PROFILE OF LACTIC DEHYDROGENASE AND ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE FROM SEVERAL STAGES OF *DROSOPHILA MELANOGASTER*

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We hereby recommend that the prepared under our supervision by Estelline Daniels Doyle entitled PROFILE OF LACTIC DEHYDROGENASE AND ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE FROM SEVERAL STAGES OF DROSOPHILA MELANOGASTER

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PROFILE OF LACTIC DEHYDROGENASE AND ALPHA-GLYCEROPHOSPHATE DEHYDROGENASE FROM SEVERAL STAGES OF *DROSOPHILA MELANOGASTER*

INTRODUCTION

The object of this research was to establish a qualitative profile for lactic dehydrogenase (LDH) and alpha-glycerophosphate dehydrogenase (α-GPDH), two enzymes existing as isozymes, which may be related to tissue or stage specificity during the development of *Drosophila melanogaster* (Wright and Shaw, 1969). Isozyme is a term used by Markert and Møller (1959) denoting the "different molecular types" of an enzyme. This qualitative profile has been established by specific biochemical-staining techniques for the enzymes, LDH and α-GPDH, separated by the microdiscelectrophoresis technique. This enzyme profile includes stage-specific enzyme assays of whole organisms during different developmental stages.

Organisms commonly synthesize many of their enzymes in multiple molecular forms to fulfill the metabolic needs in different groups of cells or in particular stages in development. Enzymatic heterogeneity gives an organism advantages in the many metabolic sequences involved during development. Isozymes commonly exhibit identical specificity, but differ from other types of the same enzyme in electrophoretic mobility and in characteristics which are

specific for each individual enzyme. The changes in pattern and spatial arrangement of isozymes during development reflect differential gene action which will aid in the correlation of gene activity with phenotypic expression in organisms. Phenotypic diversity between organisms of a given species probably results from the presence of isozymes in enzyme systems.

Development of an organism occurs through an orderly series of changes, each of which exhibits increased complexity. As development progresses the chemical composition of the fertilized egg changes in pattern and spatial arrangement. Chemical analysis of the biochemical composition in the developing organism has allowed investigators to correlate visible morphogenetic changes in the organism with changes in chemical composition.

The processes involved in the developmental phases of organisms have led investigators to three principle areas of study - structural, biochemical, and physiological changes within an organism in efforts to determine the initiators of differentiation or morphogenesis which result in new stages of development. It has been established that the differentiation process is dependent on cellular components present before differentiation occurs. If the cellular components are under gene control then these new

stages of development may be an indirect result of gene function control.

Biochemical investigators have studied qualitative changes and/or quantitative changes in these cellular components present at different times in the development of particular organisms (Claycomb and Villee, 1971; Spiegel et al. 1969; Brosemer, 1965; Spiegel et al. 1965). The soluble fraction of developing embryos is often used to study qualitative changes in proteins, particularly enzymes. Variations in patterns of isozymes in Tetrahymena as well as studies on pH and temperature stability were used by Phillips (1972) to study differences in two distantly related syngens. That cellular components often exhibit stage specificity during development was evidenced by Ilan and Ilan (1971) in their studies of m-RNA. Studies such as the correlation of the distribution of protein and enzymes to morphological studies of the flight muscle sacrosomes combined cytological and biochemical studies to assay developmental changes in the blowfly (Lennie and Birt, 1967).

Enzymes have been one of the classes of cellular components which has been studied extensively (Richmond, 1972; Ayala et al. 1971; Fox et al. 1971; and O'Brien and MacIntyre, 1969). Many of these studies have evaluated changes in the biological activity of different enzymes

which exist during metabolic sequences involved in the developmental phases (Pasteur, 1972; Dickinson, 1971; Rechsteiner, 1970; O'Brien, 1970; Wright and Shaw, 1969; Knowles and Fristrom, 1967). These studies have led to a better understanding of the role of enzymes during development. Murray and Buhse (1971) described the morphogenetic effect of inactive or missing enzymes on the production of pteridines in eye pigment in Drosophila. This lack of activity is easily seen in such mutants as scarlet, vermilion, and cinnabar strains. O'Brien and MacIntyre (1972) isolated an α-GPDH deficient group of mutants. These mutants had no visible phenotype but had low viability and were not able to sustain flight.

Enzymes can serve as important research tools for both the developmental biologist and the geneticist through the existence of isozymes during different stages of development. The developmental biologist can relate isozyme patterns to morphogenetic change and isozymes can serve as markers in ontogeny and phylogeny. Isozymes can serve as genetic markers whose loci on chromosomes are being plotted by genetic investigators in relation to loci already plotted (Sanger and Race, 1971). In addition, isozymes can be used to discover polymorphic systems and for studying gene action (Vesell, 1968).

Medvedev (1970) described a universal enzyme system which functions in metabolic processes such as glycolysis, oxidation, and phosphorylation. A special enzyme system appears during embryogenesis relating to the development of a specialized function of organs such as liver, kidney, and nerve tissue. In Drosophila, it appears that LDH and α-GPDH are associated with muscle tissue in the role of special enzymes and in other stages of development or in other organs may serve as universal enzymes.

The enzyme LDH was one of the first enzymes known to exist in multiple molecular forms. LDH catalyzes the interconversion of pyruvate and lactate, and concurrently the interconversion of reduced nicotinamide adenine dinucleotide (NADH) and oxidized nicotinamide adenine dinucleotide (NAD^+). In mammalian tissue LDH exists in five forms. LDH has been found to be under the control of two genes in mammals, with a third gene active in the production of the LDH isozymes in sperm. The M type of isozymes is predominate in muscle and embryonic tissues which utilize glycolysis for the production of energy. The H type of isozymes occur more often in aerobic and respiratory metabolism (Markert, 1968). According to Lehninger (1972) the synthesis of LDH isozymes is coded in the genetic information so that sufficient energy is

present at different stages of development or cell differentiation.

Rechsteiner (1970b) found the level of LDH greater in the larval stage of D. melanogaster while the level of ∝-GPDH increases in the adult organism. This indicates that the activity of the enzymes vary with the function of tissue with which it is associated. Since LDH functions in the metabolism of muscles in vertebrates it was concluded that LDH functions in the metabolism of larval muscles.

Holometabolous insects have a pupal period between the larval and the adult stage. Insects such as D. melanogaster live in two different habitats during their life cycles. Metamorphoses occur between the larval stage and the adult organism and result in continual morphological changes and apparent changes in the enzyme composition which can be observed colorimetrically or electrophoretically (O'Brien and MacIntyre, 1972; Rechsteiner, 1970a and 1970b; Marquardt and Brosemer, 1966; Brosemer, 1965; Estabrook and Sacktor, 1958). During the larval stage the organism is buried in the media and is actively feeding to store the nutrients necessary to maintain the organism during the pupal period. This rapid nutrient accumulation may result in a condition of oxygen deprivation. However, it is not known if this condition is totally due to oxygen

deprivation or to inadequate tracheation in the larvae (Rechsteiner, 1970b). In this stage of development the tissue and the enzyme which is associated with it in the metabolic sequences in D. melanogaster resembles the metabolic sequence in muscles of vertebrates. During the pupal period complete destruction of the larval musculature occurs and concurrently activity of LDH decreases while activity of α-GPDH increases.

α-GPDH is important in the metabolism of insect flight muscles and catalyzes the oxidation of alpha-glycerophosphate (α-PO_4) to dihydroxyacetone phosphate (DHAP) and the reverse reaction (Grell, 1967). α-GPDH serves in the glycerolphosphate shuttle in insects as a source of reducing activity from the extramitochondrial NADH to the intramitochondrial respiratory chain. This shuttle is unidirectional and transports reducing equivalents into the mitochondria particularly in insect flight muscles. This is in direct contrast to the muscle systems of vertebrates which possess the glycolytic system and utilize LDH as the source of reducing power. Adequate tracheation in insect flight muscles is thought to supply sufficient oxygen to allow α-GPDH to function efficiently. Zebe and McShan (1957) found that insect flight muscles function more efficiently over longer periods of time than other flying organisms, particularly birds. Rechsteiner (1970) found

the activity of α-GPDH to increase in late pupae. Perhaps this increase in activity can be correlated with the development of the adult flight muscle which occurs approximately eighty-four hours after puparium formation. According to Brosemer et al., (1967) the increase in enzyme activity in the adult honey bee was due to an increase in the number of enzyme molecules since the immunological properties of the six-day old bee was identical to that of bees which had recently emerged. The activity of α-GPDH seems rather unimportant in the production of energy in the vertebrates, but in insects the reactions catalyzed by α-GPDH have high physiological significance.

D. melanogaster contains three isozymic forms of α-GPDH (Wright and Shaw, 1969). GPDH-1 is most concentrated in the adult thorax while GPDH-3 is found in lesser amounts in the abdomen. The head was found to contain approximately equal amounts of GPDH-1 and GPDH-3. In the egg and larvae only GPDH-3 is present and is thus associated with LDH activity. GPDH-3 may function in periods of oxygen deprivation as does LDH. In this stage of development it is possible that GPDH-3 may function to produce precursors for lipid biosynthesis.

Many isozymes exist in fast and slow variants. Wright and Shaw (1969) observed the fast and slow form of α-GPDH in D. melanogaster. Oregon-R exhibits the slow type of

isozyme and Muller-5 exhibits the fast type of isozyme. As a result of these studies it was proposed that the isozymes of α-GPDH in Drosophila are apparently controlled by a single genetic locus.

Further evidence of the existence of fast and slow types of isozymes was observed by Aspinwall (1973) in his studies with the pink salmon. From this study he concluded that α-GPDH in the salmon is coded by a single autosomal locus with two codominant alleles. It is not known if the activity of another gene may be blocked, or if it may be tissue specific in its function. Further evidence of this theory was expressed by Apella and Zito (1968). They observed that LDH activity in the embryo may be under the control of one gene while another gene may be active in the control of LDH later in the developmental period.

D. melanogaster is often used as a research tool because of its short life cycle, ease in culturing, and its low chromosome number. As a result of the low chromosome number, it is relatively ease to produce mutants in this genus. These mutants exhibit variant characteristics that facilitate genetic and developmental studies. One such variant characteristic is vestigial (vg) wings which is due to the presence of a single pair of recessive genes. The wings of this strain lack normal development (Lindsley

and Grell, 1967), and it is classed as a biochemical mutant in the category of developmental time (Sober, 1970). Fristrom (1968) examined the "wild type" and the vestigial strains with electron microscopy during the stage when wing development is occuring. Numerous degenerating cells were observed in the wing blade region of the vestigial and none were observed in the "wild type" strain. Mutant organisms such as vestigial may make a correlation possible between morphological events in the various stages of development and biochemical changes within the developing organism. In addition, D. melanogaster is a multicellular organism whose genome is midway in complexity between that of Escherichia coli and mammals. Thus Drosophila is a good choice for developmental studies (Schneiderman, 1971).

Assays of enzyme patterns have reflected changes in morphology that occur during the life cycle of insects. These studies when used concurrently with appropriate cytological and physiological studies may identify why a cell(s) suddenly stops replicating and differentiation is initiated. Thus the mechanisms involved in the development of complex organisms may be elucidated.

MATERIALS AND METHODS

Experimental Material

Two strains a "wild type" and a mutant of *Drosophila melanogaster* routinely maintained in the Genetics Laboratory at Texas Woman's University were used in this research. Both strains have been inbred for several years and are assumed to be nearly homozygous. The "wild-type" strain Oregon-R (O-R) and the mutant strain vestigial (vg) were assayed to compile a qualitative profile of the enzymes lactic dehydrogenase (LDH) and α-glycerophosphate dehydrogenase (α-GPDH).

Synchronization and timing of different stages was achieved by transferring adult flies to fresh media twenty-four hours before the desired collection period. During the collection period the flies were allowed to remain on sucrose-yeast media for four hours thus allowing ± 2 hour variance in all stages collected except eggs. Rechsteiner (1970b) found no measurable change in the egg until the twentieth hour of development. Because of the difficulty in collecting sufficient sample the eggs collected in this research were 6 ± 3 hours in development. Eggs for different developmental stages were allowed to develop normally at 25-26 C in an environmental chamber (Lab-Line Instruments, Inc. #706). During the experimental period a normal light

day and night was maintained. Zero time for the developmental staging was the time of oviposition as described by Duke and Pantelouris (1963). Although their experimental conditions were not described, their stages of development correlates with those obtained with the experimental conditions in this laboratory. In addition to the timed staging, larvae were staged morphologically by the method of Schuellein (1954). Pupae were staged visually with the method described by Rechsteiner (1970b). His method allowed larvae to be divided into 144 hour "non-floating" and 168 hour "floating" samples. Under the experimental conditions described, larvae everted their spiracles at 130 ± 6 hours.

The material to be electrophorised was homogenized in a hand held Kimax #43910 tissue grinder for 30 seconds. Each sample was homogenized in one ml of 0.25M sucrose with the addition of a few crystals of Phenylthiourea (Sigma). The samples were as follows:

10 mg	eggs
80	first instar larvae
30	second instar larvae
20	third instar larvae
14	mid-third instar larvae
7	late-third instar larvae
15	one day old or 15 ten day old adults

In experiments involving pupae seven "non-floating" or white pupae or fourteen "floating" pupae were homogenized as described above. Throughout the experimental period, different stages were collected and used immediately or stored at -20 C.

The homogenate samples were centrifuged at room temperature in a Sorvall centrifuge, Model NSE, Rotor head M at 600 RPM for eight minutes and the speed was then increased to 3200 RPM for five minutes. Ten μl of the supernatant was applied directly to the stacking gel.

Electrophoresis

The electrophoresis procedure utilized in this research was a modification of the method used by Pasteur (1972). This method involved modifications of electrophoresis equiptment in the Genetics Laboratory and at the same time allows determinations of proteins and enzymes in small amounts of sample. With organisms such as Drosophila this technique makes it possible to analyze a few organisms, or in some stages only a single organism.

Electrophoresis was carried out with a Joyce and Loebl Disc Electrophoresis unit # DO 95270/2/J with two-hole stoppers adapted as tube holders. This adaption allowed sixteen tubes to be processed in a single buffer tank instead of the usual eight tubes. Late in the experimental

period a second buffer tank was obtained and thirty-two tubes were processed thus allowing more samples for each staining procedure used in each replication. Constant current of 0.6 mA per tube was supplied in each electrophoretic run with an average running time of 35 minutes. The assays were run at room temperature with precooled buffer (Canalco RDS-H premixed buffer) in the buffer tank to offset any heat produced during the run. The run was stopped when the tracking dye reached 2-3 mm from the bottom of the tube. When the second buffer tank was obtained all the gels were removed from the tanks and were placed in a container of cold deionized water. The gels were removed randomly and no differences was detected in the pattern of the bands obtained when compared with those run prior to this time. Dry ice was used in one experiment to prevent diffusion of the protein bands, but this procedure resulted in broken gels. The gels were removed from the glass tubes by inserting a dull one ml syringe needle which was filled with cold distilled deionized water between the wall of the tube and the gel. The gel was rotated while water was forced into the tube until the tracking dye (Bromphenol Blue, Sigma) appeared to move. The tube was then attached to a rubber tube fitted to a 2½ or 10 ml syringe and the gels were pushed from the glass tube into a container of water. The gels were usually removed with little

except for the stacking gel which was more easily broken.

Electrophoresis tubes were cut from 2 mm quartz tubing (Wale Apparatus Co.) in five and one-half cm lengths. The tubes were cleaned in chromerge-sulfuric acid solution overnight, rinsed in plain and distilled water, and finally in RDS-K tube rinse (Canalco). Tube holders were constructed as described previously by Pasteur (1972) and the tubes were inserted into parafilm in the holders in a similar manner.

The various stock solutions used in preparing the gels were prepared according to Canalco, Inc. (1968) formulations. The final concentration of separating gel was 7.5%. These gels were prepared fresh daily or stored under buffer in the refrigerator. The gels were stored no longer than three days. The 3% stacking gel was made the day the gels were run. The tubes were run within an hour after polymerization of the stacking gel. According to Maurer (1971) certain procedures must be followed to make electrophoresis meaningful as an assay technique.

Tubes were filled with separating gel solution to a level predetermined on "intramedic polyethylene" tubing attached to a 2½ ml disposable syringe. A flat gel surface is necessary if the protein discs obtained are to be useful in determining relative mobilities. Careful layering of water over the gel is necessary to remove the meniscus and

achieve the desired flat surface. After polymerization the water is removed with a finely twisted wick of Kimwipe which must not touch the gels because lint in the gels can cause distortion in the bands. One cm of stacking gel was then introduced and layered with water as previously described. Photopolymerization starts in fifteen minutes and is complete in one hour. No sample gel was used in this technique but with this method it was necessary to layer buffer carefully over the sample to prevent diffusion of the sample into the upper buffer solution.

Staining Procedure

The gels stained for non-specific proteins were rinsed in water and incubated in 12% trichloroacetic acid for fifteen minutes. They were then placed in a 0.25% (w/v) Coomassie Brilliant Blue R (Sigma) dissolved in 20% trichloroacetic acid (Kuchler, 1972) for 30 minutes. Unbound stain was diffused from the gels in a few hours in 7% acetic acid. The gels can be stored indefinitely in 7% acetic acid.

To demonstrate LDH and α-GPDH the following staining procedures (Pasteur, 1972) were adapted. The gels were incubated at room temperature for a period of two hours in the dark for each of the staining solutions used (see Table 1). These conditions were most effective in eliminating

Table 1. Preparation of stains

	LDH	α-GPDH	
		pH 8.5	pH 4.8
Tris-HCl buffer 1mM pH 8.5	3.5 ml	3.5 ml	
Sodium acetate buffer 0.05M pH 4.8			3.5 ml
Potassium Cyanide (KCN) 0.1mM (Baker)	0.5 ml	0.5 ml	0.5 ml
NAD^+ 1.5 mg/ml (Sigma)	0.5 ml	0.5 ml	0.5 ml
Nitroblue tetrazolium (NBT) 7 mg/ml (Sigma)	0.5 ml	0.5 ml	0.5 ml
Phenazine methosulfate (PMS) 2 mg/ml (Sigma)	0.07 ml	0.07 ml	0.07 ml
Sodium-DL-lactate 50 - 60% (Sigma)	360 mg		
DL- -glycerophosphate (Sigma)		1.25 mg	1.25 mg

1. KCN and NBT were mixed in separate solutions with distilled water.

2. NAD^+ was mixed in buffer of pH 8.5 or pH 4.8. The solutions were discarded after one week.

3. PMS was mixed in distilled water and stored in the dark. The solution was discarded after two weeks.

4. Substrates were weighed and the solutions mixed in the above order the day the stains were to be used.

5. All solutions were stored at 5 C until used.

bands which were not substrate specific and appeared in the absence of a substrate in the staining solution.

During staining for α-GPDH two different pHs were used in the staining mixtures (see Table 1). At a pH of 4.8 the gels were preincubated for 15 minutes in 0.05M sodium acetate buffer. No preincubation was required for staining at pH 8.5. Following the staining period the staining solutions were decanted and the gels stored in a 2% acetic acid solution. Due to the lack of information concerning the stability of these stained bands, the gels were analyzed visually within a three-day period from the time they were run. The gels were stored at 5 C in the refrigerator so the bands could be compared during the experimental period.

RESULTS

Non-Specific Proteins

Zymograms of total protein content of two strains of Drosophila melanogaster reveal both spatial and quantitative changes as the complexity of the organism increases with each successive developmental stage.

Figures 1 and 2 summarize the stages observed in zymograms stained with Coomassie Blue during the electrophoresis of whole-fly and section homogenates for Oregon-R and vestigial during the developmental stages studied.

Comparing the bands between stages of the two strains is difficult particularly when closely spaced bands are involved. No quantitation has been attempted because many closely moving bands appear as one disc and thus could be misinterpreted.

Lactic Dehydrogenase

The staining procedure for lactic dehydrogenase and α-glycerophosphate dehydrogenase involves a series of coupled reactions similar to those in Figure 3. As the reaction proceeds formazan is precipitated resulting in a blue color in the gel. The color intensity observed in this reaction is an indirect method to detect the amount of enzyme activity in each sample.

Two bands of LDH activity appear in the second instar,

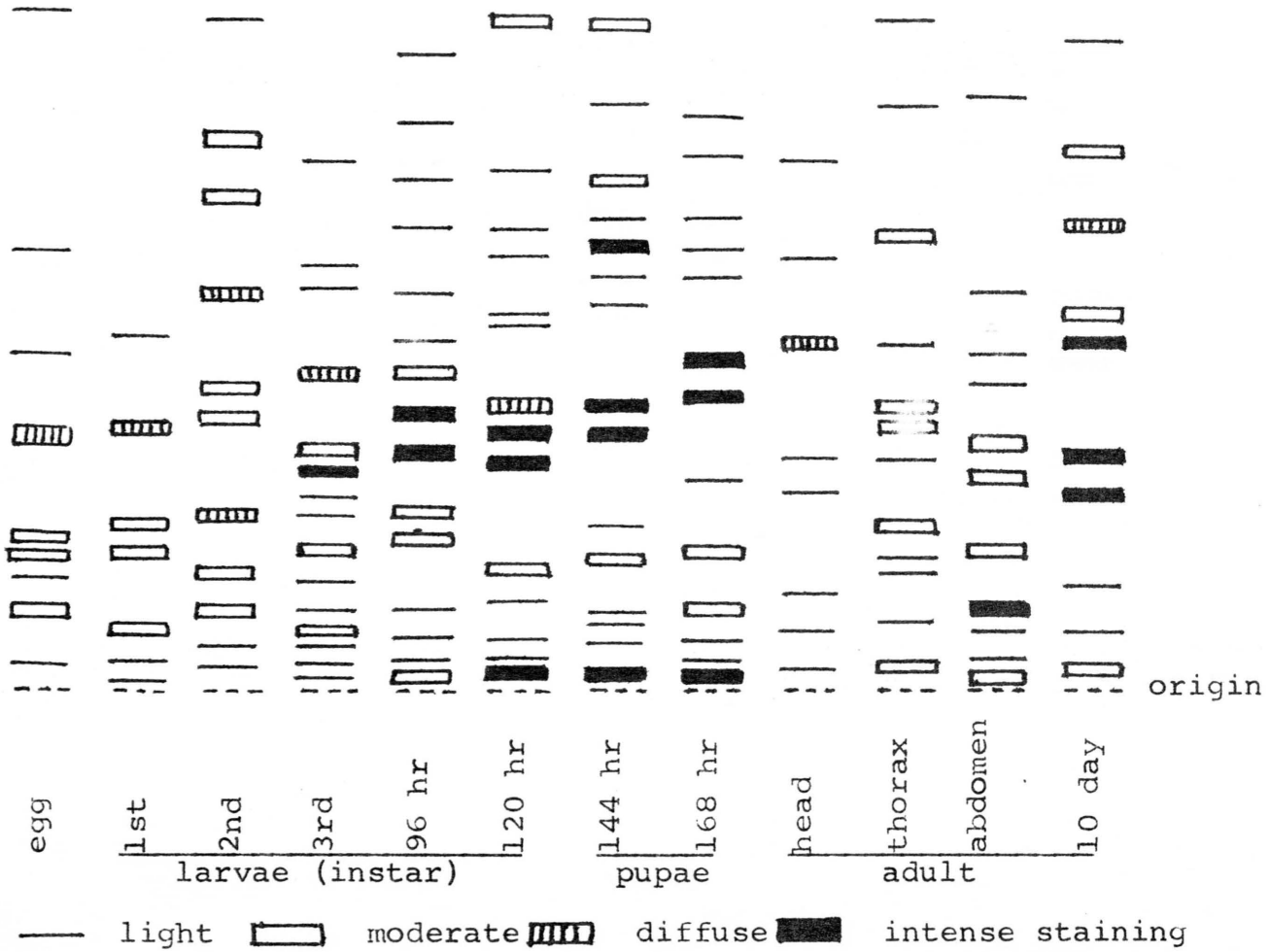

Figure 1. Staining pattern of non-specific proteins in vestigial strain

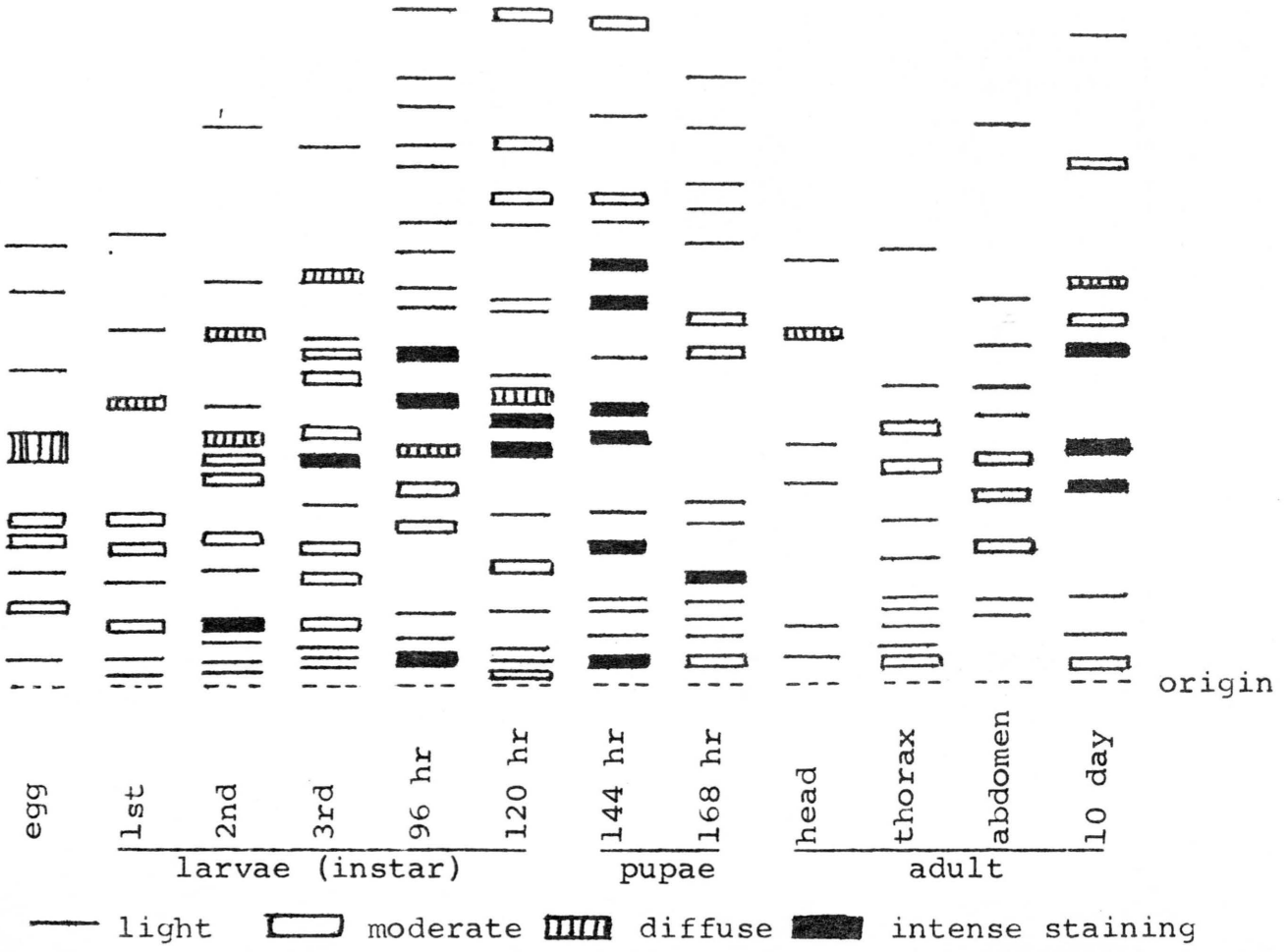

Figure 2. Staining pattern of non-specific proteins in Oregon-R strain

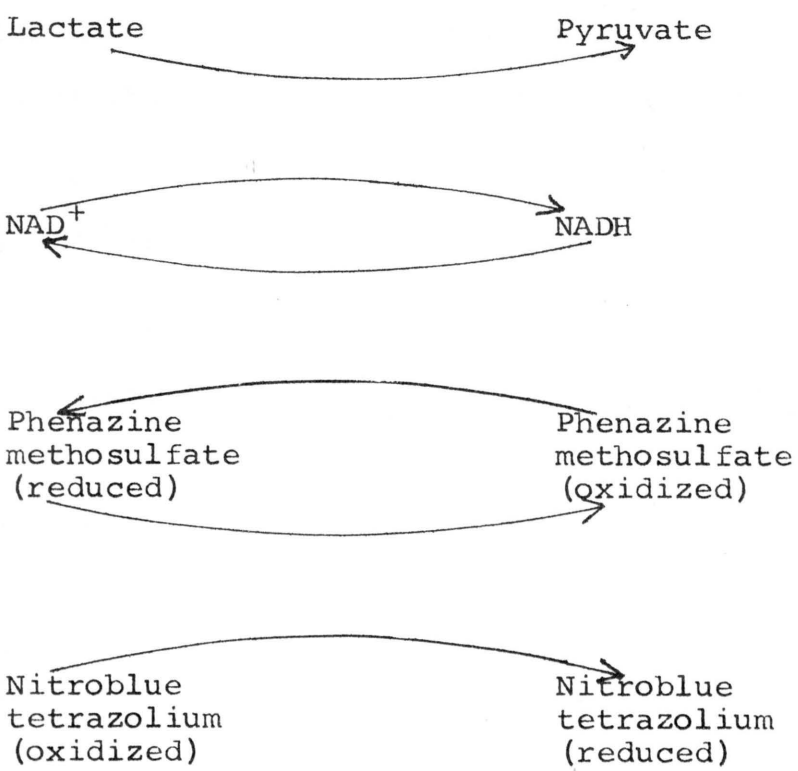

Figure 3. Series of reactions occuring during visualization of lactic dehydrogenase and α-glycerophosphate dehydrogenase.

third instar, and the adult organism. No LDH activity was detected in the egg and only one band was observed in the first instar and the pupal stages (Table 2). A visual examination of the staining intensity of the enzyme bands on a scale of 0 - 4 (Table 2 and Table 3) has been used to compare bands.

α-Glycerophosphate Dehydrogenase

Only one band of α-GPDH activity was observed in all the stages studied in this research. At a pH of 4.8 the activity of α-GPDH (Table 3) decreases which indicates that inhibition is occuring at this pH as described by Pasteur (1972). More intensely stained bands were observed in the electrophoretic run which contained samples of thoracic sections. The thoracic α-GPDH band had an R_m value of 25.3 and had an intensity of 3.0 in the Oregon-R strain. In the vestigial strain the R_m value was 31.3 and the intensity was 4.0. No activity was observed in gels containing head sections and only slight activity was observed in the gels with samples of the abdomen.

Tests for non-substrate specific bands were carried out routinely for both specific enzyme staining techniques. Since these small gels could not easily be split, whole gels from the same electrophoretic run were incubated in the absence of substrate. Other gels in the same run

Table 2. Average R_m values[1] and band intensity[2] for lactic dehydrogenase

	Strain							
	Oregon-R				vestigial			
	Band I		Band II		Band I		Band II	
Stages	R_m	Intensity	R_m	Intensity	R_m	Intensity	R_m	Intensity
egg	-	0	-	0	-	0	-	0
1st instar	32.0	1.5	-	0	34.9	2.0	57.1	1.0
2nd instar	25.0	2.0	40.0	1.0	32.0	3.0	50.0	1.0
3rd instar	30.4	3.5	46.5	1.0	32.5	3.5	46.5	1.0
96 hour	38.8	3.0	54.2	1.0	34.5	3.0	53.7	1.0
120 hour	31.3	2.5	45.2	1.0	34.2	2.5	47.8	1.0
144 hour	33.0	2.5	54.5	1.0	33.7	2.5	-	0
168 hour	32.8	1.5	-	0	32.2	2.0	-	0
1 day adult	32.8	1.5	32.0	1.0	26.7	1.5	39.8	1.0
10 day adult	28.4	1.0	44.8	2.0	28.7	1.0	42.9	1.5

1. R_m X 100
2. Staining intensity on a scale of 0 - 4

Table 3. Average R_m values[1] and band intensity[2] for α-glycerophosphate dehydrogenase

Stages	Strain							
	Oregon-R				vestigial			
	pH 8.5		pH 4.8		pH 8.5		pH 4.8	
	R_m	Intensity	R_m	Intensity	R_m	Intensity	R_m	Intensity
egg	-	0	-	0	-	0	-	0
1st instar	27.0	1.0	-	0	20.0	1.0	-	0
2nd instar	-	0	-	0	35.0	1.0	-	0
3rd instar	30.8	3.5	30.6	1.5	33.0	1.5	34.3	1.5
96 hour	37.0	2.5	34.4	1.5	37.0	3.0	34.4	1.0
120 hour	28.3	2.5	29.0	1.5	32.0	3.0	29.5	1.5
144 hour	31.0	2.5	30.2	1.0	34.3	4.0	41.7	1.0
168 hour	24.0	1.0	-	0	-	0	-	0
1 day adult	29.9	2.0	35.8	1.5	34.5	2.5	42.2	1.0
10 day adult	26.7	4.0	31.1	1.5	35.9	3.0	31.5	2.0

1. R_m X 100
2. Staining intensity on a scale of 0 - 4

were incubated and were stained specifically for the enzymes LDH and ∝-GPDH.

Gels stained in the absence of a specific substrate for LDH or ∝-GPDH exhibited only one faint slowly moving band. This band migrated approximately 0.5 mm into the separating gel and was omitted from the results. Some investigators have called this band "nothing dehydrogenase", but it was described by Hare (1973) as "formazan oxidase".

DISCUSSION AND CONCLUSION

Non-Specific Proteins

The visible changes in morphology of a developing organism may be related to changes in the biochemical composition within the organism where sufficient data exists to correlate these events. The Oregon-R and vestigial strains of Drosophila melanogaster show both qualitative and quantitative differences during the developmental stages studied from the egg to the adult.

As more research on different proteins and enzyme systems is accomplished, the non-specific protein bands shown in Figure 1 and Figure 2 may be identified and their activity correlated with specific stages of development.

Lactic Dehydrogenase

The activity of LDH reaches its highest level during the third instar and remains high until pupation occurs. LDH activity then decreases and remains low throughout the remaining life cycle studied (Figure 4). Pasteur (1972) in her studies of Drosophila pseudoobscura found two bands of activity in the adult stages. Although Rechsteiner (1970a) identified only one band of LDH in D. melanogaster, he theorized that two bands should exist. As shown in Table 2 two bands of activity were found in some stages and only one in others. The second band observed in this

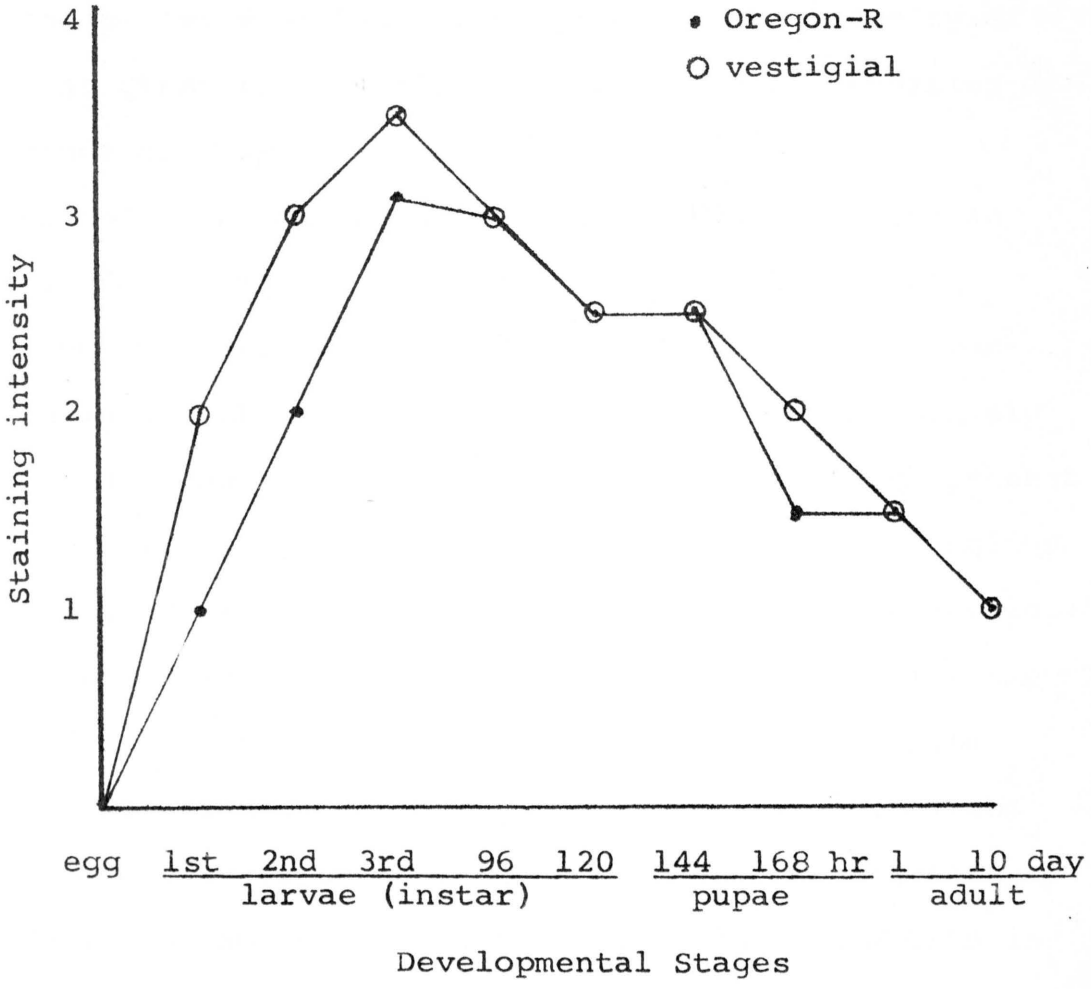

Figure 4. Staining intensity of lactic dehydrogenase in different developmental stages of Oregon-R and vestigial strains.

may be a result of a different electrophoresis technique. The isozyme designated as Band II was not easily detected in the pupal stage. However, an increase does occur such that the activity of Band II compared to the activity of Band I is greater. In this adult stage the intensities of both bands are low.

Based on the developmental pattern shown by LDH in Table 2, Band I may be involved in the metabolism of larval musculature because it is in its greatest concentration in the third instar when the organism is actively feeding and growing. Rechsteiner (1970b) found LDH present in histological sections in the brain and ventral ganglion of larvae. Thus Band II may be connected with the development of brain and nerve tissue. This may account for the increase in intensity of Band II in the adult organism. Histological studies of these same stages may reveal the site of activity of these enzymes in the whole organism.

The zymogram patterns exhibited by LDH and α-GPDH is shown in Figure 4 and Figure 5. This is nearly the same pattern observed by Rechsteiner (1970b) in spectrophotometric assays of Oregon-RCH and Chicago strains of D. melanogaster.

The low level of LDH observed in the adult organism does not fit the pattern observed in other enzyme systems such as alcohol dehydrogenase (unpublished results, Bazan,

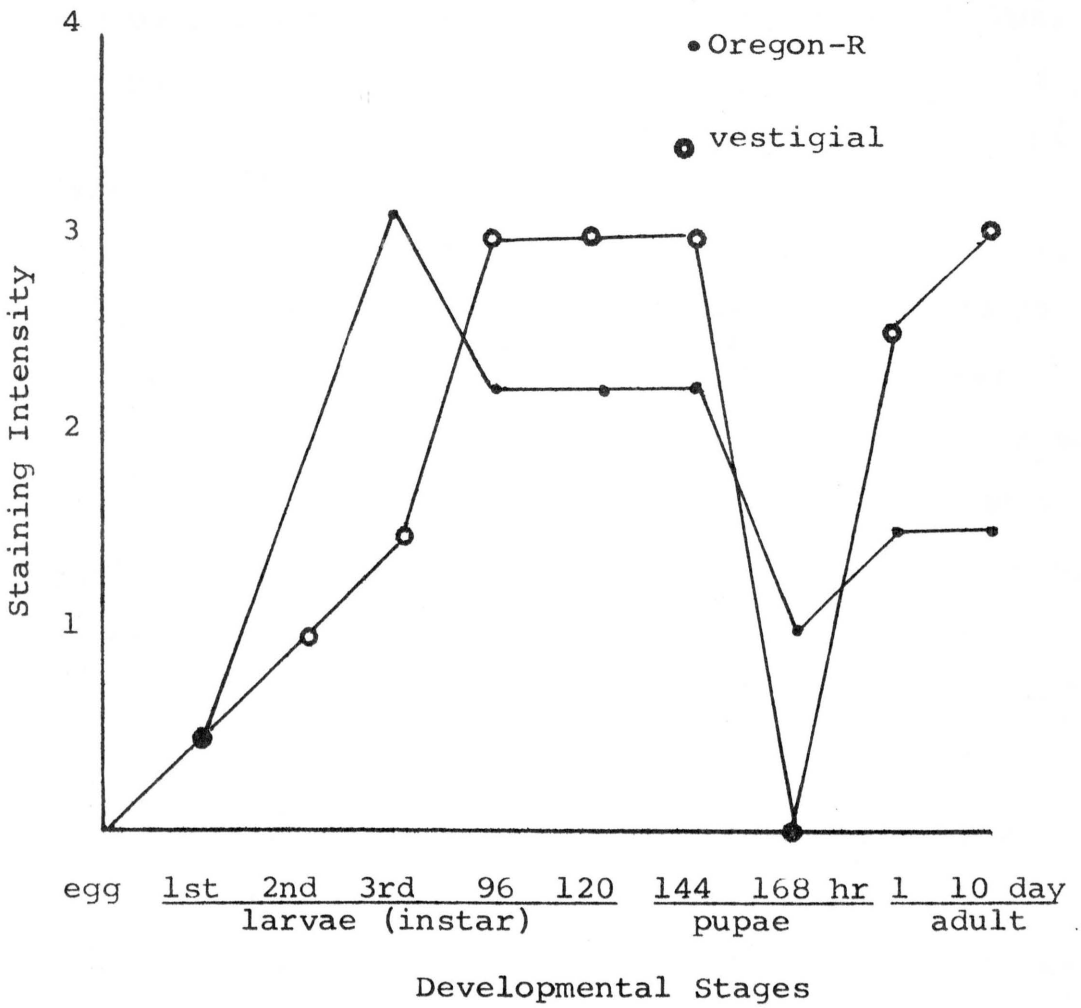

Figure 5. Staining intensity of α-glycerophosphate dehydrogenase in different developmental stages of Oregon-R and vestigial strains.

1973), α-GPDH (Rechsteiner, 1970b), and isocitric dehydrogenase (Fox, 1971). The metabolic role of LDH in the larval stage may be assumed in the adult organism by α-GPDH (Fox, 1971) in the metabolism of insect flight muscle.

α-Glycerophosphate Dehydrogenase

The band of α-GPDH observed in this research must represent the soluble form because no attempt was made to sonicate the homogenate or solublize it with detergents. The activity of α-GPDH as shown in Table 3 increases during the second and third instar larval stages (named α-GPDH-3 by Wright and Shaw, 1969), decreases during the early pupal stages, and once again increases with the eclosion of the adult. The level of α-GPDH observed soon after eclosion remains constant throughout the life cycle studied.

Lipids are being stored in the fat body during the larval stages, thus it can be assumed that α-GPDH is catalyzing the reaction which involves formation of alpha-glycerophosphate (α-GPO4), a precursor in lipid biosynthesis (Rechsteiner, 1970b). In this stage it is theorized that only the soluble fraction of α-GPDH catalyzes this reaction. Although cytoplasmic and mitochondrial α-GPDH are necessary in the coupled reactions involved in the metabolism of insect flight muscle, only one band of activity was observed in the adult organism in this research.

More research will be necessary to resolve the identity of the α-GPDH isozyme observed in this research. Such methods could include the addition of a detergent to the homogenate to solublize the mitochondrial fraction of α-GPDH and the subsequent deletion of NAD^+ from the staining solution to identify the mitochondrial band. Specific activity of this enzyme may be determined with densitometric measurements or spectrophotometric assays.

α-GPDH must portray a dual role during the development of D. melanogaster. In Figure 5 it can be seen that the enzyme α-GPDH is present in the larval stages and at the same time is probably not associated with the metabolism of larval muscles. Its activity decreases in the pupal stages when histolysis of the larval fat body is occuring. The activity of α-GPDH increases rapidly in post eclosion and remains high throughout the life cycle thus supplying the energy necessary for metabolism of insect flight muscles.

SUMMARY

Morphogenetic changes in a developing organism are reflected in changes in biochemical components within the organism. These components can be identified with biochemical assays. The variations observed during the life cycle of an organism can be correlated to morphological events.

Proteins and enzymes are the result of a gene(s) activity. Thus the patterns established by non-specific proteins and specific enzymes such as lactic dehydrogenase and α-glycerophosphate dehydrogenase reflect differential gene action during the life of an organism. LDH and α-GPDH function by supplying energy in different stages of the life cycle of insects. Only one metabolic role has been identified for LDH while α-GPDH functions in a dual role to provide energy and to supply precursors for lipid biosynthesis.

Non-specific proteins vary in qualitative and quantitative patterns during the development of two strains of Drosophila melanogaster. More research will be necessary to relate the differences observed to morphogenetic changes in the organism.

Two bands of LDH activity appeared at different stages of development. One LDH band is thought to function in larval muscles and is most concentrated in the third instar

stage. During pupation the level of LDH decreases and remains low throughout the life cycle. A second band which exhibits faint activity in the larval stages increases slightly in intensity in the adult stages. This band may be associated with energy formation in brain and nerve tissue.

α-GPDH activity is present in the second and third instar when the organism is known to be storing lipids in the fat body as a source of energy during the pupal period. The activity of α-GPDH decreases during the early pupal period and increases in the late pupal stage. The level of α-GPDH in the adult is greater than the activity of LDH in these same stages.

The enzyme profiles observed in this research need additional study to define their role in relation to morphogenetic and cytological changes occuring within the developing organism.

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