CHAPTER 1

ABSENCE OF DIURNAL VARIATION IN GLYCOGEN LEVELS OF GLYCOGEN BODY OF ADULT FERAL PIGEON, *COLUMBA LIVIA* DURING THE WARMER MONTHS.

Glycogen body is an oval, pearl-like aggregation of glycogen loaded astroglial cells, found in the mid-dorsal region of lumbosacral spinal cord of birds. The presence of such enormous quantities of glycogen in its cells have intrigued many. Attempts made at identifying the factors that would either increase or decrease the glycogen load of this tissue have not met with success. The majority of workers are of the view that the load remains unaffected after exposure of the animal to various hormones. Thus. glycogen content of glycogen body of the birds treated with either epinephrine or glucagon(Hazelwood *et al.*,1962; Snedecor *et al.*, 1961), insulin (Anderson and Hazelwood, 1969; Hazelwood and Barksdale, 1970) or thyroid hormone (Snedecor and King, 1964; Grandhi *et al.*, 1973) remains unchanged. On the other hand Buschiazzo *et al.* (1964) reported an increase after prolonged administration of epinephrine and glucocorticoids to adult ducks. Similarly, majority believe that starvation of birds for an extended period does not bring about reduction in glycogen levels of this tissue (Doyle and Watterson, 1949; Szepsenwohl and Michalski, 1951; Smith and Geiger, 1961; Hazelwood *et al.*,

1963; Houska *et al.*, 1969). Contrary to this, Graber *et al.* (1973) and Kundu and Bose (1974) have reported loss of glycogen in starved chicks. Since the reported results about the glycogen load of this tissue following various experimental manipulation has been controversial glycogen body remains an enigma to those working on the biochemical aspect of this tissue. However, the presence of glycogen in its cells does indicate some functional significance of this tissue in the central nervous system of birds. As of date, the exact role of glycogen body is not known.

Diurnal rhythms in various metabolic systems have been reported in many species but very few studies have been conducted in birds. Raheja (1973) has failed to report any diurnal variation in the liver glycogen levels in 5 week old chicks, which is in contrast to the findings of Twiest and Smith (1970) who reported a significantly higher plasma glucose levels during the day hours than during night hours. Raheja (1973) is of the view that the variation observed may be due to differences in feeding activity rather than due to light-dark cycle. Nene and Syeda (1988) have shown glycogen content of glycogen body of adult blue rock pigeon to show diurnal variation during the warmer months. Subsequent study of Syeda and Nene (1990), conducted during the colder months failed to show

any significant alteration in the levels of glycogen in the glycogen body. These studies were however, based on results obtained during only one season. Moreover, the reported results during the warmer months were quite high when compared to those obtained during the colder months. Since the values obtained during the summer months did not seem convincing, it became necessary to reassess the possible diurnal variation if any, in the glycogen content of glycogen body of adult feral pigeon, *Columba livia* during the warmer months of two successive years (1993 and 1994).

MATERIAL AND METHOD

Experimental protocol observed was same as that adopted by earlier worker (Syeda, 1989) except at places indicated by asterisk (*). Healthy adult blue rock pigeon (*Columba livia*) of both sexes weighing between 260-300 g were used for the present analysis. Birds were procured in the months of September-November of 1993 and June-August 1994. They were placed in large cages, fed jowar and bajra grains and water *ad libitum*. The ambient maximum and minimum temperature range being 32-36^{o}C and 23-26^{o}C. Pigeons were expurgated and later exsanguinated at 4 hourly interval ($\pm$ 20 min), the timings being 22-00, 02-00, 06-00, 10-00, 14-00 and 18-00 hours respectively.

Glycogen body was instantly taken for the analysis of glycogen. Since this tissue is flimsy and minute, it had to be taken on a preweighed coverslip*, blotted free of tissue fluids, weighed on an electronic balance* and the coverslip along with the attached* tissue dropped into 30% KOH to be processed for quantitative estimation of glycogen by employing the Anthrone method of Seifter *et al.* (1950). The absorbance was read at 620 nm on a UV-VIS spectrophotometer* Sicopec 200GL and the glycogen content of glycogen body expressed as mg glycogen per 100 mg wet tissue weight.

RESULTS

Table 1 indicates the glycogen levels of glycogen body of adult feral pigeon, *Columba livia* during the warmer months of 1993 and 1994. Results depict that the maximum and minimum temperature to which the birds were exposed during the two successive years almost matched with one another. Analogous to this, the respective values of glycogen content in the glycogen body of pigeon during night hours of both the years also correspond with one another (Table 1). Except for a slight drop in value at 14-00 hour, the glycogen values remained steady all throughout the 24 hour period.

Table 1 : Changes in glycogen content of Glycogen body of blue rock pigeon (*Columba livia*) during warmer months of 1993 (Max. Temp range between 32-36°C and Min. Temp range between 23-26°C) and 1994 (Max. Temp range between 30-32°C and Min. Temp range between 24-26°C).

Time (Hours)	Sept-Nov 1993	June-Aug 1994
22-00	16.20 ±1.018	14.88 ±2.237
02-00	15.37 ±2.547	14.43 ±2.598
06-00	15.90 ±2.684	14.79 ±1.465
10-00	-	13.99 ±1.330
14-00	-	# 12.50 ±0.601[a]
18-00	-	14.34 ±2.620

All values expressed are mean ± SD of 6-8 birds except # where n=2. Glycogen content of glycogen body is expressed as mg glycogen/ 100 mg wet tissue weight. Values significantly different from 22-00 hrs of 1994 by 't' test is indicated as [a]$p < 0.02$, rest of the values are nonsignificant.

DISCUSSION

Two differences become apparent when the results reported herein are compared with those reported earlier (Nene and Syeda, 1988). First, the maximum ambient temperature then varied between 38 and 42°C and minimum between 24 and 27°C. Secondly, the glycogen values obtained by earlier workers were considerably higher. On close scrutiny it appears that these high values of glycogen should be ascribed to faulty procedure rather than to high temperature as claimed by these workers. A later report of theirs (Syeda and Nene, 1990) concerning glycogen content during colder months indicate lower values. Nonetheless, despite the lower ambient temperature (minimum range being 16-18°C), the glycogen values appeared to be quite high.

Unlike in the previous study wherein steady level throughout the day followed by a rise in glycogen content during the night hours with attainment of peak values at 02-00 hour and lowest values at 06-00 hour were observed (Nene and Syeda, 1988), the present work did not reveal a change in either direction throughout the darker half of the day, the glycogen values remaining almost steady between 22-00 and 18-00 hours. The difference in results obtained in the previous and the present work can be attributed to improper method adopted in the former work

during the processing of samples rather than as a response of the bird to a higher ambient temperature. In the earlier work, all the samples collected at any one time (say 22-00 hour) formed one batch whereas in the present study, all the samples collected during the 24 hour period were processed simultaneously as one batch. On doing this, the difference in values obtained during different times of the day as reported in previous work disappeared. It is of interest that these workers in their later publication (Syeda and Nene, 1990), report maintenance of steady levels of glycogen during the night hours of colder months, the glycogen values although not as high as reported earlier were higher nonetheless. This discrepancy is not seen in their work on muscle in which several samples were processed simultaneously and therefore, there was randomness in processing of the samples. Moreover, the tissue wet weight was enormous by comparison and glycogen content of the tissue was also much less. Thus, in response to detection of certain flaws which had crept in the earlier work measures were taken during the present study to remove them and to check whether glycogen values did in fact vary during the night hours of summer months. The results obtained after making certain alterations in experimental protocol as well as by resorting to processing of the samples simultaneously clearly establish

the absence of any variation in glycogen content of glycogen body during night hours of summer months. Having established the nonvarying nature of glycogen levels during 24 hour period in glycogen body cells one is led to consider the possible role played by glycogen body tissue in the C. N. S. metabolism and body metabolism in general.

Snedecor *et al.* (1963) have shown that C^{14} labelled glycosyl units are incorporated as well as mobilized from glycogen of glycogen body tissue. The presence of phosphorylase and glycogen synthetase in this tissue has also been demonstrated biochemically (Benzo and DeGennaro, 1974; Fink *et al.*, 1975). This means that per 6-10 Glucose-1-Phosphate (G-1-P) units released by phosphorylase from time to time at least one glucose will be released by debrancher. Released G-1-P may be committed either to Embden Meyerhof Pathway or hexose monophosphate shunt. Lactate produced by the former pathway will be either used by the cell itself or it may be sent out. It is possible that in birds from colder countries use phosphorylation of Fructose-6-phosphate to Fructose-1,6-diphosphate as a heat generating system. F-1,6-diphosphate so formed would be hydrolysed to Fructose-6-phosphate by a phosphatase to get phosphorylated to F-

1,6-diphosphate once again. The process when repeated in succession can generate enough heat to keep the C.N.S. warm.

In vitro studies of Lomako *et al.* (1993) on biogenesis of glycogen in the astrocyte of the newborn rat brain have brought forth the concept of glycogenin (Kennedy *et al.*, 1985), a protein primer that covalently binds the glycogen molecule. They found glycogen to be absent in the astrocytes when exhausted of glucose in the surrounding medium. When the astrocytes were placed in a medium containing ammonium chloride and glucose, synthesis did occur but to a limited extent that is, up to proglycogen (low-molecular-weight form). However, in the unexposed astrocytes the synthesis of glycogen occurred unimpeded so as to form macroglycogen. This result raises the question as to which type of two glycogens exist in the glycogen body.

Since there is no alteration in glycogen content of this tissue either during day or night hours and the fact that the enzyme glucose-6-phosphatase (Benzo *et al.*, 1975; Fink *et al.*, 1975) is absent from this tissue should not mean that the tissue is unable to supply glucose to the surrounding neuronal elements. Glycogen body is known to

have as much activity of debranching enzyme as in liver (Fink *et al.*, 1975) which is known to be a releaser of glucose. However, unlike liver which is able to deliver glucose in large quantities, this tissue may be in a position to release a steady stream of glucose to the CSF though of course in much smaller quantities. Present study is in agreement with the earlier hypothesis that glycogen body may be functioning as a supplier of glucose to CSF(Smith and Geiger, 1961; Welsch and Wachtler, 1969). There is also a strong possibility of the glucose-1-phosphate molecule, released as a result of glycogen breakdown, to be getting used in the synthesis of amino- and acetyl-substituted sugar derivatives needed for the formation of oligosaccharides such as glycosaminoglycans. These sugar derivatives serve a variety of important functions including their role as constituents of extracellular materials (chapter 5).