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Studies on lactate dehydrogenase activity on some tissues of a scorpion *Heterometrus swammerdami*

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ABSTRACT

The lactate dehydrogenase activity of a scorpion was studied with reference to the bio chemical activity in different tissues by using gel electrophoresis. The reproductive activity in the ovary and follicles were also studied with regard to the development. The changes was observed even in the different tissues with regard to the enzyme activity

Key words: *Heterometrus swammerdami*, Lactate dehydrogenase, Cuticle, Haemolymph, Hepatopancreas, Muscle, Ovary and Testis.

INTRODUCTION

The biological significance of the occurrence of enzymes in the multiple forms is a problem which has greatly interested biochemists during the past decade. Although there is volume of information regarding the genetic determination of these enzymes, the possible physiological functions of the heteropolymorphic isoenzymes, yet to be precisely determined., the possible role of lactate dehydrogenase are regulators of glycolytic process isoenzymes (LDH) as regulators of oxidative and glycolytic processes has been obtained from tissue culture and play a major role in regulating NAD/NADH and chick embryo studies under aerobic and anaerobic conditions. (Good friend and Kaplan 1964; Bucher & Klingberg 1958; Dawson *et al.,* 1964; Goodfriend *et al.,* 1966; Guttler and Clausen 1969), Market (1963) Gleason *et al*. (1971) studied in detail the isoenzymes of crustaceans. They found that LDH from Barnacles is D (-), while in *Malacostraca* the LDH is D (+). In *Homarus americanus*, a typical 5 band pattern of vertebrate L LDH was obtained for liver tissues. Another decapod crustacean *Emerita asiatica* has been studied for its LDH pattern during different stages of moult cycle, Dhandayuthapani *et al* (1982). Besides crustaceans, arachnids have also been studied for their LDH isoenzymes. Long and Kaplan (1968) in horse shoe crab *Limulus polyphemus* showed D specific dimeric LDH with six electrophoric forms. Using starch gel electrophoresis Gleason *et al.,* (1971) isolated three fractions of LDH isoenzymes in two arachnids *Limulus Polyphemus* and *Dugesiella*.The mobility of these isoenzymes in two species varies greatly. They further showed that in arachnids LDH belongs to D (-) type. This paper deals with LDH isoenzymes of different tissues of a scorpion *Heterometrus swammerdami*. It shows remarkable variations with reference to their forms and function.

MATERIAL AND METHODS

A representative type of scorpion *Heterometrus sammerdami* was used in the present study. The animals were collected around St Thomas mount,10 miles away from the city of Madras. The

animals were reared in the laboratory by keeping them in a glass trough and feeding them with live cockroaches. The animals feeding normally were taken for the present study. The method of Subromonium and sudha varadharajan (1980) was followed for determining the stages of ovary development. Accordingly three stages namely stage I, stage II and stage III were marked and the animals showing peculiar changes to the respective stages were taken for experimental analysis of LDH isoenzymes. Lactate dehydrogenase enzymes were analysed by polyacrylamide gel electrophoresis. The enzyme extracts were prepared following Hunter and Marker (1957).

RESULTS AND DISCUSSION

In order to understand the LDH pattern of different tissues of *Heterometrus swammerdami* the tissue extracts were analysed using polyacrylamide gel electrophoresis. The results obtained are given in the table and electropherograms (Table.1 and Fig.1).

The cuticle of the *Heterometrus swammerdami* shows the presence of intensely stained single LDH fraction. For characterization of subunits of LDH isoenzymes, the method of Johnson et al., (1972) was followed and this single fraction has been designated as A3B1. It is possible that this LDH isoenzyme of cuticle is associated with the hydrolysis of cuticular components during each moult when the old cuticle is digested and new cuticle is formed. In this context, it is of interest to recall the observation of Mcwhinnie and Conkill (1964) that the isoenzymes changes during moult cycle stages are associated with metabolism involving a number of enzymes which may digest the cuticular compensate each moult. The cuticular chemical components including isoenzymes are known to be derived from haemolymph which is bathing the epidermal cells underlying the cuticle. These epidermal cells have been shown to accumulate precursors from the haemolymph and synthesize the cuticular components.

The results obtained on the isoenzymes of haemolymph are interesting. The results of electrophoretic analysis of isoenzymes of haemolymph are given in electropherograms (Table.1 and Fig.1). It is seen that in the haemolymph of *Heterometrus swammerdami* there are five fractions of LDH. Following Ezhilarasi (1982).these fractions were divided into three groups. The fractions which moved up to 1.0cms are characterized as slow moving, the fractions which moved up to 3.0cms as medium moving, and those fractions which moved beyond 3.0 cms are characterized as fast moving fractions. Based on this criterion, it is observed that there are two medium moving and three fast moving fractions of LDH in haemolymph.

The first fraction is designated asA41 the second one as A3B1;the third one as A2B2;the fourth one as A1B3and the fifth one as B4.The results are in agreement with those of Markert (1962) who observed five isoenzyme fractions of mammalian LDH and that each is made up of a group of four subunits. The subunits are polypeptide in nature and hence are written as B4 (LDH -1), A1B3(LDH -2), A2B2(LDH -3), A3B1(LDH -4) and A4(LDH -5). In *H.swammerdami* there are five fractions of which one fraction appears to be significant. The haemolymph (LDH) fraction A3B1 is similar to that of cuticle. It is reasonable to expect the haemolymph which acts as a transporting medium may show the presence of enzymes associated with various physiological process. The haemolymph enzymes and other proteins are known to be derived from other storage tissues such as hepatopancreas and muscle. The work of Goyffon (1970) and Prabhakaran (1973) revealed that the haemolymph of scorpion is acting as a medium through which the metabolites for various tissues are transported. Prabhakaran (1973) who analyzed hepatopancreatic proteins by electrophoresis found that a particular protein fraction synthesized in the hepatopancreas is transported to haemolymph from where it is taken up by the epidermal cells underlying cuticle and used for the formation of new cuticle at each moult**.**

In this study, the hepatopancreas LDH enzymes were analyzed and the results are given in (Table.1 and Fig.1). There are two LDH fractions in the hepatopancreas; one medium moving and another a fast moving one .They are designated as A4 and A2B2. A comparison of the electropherograms of hepatopancreas and

haemolymph clearly shows that the LDH fraction A4 resembles in both that the hepatopancreatic protein may be released into the haemolymph has been shown in a scorpion *Palaemneus swammerdami* by Prabakaran (1973). He reveled that that the hepatopancreatic protein may act as a precursor, which may be released into the haemolymph from where it may be transported into

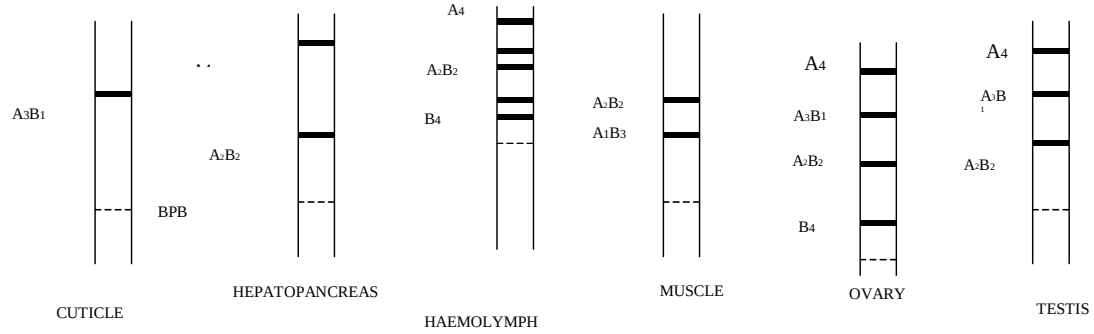

Fig. 1: Electropherograms showing the LDH Pattern in different tissues

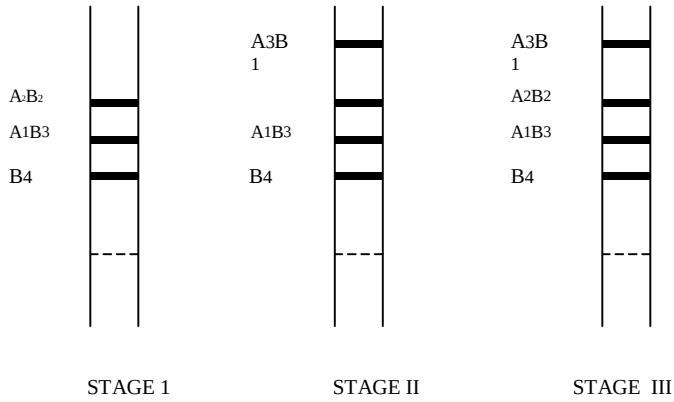

Fig. 2: Electropherograms showing the LDH Pattern and stages of ovary

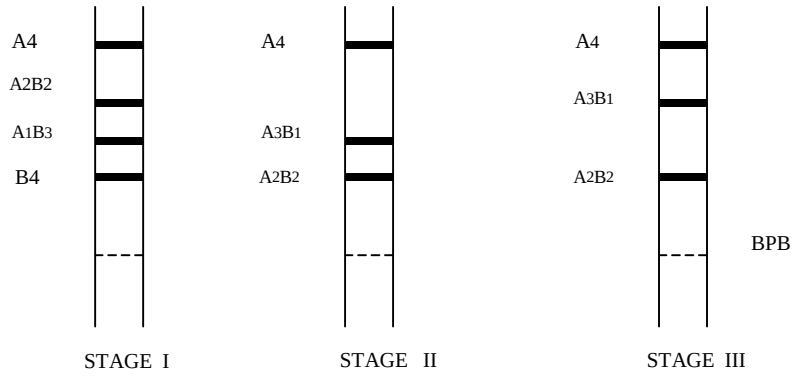

Fig. 3: Electropherograms showing the LDH Pattern in follicle region

the various tissues. The fact that the A4. LDH fraction of haemolymph is similar to A4 fraction of hepatopancreas suggests that this LDH fraction of haemolymph would have been derived from the hepatopancreas.

In addition to hepatopancreas, muscle is also known to be a storage site in arthropods.(Renaued 1949) The muscle tissue was also subjected to LDH analysis and the results are given in (Table.1 and Fig.1). In *H. swammerdami* there are two LDH fractions designated as A2B2 and A1B3 in the muscle denoted as fast moving groups. (Carlsson and Gade 1985) analysed LDH from the muscle tissues of an arachnid, *Limulus polyphemus* and compared it with that of the hepatopancreas and observed that the two muscle isoenzymes were bound by the ion exchange and therefore could be separated by ion exchange, column chromatography. The reason for such differences between the LDH isoenzymes of two different tissues may be due to the differences in the isoelectric points of the isoenzymes of isoelectrofoccusing. The electrophoretic analysis of these tissues showed only one fraction in each, but the muscle fraction moved faster than the hepatopancreatic fraction. Based on their observations they suggested that muscle D-LDH isoenzymes of *Limulus Polyphemus* function during muscular activity to reduce pyruvate and the heart type isoenzymes are kinetically suited for the oxidation of D-Lactate, The results obtained in the present investigation show two fractions in the muscle tissues of *H.swammerdami* where it is possible that a similar metabolism exists involving both the enzymes. Storey and storey (1979) and Gade (1980a) (1980b) suggested that in cephalopod muscle octopine dehydrogenase (ODH)

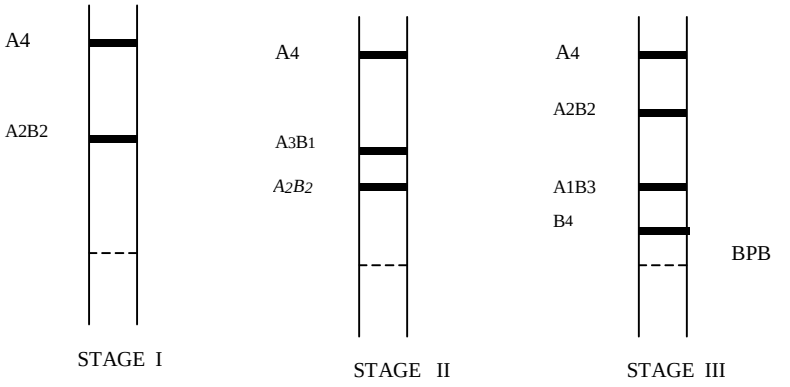

Fig. 4: Electropherograms showing the LDH Pattern of hepatopancreas

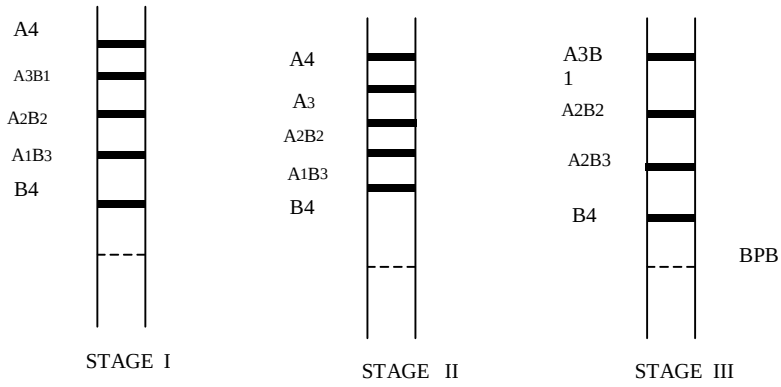

Fig. 5: Electropherograms showing the LDH Pattern of Haemolymph

shows rapid synthesis of octopine, whereas ODH from brain or optic lobe has kinetic properties favoring oxidation of octopine. In both vertebrates and cephalopods the presence of kinetically distinct L-LDH and ODH isoenzymes help with active movement in circulation necessary to transport the product like octopine or lactate to the muscle tissue during oxidation.

A close perusal of results shows that two fractions of LDH, A2B2 and A1B3 from muscle are also present in the haemolymph suggesting that these fractions from muscle may be transported to haemolymph circulating medium from where these LDH fractions may be passed on to the sites of their utilization.

In crustacea, L- lactate is transported into haemolymph from where it may be taken to sites of active metabolism. Philliphine *et.al* (1977) Lensky and Holstien (1969) and Roa (1971) suggested that the haemolymph chemical components may be utilised by various tissues such as ovary in case of female and testis in case of male.The carbohydrate compenents of haemolymph and reproductive tissues,showed a close relationship between these two suggesting the utilisation of haemolymph sugars by reproductive tissues. Similarly Prabhakaran (1973) who studied the glycoprotien component of a scorpion *H.swammerdami* suggests that haemolymph protiens may be utilised, for various physiological process.In the light of these observations, the LDH isoenzymes of reproductive tissue of female ovary were analysed and the results are given in the (Table. 1 and Fig. 1).

The results obtained clearly show the presence of three LDH fractions in the testis as given in (Table. 1 and Fig. 1), of which two belong to medium moving group and other one belongs to fast moving group. The fractions are designated as A4, A2B1 and A1B3. However in the ovary there are four LDH fractions of which two belongs to medium moving group.The fractions are designated as A4, A3B1, A2B2 and B4. Jayalectumie and Subromonium (1987) observed a high LDH activity, in the spermatozoa and equally high concentration of free carbohydrates in the seminal plasma of

Table 1: LDH fractions of different tissues *H. swammerdami*

S. No	Name of fractions	Tissues					
		Cuticle	Haemolymph	Hepatopancreas	Muscle	Ovary	Testis
1	A_4	-	+	+	-	+	+
2	A_3B_1	+	+	-	-	+	+
3	A_2B_2	-	+	+	+	+	-
4	A_1B_3	-	+	-	+	-	+
5	B_4	-	+	-	-	+	-

Table 2: LDH fracions of different tissues of *H. swammerdami* during ovarian development (Stage I)

S. No	Name of fractions	Tissues			
		Ovary	Follicle	Hepatopancreas	Haemolymph
1.	A_4	-	-	+	+
2.	A_3B_1	-	-	-	+
3.	A_2B_2	+	-	+	+
4.	A_1B_3	+	+	-	+
5.	B_4	+	+	-	+

Paratelphusa hydrodromous.They also observed difference in LDH activity of spermatozoa between posterior vasdeferens in the male and spermatheca of the females suggesting that the spermatozoa, when stored within male reproductive tract may rely mostly on anaerobic metabolism utilising the large quantity of lipids.

A similar mechanism exists in *H.swammerdami* also. One of the aspect show that LDH in testis reveals three fractions, of which one of them is compared to the fraction of LDH X of the mammalian spermatozoa, Blanco and Zinkham (1963). In *Rhinophoma kinneeri* bats, Singhvi and Lall (1979) showed six distinct isoenzymes .They were able to locate LDHX in the testicular homogenates and suggested that it denotes initiation and substance of active spermatogenesis, and androgenesis of testis. The ovary has also been studied for LDH isoenzymes in the present investigation and its results obtained from polyacrylamide gel electrophoresis showed four LDH fractions which are designated as A4, A3B1, A2B2 and B4. In a crustacean E.asiatica Prema *et.al* (1988) reported three fractions of LDH in the ovary of *S.serrata*. Prabhakaran *et al* (1988) reported the occurrence of two fractions in ovary of a spider *P.collinus*. The difference in the number of fractions of ovary between the scorpion and spider may be due to difference in the stages of oogenesis .Hence in the present investigation, the LDH isoenzymes were studied during the different stages of oogenesis. For identification of stages of ovary the method of sudha varadharajan and Subromonium (1982) was followed.

The results of electrophoretic analysis of LDH isoenzymes from various stages of oogenesis are given in the table (Table. 2, 3 and 4. Fig. 2). It is found that in stageI there are only three LDH fractions in the ovary. They are designated as A2B2, A1B3 and B4. The fraction corresponding to A4 of the normal female is absent. However in stage II and stage III there are four fractions of LDH. It is of interest to note the disappearance of A4 LDH fraction in stage1.The follicles which also form a part of ovary and where the development of the young ones takes places have also been analysed for their LDH compositions. The results are given in the (Table. 2, 3 and 4. Fig.3). The follicle consists of two LDH fractions namely A1B3 andB4.in stage II and III there are three fractions. An interesting

Table 3: LDH fraction of different tissues of *H. swammerdami* during ovarian development (Stage II)

S. No	Name of fractions	Tissues			
		Ovary	Follicle	Hepatopancreas	Haemolymph
1.	A_4	_	+	+	+
2.	A_3B_1	+	_	+	+
3.	A_2B_2	+	+	+	+
4.	A_1B_3	+	_	_	+
5.	B_4	+	_	_	+

Table 3: LDH fraction of different tissues of *H. swammerdami* during ovarian development (Stage III)

S. No	Name of fractions	Tissues			
		Ovary	Follicle	Hepatopancreas	Haemolymph
1.	A_4	_	+	+	_
2.	A_3B_1	+	+	_	+
3.	A_2B_2	+	+	+	+
4.	A_1B_3	+	_	+	+
5.	B_4	+	_	+	+

point is the disappearance of the fraction A1B3 but appearance of A3B1 in follicle of stage II and stage III. The LDH isoenzymes of the hepatopancreas have been analysed electrophoretically during the three stages of ovarian development and the results are presented in the (Table. 2, 3 and 4. Fig. 4).

The results given in the table it shows that in stage I there are two LDH fractions in the hepatopancreas of *H.swammerdami*. These two fractions are designated as A4 and A2B2 respectively. However, it is interesting to note that in stage II there are three fractions in the hepatopancreas, a new fraction A3B1 appearing in this stage. In stage III it is further significant that a fraction namely B4 also appears. This fraction is absent in stageI and II. These observations suggest that in stage I when the ovarian development starts, the hepatopancreas may supply necessary LDH enzymes in this tissue. However, once the hepatopancreatic enzymes are depleted and the hepatopancreatic cells are activated to synthesize new LDH fractions. This is probably a reason for the new proteins in the hepatopancreas and its utilization in the vitellogenesis of stage II and stage III.

It is known that haemolymph is a transporting medium through which all the chemical substances are transported to the sites of utilization. Hence the LDH composition of haemolymph has been analysed and the results are given in the (Table. 2, 3, 4 and Fig.5). It is seen that there are five LDH fractions in stage I and stage II and are designated as A4, A3B1, A2B2 and B4. It is relevant to recall the observations of (Milne and Doxey 1987) who studied the LDH activity of different tissues of mammals suggested that the isoenzymes of serum may be derived from the liver, a counter part of hepatopancreas .The transport of isoenzymes from the hepatopancreas to the ovary has been suggested by several authors, (Kerr (1969) Anilkumar and Adiyodi (1980) Prabhakaran (1988)) found that hepatopancreas and ovary of *P.collinus* posses a distinct similar LDH fraction suggesting a close relationship between the two and that this particular fraction of hepatopancreas may be transported to haemolymph from where it may be taken up by ovary and utilized for the formation of vitellogenin. In the light of this observation it is suggested that in *H.swammerdami* the hepatopancreatic isoenzymes may be transported to different tissues including ovary through the haemolymph.

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