar to glycogen body in that their cells are also filled with glycogen. However, the lobes of Lachi, as they are called, are relatively small, extending bilaterally from the periphery of the lumbosacral cord. Similar glycogen loaded cells have been observed all throughout the spinal cord in areas surrounding the ependyma of the central canal (Sansone and Lebeda, 1976; Sansone, 1977, 1980; Uehara and Ueshima, 1982). Work on glycogen body tissue started about a century ago, when Duval (1877) worked out histochemically, the nature of glycogen body cells in developing chickens and pigeons. He showed that the substance filling the cells was not lipid since he observed negative reaction with osmic acid. Next to Duval (1877) was Imof (1905) who carefully worked the presence

of this structure in many different species of birds and came to conclude that GB was composed of neuroglial cells. Major contribution to the work on GB comes from Watterson (1949) who gave the term glycogen body to this unusual struture. Several studies have been aimed at understanding the functional importance of these glycogen depots in the C. N. S. of birds. Work has been carried out biochemically, histologically, histochemically and at ultrastructural levels since then. Worth mentioning is DeGennaro (1982) whose review on glycogen body contains information about various aspects of this tissue since 1877. He has emphasized the need for future studies so that more information could be gathered about this unique avian feature. This has led to the present work on avian glycogen body.

Though glycogen content of glycogen body has been shown to be nonvarying to various agents (Doyle and Watterson, 1949; Szepsenwohl and Michalski, 1951; Hazelwood *et al.*, 1962; Hazelwood *et al.*, 1963; Snedecor *et al.*, 1964; Anderson and Hazelwood, 1969) yet the tissue is metabolically active since both synthesis and degradation of glycogen occurs (Benzo and DeGennaro, 1974, 1981) as also in utilization of glucose by way of glycolysis and pentose phosphate pathway (Benzo *et al.*, 1975; Fink *et*

al., 1975). Earlier work from our laboratory have shown diurnal variation to occur in the glycogen body of adult blue rock pigeon during summer months. However, similar study conducted during colder months did not exhibit any significant variation in their glycogen content. Since the tissue wet weight being small and glycogen content being very high, utmost care is called for in processing of samples for glycogen estimation. Glycogen is very unstable in solution. It seemed very necessary to recheck the possible variation in the glycogen content during warmer months before drawing conclusions. Using modified method for weighing the tissue and measuring O.D. it was seen that glycogen body did not show any diurnal variation in the feral pigeons.

In the last decade glia, the brain's major cell type had a very low repute and was thought to have only supporting role to the neurons. Today, glia are credited with additional roles, playing major functions in central nervous system development, homeostasis and pathology. The field of glial research has expanded as more and more information is being added to the functional role played by various glia in the developing C. N. S. Glycogen body proves to be a promising tissue owing to the presence of a pure mass of astrocytes which is easily removable in

the young post-hatched developing chicks. The advantage of working with this tissue is that there is no intervention of neurons and hence would serve a good material for carrying out various studies on this cell type. Keeping in view this point and the fact that glycogen body has a very high activity of LDH as detected histochemically and biochemically (Friede and Vossler, 1964; Syeda, 1989) work was carried out on the LDH isoenzymes in the glycogen body and different regions of spinal cord (possessing different proportion of astrocytes, oligodendrocytes and neurons) in both the post-hatched developing chicks and adult fowl as also the adult pigeon. Difference in result obtained in GB and spinal cord could be due to absence of neurons in the former and to their presence in the latter. A noteworthy result obtained only in the glycogen body tissue was the occurrence of diffuse staining in the interband and pre-anodal band 1 (anodal side of anodal band 1) regions. Zinkham *et al.* (1966) similarly observed presence of two bands pre-anodally in the heart of wild pigeons and attribute them to be allele of H (B') subunit of LDH isoenzyme. The present study facilitated comparison between different cell types in the C. N. S. of bird. Thus electrophoretic study offers ample scope for further work regarding the nature of pre-anodal material in the glycogen body.

Glycogen body is known to possess a very high activity of glutamine synthetase and moderate activity of glutamate dehydrogenase in both 2 and 10 day old chicks of domestic fowl (Nene and Syeda, 1993). Determination of amino acids in GB was done using thin layer chromatography. The result supports the above findings. Syeda (1989) is of the opinion that glycogen body cells have capacity to detoxify ammonia to some extent. Further work that needs to be carried out includes HPLC of amino acids in GB and different regions of spinal cord and brain following ammonia exposure so as to check for changes in their profile and content (i.e both qualitative and quantitative). An array of literature has shown the astrocytes to play a very important role in the detoxification of ammonia. However, these astroglial cells differ morphologically from other astroglial cells in the C. N. S. and hence their function in the nervous system of bird cannot be said with certainty. Again taking advantage of the specialized glial tissue it became imperative to study the toxic effects of ammonia on glycogen and enzymes glycogen synthase and phosphorylase in them. The work saw glycogen to increase in a dose dependent manner with glycogen synthase showing no change in its activity whereas phosphorylase enzyme activity showed a significant decline. The observed

results could be attributed to derangement in β-adrenergic cAMP producing system rendering the enzyme inactive. Ammonia has no effect on the uptake of glucose into the cells. Thus G-1-P could be formed leading to increases in the levels of glycogen in the tissue. Even though the results of the present study do not provide direct answer to the development of glycogen body in the lumbosacral spinal cord of birds, the possibility of this structure being an inevitable byproduct of an altered form of development has to be taken into account. Like mammals, and unlike fish and reptiles, birds are warm blooded. The basic difference between the development of embryo of aves to that of mammals is that their development takes place within the egg. Mammalian embryo is supported by placenta which is capable of quick removal of nitrogenous wastes generated during its development. Despite the fact that avian egg is endowed with (1) enough nutrient which will last for 2-4 weeks, (2) protection from physical forces such as turbulance, mechanical shocks etc. (3) protection from predators, (4) ability to withstand damaging effects of high/low temperatures, they are imposed on having to live with nitrogenous waste products. The necessity of putting up with the presence of these nitrogenous waste products would explain the presence of this unusual structure in

birds. A point worth noting at this juncture is that during the development of nervous system in the avian embryo, first active movements of the trunk begin by day 5 i.e stage 25 (Porvine *et al.*,1970) which means that neurons are active and their activity would in turn produce ammonia. Using the present work as a base further experiments could be tried out. It would be worthwhile to test the effect of sublethal dose of ammonia on the nerve cord especially the cells present in the roof plate during the early stages of embryonic development i.e between stages 21-30. Since ammonia poses severe threat to human beings and other animals, drugs which can provide therapeutic help could be tested in conjunction with ammonia. Since CNTF is known to prevent loss of motorneurons, it would be worthwhile seeing the effect CNTF will have on the course of development and more especially on the glycogen body. Can survival of ventral motor neurons in lumbosacral from embryonic day 6-12 prevent caving in of neural canal and surrounding glial cells and eventually formation of glycogen body tissue.

In recent years, the use of electron microscope has enabled researchers to study the ultrastructural morphology of various tissues. Consequently many reports on electron microscopy of glycogen body cells have

appeared. Matulonis (1972) carefully worked out the morphology of glycogen body cells in embryonic chicks (stage 32-44) and found a paucity of smooth endoplasmic reticulum in their cells. Later studies by DeGennaro and Benzo (1976, 1991) on day old chicks have shown some of the glycogen body cells to contain large number of smooth endoplasmic reticulum and based on these findings they have postulated that the cells play a functional role in the synthesis of lipids for the formation of myelin in the developing nervous system. There have been reports emphasising astrocytes to play a secretory role in the C.N.S.. Glycogen body being a specialized neuroglial astrocyte, work was aimed at understanding its ultrastructural details. An abundance of secretory vesicles in different formative stages were visible amidst the golgi complex in many of the cells. Histochemical observation made on glycogen body revealed the perinuclear space to contain RNA. The results of the present ultrastructural study on two week post-natal chick and adult pigeon are more in line with the conclusions drawn by Matulonis (1972). It seems that glycogen body is involved in production of various components that constitute the extracellular matrix. Future work aimed at discerning the nature of such vesicles using specific stains for electron microscopy

should be tried out. Even use of certain tracer dyes like lanthanum would help in better understanding of their cell morphology.

Lately glycogen body tissue has been used for the study of sublethal effects of sodium fluoride on various metabolic parameters in developing *ex-ovo* chicks (1-30 days of age) of domestic fowl. It was observed that glycogen showed a decline, with SDH activity also decreasing. On the other hand, LDH activity was seen to increase towards the end of experimentation. Thus the results of this study is indicative of an altered oxidative metabolism.

The present work is an attempt to shift the focus on certain aspects not explored earlier. Instead of viewing the glycogen body as a specialized tissue, functioning as a supplier of lipids during early embryonic development, it should be perceived as a mere mass of ordinary unspecialized glial cells that perform the routine, mundane, day-to-day functions carried out by any other glial cell. The mass initially might have evolved as a means to withstand the adverse internal environment the embryo was faced with, rather than because of any specific program. Having formed such a mass, and being

placed away from the main stream of neurons, its only function seems to be to help C. N. S. in detoxification process, store glycogen and supply glucose as and when required or supply oligosaccharides or proteoglycans or glycoproteins as per needs, in short, to take the burden off the other glial cells which associate themselves with specific groups of neurons.