CHAPTER 2

LACTATE DEHYDROGENASE ISOENZYMES IN THE GLYCOGEN BODY AND SPINAL CORD DURING POST-HATCHED DEVELOPMENT OF DOMESTIC FOWL *GALLUS DOMESTICUS* AND ADULT FERAL PIGEON *COLUMBA LIVIA*.

Lactate dehydrogenase (LDH;L-lactate : NAD-oxidoreductase, E.C.1.1.1.27) is known to occur in 5 molecular forms in most avian and mammalian species (Markert and Moller, 1959). Nearly every tissue of adult mice have all 5 LDH isoenzyme but in different proportions (Markert and Ursprung, 1962). The distribution pattern of these 5 isoenzyme types varies in different tissues and also varies during different developmental stages (Cahn *et al.*, 1962; Markert and Ursprung, 1962; Markert, 1963). Glycogen body, a unique structure found only in birds and composed of an astroglial mass is found in the lumbosacral spinal cord. Its cells are loaded with enormous quantities of glycogen, the amount present being many fold more even to that in liver. Studies by Friede and Vossler (1964) have demonstrated histochemically the presence of lactate dehydrogenase activity in the glycogen body of turkey. Syeda (1989) has shown the glycogen body of 2 and 10 day old neonatal chicks of fowl to have a very high LDH activity. Anterior thoracic (AT) and lumbosacral (LS) regions of spinal cord which are rich in neurons but

poor in nerve tracts also were shown to have greater LDH activity to that of posterior thoracic (PT) and cervical (CR) regions, poorer in neurons but richer in nerve tracts. Based on these observations it was decided to carry out LDH electrophoresis to see whether there is any difference in the isoenzyme distribution in glycogen body, a primarily astroglial mass and spinal cord - a mix of neurons and glia. It was also decided to see whether this pattern changed during different stages of post-hatched development in glycogen body and spinal cord of domestic fowl, *Gallus domesticus*. For comparison, same tissues from adults of blue rock pigeon *Columba livia* were also studied.

MATERIALS AND METHOD

Day old chicks of domestic fowl, Rhode Island Red variety (*Gallus domesticus*) were brought from a nearby poultry farm and maintained in large cages, fed chick mash and water on *ad libitum* basis till the end of experimentation. On days 1, 5, 10, 20, 30, 40 , 50 (juvenile) and adult birds were killed for experimentation. Adult healthy, feral pigeons were also used for the present study. Tissues, namely, glycogen body (GB) and four regions of spinal cord *viz.* cervical (CR), anterior thoracic (AT),

posterior thoracic (PT) and lumbosacral (LS) as defined by Syeda (1989) were quickly removed and homogenized in cold distilled water. M. *adductor longus* which yields 3 anodal bands (Syeda, 1989) was also processed and run simultaneously as a reference material. Later the samples were subjected to centriugation at 2,330 r.p.m in a refrigerated centrifuge for 30 minutes and the clear supernatant used for LDH vertical slab gel as well as tube gel polyacrylamide electrophoresis. The method adopted was that of Dietz and Labrano, (1967) as modified by McIndoe and Mitchell, (1978). Of the various buffers with different pH tried out, citrate phosphate buffer (pH 7.5) was found to be better suited as electrode buffer yielding far better results and accordingly used in studies on fowl. Tris-glycine buffer (pH 8.3) prescribed by Dietz and Labrano (1967) served well for tissues from pigeon. Electrophoretograms obtained from tissues of a minimum of 5 different animals from both species were studied. Stained gels were either photographed immediately or a hand drawn representation of the staining pattern of gels was made immediately. To ascertain that the staining was due to lactate dehydrogenase, some of the gels after electrophoretic run were also incubated in substrate blank incubation medium. Since glycogen body tissue yielded staining in interband and other areas in

addition to the usual bands, it was decided to stain GB by incubating it in a medium which contained urea, a compound known to inactivate LDH enzyme having one or more M subunits (van Wijhe *et al.*, 1964).

RESULTS

Throughout the post-hatched development, glycogen body of *Gallus* yielded anodal bands 1 and 2 representing H_4 and H_3M LDH isoenzyme types (Fig.1, 4, 6, 8 and 9). Darkly stained material was observed in the interband area (i.e between band 1 and 2) and anodal side of anodal band 1 or pre-band 1 regions in the case of fowl, with the result, discrete bands were not discernible (Fig. 6 and 8). On staining the gel in a medium containing urea, GB yielded a cleaner picture, for, band 2 and the interband material did not appear at all: only band 1 (H_4) was present. [Fig. 6].

In adult pigeon, the glycogen body shows presence of 3 anodal LDH isoenzyme bands instead of two of domestic fowl (Fig. 5 and 7). Pigeon glycogen body showed similar results to that of fowl in that interband material was present between bands 1, 2 and 3. However, it was much lighter as compared to that of fowl. A noteworthy

PLATE I

Figs 1-6. Electrophoretograms of lactate dehydrogenase isoenzymes in glycogen body and spinal cord of developing chicks and adult of domestic fowl and adult pigeon.

Fig. 1 Gels of glycogen body (GB), LS and AT regions of spinal cord and reference material (R) from 10 day post-hatched chick. Note the presence of interband and pre-band 1 in GB.

Fig. 2 LS, AT, PT and CR from 20 day old chick. 3 bands are visible in all four regions of spinal cord.

Fig. 3 CR, LS, AT and PT of 40 day old growing chick.

Fig. 4 LS, GB, AT and CR from juvenile chicken.

Fig. 5 Gels of CR, AT, PT, LS and GB from adult pigeon. Note that GB shows 3 bands with light staining pre-band 1 while spinal cord regions show presence of 4 bands.

Fig. 6 Electrophoretogram of glycogen body of adult fowl. The gel on the left is of urea treated GB.

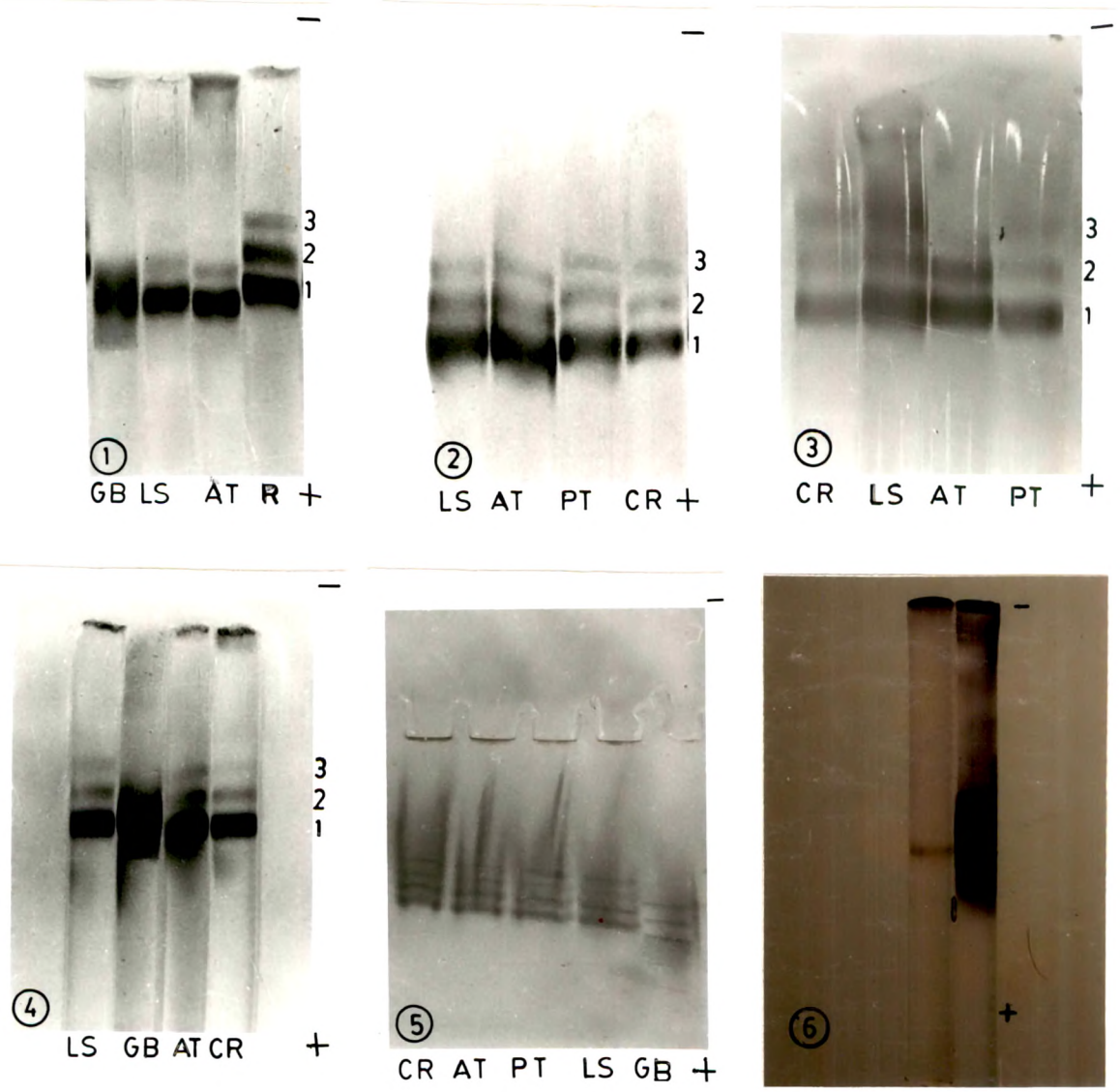


PLATE 1

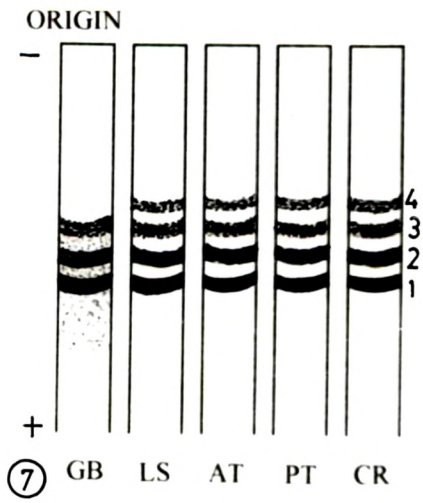
difference in the staining pattern of GB from the rest of the tissues is the presence of a thick additional faint band towards anodal side of anodal band 1 or pre-band 1 (Fig. 6, 7 and 8). In the glycogen body of post-hatched developing and adult fowl this staining material was darker in appearance while in the adult pigeon it was lighter (Fig. 7 and 8). Gels incubated in the substrate blank incubation medium did not yield any reaction, the usual as well as additional bands both being absent.

In fowl, all the four regions of spinal cord, also yielded these two bands till 10 days of *ex-ovo* development (Fig. 8); however, 3rd band representing H_2M_2 molecular species appeared on 20th day and persisted till the rest of the development (Fig. 2, 3, 4 and 9). Anodal band 1 is very thick in LS, the width of band 2 and 3 being almost half the width of band 1. In the remaining three regions of spinal cord (CR, AT and PT) the width of all the bands were more or less alike. Staining intensity of 2nd and 3rd band in all the regions of spinal cord is lighter than that of the 1st band. All the regions of spinal cord of adult pigeon exhibited presence of 4 LDH isoenzyme bands in place of three of fowl(Fig. 5 and 7), the 1st being darker than the rest. The staining intensity was seen to become lighter in decreasing order from band 1 to 4.

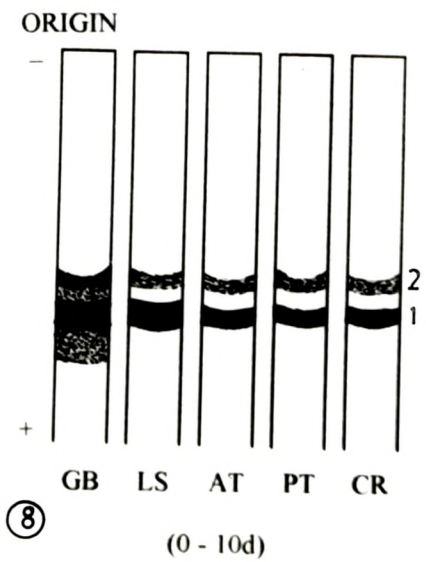
PLATE II

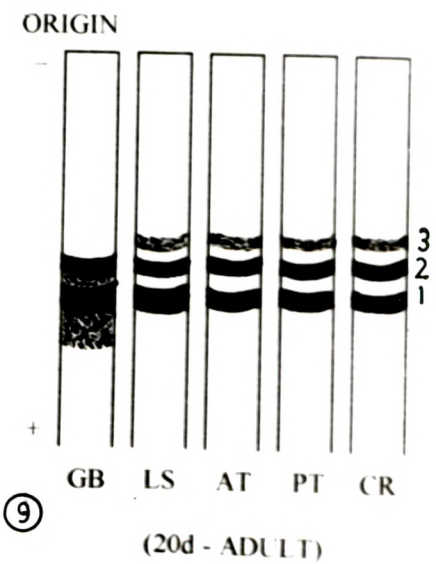
Figs 7-9 Zymograms of lactate dehydrogenase isoenzymes in glycogen body (GB) and four regions of spinal cord *viz.* lumbosacral (LS), anterior thoracic (AT), posterior thoracic (PT) and cervical (CR) of developing and adult of domestic fowl and pigeon.

ELECTROPHORETOGRAM OF LACTATE DEHYDROGENASE ISOENZYMES OF DOMESTIC FOWL AND FERAL PIGEON



PIGEON





FOWL

PLATE 2

DISCUSSION

Though heterogeneous in cell population, glycogen body comprises mainly of astroglial cells. The other cell types or cell parts present are a few axons, accompanying oligodendroglia, axon terminals, microglia and cells forming and from blood vessels and capillaries. In contrast to this, spinal cord will not only have all these cell types but also cell bodies of neurons - the main cell type not represented in glycogen body. Therefore, any difference in pattern of distribution of LDH isoenzyme types represented in the form of specific stained bands in these two set of tissues *viz.* the glycogen body and the spinal cord should be ascribed mainly to neuronal cell population and to some extent to oligodendroglia.

Throughout the post-hatched development, glycogen body of fowl yielded anodal bands 1 and 2 representing H_4 and H_3M isoenzyme types (Fig. 1, 4, 6, 8 and 9). On the contrary, all the four regions of spinal cord namely CR, AT, PT and LS did yield these two bands till 10 days of ex-ovo development, a 3rd band representing H_2M_2 molecular species appeared on 20th day in them and persisted till the rest of the development as also in adult fowl (Fig. 2, 3, 4, and 9). Absence of anodal band 3

representing H_2M_2 in glycogen body till day 50 and onwards denotes the absence of these isoenzyme type in the astroglial cells of at least glycogen body. Their presence in spinal cord therefore could be ascribed to nonastroglial material, namely, neuronal cell bodies and their axons as well as the accompanying oligodendroglia that envelope axons. Furthermore, slight difference observed either in relative staining intensity or width of each band should be considered not only as an index of number of enzyme molecules present in different parts of spinal cord but will also be indicative of the type of cells and relative numbers in which they are represented in respective regions. Despite the presence of different cell types, only first and second anodal bands appear upto 10 days [illegible] development in spinal cord of fowl indicates that neuronal cells are yet not fully differentiated and it is only by 20th day that a 3rd band appears and lasts till the completion of development and also persists in adults. As seen earlier, anodal band 1 is very thick in LS, the width of band 2 and 3 being almost half the width of band 1. The isoenzyme distribution pattern in the rest of spinal cord (CR, AT and PT) almost matches with that of LS except that bands 1 and 2 are uniformly thick and their thickness matches with that of band 2 of LS. The reason for LS having thickest band 1 could be due either to the presence of astroglial cell

mass which forms the tip of the wedge shaped glycogen body around the central canal of lumbosacral spinal cord or due to the presence of astroglia that surrond synapses. In the lumbosacral region, an enormous number of neuronal cell bodies for controlling hind limb musculature are present. Each cell body either directly or through its dendrites is expected to have even greater number of synapses. each being associated with several astrocytes. The total number of astrocytes present would thus be enormous. The additional mass of these astroglial cells probably adds H_4 type of molecules to the sample preparation and hence a wider band 1. Since AT houses neurons that control wing musculature it will also have enormous number of neuronal cell bodies, synapses and astrocytes. Despite this it does not yield a thick band 1. Here it has to be noted that the *proportion* of different cell types also influences the electrophoretic pattern. Unlike in LS, in this part of spinal cord ascending and descending tracts being more, relative proportion of axons, myelin and oligodendroglia is more as compared with the one in LS. CR and PT will have neurons controlling neck muscles and dorsal body wall muscles respectively as also ascending and descending nerve tracts producing an electrophoretogram pattern similar to the one in AT. A pattern more or less similar to the one in adult fowl was

also observable in glycogen body and four regions of spinal cord of pigeon. Glycogen body in the adult of pigeon had 3 anodal band while four bands were discernible in all the regions of spinal cord. Glycogen body of both fowl and pigeon showed presence of a thick but uniform band on the anodal side of anodal band 1 or pre-band 1. However, the glycogen body of developing and adult fowl had this material darker in appearance while in the adult pigeon it was lighter. Zinkham *et al.* (1966) who studied several different species of pigeon showed occurrence of two extra bands beyond anodal band 1 towards anode in heart but not any other somatic tissues of pigeon. According to them, the occurrence of these bands were due to the presence of allele of B unit (H) namely B' in the wild pigeons. It is known that tissue and organ formation, during early development, is dependent on large scale cell migration. Within C.N.S., it is the astroglia which lay down the foundation and provide a substratum to the neurons and are subsequently overlaid by axonal sprouts that form nerve tracts. Accordingly, during development, between 1 and 10 days after hatching in fowl for instance, it is the bands 1 and 2 which appear first, followed by the appearence of a sharp band 3. It is ill-defined at first but turns into a sharp one by day 20. In pigeon, however, the spinal cord expresses HM_3. It is of

interest that even in developing brain of fowl, a similar sequence in appearance of LDH band 4 (HM_3) follows with the exception of optic lobes and thalamus (which represents the whole of diencephalon and dorso-medial and ventro-medial portion of mid-brain) where a band, namely, M_4 appear on the 20th day, after which the usual 4 band pattern is seen in juvenile and it persists in adult (unpublished data). Similar results were observed in different brain regions of the adult pigeon (unpublished observation).

Data are available on the breast muscle of developing and adult chicken, a muscle made up of only white fibres, where it is band 1 and 2 that develops first and gradually as development progresses, shows presence of bands 3, 4 and 5 and finally in the adult it is the band 5 that persists (Lindsay, 1963). Studies on distribution pattern of LDH in avian skeletal muscle shows a similar trend as seen in the nervous tissue. The skeletal muscle having tonic (Type I) fibres yield anodal bands 1 and 2 of LDH (Bock and Hikida, 1968; Nene and Naik, 1986, 1988; Syeda and Nene, 1989). Muscles containing greater proportion of red fast twitch fibres (Type IIA) yield bands 2 and 3 while those having larger proportion of white fast twitch fibres (Type IIB) show bands 4 and 5 (Nene *et al.*, 1988). If tonic muscle fibres are to be

considered as representative of early stage of differentiation, the red fast twitch (Type IIA/FOG*) as the later and the white fast twitch (Type IIB/FG*) as the latest (Hikida, 1972) then the sequence in which various bands will appear would match with the sequence in which various bands appear in C.N.S..

In conclusion it can be said that as the tissues move from a more oxidative metabolic type to that of glycolytic type, they begin to express the anodal bands 3, 4 and 5 and glycogen body, the only tissue in the nervous system to express staining in the interband and pre-band 1 regions in both the domestic fowl and pigeon exhibits presence of an allele of B unit (H) namely B' similar to tissues such as heart muscle that expresses two additional bands beyond anodal band 1 towards anode in wild pigeons.

*FOG - Fast oxidative glycolytic, FG - Fast glycolytic.