CHAPTER 3

PATTERN OF AMINO ACIDS IN THE GLYCOGEN BODY AND SPINAL CORD OF 2 WEEK OLD DEVELOPING CHICKS OF DOMESTIC FOWL, *GALLUS DOMESTICUS*.

That the glycogen body cells supply glucose to the CSF and also has a limited capacity to detoxify ammonia is the contention of Syeda (1989). The enzyme studies carried out on glycogen body and various regions of spinal cord of 2 and 10 day old chick (Nene and Syeda, 1993) revealed a moderate activity of glutamate dehydrogenase (GDH) and relatively high activity of glutamine synthetase. Further work on dehydrogenases wherein a moderate activity of isocitrate dehydrogenase, a low activity of succinate dehydrogenase and high activity of malate dehydrogenase indicated the siphoning of isocitrate from TCA cycle for the generation of glutamate during GDH action (Syeda, 1989). Moreover, high aspartate aminotransferase as against alanine aminotransferase was indicative of an operative malate-aspartate shuttle (Syeda, 1989). Based on these observations it was felt that the determination of amino acids (both free and bound) in the glycogen body tissue would be of interest. The following is an attempt to study the amino acids of the glycogen body and compare them with the two regions of spinal cord *viz.* lumbosacral

and cervical of the 2 week old (15 days post-hatched) chicks of domestic fowl, *Gallus domesticus* using thin layer chromatography (TLC). These two regions were selected due to the fact that the former has greater population of large-sized ventral motor and other neurons and comparatively fewer ascending and descending fibre tracts while the latter has comparatively fewer number of neurons and many more of these fibre tracts.

The most basic and yet a neglected issue, so far as the available information on glycogen body is concerned is its water content. The same has been worked in glycogen body of 15 and 50 days post-hatched chicks.

MATERIAL AND METHODS

Day old chicks of domestic fowl, *Gallus domesticus* were purchased from local Government poultry farm. They were placed in large metal cages till the day of experimentation. The chicks were provided chick mash and water on an *ad libitum* basis.

Amino acid Analysis :

Sample preparation - 15 day old chicks were decapitated and the tissues, namely, glycogen body, lumbosacral and

cervical spinal cord were quickly dissected out and placed in the freezer till the time of homogenization. Since the glycogen body tissue mass is very little, material had to be pooled from an average of 10 - 15 chicks for the preparation of sample solution. In case of spinal cord, however, pooling of tissues from only 4 animals sufficed. The tissue samples for both free and bound amino acids were homogenized in cold distilled water (approx. 1:4 w/v). TLC was performed using the procedure as described by Stahl (1969).

Determination of free amino acids :

The tissue samples after homogenization were mixed with acetone (twice the amount of distilled water in tissue sample) and allowed to stand in refrigerator for few hours. The use of acetone during sample preparation removes the protein and polysaccharides from the tissue samples (Hais and Macek, 1958 and Crawford, 1968). The tissue extracts were then centrifuged at 3000 r.p.m for 15 minutes, following which the supernatant liquid were washed again in ice cold acetone, evaporated and resuspended in 80% alcohol as described by Awapara (1948). For removal of lipids from the tissue samples, the supernatant liquid in 80% alcohol was extracted by vigorous shaking with 3 parts of chloroform by volume and

allowed to stand for few hours. The upper aqueous phase containing amino acids were taken for amino acid analysis after placing them in an oven at 65°C for evaporation to near dryness. Finally prior to loading of samples onto TLC plates, 1 to 2 drops of 0.1N HCl was added.

Determination of low M.W. peptides in glycogen body :

The tissue was homogenized as explained above and centrifuged. The supernatant fluid was collected and evaporated to dryness. The dried residue was suspended in 5 ml of 6N HCl and hydrolysis carried out at 100°C for 2-3 hours. The sample was then cooled and dried in the oven at 65°C. The hydrolysed sample of glycogen body was then washed twice in distilled water to remove impurities before loading of samples on TLC plates.

For thin layer chromatography silica gel G was used as an adsorbent. The solvent system found suitable for the development of chromatograms were n-butanol-acetic acid-water used as first solvent system (60:20:20 v/v/v) and phenol-water (75:25 v/v) used as second solvent system, re-run in the same direction as the first. The developed chromatograms were dried and sprayed with 0.25% (w/v) ninhydrin (*triketohydrindene hydrate*) in acetone and set in

the dark for a standardized interval until the colour had developed. The different amino acids in glycogen body, lumbosacral and cervical spinal cords were identified by co-chromatography with authentic samples.

Water content of Glycogen body :

15 day old ~~ex-ovo~~ chicks and 50 day old juvenile chicken were used for the determination of total water content of glycogen body. Glycogen bodies were then quickly dissected out from the birds and used for desiccation determination by placing them dried preweighed vial and the wet weight of glycogen body noted down. The tissue was then placed in an oven at 100°C, weighed at intervals till complete drying to constant weight was achieved. Water content of glycogen body is expressed as percent wet weight of the samples.

RESULTS

The quantitation of amino acids was done based roughly on the area of the various spots on the chromatograms. The colour reactions of amino acids detected on thin layer chromatograms using ninhydrin reaction in the glycogen body, lumbosacral and cervical regions of the spinal cord of 15 day old chicks are depicted in table 1. The amino

Table 1: Amino acids detected on Thin Layer Chromatogram (silica gel G) using the ninhydrin reaction, in glycogen body (GB), lumbosacral (LS) and cervical (CR) regions of the spinal cord of 15 day post-hatched chicks of domestic fowl, *Gallus domesticus*.

AMINO ACIDS	UNHYDROLYSED			HYDROLYSED
	GB	LS	CR	GB
Alanine	+	+++	++	++
Arginine	+	++	+	++
Asparagine	++	++	+	-
Aspartate	+++	+++	+++	++++
Cysteine	++	++	+	+++
Glutamine	++	++	+++	-
Glutamate	+++	++++	+++	++++
Glycine	++	++	++	+++
GABA	-	+++	++	-
Histidine	+	++	+	+
Lysine	+	++	++	++
Methionine	-	++	+	++
Phenylalanine	-	++	+	++
Serine	+	+	+	++
Peptide 1	-ve	+ve	-ve	-ve
Peptide 2	-ve	-ve	+ve	-ve

The amount of tissue taken was different in each case. The values given are therefore not comparable between tissues. (+) = low, (++) = moderate, (+++) = high and (++++) = very high

acids that were detected in high concentration in free state in glycogen body were glutamate and aspartate. On the other hand, amino acids glycine, cysteine, asparagine, glutamine and arginine appeared in moderate quantities. Low levels of alanine, serine, lysine and histidine were also detected in the glycogen body. The pattern of free amino acids in the lumbosacral and cervical spinal cord were more or less similar to that of glycogen body except for the presence of amino acid phenylalanine, methionine and gamma amino butyric acid (GABA).

The pattern of amino acids in hydrolysed samples of glycogen body differed from that of free amino acids in glycogen body (Table 1) in 3 ways (1) There were present additional amino acids *viz.* phenylalanine, methionine and GABA. (2) Glutamine and asparagine were absent altogether and (3) Certain amino acids such as glutamate, aspartate, glycine, alanine, cysteine and lysine were present in increased conentrations. The presence of spots of low Rf in chromatograms and the appearance of additional amino acids in hydrolysed extracts of glycogen body indicates the presence of low molecular weight peptides.

As far as the water content of glycogen body tissue is concerned, the same was seen to decrease from 15 to 50 day

old chicks [$p<0.01$]. Water content of 50 day old chicks were 60.40 % ($\pm$ 2.49, n=10) of its wet tissue weight while that of 15 day old chick was 63.70 % ($\pm$ 2.59, n=8).

DISCUSSION

The data obtained on amino acids in the glycogen body using TLC confirms the suggestion of Syeda (1989). Quantitation of free amino acids revealed glutamate and aspartate in high concentrations, with alanine in low quantities which does indicate the functioning of malate-aspartate shuttle in the glycogen body. Moreover, the amino acids glutamine and aspartate are needed for the synthesis of uridine which is required for the formation of UDPG and thus in the synthesis of glycogen and hexosamines.

The occurrence of amino acids glutamate, cysteine and glycine in free state and their increased concentration in the hydrolysed extracts probably indicate the presence of glutathione in the glycogen body tissue. Glutathione, a tripeptide found in all life forms is required especially for the maintenance of optimum redox potential in the cell thus preventing peroxide formation in membranes.

Glutathione also plays an important role in transportation of amino acids through membranes. According to De Gennaro (1982), the major metabolic activity in the glycogen body involves the degradation of glucose *via* the hexose monophosphate shunt pathway. Benzo *et al.* (1975) have shown the glycogen body of both the embryonic and day old post-hatched chicks to possess high activities of enzyme glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The activites of both the enzymes were as much as 2 to 5 times more than that of liver and skeletal muscle respectively. Based on these findings, they assumed the glycogen body to generate sufficient amounts of NADPH, required for the biosynthesis of fatty acids. It seems possible that the NADPH produced as a result of pentose phosphate shunt may also be getting used for the reduction of oxidised glutathione (G-S-S-G) to reduced glutathione (2G-SH), which in turn would eradicate oxygen radicals especially superoxide (O_2^-) and singlet oxygen (O_2) that cause cellular damage.

It is also probable that the tissue relies on HMP shunt for yielding pentoses needed for the synthesis of nucleotides and nucleic acid especially RNA. Histochemical localization of RNA (chapter 5) have shown it to occur in large amounts in the perinuclear space. Ribosomes, both in

free and polysomal cluster have been found to occur in the perinuclear space (Matulionis, 1972; Lyser, 1973). The presence of ribosomes in free state would indicate their role in formation of proteins (enzymes) for the intracellular use. On the other hand, occurrence of ribosomes on endoplasmic reticulum, would indicate their involvement in the synthesis of proteins for export from the cells. During the stages of development, the processing of pre-r-RNA and t-RNAs involves its methylation. S- adenosyl methionine, the active form of methionine acts as the methyl donor during the processing of pre-r-RNA in the nucleolus, which prevent the molecule from degradation. Methionine is very essential during the early stages of development of glycogen body. However, its absence in free state in the glycogen body of 2 week old chicken does not mean that they have no role in methylation. The abundance of ribosomes in adult birds provides proof that some amount of r-RNA must be getting synthesized in the cells. Hydrolysis of glycogen body tissue yielded methionine.

Presence of amino acids glutamate and aspartate in abundance suggests that they are being synthesized de novo in this tissue. Occurrence of amino acid serine in low levels and glycine in moderate quantities may indicate

its use in the synthesis of tetrahydrofolate which is required for the thymidine and purine nucleotides during the early stages of development (i.e cell multiplication and growth) of glycogen body. Terni (1924) and Watterson (1949) observed rapid increase in the number of glycogen body cells in the roof plate of avian nerve cord between 10-14 days of incubation, with peak mitotic activity occurring at about 12 days after which the rate of proliferation was seen to drop. Low levels of serine in free pool amino acids of glycogen body indicates that some amount of synthesis of folate may be occurring.

Further work will be needed to find out what peptides have contributed to increase in the pool of free amino acids; glutathione certainly seems to be one of them. One must also look for the presence of amino acids not covered here, amino acids such as taurine for example which is known to be present in increased amounts in the brain.